Hepatic Overexpression of Endothelial Lipase Lowers High-Density Lipoprotein but Maintains Reverse Cholesterol Transport in Mice

Role of Scavenger Receptor Class B Type I/ATP-Binding Cassette Transporter A1-Dependent Pathways

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Objective—Reverse cholesterol transport (RCT) is a major mechanism by which HDL (high-density lipoprotein) protects against atherosclerosis. Endothelial lipase (EL) reportedly reduces HDL levels, which, in theory, would increase atherosclerosis. However, it remains unclear whether EL affects RCT in vivo.

Approach and Results—Adenoviral vectors expressing EL or luciferase were intravenously injected into mice, and a macrophage RCT assay was performed. As expected, hepatic EL overexpression markedly reduced HDL levels. In parallel, plasma ^{3}H-cholesterol counts from the EL-expressing mice decreased by 85% compared with control. Surprisingly, there was no difference in fecal ^{3}H-cholesterol excretion between the groups. Kinetic studies revealed increased catabolism/hepatic uptake of ^{3}HDL-cholesteryl ether, resulting in no change in fecal HDL-cholesteryl ester excretion in the mice. To explore underlying mechanisms for the preservation of RCT despite low HDL levels in the EL-expressing mice, we investigated the effects of hepatic SR-BI (scavenger receptor class B type I) knockdown. RCT assay revealed that knockdown of SR-BI alone reduced fecal excretion of macrophage-derived ^{3}H-cholesterol. Interestingly, hepatic EL overexpression under SR-BI inhibition further attenuated fecal tracer counts as compared with control. Finally, we observed that EL overexpression enhanced in vivo RCT under pharmacological inhibition of hepatic ABCA1 (ATP-binding cassette transporter A1) by probucol.

Conclusions—Hepatic EL expression compensates for reduced macrophage-derived cholesterol efflux to plasma because of low HDL levels by promoting cholesterol excretion to bile/feces via an SR-BI pathway, maintaining overall RCT in vivo. In contrast, EL-modified HDL might negatively regulate RCT via hepatic ABCA1. Despite extreme hypoalphalipoproteinemia, RCT is maintained in EL-expressing mice via SR-BI/ABCA1-dependent pathways.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2018;38: 1454-1467. DOI: 10.1161/ATVBAHA.118.311056.)**

Key Words: ATP-binding cassette transporter 1 ■ endothelial lipase ■ high-density lipoproteins ■ scavenger receptors, class B

Low plasma levels of HDL-C (high-density lipoprotein-cholesterol) have been consistently associated with increased risk of atherosclerotic cardiovascular diseases, and it is therefore considered to be an antiatherogenic lipoprotein.[1] The development of novel therapies to enhance the atheroprotective properties of HDL may further reduce the residual risk. Reverse cholesterol transport (RCT) is believed to be a primary atheroprotective property of HDL and its major protein, apoA-I (apolipoprotein A-I).[2] HDL and apoA-I have been shown to promote efflux of excess cholesterol from macrophage-derived foam cells via the cholesterol transporters, ABCA1 (ATP-binding cassette transporter A1), ABCG1 (ATP-binding cassette transporter G1), and SR-BI (scavenger receptor class B type I), and then transport it back to the liver for excretion into the bile and eventually into feces.[3]

In this regard, a validated murine assay that quantifies macrophage RCT may be a better predictor of atherosclerosis than measuring the steady state plasma concentration of HDL-C.[3] Indeed, a recent clinical study by Khera et al[4] demonstrated that the ability of serum HDL to mediate cholesterol efflux from macrophages was independently and negatively associated with cardiovascular diseases risk even after adjustment for HDL-C levels, suggesting that HDL functionality is more important rather than its quantity.

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Nonstandard Abbreviations and Acronyms

ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
Ad-hEL	adenovirus expressing human EL
Ad-Luc	adenoviral vectors harboring luciferase
apoA-I	apolipoprotein A-I
CE	cholesteryl ester
CEs	cholesteryl oleate
CEther	cholesteryl ether
CETP	CE transfer protein
EL	endothelial lipase
HDL-C	high-density lipoprotein-cholesterol
IDL	intermediate-density lipoprotein
LDLR	low-density lipoprotein receptor
LSC	liquid scintillation counting
RCT	reverse cholesterol transport
SR-BI	scavenger receptor class B type I
SREBP-2	sterol responsive element-binding protein-2

Endothelial lipase (EL) is an extracellular lipase with robust phospholipase but little triglyceride lipase activity.[5] EL preferentially hydrolyzes lipids in HDL, which in turn enhances catabolism of HDL particles, resulting in reduced circulating HDL levels in mice.[6] Conversely, loss of EL activity in mice significantly raises plasma HDL.[6,7] In humans, N396S, an EL variant with lower phospholipase activity, reportedly had a higher HDL-C level phenotype as compared with the wild-type EL.[8] However, it is still controversial whether EL-mediated HDL-C changes are associated with atherogenesis in mice[9,10] and humans,[11,12] because decreasing (or raising) HDL-C levels does not always translate into corresponding changes in atherogenesis. A typical illustration of this concept is the effect of hepatic SR-BI on atherosclerosis and RCT. Liver-specific SR-BI overexpression in mice promoted RCT, despite reduced HDL-C levels,[13] resulting in the attenuated development of atherosclerosis.[14] However, it remains unclear whether EL-mediated HDL reduction affects RCT in vivo.

Here, we demonstrated for the first time that hepatic overexpression of EL because of adenoviral vectors induced markedly low plasma HDL levels but maintained macrophage RCT in an SR-BI–dependent fashion in mice.

Materials and Methods

Data available on request from the authors.

Animals and Diet

C57BL/6J mice were obtained from Clea Japan (Tokyo, Japan) and fed a standard chow diet. The experiments were performed using mice aged 6 to 8 weeks. Mice were handled according to the guidelines of National Defense Medical College Institutional Animal Care and Use Committee. According to the details in a previous study by Yamamoto et al,[15] we used a 0.5% probucol (WAKO, Osaka, Japan)-containing diet (Oriental Yeast, Tokyo, Japan) for 14 days for pharmacological suppression of ABCA1.

Generation of Recombinant Adenoviruses Encoding Human EL and Synthetic MicroRNAs Targeting Mouse SR-BI

Recombinant adenovirus expressing human EL (Ad-hEL) was produced using the ViraPower Adenoviral Expression System (Invitrogen), according to the manufacturer's instructions. Briefly, to generate an entry clone of the Gateway system (Invitrogen), cloning of the open reading frame into a pENTR/D-TOPO vector (Invitrogen) was performed using first-strand complementary DNA (cDNA) derived from human monocyte-derived macrophages as a template and the following specific primers: forward: 5′-ATG AGC AAC TCC GTT CCT CTG CTC TGT T-3′; reverse: 5′-C AGT CCC ACT GTG GAG CTT CCC TGA-3′. Adenoviruses expressing catalytically inactive human EL (Ad-hEL^{S149A}) were generated as described previously.[16] Briefly, a cDNA of human EL containing the S149A mutant (rendering the protein enzymatically inactive) was generated by site-directed mutagenesis using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) according to manufacturer's instructions to substitute serine for alanine at position 149 of human EL.

An expression clone for adenoviral vector was then generated by performing an LR recombination reaction between the entry clone and a pAd/CMV/V5-DEST (Invitrogen) according to the manufacturer's protocol. The recombinant adenoviral plasmid was purified and then transfected into 293A cells. After a sufficient cytopathic effect was observed in 293A cells, the adenovirus was purified using the Adeno-X Virus Purification Kit (Clontech, Palo Alto, CA). Adenoviral vectors harboring luciferase (Ad-Luc) was kindly donated by Dr Santamarina-Fojo S of National Institute of Health and used as a control.

The pre-microRNA (miR) sequences for mouse SR-BI were designed using an RNAi designer online tool, Invitrogen's RNAi Designer.[17] Three different double-stranded oligo duplexes encoding desired miRNA target sequences were selected and cloned into pcDNA6.2/EmGFP-GW vector (Invitrogen).

The sequences of one of the most efficient oligo duplexes are as follows: top sequence 5′- TGC TGT GAG GAT CAT GAC AAC GCC GAG TTT TGG CCA CTG ACT GAC TCG GCG TTC ATG ATC CTC A-3′ and bottom sequence 5′- CCT GTG AGG ATC ATG AAC GCC GAG TCA GTC AGT GGC CAA AAC TCG GCG TTG TCA TGA TCC TCA C -3′. The sequences of miR against LacZ duplex are as follows: top sequence 5′-GCT AAA GTA TGC CGT GAG CGT TTG CCA TGA TGA GTC CCA GCA TAC TTT-3′ and bottom sequence 5′-CCT AAA GTA TGC TGG GAC TCA TCA TGG CAA ACG CTC CGC CAG ACA TTC-3′.

The miR flanking sequence was transferred into pDONR221 vector (Invitrogen) to generate an entry vector, which was used to generate adenoviruses encoding for synthetic miRs (adenoviral vectors expressing miR against mouse SR-BI and adenoviral vectors expressing miR against LacZ) as described above.

The adenovirus titer in plaque-forming units (PFU) was determined by a plaque formation assay after infection of HEK293 cells. The multiplicity of infection was defined as the ratio of the total number of PFU to the total number of cells that were infected.

In a pilot experiment, mice were injected with increasing levels of adenovirus (10^7 to 10^9 PFU for each mouse), and EL expression was monitored. The dose of 10^9 PFU was lethal; the highest hepatic EL expression with no complications was observed in mice injected with 5.0×10^8 PFU (data not shown). Male C57BL/6J mice were injected intravenously via the tail vein with 5.0×10^8 PFU of purified recombinant adenovirus on day 0 of the study. Blood was obtained from the tail vein at several time points after injection. Postheparin plasma was obtained on day 4 after virus injection 10 minutes after intravenous injection of 100 U/kg of heparin. Aliquots of serum were stored at −80°C for subsequent lipid analysis.

Plasma Lipid and Lipoprotein Analysis

Blood sampling was performed on the indicated days after injection of Ad-hEL or Ad-hEL^{S149A}, Ad-Luc (5×10^8 PFU) into the mice. Plasma total cholesterol, triglyceride, and phospholipids levels were measured using commercially available assay kits (Wako Pure Chemical Industries, Ltd, Osaka, Japan). For lipoprotein fractionation analysis, equal volumes of plasma samples were pooled from mice for each group (total volume of 400 μL). Lipoproteins were fractionated using a Superose 6 10/300 GL fast protein liquid chromatography column (Amersham Biosciences, Piscataway, NJ). Fractions (500 μL) were collected and used for lipid measurement.

Isolation of Mouse Peritoneal Macrophages and Livers

Four days after virus injection, the mice were euthanized to harvest mouse peritoneal macrophages and livers. Ice-cold phosphate-buffered saline (PBS) was injected into the peritoneal cavity. Fluid was then carefully collected and centrifuged at 3000 rpm. After suspending cells in Dulbecco's modified Eagle's medium (DMEM), they were plated onto 6-well cell culture plates for 1 hour, harvested and subjected to Western blot analyses.

Western Blot Analyses

Protein extracts from livers and mouse peritoneal macrophages were prepared with T-PER (Pierce Chemical Co, Rockford, IL) in the presence of protease inhibitors (Roche Applied Science, Barcelona, Spain), and subjected to Western blot analyses as described previously.[18] They were then subjected to Western blot analyses (10% SDS-PAGE; 25 µg protein per lane) with anti-rat anti-ABCA1 antiserum (kindly donated by Dr S. Yokoyama of Nagoya City University),[19] anti-rabbit anti-ABCG1, anti-rabbit anti–SR-BI, rabbit anti-EL (Novus Biologicals, Littleton, CO), anti-LDLR (low-density lipoprotein receptor; Abcam, Cambridge, United Kingdom), anti–SREBP-2 (sterol regulatory element-binding protein-2; Santa Cruz, Santa Cruz CA), and anti–β-actin (Santa Cruz) specific antibodies. The proteins were visualized by a chemiluminescence method (ECL Plus Western Blotting Detection System; Amersham Biosciences, Foster City, CA).

Real-Time Quantitative RT-PCR

Total RNA was extracted from the livers, and first-strand cDNA was synthesized from the total RNA (250 ng) by placing in a Reverse Transcription Reagent (Applied Biosystems, Foster City, CA). Quantitative PCR was performed with a Perkin–Elmer 7900 PCR machine, TaqMan PCR master mix, and FAM-labeled TaqMan probes (Assays-on-Demand, Applied Biosystems) for mouse ABCA1, ABCG1, ABCG5, ABCG8, SR-BI, CYP7A1, ABCB11, LDLR, HMG-CoA reductase, SREBP-2, EL, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Expression data were normalized for GAPDH levels.

In Vivo Macrophage RCT Studies

Acetylated LDL was prepared according to the methods reported previously.[20] RAW264.7 (RAW) cells were grown in RPMI1640 supplemented with 10% fetal bovine serum (FBS), and then radiolabeled with 5 µCi/mL ^{3}H-cholesterol and cholesterol enriched with 100 µg/mL of acetylated LDL for 48 hours. These foam cells were washed, equilibrated, detached with cell scrapers, resuspended in RPMI 1640, and pooled before being injected into mice.

The mice were caged individually with unlimited access to food and water. Four days after intravenous injection of adenoviral vectors, ^{3}H-cholesterol-labeled and acetylated LDL-loaded RAW cells (typically 5.0×10^6 cells containing 7.5×10^6 counts per minute [dpm] in 0.5 mL RPMI 1640) were injected intraperitoneally as described previously.[21] Blood was obtained at 24 and 48 hours, and plasma was subjected to liquid scintillation counting (LSC). Feces were collected continuously from 0 to 48 hours and stored at 4°C before being counted. At 48 hours after injection, mice were exsanguinated and livers and bile were removed.

Liver lipids were extracted according to the procedure of Bligh and Dyer.[22] Briefly, a 50 mg piece of tissue was homogenized in water, and lipids were extracted with a 2:1 (vol/vol) mixture of chloroform/methanol. The lipid layer was collected, evaporated, resuspended in a 3:2 (vol/vol) mixture of hexane/isopropanol, and counted in an LSC. The bile was directly counted in an LSC. The total feces collected from 0 to 48 hours were weighed and soaked in Millipore water (1 mL water per 100 mg feces) overnight at 4°C. An equal volume of ethanol was added the next day, and the samples were homogenized; 200 µL of the homogenized samples was counted in an LSC. Results were expressed as a percentage of dpm injected. To extract the ^{3}H-neutral sterols and ^{3}H-bile acid fractions, 2 mL of the homogenized samples was combined with 2 mL ethanol and 400 µL sodium hydroxide. The samples were saponified at 95°C for 2 hours and cooled to room temperature, and then ^{3}H-cholesterol was extracted 3× with 9 mL hexane. The extracts were pooled, evaporated, resuspended in toluene, and then counted in an LSC. To extract ^{3}H-bile acids, the remaining aqueous portion of the feces was acidified with concentrated hydrogen chloride and then extracted 3× with 9 mL ethyl acetate. The extracts were pooled together, evaporated, resuspended in ethyl acetate, and counted in an LSC.

HDL Metabolic Studies

Human HDL_3 was isolated from pooled human plasma by sequential ultracentrifugation (density 1.063 < density 1.21 g/mL). Dialyzed human HDL_3 was labeled with ^{3}H-cholesteryl ether (CEther) or ^{3}H-cholesteryl oleate (CEs). Fifty µCi of CEther or oleate in toluene were dried down under nitrogen. Ethanol was then added, and the solution taken up by a pipette. This was added to the HDL_3 solution (1.07 mL of dialyzed HDL_3 containing 10 mg of protein) over a period of 5 minutes while gently shaking with short interruptions for a brief vortex. The HDL_3 solution was incubated for 24 hours at 37°C. The HDL_3 from the solution was reisolated by ultracentrifugation (40 000 rpm, 48 hours) at the original density and the ^{3}H-HDL_3 was dialyzed overnight against PBS containing 0.01% EDTA. Finally, the ^{3}H-HDL_3 was filter-sterilized and stored at 4°C until injection.

For the metabolic study, 1 million dpm of HDL_3 labeled with ^{3}H-CEther or ^{3}H-CEs were injected intravenously via tail veins into C57BL/6J mice at day 4 after Ad-Luc or Ad-hEL injection. Blood was collected from the tail vein at 2 minutes, 1, 2, 6, 9, 24, and 48 hours. Feces were collected continuously up to 48 hours. Aliquots of 10 µL of plasma were counted for each time point by LSC. After correction with blank values, plasma decay curves for both tracers were normalized to the plasma counts at the initial 2 minutes time point after tracer injection. The fractional catabolic rates were determined using the SAAM II program.[23] After the 48 hours, blood samples were collected, livers were harvested. Results were expressed as a percentage of the injected dose.

Construction of Expression Plasmids Encoding SR-BI

To obtain open reading frames of human SR-BI, PCR was performed using first-strand cDNA derived from human monocyte-derived macrophages as a template and the specific primers. The sequences of primers with NotI sites used for vector construction were indicated as follows: forward: 5′-GCG GCC GCC ACC ATG GGC TGC TCC GCC CAA GC-3′; reverse: 5′-GCG GCC GCC TAC AGT TTT GCT TCC TGC AGC AC-3′. The PCR product was ligated in NotI sites of pcDNA3.1 (+) (Invitrogen), designated as phSR-BI. All constructs were verified by DNA sequencing.

Generation of Stable Cell Lines Expressing Human SR-BI

BHK-21 (Riken Cell Bank, Tsukuba, Japan) cells were maintained in DMEM containing 10% FBS. To generate SR-BI-expressing cells, BHK-21 cells were transfected with phSR-BI using the Lipofectamine LTX reagent (Invitrogen). Stable cell clones (BHK-SR-BI) were selected and maintained under DMEM supplemented with 10% FBS and G418 sulfate (0.8 mg/mL, Invitrogen). In control cells that were transfected with the parental vector pcDNA3.1 (+), no expression of SR-BI was detectable at the protein level as determined in specific immune blots.

HDL-CEther Uptake In Vitro

HepG2 cells (Riken Cell Bank) and BHK-SR-BI were infected with 30 multiplicity of infection of Ad-hEL or Ad-Luc for 24 hours. Then, the cells were incubated in DMEM containing 0.1% BSA and 10 µg/mL of ^{3}H-CEther-labeled HDL_3 in the presence or absence of 10 µmol\L of BLT-1, an SR-BI inhibitor,[24] for 24 hours. The percentage of HDL-CEther

uptake was calculated by dividing the cell-derived radioactivity by the sum of the radioactivity in the media and the cells.

Statistical Analysis

All the statistical analyses were conducted using the 2-tailed Student *t* test and ANOVA using GraphPad Prism Software. Results are presented as mean±SD. *P* values of <0.05 were considered to be statistically significant.

Results

Intravenous Injection of Adenoviral Vectors Harboring EL Resulted in Liver-Specific Overexpression of EL

First, we intravenously injected adenoviral vectors harboring human wild-type EL, an enzymatically inactive mutant, EL^{S149A} and luciferase (Ad-Luc), as a control, to C57BL/6J mice and assessed EL expression in the liver, peritoneal macrophages and pre- and postheparin plasma 4 days after the injection. As shown in Figure 1A, transduction with Ad-hEL and Ad-hEL^{S149A} resulted in EL protein overexpression in the liver, but not in peritoneal macrophages or other organs (Figure IA in the online-only Data Supplement), indicating that intravenous injection of adenoviruses achieved liver-specific overexpression of EL. In livers transduced with wild-type EL, 2 bands of EL protein were visualized in Western blot analysis:full-length protein (≈60 kDa), and a cleaved type (≈50 kDa) because of proprotein convertase as previously reported,[25] while in contrast, Ad-hEL^{S149A} generated only the upper band. There was no difference in human EL mRNA levels between the Ad-hEL and Ad-hEL^{S149A} groups (Figure II in the online-only Data Supplement). Figure 1A also shows the EL protein in plasma before and after heparin injection. Both EL viruses induced appearance of EL in the circulation. The experiment also revealed that Ad-hEL^{S149A} caused greater protein expression as compared with the wild-type, which was consistent with the previous observations[16] and heparin injection increased expression of the upper band. ABCA1 expression was not affected by Ad-hEL and Ad-hEL^{S149A} transduction. There was a decrease in the upper band of SR-BI (N-glycosylation form, ≈80 kDa; nonglycosylation form, ≈50 kDa) because of Ad-hEL but not Ad-hEL^{S149A}. We also measured hepatic expression of other molecules involved in cholesterol metabolism. Cleaved fragments (≈70 kDa; full-length protein, ≈140 kDa) of SREBP-2 were slightly reduced by hEL/hEL^{S149A} overexpression; however, LDLR expression was unchanged. Figure III in the online-only Data Supplement demonstrates that there were no significant differences in the hepatic mRNA expression of various molecules related to cholesterol metabolism, including mouse endogenous EL, between the groups. Overall, cholesterol homeostasis was maintained under EL overexpression.

Hepatic Overexpression of EL in Mice Induced a Marked Reduction in Cholesterol and Phospholipid Levels

Figure 1B and Figure IVA through IVC in the online-only Data Supplement show time courses of the effects of hepatic EL overexpression on plasma lipids. Wild-type EL induced a marked decrease in total cholesterol/phospholipids and a slight decrease in triglyceride as reported previously.[5,26] In contrast, overexpression of hEL^{S149A} did not affect plasma lipids. Fast protein liquid chromatography analysis of plasma obtained 4 days after virus injection revealed that cholesterol levels were markedly reduced in all lipoprotein fractions in EL-overexpressing mice. In contrast, Ad-hEL^{S149A} did not affect cholesterol levels in lipoprotein fractions, which was consistent with a previous study.[16]

Hepatic Overexpression of EL Dramatically Reduced HDL-C Levels but Maintained Overall RCT

We hypothesized that the marked reduction in HDL induced by EL overexpression would attenuate cholesterol efflux from macrophage to plasma. If this were the case, the decreased efflux would in turn inhibit cholesterol transfer to the liver and its excretion into feces, thereby decreasing overall in vivo RCT. To test this hypothesis, we performed a macrophage RCT study in EL-overexpressing mice. Four days after virus injection, ^{3}H-cholesterol labeled RAW264.7 (RAW) cells were intraperitoneally injected into the mice. As shown in Figure 1C, EL overexpression induced a dramatic reduction in the appearance of macrophage-derived ^{3}H-cholesterol in the systemic circulation, partly supporting our hypothesis. However, to our surprise, EL overexpression did not affect ^{3}H-cholesterol levels in the liver, bile, and feces (Figure 1D through 1G), despite a marked decrease in macrophage-derived ^{3}H-cholesterol in plasma. Fecal excretion of the tracer in neutral sterols and bile acids did not differ between the groups.

These results indicate that severe hypoalphalipoproteinemia induced by EL overexpression does not directly translate into inhibition of overall RCT from macrophage to feces in mice.

Despite Increased Cholesterol Uptake by Liver, Hepatic EL Overexpression Did Not Enhance HDL-Derived Cholesterol Excretion Into Bile and Feces

Based on the previous observation that HDL particles were rapidly catabolized in the livers of EL-expressing mice,[26] we next hypothesized that promotion of cholesterol delivery to the liver by EL-modified HDL particles compensates for the low efflux capacity from macrophages to plasma, thereby maintaining RCT. To test this hypothesis, we performed an HDL turnover study using ^{3}H-CEther, an analog of cholesteryl ester (CE). In plasma, CEther behaves like CE, but has the advantage that it is not hydrolyzed in tissues, allowing assessment of hepatic uptake. Figure 2A shows that HDL-CEther was catabolized much faster in EL-overexpressing mice than in controls, with fractional catabolic rates of 0.94±0.23 and 0.3±0.05 pools/day, respectively (Figure 2B). About hepatic uptake of HDL-derived ^{3}H-CEther, EL overexpression significantly increased uptake at early (6 hours, by 55%) and late (48 hours, by 36%) time points (Figure 2C), supporting our hypothesis.

To further assess whether enhanced uptake of HDL-derived cholesterol by the liver translates into the promotion of

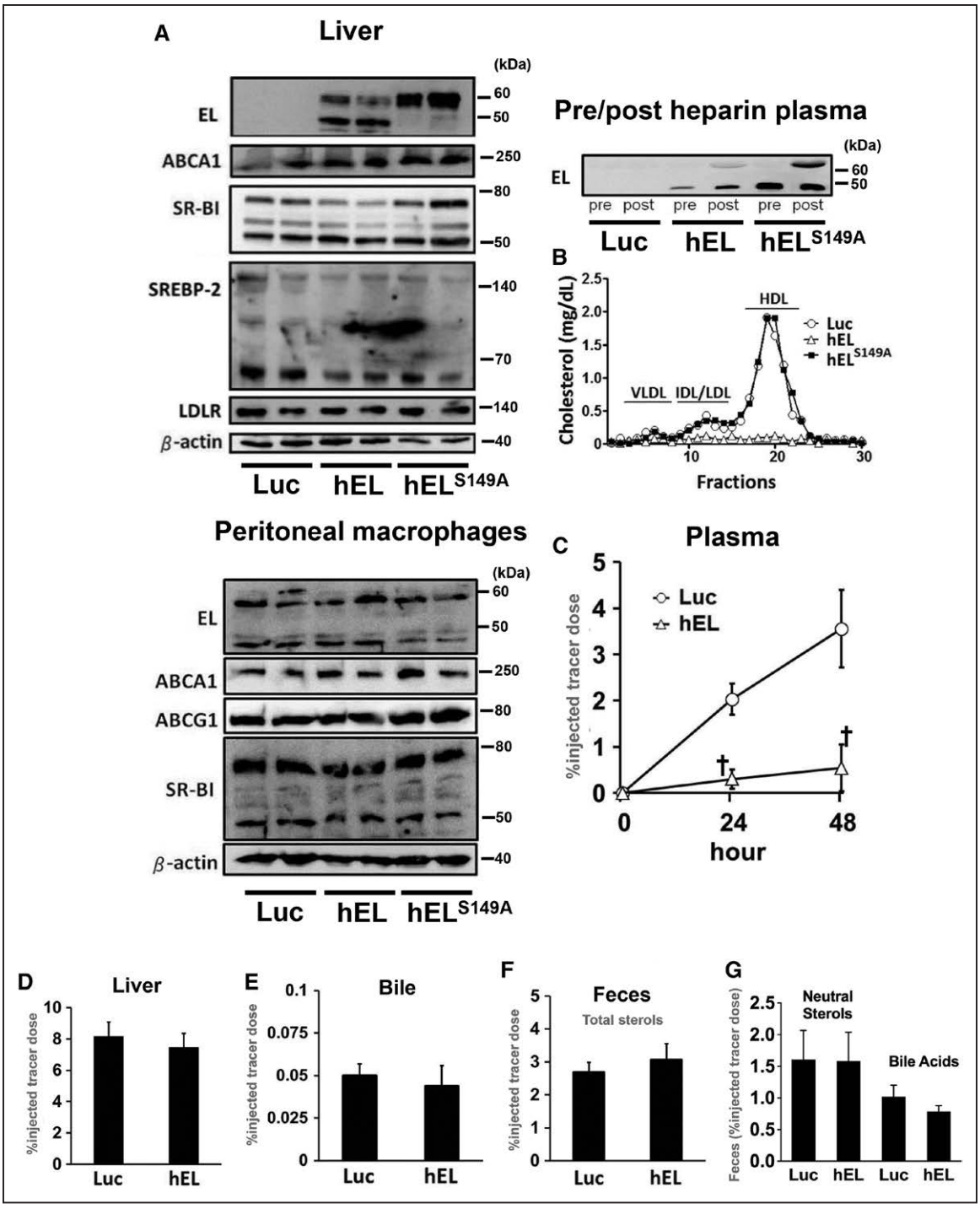

Figure 1. Macrophage reverse cholesterol transport (RCT) is not affected by hepatic endothelial lipase (EL) overexpression in mice despite markedly reduced HDL (high-density lipoprotein) levels. **A**, Four days after intravenous injection of adenovirus expressing human EL (Ad-hEL), Ad-hELS149A, or adenoviral vectors harboring luciferase (Ad-Luc) into C57BL/6J mice, liver, peritoneal macrophage and pre/postheparin plasma samples were obtained and subjected to Western blot analyses as described in Materials and Methods. **B**, Four days after intravenous injection of Ad-hEL, Ad-hELS149A, or Ad-Luc into C57BL/6J mice, plasma was obtained and subjected to lipid determination as described in Methods. Data are given as mean±SD. **C**–**G**, Four days after intravenous injection of Ad-hEL, Ad-hELS149A, or Ad-Luc into C57BL/6J mice, plasma was obtained and separated into lipoprotein fractions by fast protein liquid chromatography (FPLC). Cholesterol levels in each fraction were determined. Four days after intravenous injection of Ad-Luc or Ad-hEL into mice, ^{3}H-cholesterol-labeled RAW cells were intraperitoneally injected. At the indicated hours after injection, plasma was obtained and subjected to ^{3}H-tracer analysis (**C**). Forty-eight hours after injection, the liver (**D**) and bile (**E**) were isolated and then subjected to 3H-tracer analysis. Feces (**F**, **G**) collected continuously from 0 to 48 h were subjected to ^{3}H-counting. Neutral sterols and bile acids were separated as described in Materials and Methods. Data are expressed as percent counts relative to total injected tracer (means±SD, n=6 for each group). †P<0.01 vs Ad-Luc. ABCA1 indicates ATP-binding cassette transporter A1; IDL, intermediate-density lipoprotein; LDLR, low-density lipoprotein receptor; SR-BI, scavenger receptor class B type I; SREBP-2, sterol responsive element-binding protein-2; and VLDL, very-low-density lipoprotein.

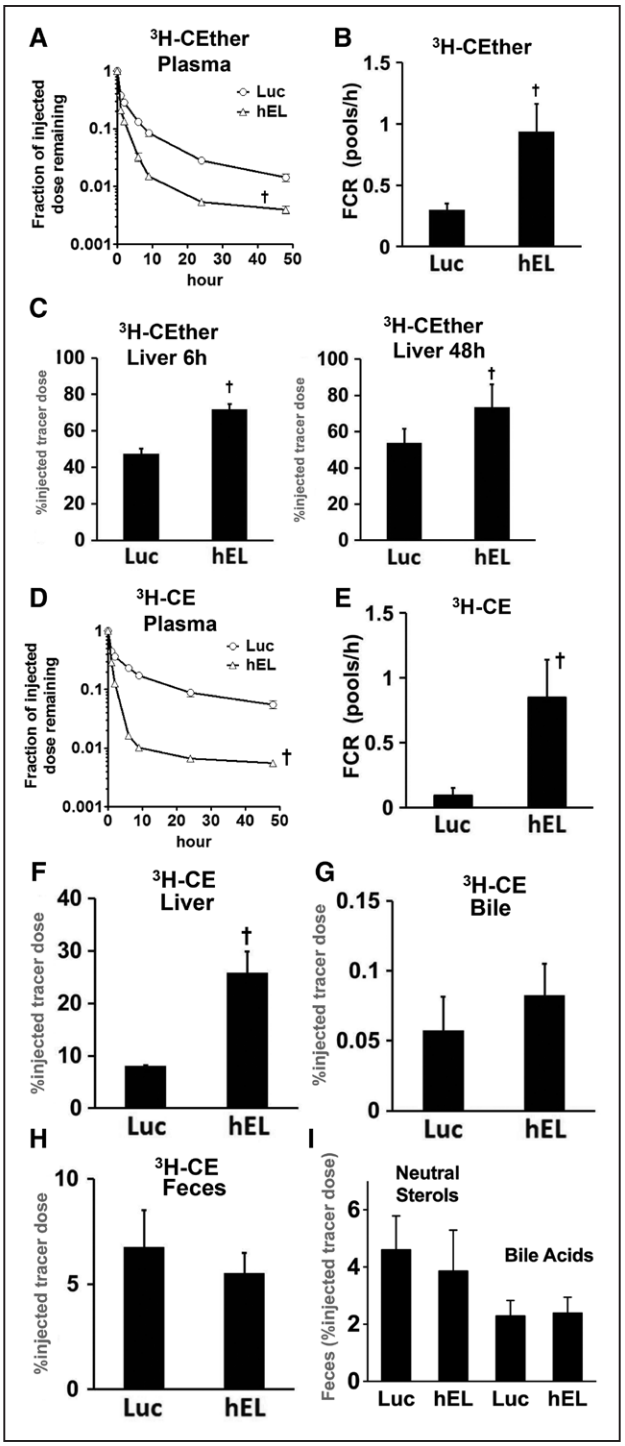

Figure 2. Hepatic overexpression of endothelial lipase (EL) accelerates HDL (high-density lipoprotein) catabolism and increases hepatic uptake of HDL-cholesteryl ether (CEther)/cholesteryl oleate (CEs) but does not alter biliary and fecal excretion. **A**, Four days after intravenous injection of adenoviral vectors harboring luciferase (Ad-Luc) or adenovirus expressing human EL (Ad-hEL), the mice were intravenously injected with ^{3}H-CEther-labeled HDL_3. Plasma was obtained after the indicated times and then subjected to ^{3}H-tracer analysis. Plasma decay curves were normalized to radioactivity at the initial 2-min time point after tracer injection. Data are expressed as percent counts relative to total injected tracer, mean±SD. **B**, The fractional catabolic rates (FCR) were determined from the area under the plasma disappearance curves fitted to a bicompartmental model using the SAAM II program. Six (**C**) or 48 (**D**) h after injection with ^{3}H-CEther-HDL_3, livers were isolated and then subjected to ^{3}H-tracer analysis. (*Continued*)

its excretion into bile and feces, we next performed a separate kinetic experiment using HDL_3 labeled with ^{3}H-CEs, which is metabolized to free cholesterol and bile acids in the liver. The results revealed that EL overexpression again accelerated catabolism of HDL-CEs and hepatic cholesterol uptake, similarly to HDL-CEther (Figure 2D through 2F). However, there was no difference in excretion of ^{3}H-CEs in bile (Figure 2G; 0.057±0.02% versus 0.08±0.02%, Ad-hEL versus Ad-Luc, respectively) or in feces (Figure 2H; 5.9±1.48% versus 4.5±0.87%, Ad-hEL versus Ad-Luc, respectively) between experimental groups. Again, fecal excretion of the tracer in neutral sterols and bile acids did not differ between the groups (Figure 2I).

Taken together, these results indicate that despite increased cholesterol uptake by the liver, hepatic EL overexpression did not enhance HDL-derived cholesterol excretion into bile and feces.

EL With No Lipase Activity Promoted Uptake of Macrophage-Derived Cholesterol by Liver but Did Not Affect RCT

Independently of its lipase activity, EL reportedly functions as a ligand by promoting lipoprotein uptake both in vitro[27] and in vivo.[16] This observation raises 2 questions (1) whether this nonlipolytic function contributes to the observed enhanced uptake of HDL-C because of hepatic EL overexpression and (2) whether it affects RCT.

To try to answer these questions, we performed an in vivo macrophage RCT study in mice injected with Ad-hEL^{S149A}, an inactive mutant for lipolysis. Interestingly, overexpression of the mutant EL resulted in reduced appearance of plasma ^{3}H-cholesterol derived from macrophages 24 hours (by 22%) and 48 hours (by 17%) after the injection of the macrophages (Figure 3A), despite having no effect on plasma HDL-C levels (Figure 1B). In sharp contrast to the observed reduction in circulating ^{3}H-cholesterol, hepatic tracer counts were significantly increased (by 68%) in EL^{S149A}-expressing mice (Figure 3B) as compared with controls. However, there was no significant difference in excretion of ^{3}H-cholesterol into bile (Figure 3C) and feces (Figure 3D) between the groups. Fecal excretion of the tracer in neutral sterols and bile acids was comparable between the groups (Figure 3E).

Given that EL did not affect RCT from HDL to feces despite increased delivery of HDL-derived cholesterol to the liver (Figures 2 and 3), and taking this together with the recent observation that EL facilitates selective uptake of HDL-CE

Figure 2 Continued. Values are expressed as a percentage of the total ^{3}H-CEther-HDL_3 injected (means±SD, n=5 for each group). **D**, After intravenous injection of Ad-Luc or Ad-hEL, the mice were intravenously injected with 3H-CEs-labeled HDL_3. Obtained plasma was subjected to ^{3}H-tracer analysis as described above. **F–I**, Forty-eight hours after injection with ^{3}H-CEs-HDL_3, livers (**F**) and bile (**G**) were isolated and then subjected to ^{3}H-tracer analysis. Feces collected continuously from 0 to 48 h were also subjected to ^{3}H-counting (**H**, **I**). Neutral sterols and bile acids were separated as described in Materials and Methods. Values are expressed as a percentage of the total ^{3}H-CEs-HDL_3 injected (means±SD, n=5 for each group). **P*<0.05, †*P*<0.01 vs Ad-Luc.

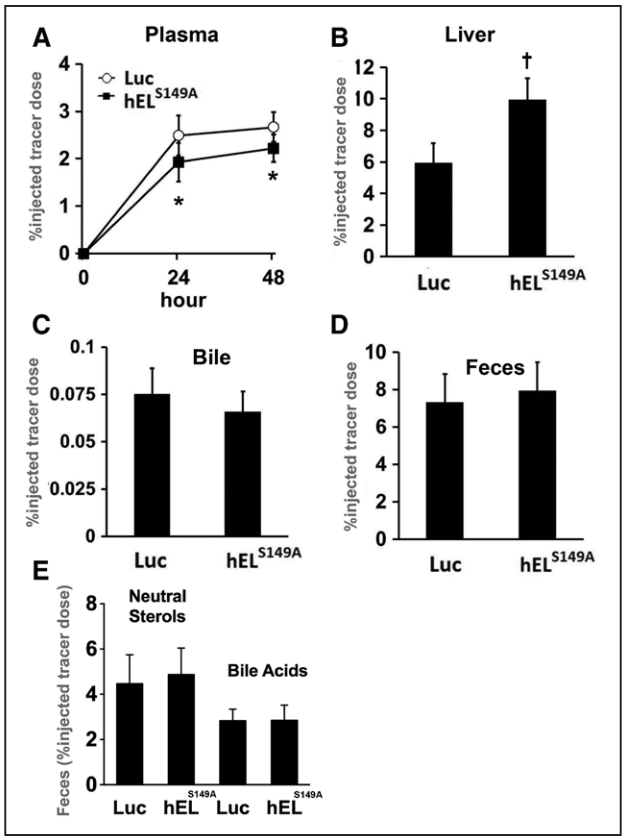

Figure 3. Endothelial lipase (EL) with nonlipase activity promotes uptake of macrophage-derived cholesterol by the liver but does not affect reverse cholesterol transport (RCT). Four days after intravenous injection of adenoviral vectors harboring luciferase (Ad-Luc) or adenovirus expressing human EL (Ad-hELS149A) into mice, ^{3}H-cholesterol-labeled RAW cells were intraperitoneally injected. At the indicated hours after injection, plasma was obtained and subjected to ^{3}H-tracer analysis (**A**). Forty-eight hours after injection, livers (**B**), and bile (**C**) were isolated and then subjected to ^{3}H-tracer analysis. Feces (**D**, **E**) collected continuously from 0 to 48 h were subjected to ^{3}H-counting. Neutral sterols and bile acids were separated as described in Materials and Methods. Data are expressed as percent counts relative to total injected tracer (means±SD, n=6 for each group). **P*<0.05, †*P*<0.01 vs Ad-Luc.

through SR-BI in the liver,[28] we hypothesized that SR-BI affects EL-mediated HDL uptake by the liver.

EL Promoted HDL-C Uptake from Cells in SR-BI–Dependent Fashion

To test the above hypothesis, we performed an in vitro cholesterol uptake study using HepG2 and BHK-SR-BI, a stable SR-BI expressing cell line. Figure 4 shows that adenoviral transduction of EL attenuated upper bands (N-glycosylated, ≈80 kD) and enhanced lower bands (non-N-glycosylated, ≈50 kD) of SR-BI as compared with controls in both HepG2 and BHK cells. EL overexpression in HepG2 cells resulted in enhancement of HDL-CEther uptake, by 16%, which was completely canceled in the presence of the SR-BI inhibitor BLT-1[29] (Figure 4A). In the case of BHK-SR-BI cells, BLT-1 inhibited HDL-CEther uptake to a greater extent in EL-expressing cells as compared with controls (Figure 4B; 23% versus 39%, Ad-hEL versus Ad-Luc). These data suggest that EL contributes to HDL-CE uptake by the liver in an SR-BI–dependent fashion.

RCT Maintained by Hepatic EL Overexpression Is Dependent on SR-BI Expression in Liver

We next performed a macrophage RCT study in mice, in which adenoviral vectors expressing EL and miR against mouse SR-BI (adenoviral vectors expressing miR against mouse SR-BI) were coadministered to investigate whether the above in vitro observations translate into the in vivo setting. As shown in Figure 5A, we first confirmed that intravenous injection of adenoviral vectors expressing miR against mouse SR-BI achieved marked knockdown of hepatic SR-BI (both N-glycosylated and nonglycosylated protein) without change of expression in peritoneal macrophages (Figure 5B). Under transduction of adenoviral vectors expressing miR against mouse SR-BI, exogenous EL expression (both full-length protein and cleaved type) was attenuated as compared with the controls injected with adenoviral vectors expressing miR against LacZ. Fast protein liquid chromatography lipoprotein analysis demonstrated that hepatic SR-BI knockdown increased cholesterol levels across all lipoprotein fractions (Figure 5C and 5D), an observation consistent with that for liver-specific SR-BI knockout mice.[30] Similar to the control mice, hepatic overexpression dramatically reduced lipoprotein-cholesterol levels, excepting IDL (intermediate-density lipoprotein)/LDL for which a slight peak remained (Figure 5D).

As shown in Figure 5E through 5H, the in vivo macrophage RCT study revealed that hepatic SR-BI knockdown increased plasma macrophage-derived ^{3}H-cholesterol (by 67%) as compared with controls, and reduced ^{3}H-tracer counts in bile (by 35%) and feces (by 54%), but hepatic tracer levels were not changed by SB-BI knockdown. These findings indicate that inhibition of hepatic SR-BI attenuated RCT in vivo, which is consistent with the results of a previous study using knockout mice.[13]

Thus, about the effects of EL overexpression, we obtained data supporting our in vitro observations; namely that only under SR-BI knockdown did hepatic EL overexpression further reduce excretion of macrophage-derived ^{3}H-cholesterol into bile (Figure 5G) and feces (Figure 5H). Both neutral sterols and bile acids derived from fecal ^{3}H-tracer were equally decreased in hEL/miR mSR-BI mice. Also, the slightly greater appearance of ^{3}H-tracer in plasma (in hEL/miR mSR-BI mice compared with hEL/miR LacZ mice; Figure 5E) might be associated with reduced excretion of ^{3}H-tracer into feces.

Collectively, these results indicate that hepatic overexpression of EL induces severe hypoalphalipoproteinemia but maintains macrophage RCT in an SR-BI–dependent fashion in mice.

Hepatic Overexpression of EL Facilitates RCT Under Pharmacological Inhibition of ABCA1

Finally, to investigate whether hepatic ABCA1 expression contributed to EL-mediated RCT promotion, we performed an in vivo RCT assay under pharmacological inhibition of ABCA1 with probucol.[15] In this experiment, we used a lower dose (1×10^{8} PFU) of adenoviral vectors to achieve a milder

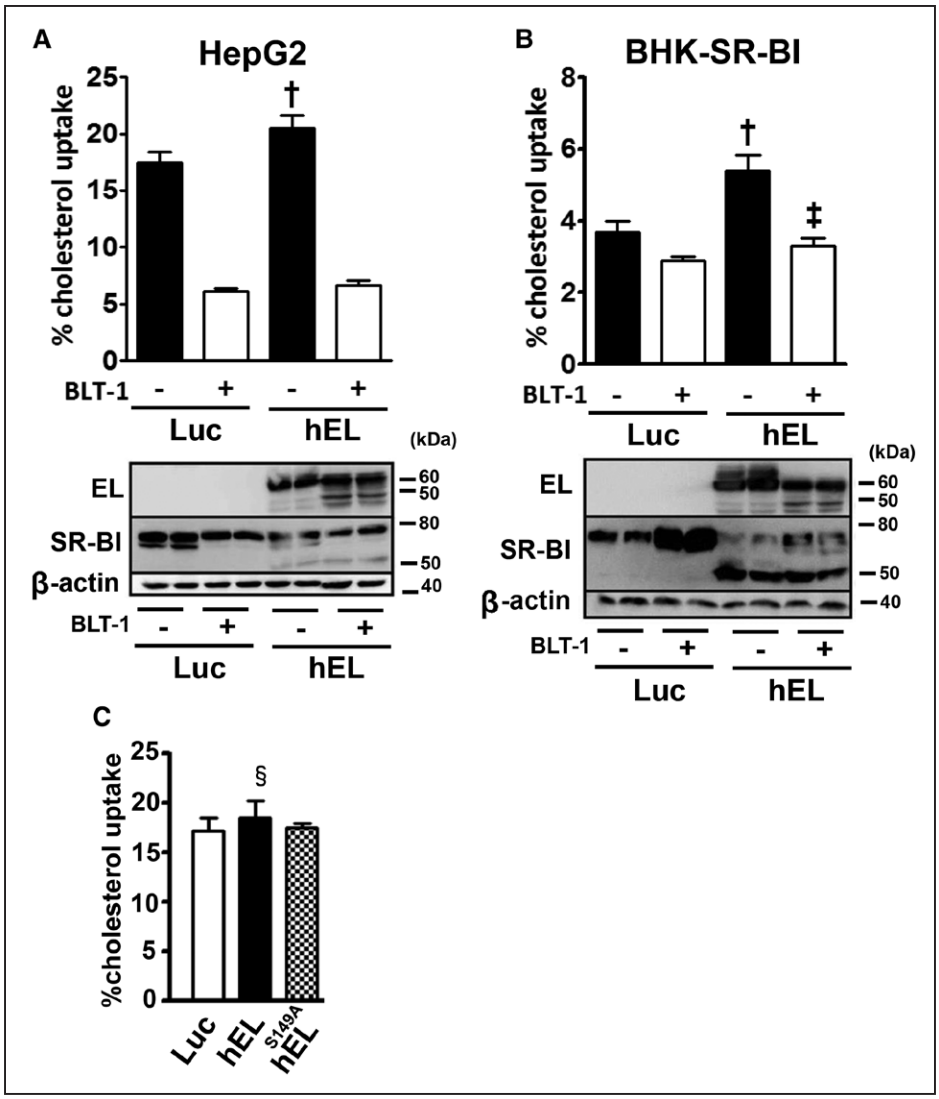

Figure 4. Endothelial lipase (EL) promotes HDL-C (high-density lipoprotein-cholesterol) uptake from cells in an SR-BI (scavenger receptor class B type I)–dependent fashion. HepG2 cells (**A**, **C**) and BHK-SR-BI (**B**) were infected with 30 multiplicity of infection of adenovirus expressing human EL (Ad-hEL)/hEL^{S149A} or adenoviral vectors harboring luciferase (Ad-Luc) for 24 h. Then, the cells were incubated in DMEM containing 0.1% BSA and 10 μg/mL of ^{3}H-cholesteryl ether (CEther)-labeled HDL_3 in the presence or absence of 10 μmol\L of BLT-1 for 24 h. The percentage of HDL-CEther uptake was calculated as described in Materials and Methods. †P<0.01 vs Ad-Luc in the absence of BLT-1, ‡P<0.01 vs Ad-hEL in the absence of BLT-1, §P<0.01 vs Ad-Luc. Using the cell lysates obtained just before ^{3}H-CEther counting, Western blot analyses were performed.

effect on HDL reduction as compared with that in the above experiments (Figures 1 through 5: 5×10^{8} PFU). Indeed, Ad-hEL injection reduced HDL-C levels but a peak was still visible (Figure 6A). ABCA1 inhibition by the probucol diet also resulted in a reduction in HDL-C level comparable to that because of EL overexpression, and both interventions synergistically exacerbated hypoalphalipoproteinemia. The appearance of ^{3}H-tracer derived from macrophages in plasma was in parallel with nonhot cholesterol levels in the HDL fractions (Figure 6A and 6C). About tracer levels in the liver and bile (Figure 6D and 6E), there were no significant differences between adenoviruses/diets, except for increased tracer in the liver because of probucol. In contrast, we found dramatic differences in the tracer in feces; EL overexpression markedly increased fecal ^{3}H-tracer excretion only under the probucol diet (Figure 6F). Both neutral sterols and bile acids derived from fecal ^{3}H-tracer were equally increased in hEL/probucol mice (Figure VI in the online-only Data Supplement). These findings suggest that hepatic ABCA1 attenuates excretion of cholesterol derived from EL-modified HDL into bile/feces as compared with normal HDL.

Discussion

The large burden of atherosclerotic disease remains despite currently available optimum medical therapies. LDL has been the primary target in lipid management and HDL has been considered as the next target to further reduce the residual risk. In this regard, in spite of achieving modest[31] to marked[32] increases in HDL-C levels, CETP (CE transfer protein) inhibitors have not yet provided clinical benefits. In this context, one would expect that enhancing HDL functionality, specifically about RCT, could be the next target for preventing

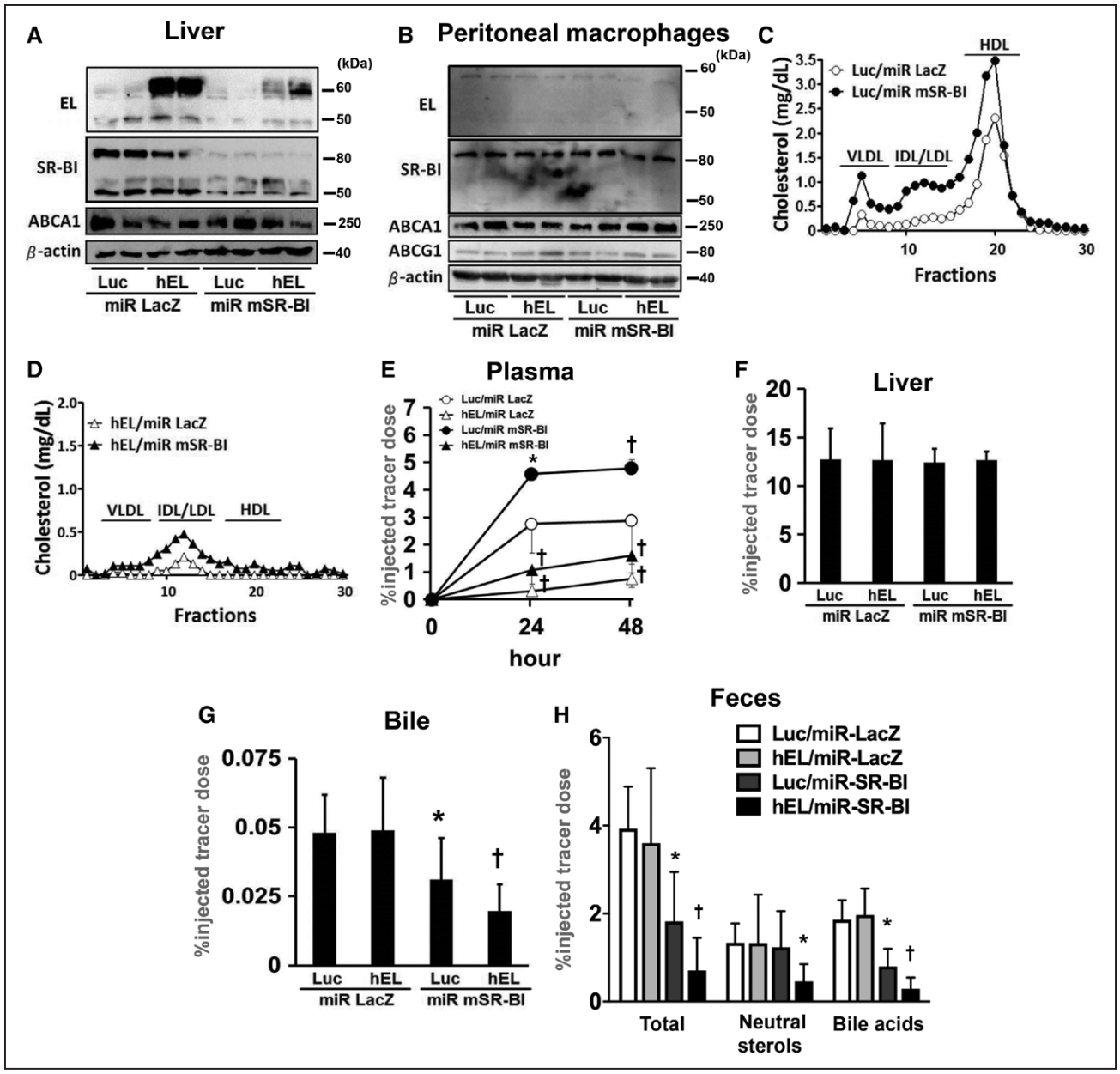

Figure 5. Reverse cholesterol transport (RCT) maintained by hepatic endothelial lipase (EL) overexpression is dependent on SR-BI (scavenger receptor class B type I) expression in liver. **A**–**D**, Four days after intravenous injection of adenoviral vectors expressing miR against LacZ (Ad-miR LacZ) or Ad-miR SR-BI together with adenoviral vectors harboring luciferase (Ad-Luc) or adenovirus expressing human EL (Ad-hEL) into mice, livers (**A**), peritoneal macrophages (**B**), and plasma were obtained and subjected to Western blot analyses as described in Materials and Methods. The plasma was separated into lipoprotein fractions by fast protein liquid chromatography (FPLC). Cholesterol levels in each fraction were determined (**C**, **D**). **E**–**H**, Four days after intravenous injection of Ad-miR LacZ or Ad-miR SR-BI together with Ad-Luc or Ad-hEL into mice, ^{3}H-cholesterol-labeled RAW cells were intraperitoneally injected. At the indicated hours after injection, plasma was obtained and subjected to ^{3}H-tracer analysis (**E**). Forty-eight hours after injection, liver (**F**) and bile (**G**) were isolated and then subjected to ^{3}H-tracer analysis. Feces (**H**) collected continuously from 0 to 48 h were subjected to ^{3}H-counting. Neutral sterols and bile acids were separated as described in Materials and Methods. Data are expressed as percent counts relative to total injected tracer (means±SD, n=6 for each group). *P<0.05, †P<0.01 vs Ad-Luc/Ad-miR LacZ. ABCA1 indicates ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein receptor; and VLDL, very-low-density lipoprotein.

atherosclerosis. Supporting this concept, we[33] and Khera et al[4] have demonstrated that ability of serum HDL to promote cholesterol efflux from macrophages was a better negative risk predictor for coronary artery disease as compared with HDL-C levels (However, one study[34] reported an opposite result). As described above, inhibition of EL has been shown to achieve an increase in HDL-C levels, and EL is reportedly involved in inflammation,[35] which is another important mechanism for atherogenesis. Therefore, like CETP, EL is an emerging target for atherosclerotic diseases where HDL-C levels are raised.

Conversely, we demonstrated, for the first time, that hepatic EL expression resulted in extremely low circulating

HDL levels but maintained overall RCT in vivo by promoting excretion of cholesterol to bile/feces via an SR-BI pathway. As previous studies using mouse models[36,37] have demonstrated, low HDL levels at steady state are not always associated with enhanced atherogenesis and the present study is another example of this. Two such studies reported opposing results using EL knockout mice; one[38] noted that EL deficiency promotes RCT, whereas the other[39] found no difference from the control. The precise mechanisms for this discrepancy are unknown, but we might not be able to directly compare the effect of EL on RCT between knockout and overexpression mice models.

Hepatic cholesterol uptake is recognized as a pivotal step of RCT. EL reportedly mediates hepatic uptake of lipoproteins, including HDL, by bridging lipoproteins with heparan sulfate proteoglycans on sinusoidal endothelial cells via the corresponding binding domains.[27] EL contributes to not only HDL holoparticle uptake[27] but also to selective uptake of CE from HDL, a function independent of its enzymatic activity.[27,40] Indeed, the present study demonstrated that EL overexpression promoted HDL-CEs uptake in vivo (Figure 2) and in vitro (Figure 4). Although the steady state HDL-C levels were not changed by Ad-hEL^{S149A} (Figure 1B), the appearance of macrophage-derived ^{3}H-cholesterol was reduced in plasma (Figure 3A) and

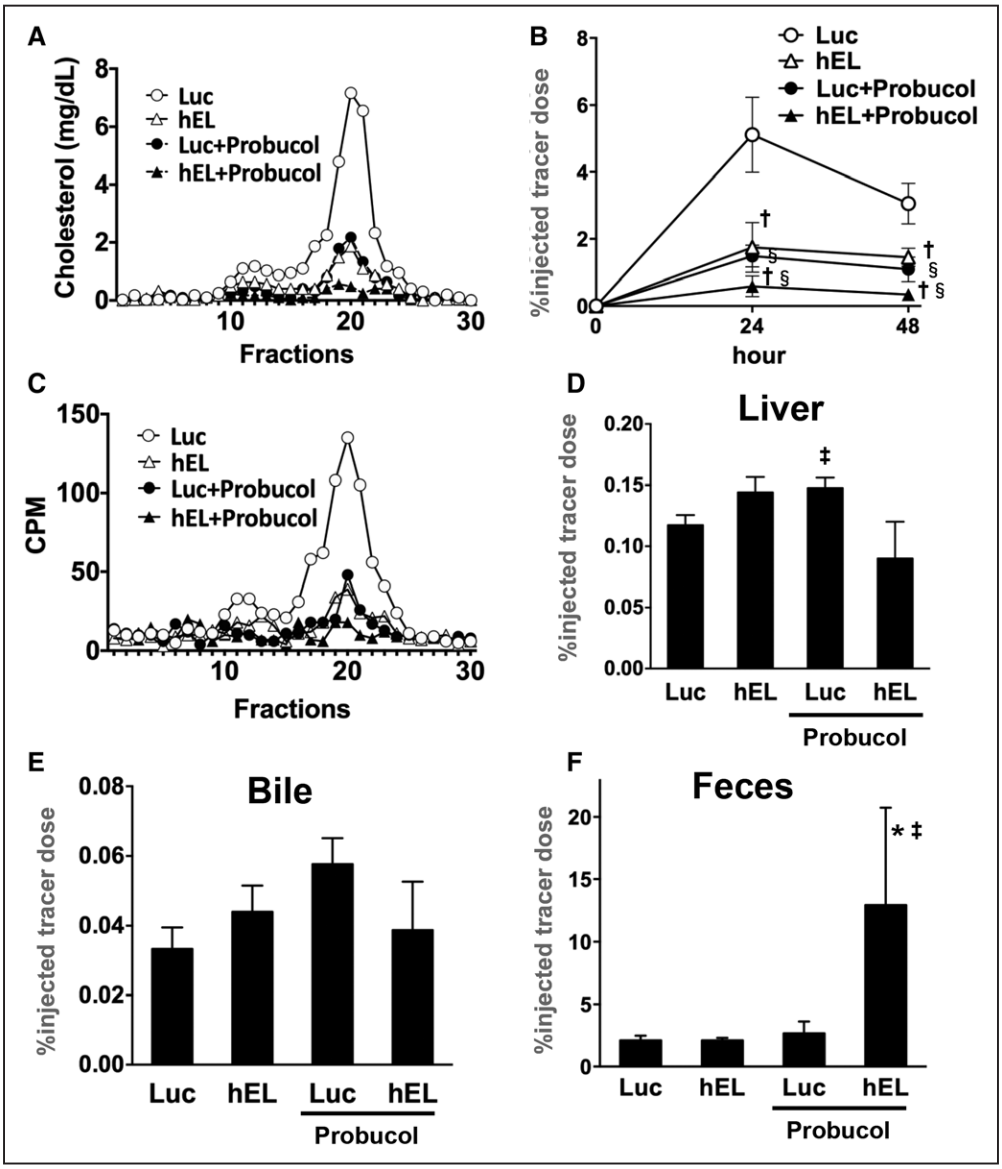

Figure 6. Hepatic endothelial lipase (EL) overexpression enhances reverse cholesterol transport (RCT) under pharmacological ABCA1 (ATP-binding cassette transporter A1) inhibition. **A**–**D**, Fourteen days after feeding with a 0.5% probucol-containing diet or the vehicle, adenoviral vectors harboring luciferase (Ad-Luc) or adenovirus expressing human EL (Ad-hEL) was injected intravenously into mice. Four days afterward, ^{3}H-cholesterol-labeled RAW cells were intraperitoneally injected. We measured cholesterol (**A**) and ^{3}H-tracer activity (**C**) in fractions separated by fast protein liquid chromatography (FPLC) from plasma obtained at 48 h, and ^{3}H-tracer activity in whole plasma (**B**) obtained at the indicated times, as described in Materials and Methods. Livers (**D**) and bile (**E**) were isolated, and then subjected to ^{3}H-tracer analysis. Feces (**F**) collected continuously from 0 to 48 h were subjected to ^{3}H-counting. Data are expressed as percent counts relative to total injected tracer (means±SD, n=6 for each group). *P<0.05, †P<0.01 Ad-Luc vs Ad-hEL; ‡P<0.05, §P<0.01 probucol vs the vehicle. (*Continued*)

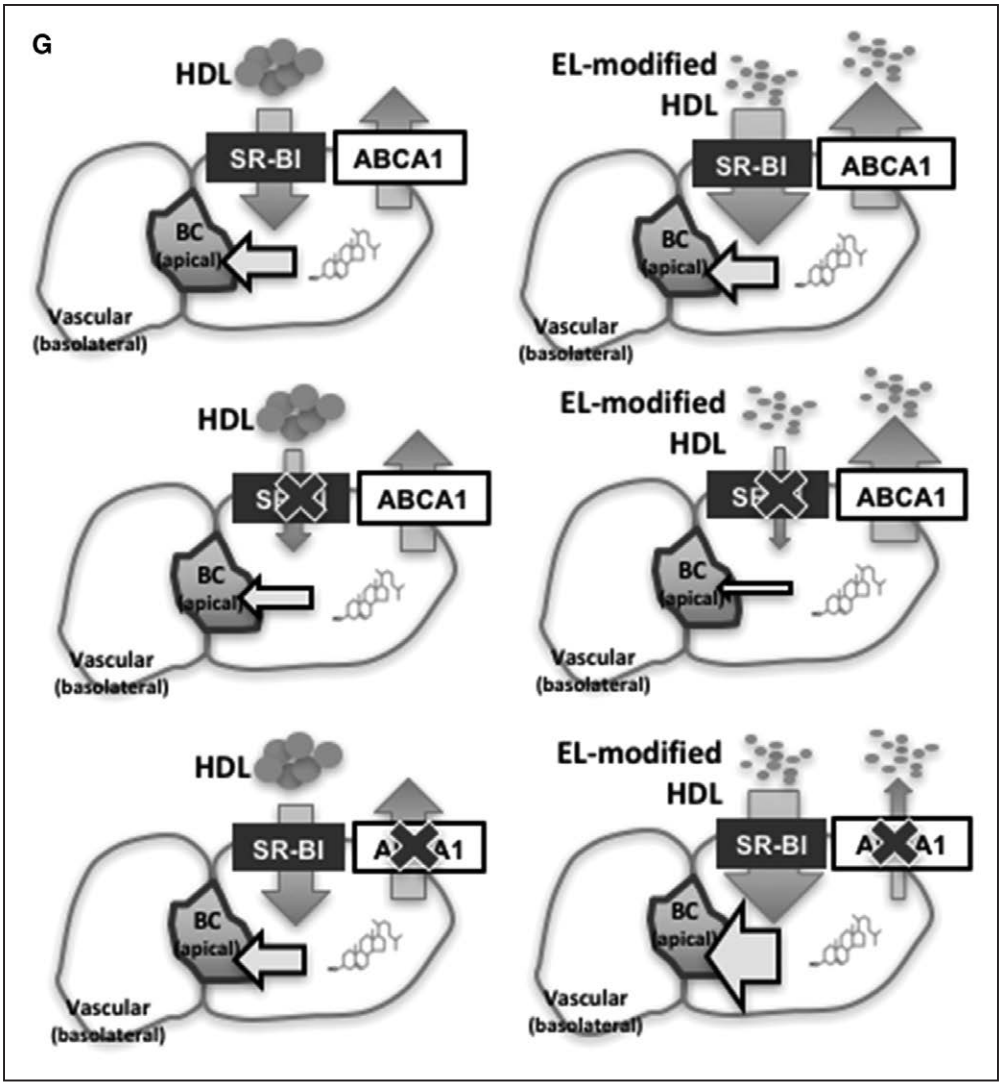

Figure 6 Continued. G, Schematic hypothesis for SR-BI (scavenger receptor class B type I)/ABCA1 (ATP-binding cassette transporter A1)-dependent regulation of RCT by hepatic EL overexpression. Figure shows that hepatocytes form bile canalicule (BC) and SR-BI/ABCA1 are located at basolateral side. Arrows indicate the flow of cholesterol and its metabolites and their size the amount of flow. HDL indicates high-density lipoprotein.

increased in the liver (Figure 3B). These observations suggest that EL exerted its nonlipolytic ligand function by promoting hepatic uptake of HDL-CE derived from macrophages. A putative mechanism by which only mutant EL (Figure 3B), but not wild-type (Figure 1D), increased hepatic uptake of macrophage-derived ^{3}H-cholesterol might be that extremely low HDL-C levels because of wild-type EL overexpression counteracted the promoted hepatic uptake of ^{3}H-cholesterol. Strauss et al[40] reported that EL protein could be released from the cell surface of endothelial cells by free fatty acids produced during lipolysis of phospholipids by EL itself. This supports the finding shown in Figure 1C that heparin injection-induced greater release of endothelial-bound mutant EL as compared with wild-type. However, it remains unclear why only wild-type, but not mutant, EL protein was cleaved in the liver (Figure 1A), so further study is needed to clarify this issue.

In the macrophage RCT study using Ad-hEL^{S149A} (Figure 3) and HDL-CEs kinetic study using Ad-hEL (Figure 2), there were no differences in fecal ^{3}H-cholesterol excretion despite increases in ^{3}H-cholesterol levels in the liver. There was no difference in excretion of both neutral sterols and bile acids derived from fecal ^{3}H-tracer between mice treated with Ad-hEL$^{(S149A)}$ and control adenovirus (Figures 2I and 3E). Also, expressions of the hepatic cholesterol transporters ABCG5/G8, and the bile acids transporter ABCB11 were not changed by EL overexpression (Figure III in the online-only Data Supplement). These observations suggest that there are unknown gatekeepers that regulate cholesterol traffic from the liver to bile. Because EL reportedly facilitates selective uptake of HDL-CE via SR-BI in the liver,[28] we focused on SR-BI, an HDL receptor that mediates the selective uptake of HDL-CE in the liver without degradation of lipoproteins.[41] Zhang et al[13] demonstrated that, through this property, hepatic SR-BI expression contributes to decreasing plasma HDL-C levels and increasing RCT and in sharp contrast, in SR-BI deficient mice, plasma HDL-C levels were increased, but RCT was reduced. From this, they concluded that hepatic SR-BI

positively contributes to RCT despite decreasing HDL-C levels. The present study confirmed these observations. Namely, hepatic knockdown of SR-BI resulted in increased HDL-C levels (Figure 6C) and tracer counts in plasma (Figure 6E), and reduced counts in feces (Figure 6H) Moreover, our detailed results were similar to those of Zhang et al,[13] marked increase/decrease in tracer counts in plasma/feces, and small changes in liver.

As described above, EL reportedly stimulates the selective uptake of CE and holoparticles of HDL, whose mechanisms are dependent and independent of SR-BI, respectively.[28] The EL-mediated promotion of HDL-CE selective uptake is completely canceled in SR-BI knockout mice.[28] Cholesterol in HDL, as compared with other lipoproteins, is thought to represent a preferred source of sterols secreted into bile after hepatic uptake by SR-BI.[31] Utilizing polarized cells, Silver et al[41] found that HDL protein and cholesterol undergo selective sorting with recycling of HDL protein from the basolateral membrane and secretion of HDL-derived cholesterol through the apical membrane in parallel with movement of SR-BI. These findings led them to hypothesize that HDL particles undergo a novel process of selective transcytosis dependent on SR-BI, resulting in preferential transport of cholesterol to bile and eventually feces. Taking these observations and our findings together (Figure 5), SR-BI is considered to be an important regulator of cholesterol traffic from the liver to bile/feces and might take up EL-modified HDL particles more effectively than normal HDL particles.

Based on our results and the observations of Nijstad et al,[28] RCT might be maintained under EL overexpression through the promotion of selective uptake of CE via SR-BI from modified, smaller HDL (Figure 6G), rather than by HDL holoparticle uptake by EL itself,[27] a function independent of its enzymatic activity. If SR-BI expression is deficient, this compensative effect is abolished, resulting in reduced RCT (Figure 6G). This mechanism might be the main reason why EL overexpression maintains in vivo RCT despite the marked hypoalphalipoproteinemia. Indeed, Brown et al[39] reported that, in EL knockout mice, plasma HDL-C levels were elevated but in vivo RCT remained unchanged as compared with controls. In the present study, we found that SR-BI knockdown reduced exogenous EL expression. Human EL mRNA levels were not affected, and serum EL levels in the SR-BI knockdown mice were slightly upregulated as compared with the control (data not shown). EL overexpression also affected post-translational protein modifications of SR-BI (Figure 4), which are reportedly critical for localization at caveolae.[42] Although the precise mechanisms for these phenomena remain unclear, the above observations might shed more light on the interaction between SR-BI and EL.

Cholesterol efflux from macrophages via ABCA1 is the initial, and very important, the step of RCT, and is associated with atherogenesis and HDL generation. Qiu et al[43] reported that overexpression and knockdown of EL in macrophages respectively increased and reduced ABCA1-mediated cholesterol efflux. This is mainly attributable to a catalytic mechanism by which smaller HDL particles are formed, supporting the previous observation that mouse HDL remodeling because of hepatic EL overexpression resulted in promoted ABCA1-mediated cholesterol efflux accompanied by a shift in the HDL particle distribution toward lipid-poor fractions.[44] Having high capacity for cholesterol efflux from macrophages, such HDL particles could in part counteract a reduction in RCT because of extremely low HDL-C levels induced by EL. About the role of ABCA1 in the liver, it still remains unclear whether it affects overall RCT.[15]

Yamamoto et al[15] reported that pharmacological ABCA1 inhibition with probucol did not affect in vivo RCT in wild-type mice, while in sharp contrast, it promoted RCT in SR-BI deficient mice. In the present study, we observed RCT promotion by probucol only under hepatic EL overexpression (Figure 6F). Because ABCA1 is reportedly located at the basolateral (vascular) side, similar to SR-BI (Figure 6G), it may theoretically attenuate cholesterol excretion into bile/feces, resulting in inhibition of overall RCT by returning cholesterol to the circulation to form nascent HDL particles. It is conceivable that EL-modified smaller HDL particles are better acceptors in ABCA1-mediated HDL neogenesis at the vasolateral side of hepatocytes (Figure 6G). Such a mechanism might maintain RCT homeostasis. Annema et al[45] reported that probucol treatment promoted in vivo macrophages-to-feces RCT in apoE3 overexpressed mice. Taking this result together with our findings and those of Yamamoto et al,[15] the impact of probucol on RCT seems to differ with the molecular expression involved in cholesterol homeostasis. Because pharmacological inhibition of ABCA1 using probucol possibly results in off-target effects, experiments with it should be ideally performed in liver-specific ABCA1 knockout mice.

Consistent results about the role of RCT on atherogenesis have been obtained in mice lacking/overexpressing SR-BI in the liver; SR-BI contributes to antiatherogenesis by promoting RCT.[37] In contrast, CETP overexpression because of transgene has produced mixed results in relation to atherosclerosis in CETP-lacking mice.[32] Furthermore, the effect of CETP on atherogenesis is inconclusive in humans.[32] With regard to EL, as already described, it is controversial whether EL-mediated HDL changes are associated with atherogenesis in mice[9,10] and humans.[11,12] Although exact underlying mechanisms that explain these discrepancies remain unknown, based on the observations in this study, we hypothesize that they may be accounted for by the complex nature of the impacts of EL and CETP on HDL metabolism and RCT. The present study also indicates that hepatic SR-BI plays a central role in RCT as a cholesterol gatekeeper between the liver and bile/feces, at least in mice. The matter of whether SR-BI plays an important role in humans is unclear at present and thus deserves future studies.

In conclusion, hepatic overexpression of EL resulted in markedly low plasma HDL levels but maintained macrophage RCT in an SR-BI/ABCA1-dependent fashion in mice. These observations seem to support the concept that not only HDL quantity, but also its quality and the environments involved in HDL metabolism/RCT are important.

Therefore, future development of HDL-targeted therapy should take both aspects into consideration to further reduce the residual risk.

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Disclosures

None.

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Highlights

- Endothelial lipase reduces HDL (high-density lipoprotein) levels which is, in theory, proatherogenic; however, it remains unclear whether endothelial lipase-mediated HDL reduction affects reverse cholesterol transport in vivo.
- We demonstrated that, in mice, hepatic endothelial lipase expression compensated for quantitatively low HDL levels by promoting excretion of cholesterol to bile/feces via a pathway regulated by SR-BI (scavenger receptor class B type I) and ABCA1 (ATP-binding cassette transporter A1), thereby maintaining overall reverse cholesterol transport in vivo.
- These results, therefore, extend our knowledge of HDL and antiatherogenicity and lead to the concept of quality rather than quantity approach toward HDL, which would have a substantial impact in HDL-targeted therapies for human atherosclerotic diseases.