Endothelial Lipase Promotes the Catabolism of ApoB-Containing Lipoproteins

Uli C. Broedl, Cyrille Maugeais, John S. Millar, Weijun Jin, Ryan E. Moore, Ilia V. Fuki, Dawn Marchadier, Jane M. Glick, Daniel J. Rader

Abstract—Endothelial lipase (EL) has been found to be a key enzyme in high-density lipoprotein (HDL) metabolism in mice, leading to the concept that inhibition of EL could be a novel strategy for raising HDL cholesterol levels. However, mice are "HDL animals" and the effect of EL on atherogenic apoB-containing lipoproteins has not been elucidated. We previously found that EL is capable of hydrolyzing very low-density lipoprotein (VLDL) and LDL lipids ex vivo. To investigate the role of EL in the metabolism of apoB-containing lipoproteins in vivo, we expressed human EL in three mouse models of elevated apoB-containing lipoproteins: apoE-deficient, LDL receptor–deficient, and human apoB transgenic mice. Unexpectedly, hepatic expression of EL resulted in markedly decreased levels of VLDL/LDL cholesterol, phospholipid, and apoB accompanied by significantly increased LDL apolipoprotein and phospholipid catabolism. To determine whether lipolytic activity is required for this effect, we also expressed a catalytically inactive form of human EL (EL^{S149A}); unexpectedly, expression of EL^{S149A} did not lower and in fact increased plasma lipids. Coexpression and coimmunoprecipitation studies suggested that catalytically inactive EL^{S149A} inhibits endogenous mouse EL, accounting for the increased lipid levels. We conclude that (1) in addition to its known effects on HDL metabolism, EL influences the metabolism of apoB-containing particles; (2) catalytic activity of EL is required for its effects on apoB-containing lipoproteins; and (3) overexpressed catalytically inactive EL inhibits endogenous mouse EL, resulting in increased levels of plasma lipids. In light of these results, inhibition of EL has the potential to raise levels of atherogenic lipoproteins in addition to HDL-C levels. **(*Circ Res*. 2004;94:1554-1561.)**

Key Words: lipase ■ lipids ■ lipoproteins

Endothelial lipase (EL), a new member of the triglyceride lipase gene family,[1,2] has been recently shown to be a key enzyme affecting high-density lipoprotein (HDL) metabolism. Hepatic overexpression of EL using adenoviral vectors resulted in markedly reduced HDL cholesterol (HDL-C) levels in mice.[1] Transgenic overexpression of EL under the control of the endogenous promoter resulted in modestly reduced HDL-C levels.[3] Conversely, inhibition of mouse EL activity in wild-type, apoA-I, and hepatic lipase (HL) knockout mice using a specific antibody resulted in significantly increased HDL-C and phospholipid levels.[4] In the EL knockout mouse model, total cholesterol, HDL-C, and phospholipids were significantly increased.[3,5] Therefore, EL is considered to be an attractive target for pharmacological inhibition as a novel approach to raising HDL cholesterol levels.

Surprisingly little data, however, are currently available about the potential effects of EL on the metabolism of apoB-containing lipoproteins in vivo. In vitro, EL has been shown to be capable of hydrolyzing chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL[6] and of bridging VLDL and LDL to heparan sulfate proteoglycans (HSPGs).[7] In vivo studies of EL overexpression and loss-of-function have been largely performed in mice that have very low levels of apoB-containing lipoproteins, making it difficult to assess the effects of EL on the metabolism of this class of lipoproteins. Conflicting results have been reported for the EL knockout mouse models. In one report, LDL cholesterol (LDL-C) levels in male (but not female) EL knockout mice were increased,[3] but in another, there was no significant difference in levels of apoB containing lipoproteins, even when fed a high-cholesterol diet.[4]

The present study was therefore designed to (1) investigate the effects of EL on the metabolism of apoB-containing lipoproteins in appropriate mouse models and (2) to determine whether catalytic activity of EL is required for its possible effects on apoB-containing lipoproteins. We used recombinant adenovirus to express both wild-type EL and catalytically inactive EL in apoE-deficient, LDL receptor knockout, and human apoB transgenic mice. Overexpression of EL resulted in significantly increased postheparin plasma phospholipase activity associated with significantly reduced VLDL/LDL cholesterol and phospholipid as well as apoB levels, a shift to smaller lipid poor LDL particles, and

Original received November 25, 2003; revision received April 20, 2004; accepted April 22, 2004.

From the University of Pennsylvania, Philadelphia, Pa.

Correspondence to Daniel J. Rader, MD, University of Pennsylvania Medical Center, 654 BRB II/III, 421 Curie Blvd, Philadelphia, PA 19104-6160. E-mail rader@mail.med.upenn.edu

Circulation Research **is available at http://www.circresaha.org** **DOI: 10.1161/01.RES.0000130657.00222.39**

accelerated catabolism of LDL apolipoprotein and LDL phospholipids. Unexpectedly, overexpression of catalytically inactive EL resulted in increased levels of VLDL/LDL cholesterol and phospholipid and decreased catabolism of LDL-phospholipids, possibly because of inhibition of endogenous mouse EL.

Materials and Methods

Generation of Adenoviral Constructs

The catalytically inactive EL (EL^{S149A}) was generated by mutation of the catalytic triad (Ser149-Asp173-His254) replacing the active serine 149 by alanine as previously described.[8] Recombinant adenoviral vectors encoding human EL (AdEL), catalytically inactive human EL ($AdEL^{S149A}$), and mouse EL (AdmEL) were constructed as previously described.[1,4,8]

Animals

ApoE (n=4/group) and LDL receptor knockout mice (n=4/group) were obtained from the Jackson Laboratory (Bar Harbor, Maine). The protocols for mice have been approved by the University of Pennsylvania Animal Care and Use Committees (IACUC) and meet their standard guidelines. Human apoB transgenic mice (n=5/group) were originally obtained as a gift from S.G. Young (Gladstone Institute of Cardiovascular Disease, San Francisco, Calif). All mice were fed a chow diet. 1×10^{11} particles of AdEL, $AdEL^{S149A}$, or a control virus (Adnull) were administered via the tail vein on day 0 of the study. For blood sampling, mice were fasted for 4 hours. Mice were bled from the retroorbital plexus 1 day before and several time points after virus injection. Postheparin plasma samples were obtained at day 3 after virus injection 5 minutes after tail-vein injection of heparin (100 U/kg). EDTA (Invitrogen) to a final concentration of 8 mmol/L was added to the tubes as an anticoagulant.

In Vivo LDL Metabolic Studies

LDL apolipoprotein and phospholipid turnover studies were performed by injecting LDL receptor knockout mice (n=4/group) with a doubly labeled human LDL. Human LDL (d=1.019 to 1.063 g/mL) was prepared by ultracentrifugation as previously described.[9] Dialyzed human LDL was labeled with ^{125}I by the iodine monochloride method as described.[10] Dialyzed [^{125}I]-LDL was subsequently labeled with [^{3}H]methylcholine-dipalmitoyl phosphatidylcholine (DPPC) as previously described.[4] Briefly, 50 μCi of [^{3}H]methylcholine-DPPC (PerkinElmer Life Sciences) was dried under nitrogen in a glass tube and resuspended in 50 μL of ethanol. Two mg of LDL protein were mixed with approximately 100 mg of heat-inactivated lipoprotein-deficient plasma (d>1.21 g/mL) in a glass tube, and the [^{3}H] methylcholine-DPPC was added dropwise with intermittent vortexing. The tube was purged with nitrogen, sealed, and placed in a shaking water bath at 37°C for 24 hours. After incubation, labeled LDL was reisolated at the original density, dialyzed against PBS, and filter sterilized before injection into mice. LDL receptor knockout mice (n=4/group) were injected with 1×10^{11} particles of AdEL, $AdEL^{S149A}$, or control. On day 5 after virus injection, an LDL turnover study was performed using [^{3}H]methylcholine-DPPC/[^{125}I]-human LDL (approximately 1×10^{6} dpm [^{3}H] in 100 μL of PBS). Blood samples were drawn from the retroorbital plexus at 1 and 15 minutes and at 1, 2, 4, 6, and 24 hours after injection. [^{125}I] counts were measured on a Cobra II gamma counter (Packard Instrument Co.) using 6 μL of plasma. To measure [^{3}H] counts, 6 μL of plasma taken at each time point and [^{125}I] standards were counted using a Beckman Coulter LS 6500 (Beckman). [^{3}H] counts were determined by correcting plasma counts for [^{125}I] contribution from each time point. A complementary experiment to determine [^{3}H] counts with lipid extraction of plasma samples[11] validated this approach. The fractional catabolic rates of LDL apoB were calculated with the SAAM II program (SAAM Institute) by fitting a biexponential curve to the [^{125}I] counts normalized to the 1-minute time point. Similarly, the fractional catabolic rates of LDL phospholipid were calculated by fitting a biexponential curve to the corrected [^{3}H] counts normalized to the 1-minute time point.

Lipoprotein Analysis

Plasma total cholesterol, HDL-C, triglycerides, and phospholipids were measured on a Cobas Fara (Roche Diagnostics System Inc) using Sigma Diagnostics reagents. Human apoB levels in apoB transgenic mice were quantified using a turbidimetric immunoassay (Wako Pure Chemical Industries). LDL from human apoB transgenic mice was isolated by ultracentrifugation (1.019 to 1.063) 7 days after virus injection. Lipoprotein composition was determined using commercially available enzymatic kits from WAKO (Cholesterol CII kit, Free Cholesterol kit, Phospholipids B kit, Triacylglycerol kit) and Pierce Biotechnology (Rockford) (Micro BCA Protein Assay kit).

NMR Lipoprotein Analysis

Human apoB transgenic mice (n=4/group) were injected with 3×10^{10} particles of AdEL or control adenovirus. Blood was obtained from the retroorbital plexus at baseline and at days 3, 5, 7, and 10 after injection. Lipoprotein subclass profiles were determined on 100 μL of pooled plasma by proton NMR spectroscopy at LipoScience as previously described.[12]

Gel Filtration Analysis

Pooled plasma samples from mice of the same experimental group were subjected to fast protein liquid chromatography (FPLC) gel filtration by using 2 Superose 6 columns (Amersham Pharmacia Biotech). Samples were chromatographed at a flow rate of 0.5 mL/min, and fractions of 500 μL each were collected. Individual fractions were assayed for cholesterol and phospholipid concentrations by using commercially available enzymatic kits (Wako Pure Chemical Industries).

Analysis of mEL RNA Expression In Vivo

Liver RNA was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer's protocol. After DNase digest (Invitrogen), 500 ng of total RNA was converted into cDNA using the SuperScript first-strand synthesis system for reverse-transcription polymer chain reaction (PCR; Invitrogen). Real-time PCR was performed using the ABI Prism 7700 Sequence Detector System (Applied Biosystems). The sequences of primers and TaqMan probes for mEL and mGAPDH cDNA were as follows: mEL, 5′-ACGCTGTCCTTTGGCTTGA-3′ (forward primer), 5′-TCACCGCCATTGGGATAGA-3′ (reverse primer), and 5′-[6≈FAM]TCAATGTGACCCACAGGCATCCGA[TAMRA≈FAM]-3′ (TaqMan probe); mGAPDH, 5′-GCCTCGTCCCGTAGACAAAA-3′ (forward primer), 5′-TGGCAACAATCTCAACTTTGC-3′ (reverse primer), and 5′-[6~FAM]CAGGCGCCCAATACGGCCAA[TAMRA≈FAM]-3′ (TaqMan probe). Quantitation of mEL was performed using the standard curve method. Determination of mouse GAPDH mRNA was used to standardize the amount of sample RNA added to the reaction.

In Vitro Tissue Culture Experiments

A Cos-7 cell line stably expressing mouse EL (mEL) protein was generated by transfecting mEL cDNA into Cos-7 cells using lipofectamine (Invitrogen) according to the manufacturer's protocol. The cells were then selected in the presence of 1000 μg/mL G418 (Invitrogen). Expression of mEL protein was determined by Western blot analysis. The clone with the highest expression level of mEL was selected for further study. Stably transfected Cos-7 cells in 60-mm dishes were infected in duplicates with 8×10^{9} particles of $AdEL^{S149A}$, AdGFP, or AdlacZ. Heparin to a final concentration of 10 U/mL was added 47.5 hours after infection. Media for Western blot analysis and phospholipase activity assay was harvested 48 hours after infection and stored at −80°C.

Coimmunoprecipitation of Mouse and Human EL

Conditioned media from Cos-7 cells coinfected with AdmEL and AdELmyc (myc-tagged human EL), AdmEL and AdGFP, or AdELmyc and AdGFP (1×10^{10} particles of each adenovirus) and a 1:1 mixture of media from Cos-7 cells infected with AdmEL/AdGFP or AdELmyc/AdGFP were incubated with mouse anti-myc IgG at 4°C in the presence of 0.1% Triton X-100 (Fisher Scientific International) for 2 hours. Protein A magnetic beads (New England Biolabs) were added to each sample, and samples were incubated overnight at 4°C. Samples were washed three times with PBS and eluted from the beads with 4× loading buffer and 10× DTT (Invitrogen). Western blot analysis of samples was performed using a rabbit anti-mouse EL antibody as described later.

Immunoblotting

Postheparin plasma samples after heparin-Sepharose treatment or conditioned media from Cos-7 cells were mixed with 4× loading buffer (Invitrogen), subjected to 10% SDS-PAGE (Invitrogen) under reducing conditions and electroblotted to Hybond-P (PVDF) membrane (Amersham Pharmacia Biotech). The detection of EL and EL^{S149A} was performed using a (species-specific) rabbit anti-human EL antibody at 1:3000 dilution as primary antibody as previously described.[1] Detection of mEL in homogenized liver lysate (30 μg total protein) or conditioned media was performed using a (species-specific) rabbit anti-mouse EL antibody at 1:2500 dilution as primary antibody as previously described.[4] A goat anti-rabbit antibody at 1:5000 dilution was used as the secondary antibody. Detection was performed by the ECL protocol (Amersham Pharmacia Biotech) according to the manufacturer's instruction.

Lipase Assays

Phospholipase activity was determined as previously described.[6] Briefly, an emulsion of cholesteryl oleate (150 mg) and DPPC (8.88 mg unlabeled and 17.15 μCi [1,2-^{14}C]-DPPC, 110 mCi/mmol) was prepared by sonication in 2.5 mL glycerol. The assay tubes contained, in a total volume of 0.3 mL, 0.05 mol/L Tris-HCl, pH 8.0, 0.75% bovine serum albumin, 4.6 mmol/L cholesteryl oleate, 245 μmol/L DPPC, 0.15 mol/L NaCl, conditioned media or postheparin plasma samples. Samples were incubated for 15 minutes at 37°C. Reactions were stopped and products were extracted by the method of Belfrage and Vaughan[13] using 100 μg lysopalmitoylphosphatidylcholine per mL as carrier in the organic extraction mix.

Statistical Analysis

Values are presented as mean±SD. Turnover study data were subjected to a 1-way ANOVA and the Newman-Keuls multiple comparison test. Statistical significance for all comparisons was assigned at $P<0.05$.

Results

EL Expression Markedly Reduces VLDL and LDL Levels

To study the effect of wild-type EL and catalytically inactive EL^{S149A} on the metabolism of apoB-containing particles, we injected apoE-deficient, LDL receptor knockout, and human apoB transgenic mice with 1×10^{11} particles of AdEL, $AdEL^{S149A}$, or control (Adnull). Wild-type EL and EL^{S149A} protein were substantially increased in postheparin plasma compared with preheparin, indicating that both the wild-type and mutant EL were bound to cell surface HSPGs (Figure 1A). As expected, overexpression of wild-type EL resulted in significantly increased postheparin plasma phospholipase activity compared with control in all animal models, whereas no increase was observed in EL^{S149A} expressing mice (Figure 1B). In addition to reducing HDL-C levels, overexpression of EL significantly reduced total cholesterol, non HDL-C, triglyceride, and phospholipid levels in apoE-deficient (Figure 2), LDL receptor knockout (Figure 3), and human apoB transgenic mice (Figure 4) as well as human apoB levels in human apoB transgenic mice (Figure 4F). Unexpectedly, overexpression of catalytically inactive EL^{S149A} resulted in significantly increased levels of total cholesterol, HDL-C, non–HDL-C, and phospholipids 5 to 7 days after virus injection in all 3 mouse models examined (Figures 2 through 4).

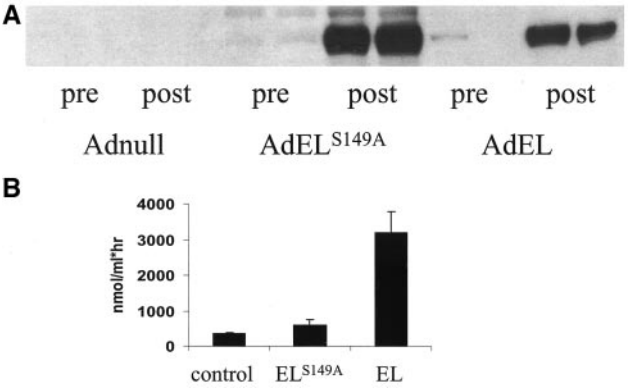

Figure 1. A, Determination of EL^{S149A} and wild-type EL protein in pre- and postheparin plasma 3 days after injection of LDL receptor knockout mice with 1×10^{11} particles of AdEL, $AdEL^{S149A}$, or control (Adnull) (2 slots for each type of injection). B, Phospholipase activity determined in postheparin plasma at day 3 after virus injection of LDL receptor knockout mice. Data from LDL receptor knockout mice are representative for apoE-deficient and apoB transgenic mice.

FPLC analysis of lipoprotein distribution demonstrated that overexpression of EL dramatically lowered VLDL/IDL cholesterol in the apoE-deficient mice (data not shown) as well as LDL-C in the LDL receptor knockout (data not shown) and human apoB transgenic mice (Figure 5A), in addition to its known effects on HDL levels in all three models. Consistent with the lipid data, the FPLC profile of EL^{S149A} expressing mice showed increased levels of VLDL/LDL cholesterol with a shift toward larger LDL particles compared with control (Figure 5A).

EL Expression Generates Lipid-Depleted, Smaller LDL Particles

LDL isolated from human apoB transgenic mice 7 days after injection of AdEL showed a significant decrease in the percentage of phospholipids and triglycerides compared with control whereas the free and esterified cholesterol content was not changed and the percentage of protein increased (data not shown). The change in the ratio of LDL core (cholesterol esters, triglycerides) to surface constituents (free cholesterol, phospholipids, protein) ($ratio_{AdEL}$, 0.75 versus $ratio_{Adnull}$, 0.96) suggests a shift in LDL size toward smaller LDL particles. The FPLC profile of human apoB transgenic mice expressing EL confirms this shift in LDL size (Figure 5A). NMR analysis of mouse plasma from human apoB transgenic mice injected with 3×10^{10} particles of AdEL and control adenovirus demonstrates that overexpression of EL resulted in reduced LDL-C levels, and decreased LDL size and particle number (Figure 5B through 5E).

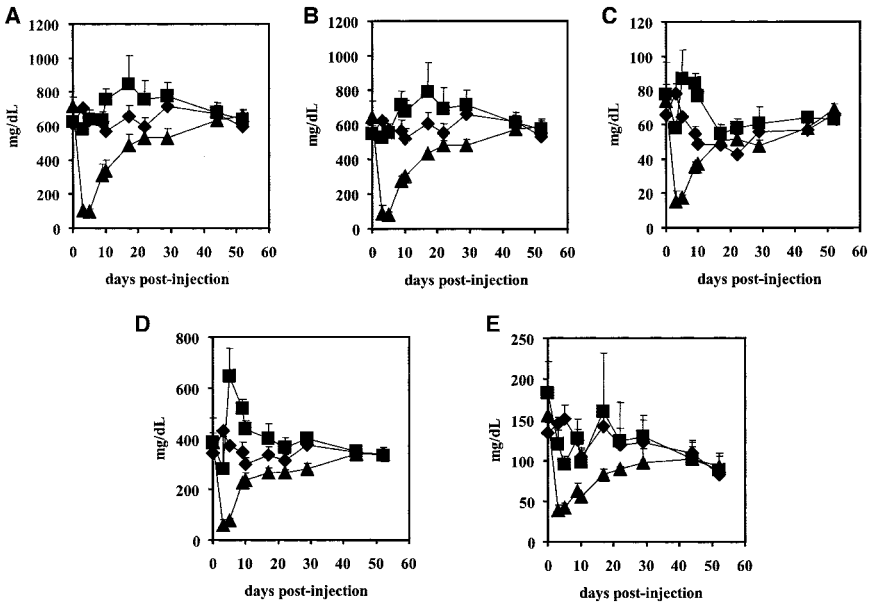

Figure 2. Total cholesterol (A), non–HDL-C (B), HDL-C (C), phospholipids (D), and triglycerides (E) in apoE-deficient mice injected with AdEL (▲), AdEL^{S149A} (■), and control (◆) over the course of the study.

EL Expression Accelerates LDL Apolipoprotein and Phospholipid Turnover

To determine the mechanism by which EL can reduce apoB-containing particles, we performed a kinetic study in LDL receptor knockout mice using [^{125}I]-apoB and [^{3}H]-phospholipid (PL) doubly radiolabeled human LDL 5 days after injection of 1×10^{11} particles of AdEL, AdEL^{S149A}, and Adnull. [^{125}I]-LDL was cleared significantly faster in the EL-expressing mice compared with both AdEL^{S149A}- and Adnull-injected mice (0.200 ± 0.036 versus 0.066 ± 0.010 pools per hour; $P<0.001$). No significant difference was observed for the fractional catabolic rates of apoB in EL^{S149A}- and Adnull-injected mice (0.060 ± 0.007 versus 0.066 ± 0.010 pools per hour; $P>0.05$) (Figure 6A). Furthermore, [^{3}H]-PL-LDL was cleared significantly faster in EL-expressing mice than in control mice (1.265 ± 0.117 versus 0.484 ± 0.017 pools/hour; $P<0.01$). Interestingly, [^{3}H]-PL-LDL was cleared significantly slower in EL^{S149A}-expressing mice compared with control mice (0.250 ± 0.098 versus 0.484 ± 0.017 pools/hour; $P<0.05$) (Figure 6B).

Inhibition of Endogenous Mouse EL May Account for the Effects of Catalytically Inactive EL

To determine the mechanism by which expression of catalytically inactive EL resulted in increased lipid levels and decreased LDL-phospholipid catabolism compared with control, we tested whether expression of catalytically inactive EL^{S149A} reduces the expression or function of endogenous mouse EL. Real-time PCR and Western blot analysis of mouse EL expression in the liver of LDL receptor knockout mouse mice 5 days after injection with 1×10^{11} particles of AdEL, AdEL^{S149A}, or control did not reveal any differences in EL mRNA or protein abundance among the groups (data not shown). To determine whether EL^{S149A} may affect mouse EL activity at a posttranslational level, COS-7 cells, stably transfected with mouse EL, were infected with AdEL^{S149A} or controls

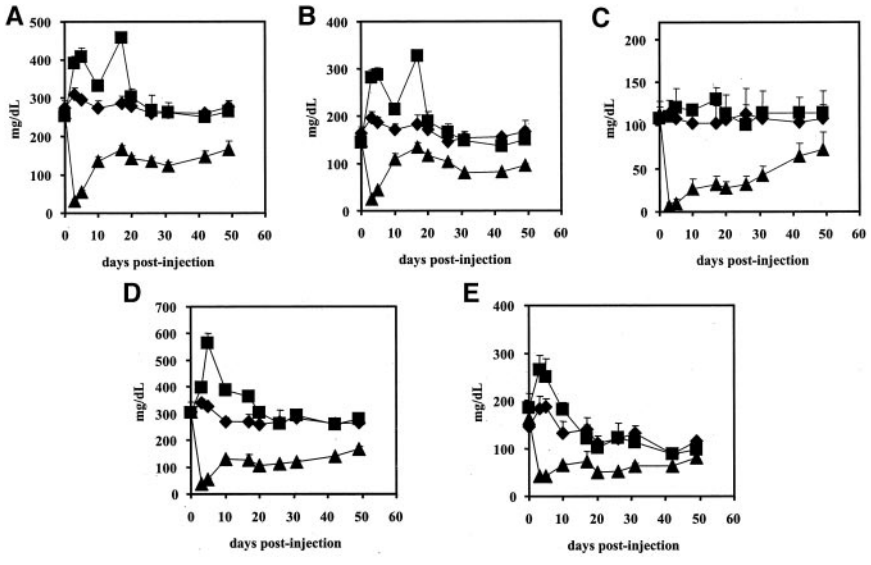

Figure 3. Total cholesterol (A), non–HDL-C (B), HDL-C (C), phospholipids (D), and triglycerides (E) in LDL receptor knockout mice injected with AdEL (▲), AdEL^{S149A} (■), and control (◆) over the course of the study.

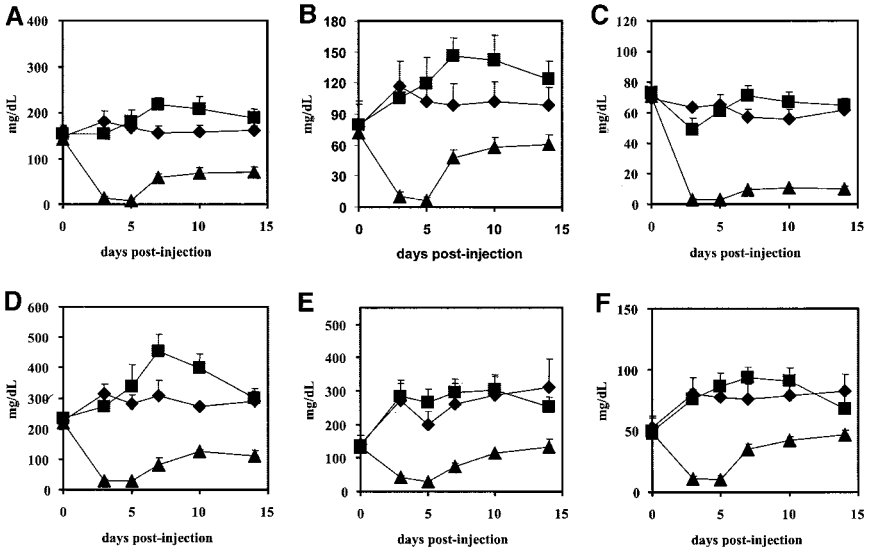

Figure 4. Total cholesterol (A), non–HDL-C (B), HDL-C (C), phospholipids (D), triglycerides (E), and apoB levels (F) in human apoB transgenic mice injected with AdEL (▲), AdEL^{S149A} (■), and control (◆) over the course of the study.

(AdGFP, AdlacZ) (Figure 7A and 7B). Interestingly, despite similar mouse EL protein expression levels, phospholipase activity in EL^{S149A}-expressing cells was significantly reduced compared with control (Figure 7C). Furthermore, human and mouse EL could be coimmunoprecipitated when coexpressed in vitro, yet could not be coimmunoprecipitated when conditioned media from cells expressing either mouse or human EL were mixed together (Figure 7D), suggesting that human and mouse EL form specific protein–protein interactions, possibly as heterodimers. Therefore, inhibition of endogenous mEL by EL^{S149A} may be the cause of the decreased LDL-phospholipid turnover and increased lipid levels.

Discussion

EL has been shown to influence HDL metabolism and levels in mice,[1,3–5] yet its role in the metabolism of apoB-containing lipoproteins remained to be elucidated. In the present study, we demonstrate that expression of EL in mouse models with elevated levels of apoB-containing lipoproteins significantly reduced VLDL/LDL cholesterol, phospholipid, and apoB levels. Expression of EL resulted in increased phospholipase activity in vivo, thus generating phospholipid depleted, smaller LDL particles that were more rapidly catabolized. Unexpectedly, expression of catalytically inactive EL resulted in increased levels of VLDL/LDL cholesterol and phospholipid as well as reduced catabolism of LDL-phospholipids, possibly because of inhibition of endogenous mouse EL activity. These data suggest that in addition to its role in HDL metabolism, EL may have a role in the metabolism of apoB-containing lipoproteins.

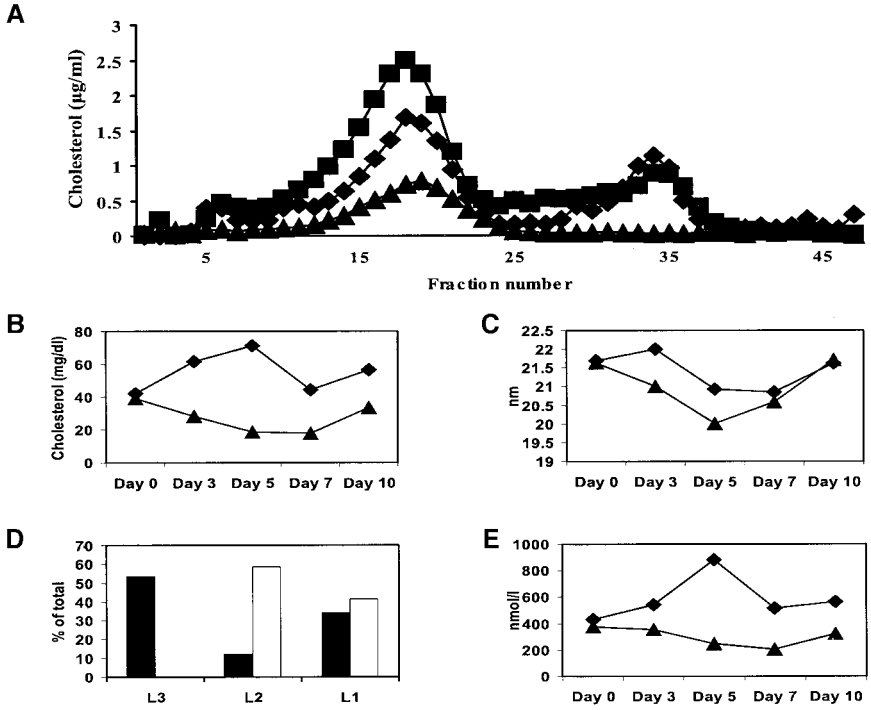

Figure 5. FPLC profile (A) of apoB transgenic mice 7 days after injection of 1×10^{11} particles of AdEL (▲), AdEL^{S149A} (■), and control (◆). LDL-cholesterol (B), mean LDL particle diameter (nm) (C), percentage of plasma LDL subclass concentration (day 5 after injection; L3, large LDL; L2, medium LDL; L1, small LDL) (D), and LDL particle concentration (nmol/L) (E) in apoB transgenic mice injected with 3×10^{10} particles of AdEL (▲, white bars) and control (◆, black bars).

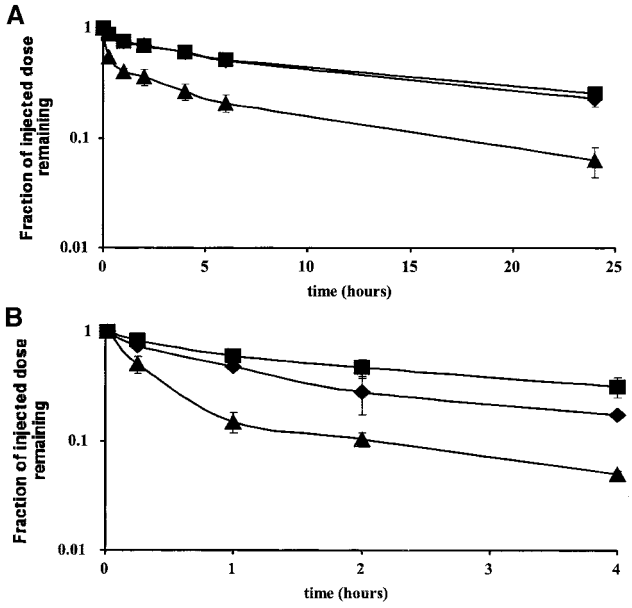

Figure 6. Plasma clearance of human ^{125}I -LDL (A) and human ^{3}H-PL-LDL (B) in LDL receptor knockout mice 5 days after injection of AdEL (▲), AdEL^{S149A} (■), and control (◆).

Overexpression of EL resulted in major changes in the structure of LDL particles. EL-modified LDL had a decreased content of phospholipids and triglycerides, whereas the protein content was increased. The shift in the ratio of LDL core to surface constituents and in the FPLC profile as well as the NMR analysis of lipoprotein distribution and size suggest that expression of EL may contribute to the generation of small dense LDL particles. HL is hypothesized to play a critical role in the generation of small dense LDL.[14] HL activity in normolipidemic subjects was reported to be significantly positively correlated with plasma triglyceride, apoB, and mass of large VLDL and small dense LDL.[15] Transgenic overexpression of HL in rabbits was shown to reduce levels of IDLs with a shift toward smaller, denser LDL particles.[16] Conversely, buoyant, triglyceride-rich LDL particles accumulate in patients with HL deficiency.[17,18] We speculate that HL might act primarily on the TG-enriched LDL particle resulting in the reduction of LDL size, whereas EL may primarily act on LDL phospholipids as a critical step in the formation of small dense LDL.

To determine whether the EL-mediated decrease of LDL phospholipids and apoB levels was because of increased catabolism, we performed a turnover study using doubly labeled human LDL in LDL receptor knockout mice. The fractional catabolic rates of both LDL phospholipids and apoB were significantly increased in EL-expressing mice compared with control. We propose that the phospholipase activity of EL depletes the LDL particle of phospholipids, resulting in smaller LDL particles that are more rapidly cleared from plasma. The major cellular pathway for tissue catabolism of LDL particles is that of the LDL receptor.[19] Still, up to 50% of LDL is removed from the plasma by LDL receptor–independent pathways.[20,21] It has been suggested that the intermediate size LDL subspecies constitute the optimal ligand for the LDL receptor among the human LDL particle subpopulations,[22] whereas small, dense LDL are cleared from plasma to a large extent by LDL receptor–independent pathways,[23] a process mediated, in part, by cell surface proteoglycans.[24] LDL cell binding to LDL receptor–independent binding sites could also be related to other cell surface binding sites such as LDL receptor–related protein (LRP) or scavenger receptor class B type I (SR-BI). Interaction of small, dense LDL and HDL_3 particles with consecutive enrichment in apoE may lead to uptake of LDL by LRP. Ueda et al[25] reported a significant reduction of non–HDL-C and apoB levels along with increased LDL-apoB clearance in transgenic mice overexpressing SR-BI. Recent in vitro studies by Rhainds et al[26] highlight the importance of SR-BI in selective LDL cholesterol ester (CE) and phospholipid uptake as well as possibly LDL holoparticle uptake: inhibition of SR-BI function using a specific blocking antibody as well as inhibition of SR-BI expression using RNA antisense strategy suggested that SR-BI mediated up to 87% of LDL-CE uptake and 75% of LDL-PL uptake in HepG2 cells.

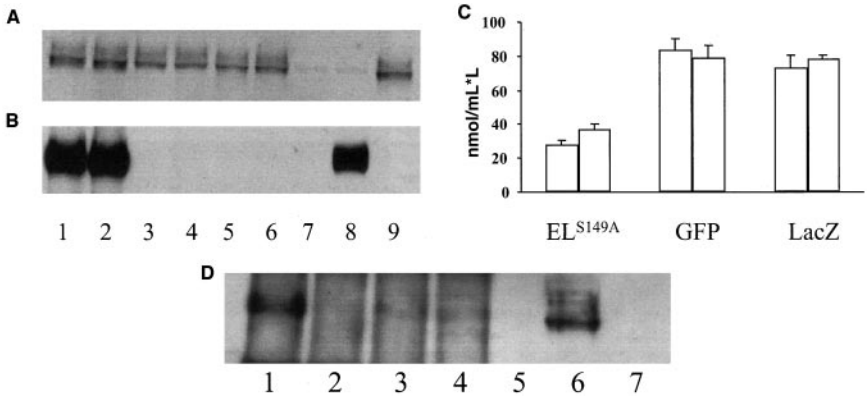

Figure 7. Mouse (A) and human EL (B) protein expression in stably mouse EL expressing Cos-7 cells after infection with AdEL^{S149A} (lanes 1 and 2), AdGFP (lanes 3 and 4), or AdlacZ (lanes 5 and 6). Lanes 7 through 9 represent Western blot controls (7, GFP; 8, human EL; 9, mouse EL). Experiments were performed in duplicates. Phospholipase activity (nmol/mL×h) (C) in stably mouse EL–expressing Cos-7 cells after infection with AdEL^{S149A}, AdGFP, or AdlacZ. Experiments were performed in duplicates. Immunoprecipitation (D) of human EL with subsequent determination of mouse EL protein in conditioned media from Cos-7 cells coexpressing mouse and human EL (lane 1), expressing either mouse (lane 2), or human EL (lane 3), and in a mixture of media from cells expressing either mouse or human EL (lane 4). Lanes 6 and 7 represent Western blot controls (5, blank lane; 6, mouse EL; 7, human EL).

Independent of their catalytic activity, lipoprotein lipase (LPL) and HL can act in cellular lipoprotein metabolism as ligands that mediate the binding and uptake of lipoproteins via proteoglycans and/or receptor pathways.[27–31] Overexpression of catalytically inactive LPL in transgenic mice resulted in increased triglyceride-rich lipoprotein particle uptake and reduced triglyceride levels.[27,28] Overexpression of catalytically inactive HL significantly lowered apoB-containing lipoproteins.[29,30] To determine whether catalytic activity of EL was required for its effects on apoB-containing particles, a catalytically inactive form of EL was overexpressed in apoE-deficient, LDL receptor knockout, and apoB transgenic mice. EL was previously shown to mediate binding of VLDL and LDL to cell surface HSPGs in vitro.[7] Unexpectedly, expression of catalytically inactive EL did not reduce VLDL/LDL cholesterol and phospholipid levels but instead resulted in a significant increase of these parameters. This increase was associated with a decreased LDL-phospholipid catabolism and a shift of LDL particle size toward larger LDLs. Our in vitro data suggest that catalytically inactive EL may interfere with the function of endogenous active mouse EL at a posttranslational level, thus reducing the activity of endogenous mouse EL, resulting in decreased LDL phospholipid turnover and increased LDL cholesterol and phospholipid levels as well as larger, buoyant LDL particles. Radiation inactivation analysis as well as sedimentation equilibrium studies of LPL and HL revealed that both enzymes are functionally active as dimers.[32–35] The smallest functional unit of endothelial lipase capable of lipolytic activity or bridging of lipoproteins has not been determined, but given the degree of identity among the members of the triacylglycerol lipase gene family, it is reasonable to speculate that EL may also be functionally active as a dimer. In light of the finding that human and murine EL coimmunoprecipitate, we speculate that catalytically inactive EL may form a heterodimer with endogenous hepatic mouse EL, thus reducing its function. Studies in EL-deficient mice will be needed to verify the effect of endogenous mouse EL on catalytically inactive EL in vivo.

In summary, hepatic expression of EL resulted in significantly increased postheparin plasma phospholipase activity associated with reduced VLDL/LDL cholesterol and phospholipids, reduced apoB levels, a shift toward smaller lipid poor LDL particles, and accelerated catabolism of LDL apolipoprotein and phospholipids. These results suggest that in addition to its role in HDL metabolism, EL plays a significant role in the metabolism of apoB-containing lipoproteins. Unexpectedly, hepatic expression of catalytically inactive EL resulted in increased VLDL/LDL cholesterol and phospholipid levels associated with reduced catabolism of LDL-phospholipids, possibly because of inhibition of endogenous active mouse EL.

Acknowledgments

This work was supported by grant HL55323 (D.J.R.) from the National Heart Lung and Blood Institute, an Established Investigator Award from the American Heart Association (D.J.R.), and a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research (D.J.R.). U.C.B. was supported by a grant from the American Heart Association, Pennsylvania Delaware Affiliate. C.M. was supported by a grant from the American Heart Association, Pennsylvania Delaware Affiliate. I.V.F. and W.J. are supported by a Scientist Development Grant from the American Heart Association. We thank Jeffrey Billheimer for helpful discussions. We are indebted to Anna Lillethun, Linda Morrell, Nicholle Campbell, Nadine Blanchard, and Anthony Secreto for excellent technical assistance.

References

1. Jaye M, Lynch KJ, Krawiec J, Marchadier D, Maugeais C, Doan K, South V, Amin D, Perrone M, Rader DJ. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat Genet*. 1999;21:424–428.
2. Hirata K, Dichek HL, Cioffi JA, Choi SY, Leeper NJ, Quintana L, Kronmal GS, Cooper AD, Quertermous T. Cloning of a unique lipase from endothelial cells extends the lipase gene family. *J Biol Chem*. 1999;274:14170–14175.
3. Ishida T, Choi S, Kundu RK, Hirata K, Rubin EM, Cooper AD, Quertermous T. Endothelial lipase is a major determinant of HDL level. *J Clin Invest*. 2003;111:347–355.
4. Jin W, Millar JS, Broedl U, Glick JM, Rader DJ. Inhibition of endothelial lipase causes increased HDL cholesterol levels in vivo. *J Clin Invest*. 2003;111:357–362.
5. Ma K, Cilingiroglu M, Otvos JD, Ballantyne CM, Marian AJ, Chan L. Endothelial lipase is a major genetic determinant for high-density lipoprotein concentration, structure, and metabolism. *Proc Natl Acad Sci U S A*. 2003;100:2748–2753.
6. McCoy MG, Sun GS, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. *J Lipid Res*. 2002;6:921–929.
7. Fuki IV, Blanchard N, Jin W, Marchadier DH, Millar JS, Glick JM, Rader DJ. Endogenously produced endothelial lipase enhances binding and cellular processing of plasma lipoproteins via HSPG-mediated pathway. *J Biol Chem*. 2003;278:34331–34338.
8. Broedl UC, Maugeais C, Marchadier D, Glick JM, Rader DJ. Effects of nonlipolytic ligand function of endothelial lipase on HDL metabolism in vivo. *J Biol Chem*. 2003;278:40688–40693.
9. Hatch FT. Practical methods for plasma lipoprotein analysis. *Adv Lipid Res*. 1968;6:1–68.
10. Tietge UJ, Maugeais C, Cain W, Grass D, Glick JM, de Beer FC, Rader DJ. Overexpression of secretory phospholipase A(2) causes rapid catabolism and altered tissue uptake of high density lipoprotein cholesteryl ester and apolipoprotein A-I. *J Biol Chem*. 2000;275:10077–10084.
11. Dole VP. A relation between non-esterified fatty acids in the plasma and the metabolism of glucose. *J Clin Invest*. 1956;35:150–154.
12. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem*. 1992;38:1632–1638.
13. Belfrage P, Vaughan M. Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J Lipid Res*. 1969;10:341–344.
14. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res*. 2002;43:1363–1379.
15. Campos H, Dreon DM, Krauss RM. Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. *J Lipid Res*. 1995;36:462–472.
16. Barbagallo CM, Fan J, Blanche PJ, Rizzo M, Taylor JM, Krauss RM. Overexpression of human hepatic lipase and ApoE in transgenic rabbits attenuates response to dietary cholesterol and alters lipoprotein subclass distributions. *Arterioscler Thromb Vasc Biol*. 1999;19:625–632.
17. Breckenridge WC, Little JA, Alaupovic P, Wang CS, Kuksis A, Kakis G, Lindgren F, Gardiner G. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis*. 1982;45:161–179.
18. Auwerx JH, Marzetta CA, Hokanson JE, Brunzell JD. Large buoyant LDL-like particles in hepatic lipase deficiency. *Arteriosclerosis*. 1989;9:319–325.
19. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science*. 1981;212:628–635.
20. Shepherd J, Packard CJ. Receptor-independent low-density lipoprotein catabolism. *Methods Enzymol*. 1986;129:566–590.
21. Spady DK, Woollett LA, Dietschy JM. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Annu Rev Nutr*. 1993;13:355–381.

22. Lund-Katz S, Laplaud PM, Phillips MC, Chapman MJ. Apolipoprotein B-100 conformation and particle surface charge in human LDL subspecies: implication for LDL receptor interaction. *Biochemistry*. 1998;37: 12867–12874.
23. Shepherd J, Caslake MJ, Lorimer AR, Vallance BD, Packard CJ. Fenofibrate reduces low density lipoprotein catabolism in hypertriglyceridemic subjects. *Arteriosclerosis*. 1985;5:162–168.
24. Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *J Lipid Res*. 1998;39:1263–1273.
25. Ueda Y, Royer L, Gong E, Zhang J, Cooper PN, Francone O, Rubin EM. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL) and non-HDL cholesterol in scavenger receptor class B type I transgenic mice. *J Biol Chem*. 1999;274:7165–7171.
26. Rhainds D, Brodeur M, Lapointe J, Charpentier D, Falstrault L, Brissette L. The role of human and mouse hepatic scavenger receptor class B type I (SR-BI) in the selective uptake of low-density lipoprotein-cholesteryl esters. *Biochemistry*. 2003;42:7527–7538.
27. Merkel M, Kako Y, Radner H, Cho IS, Ramasamy R, Brunzell JD, Goldberg IJ, Breslow JL. Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: direct evidence that lipoprotein lipase bridging occurs in vivo. *Proc Natl Acad Sci U S A*. 1998;95:13841–13846.
28. Merkel M, Heeren J, Dudeck W, Rinninger F, Radner H, Breslow JL, Goldberg IJ, Zechner R, Greten H. Inactive lipoprotein lipase (LPL) alone increases selective cholesterol ester uptake in vivo, whereas in the presence of active LPL it also increases triglyceride hydrolysis and whole particle lipoprotein uptake. *J Biol Chem*. 2002;277:7405–7411.
29. Dichek HL, Brecht W, Fan J, Ji ZS, McCormick SP, Akeefe H, Conzo L, Sanan DA, Weisgraber KH, Young SG, Taylor JM, Mahley RW. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. Evidence that hepatic lipase acts as a ligand for lipoprotein uptake. *J Biol Chem*. 1998;273: 1896–1903.
30. Dichek HL, Johnson SM, Akeefe H, Lo GT, Sage E, Yap CE, Mahley RW. Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice. *J Lipid Res*. 2001;42:201–210.
31. Dugi KA, Amar MJ, Haudenschild CC, Shamburek RD, Bensadoun A, Hoyt RF Jr, Fruchart-Najib J, Madj Z, Brewer HB Jr, Santamarina-Fojo S. In vivo evidence for both lipolytic and nonlipolytic function of hepatic lipase in the metabolism of HDL. *Arterioscler Thromb Vasc Biol*. 2000; 20:793–800.
32. Garfinkel AS, Kempner ES, Ben-Zeev O, Nikazy J, James SJ, Schotz MC. Lipoprotein lipase: size of the functional unit determined by radiation inactivation. *J Lipid Res*. 1983;24:775–780.
33. Olivecrona T, Bengtsson-Olivecrona G, Osborne JC Jr, Kempner ES. Molecular size of bovine lipoprotein lipase as determined by radiation inactivation. *J Biol Chem*. 1985;260:6888–6891.
34. Osborne JC Jr, Bengtsson-Olivecrona G, Lee NS, Olivecrona T. Studies on inactivation of lipoprotein lipase: role of the dimer to monomer dissociation. *Biochemistry*. 1985;24:5606–5611.
35. Hill JS, Davis RC, Yang D, Wen J, Philo JS, Poon PH, Phillips ML, Kempner ES, Wong H. Human hepatic lipase subunit structure determination. *J Biol Chem*. 1996;271:22931–22936.