Scavenger Receptor BI-mediated Selective Uptake Is Required for the Remodeling of High Density Lipoprotein by Endothelial Lipase*

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Endothelial lipase (EL) is a negative regulator of high density lipoprotein (HDL) cholesterol plasma levels, and scavenger receptor BI (SR-BI) is involved in remodeling of HDL. The present study investigates the requirement of SR-BI for the effects of EL-mediated phospholipid hydrolysis on HDL metabolism in vivo. In vitro, selective uptake from EL-modified HDL was 129% higher than selective uptake from control HDL in SR-BI-overexpressing cells (p = 0.01). In vivo overexpression of human EL by means of recombinant adenovirus decreased HDL plasma levels significantly (p < 0.01). Fast protein liquid chromatography analysis and agarose gel electrophoresis revealed that EL expression resulted in the generation of small pre-β HDL particles in wild-type mice, whereas in SR-BI−/− mice small HDL were preferentially removed. In kinetic experiments the fractional catabolic rate (FCR) of HDL cholesteryl ester increased by 110% (p < 0.001), and the FCR of HDL apolipoproteins increased by 64% (p < 0.001) in response to EL overexpression in wild-type mice. In SR-BI−/− mice a similar increase in the HDL apolipoprotein FCR occurred (p < 0.001); however, there was no further increase in HDL cholesteryl ester catabolism. The apparent whole body selective uptake was increased 3-fold by EL in wild-type mice (p < 0.001), whereas there was no selective uptake in SR-BI knock-out mice. EL overexpression increased hepatic selective uptake as well as holoparticle uptake (each p < 0.01) in wild-type mice, whereas in SR-BI knock-out mice only holoparticle uptake increased (p < 0.01). Our results indicate that SR-BI-mediated selective uptake of HDL cholesteryl ester is essential for the remodeling of large α-migrating HDL particles by EL.

Plasma levels of high density lipoprotein (HDL) cholesterol and its major apolipoprotein apoA-I are inversely correlated with the risk of atherosclerotic cardiovascular disease, a major cause of mortality in developed countries (1, 2). The factors responsible for the considerable variation in HDL cholesterol plasma levels are still incompletely understood. However, metabolic studies of HDL and apoA-I in humans have established that the substantial variation in their levels is primarily due to variation in the rate of apoA-I catabolism (3).

Among the factors impacting on the remodeling and catabolism of HDL particles within the plasma compartment, the recently discovered endothelial lipase (EL) is of prime importance (4). EL is expressed in endothelial cells and macrophages, as well as in hepatocytes (5). EL has merely phospholipase and very little apparent triglyceride activity (6), and HDL phospholipids represent a preferred substrate for the enzyme in in vitro assays (6, 7). The physiological relevance of EL-mediated hydrolysis of HDL particles for determining the plasma levels of HDL cholesterol has been established in experimental animals using both overexpression (5, 8) as well as loss-of-function models (9–11). Overexpression of EL resulted in significantly decreased HDL cholesterol plasma levels by increasing the catabolic rate of HDL apolipoproteins (8), whereas inhibition (10) or genetic ablation (9, 11) of EL raised plasma HDL cholesterol.

Another key determinant of HDL remodeling and catabolism is the scavenger receptor BI (SR-BI) (12, 13). SR-BI knock-out mice and mice with attenuated hepatic SR-BI expression have increased plasma HDL cholesterol levels (14, 15), whereas hepatic overexpression of SR-BI decreased HDL cholesterol (16, 17). Importantly, SR-BI mediates selective uptake of cholesteryl ester out of HDL particles into the liver and the adrenals in vivo (15, 18, 19). Although the selective uptake by SR-BI might play a central role in the remodeling of HDL (20), the dependence of EL-mediated remodeling and catabolism of HDL on functional SR-BI expression has not been experimentally addressed thus far.

Therefore, the aim of the present study was to investigate the requirement of SR-BI for the effects of EL-mediated phospholipid hydrolysis on HDL metabolism in vivo by overexpressing human EL in wild-type and SR-BI knock-out mice. Our results indicate that SR-BI-mediated selective uptake of HDL cholesteryl ester represents a key component of the remodeling of large HDL particles by EL.

EXPERIMENTAL PROCEDURES

Animals—C57BL/6J control mice were purchased from Charles River (Sulzfeld, Germany). SR-BI knock-out mice were obtained from Jackson (Bar Harbor, ME) and backcrossed to

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§ The abbreviations used are: HDL, high density lipoprotein; EL, endothelial lipase; SR-BI, scavenger receptor BI; FCR, fractional catabolic rate; CE, cholesteryl ester; sPLA₂, type IIa secretory phospholipase A₂; LRP, low density lipoprotein receptor-related protein; n.s., not significant.
the C57BL/6J genetic background for a total of eight generations. The animals were kept in animal rooms with alternating 12-h periods of light (from 7:00 a.m. to 7:00 p.m.) and dark (from 7:00 p.m. to 7:00 a.m.), with ad libitum access to water and mouse chow diet (Arie Blok, Woerden, The Netherlands). The animal experiments were performed in accordance with the national laws. All of the protocols were approved by the responsible ethics committees of the Landesamt für Gesundheit, Ernährung und technische Sicherheit Berlin and the University of Groningen.

Generation of Recombinant Adenoviruses—The human EL cDNA was amplified from HepG2 cells (ATCC via LGC Promoch, Teddington, UK) by PCR using specific primers according to the human EL sequence (NM_006033, GenBank®) and subcloned into pcDNA3.1 (Invitrogen). Recombinant adenovirus (AdhEL) was generated using the Adeno-X kit (Clontech, Mountain View, CA) according to the manufacturers instructions. An empty adenovirus (AdNull) was used as control. Recombinant adenoviruses were amplified and purified as described previously (21). The in vivo experiments described were carried out on day 5 following injection of the recombinant adenoviruses using a total dose of 1×1011 particles for each mouse. In the case of EL expressing adenovirus, 3×1010 particles thereof consisted of AdhEL. This dose of AdhEL has been confirmed in pilot experiments to cause an intermediate decrease in plasma HDL cholesterol levels in control mice.

Expression of human EL was checked by quantitative real time PCR (see below) as well as by Western blot and activity assays performed on post-heparin plasma. Post-heparin plasma was generated as described (8). Western blots for EL were carried out using a commercially available polyclonal rabbit anti-human EL antibody (Novus Biologicals, Littleton, CO) to visualize EL followed by the appropriate secondary antibody. Phospholipase activity assays were performed using a 1,2-dithio analog of diheptanoyl phosphatidylcholine (Cayman Chemical, Ann Arbor, MI) as a substrate, resulting in the liberation of free thiols by the hydrolytic activity of EL that were subsequently detected using 5,5’-dithiobis(2-nitrobenzoic acid).

Plasma Lipid and Lipoprotein Analysis—The mice were bled from the retroorbital plexus after a 4-h fast using heparinized capillary tubes. Aliquots of plasma were stored at −20°C until analysis. Plasma total cholesterol, triglycerides, and phospholipids were measured enzymatically using commercially available reagents (Wako Pure Chemical Industries, Neuss, Germany). Pooled plasma samples were subjected to fast protein liquid chromatography gel filtration using a Superose 6 column (GE Healthcare) as described (22). Individual fractions were assayed for cholesterol concentrations as described above.

To assess α and pre-β HDL, agarose gel electrophoresis was performed using the Paragon electrophoresis system (Beckman Instruments, Brea, CA). Briefly, 3 μL of plasma were electrophoresed and blotted by absorption onto nitrocellulose membranes. After blocking, the membranes were incubated with the respective first antibodies to detect apoA-I (goat anti-mouse apoA-I; Biodesign, Saco, ME) and apoE (rabbit anti-mouse apoE; Biodesign, Saco, ME) followed by incubation with the appropriate secondary antibodies.

For the analysis of HDL composition, HDL was isolated from 100 μL of mouse plasma by tabletop sequential ultracentrifugation (1.063 < d < 1.21) as described (22), and concentrations of total and free cholesterol, phospholipids, and triglycerides were determined enzymatically as described above. Protein concentrations were measured using the BCA assay (Pierce).

Cell Culture and HDL Selective Uptake Experiments—LdlA cells lacking low density lipoprotein receptor expression as well as LdlA cells stably transfected with a murine SR-BI cDNA (LdlA[mSR-BI]) were kindly provided by Dr. Monty Krieger (Massachusetts Institute of Technology) and cultured as described (23). For HDL uptake experiments, 5% lipoprotein-depleted serum was used. Ten μg/ml of 125I/3H-CE HDL isolated and radioactively labeled as described below was added to the cells. After a 5-h incubation, the cells were washed three times with phosphate-buffered saline (pH 7.4) and lysed with 0.5 ml of 0.1 M NaOH. Tracer uptake was calculated as counts recovered from the cells as a percentage of the total dose (counts from cells added to the counts from media). Selective HDL CE uptake was determined by subtracting the percentage of the total dose of 125I recovered from the cells from the percentage of the total dose of 3H recovered from the cells.

HDL Kinetic Studies—Autologous HDL isolated by sequential ultracentrifugation (density, 1.063 < d < 1.21) was prepared from pooled mouse plasma and labeled with 125I-tyramine-cellobiose and cholesteryl hexadecyl ether (cholesteryl-1,2-3H; PerkinElmer Life Sciences) essentially as described (24), and 1 μCi of the 125I-tyramine-cellobiose-HDL and 1 million dpm of the 3H-CE-HDL were injected via the tail vein. Plasma decay curves for both tracers were generated by dividing the plasma radioactivity at each time point by the radioactivity at the initial 5-min time point after tracer injection. Fractional catabolic rates (FCRs) were determined from the area under the plasma disappearance curves fitted to a bicompartamental model by use of the SAAM II program (22). The use of 3H-cholesteryl ether affects neither turnover rates in vivo nor SR-BI-mediated uptake of the label into LdlA[mSR-BI] cells in vitro compared with 3H cholesteryl ester–labeled HDL (data not shown). Organ uptake of HDL apolipoproteins (125I) and HDL-CEs (3H-cholesterol ether) was determined and expressed as a percentage of the injected dose calculated by multiplying the initial plasma counts (5-min time point) with the estimated plasma volume (3.5% of total body weight). Selective uptake into organs was determined as the percentage of the injected dose of 125I-HDL recovered in each organ subtracted from the percentage of the injected dose of 3H-HDL-CR corrected for tissue weight.

Analysis of Gene Expression by Real Time Quantitative PCR—Total RNA from mouse livers was isolated using TRizol (Invitrogen) and quantified with a NanoDrop ND-100 UV-visible spectrophotometer. cDNA synthesis was performed from 1 μg of total RNA using reagents from Applied Biosystems (Darmstadt, Germany). Real time quantitative PCR was carried out on an ABI-Prism 7700 (Applied Biosystems) sequence detector with the default settings. PCR primers and fluorogenic probes were designed with the Primer Express Software (Applied Biosystems) and synthesized by Eurogentec (Seraing, Belgium). mRNA expression levels presented were calculated
relative to the average of the housekeeping gene cyclophilin and further normalized to the relative expression level of the respective controls (wild-type mice receiving the control adenovirus AdNull).

Statistical Analysis—Statistical analyses were performed using the statistical package for social sciences (SPSS; SPSS Inc., Chicago, IL). The data are presented as the means ± S.E. * indicates statistically significant differences compared with the respective AdNull-injected controls (p < 0.05) as assessed by Mann-Whitney U test.

RESULTS

EL Modification Increases SR-BI-mediated Selective Uptake of HDL Cholesterol Ester in Vitro—To test the hypothesis that cholesteryl ester within EL-modified HDL are more susceptible toward SR-BI-mediated selective uptake, HDL was isolated from AdNull as well as AdhEL injected wild-type mice, radiolabeled with $^{125}$I-tyramine cellobiose and $^3$H-cholesteryl ether, and selective uptake was assessed as described under “Experimental Procedures” (n = 3). The data are presented as the means ± S.E. * indicates statistically significant differences compared with the respective AdNull-injected controls (p < 0.05) as assessed by Mann-Whitney U test.

EL Expression Differentially Impacts on HDL Composition in Wild-type and SR-BI Knock-out Mice—Overexpression of EL resulted in dramatic changes of HDL composition in wild-type mice. Consistent with the role of EL as phospholipase, HDL phospholipid content decreased significantly (p < 0.001; Table 1) as did HDL cholesteryl ester content (p < 0.001; Table 1). On the other hand, HDL triglyceride content (p < 0.001; Table 1) as well as protein content (p < 0.001; Table 1) increased in EL modified HDL. Free cholesterol content remained unaltered (Table 1). These data are consistent with the generation of smaller HDL particles in response to AdhEL injection either in hepatic mRNA expression (data not shown) or post-heparin EL protein plasma levels as judged by Western blot (data not shown) or post-heparin phospholipase activity (wild type, 263 ± 11; SR-BI knock-out, 280 ± 12 nmol/min/ml). Overexpression of EL in wild-type controls resulted in a 54% reduction in plasma cholesteryl levels compared with AdNull-injected animals (85 ± 8 versus 39 ± 11 mg/dl, p < 0.01; Fig. 2A). Similarly, AdhEL injection resulted in a 55% decrease in plasma cholesteryl levels in SR-BI knock-out mice compared with SR-BI knock-out mice receiving the control virus AdNull (229 ± 12 versus 104 ± 18 mg/dl, p < 0.001; Fig. 2A). Plasma phospholipids were reduced by 53% in wild-type mice by EL overexpression (211 ± 9 versus 100 ± 14 mg/dl, p < 0.001) and by 52% in SR-BI knock-out mice (283 ± 13 versus 137 ± 16 mg/dl, p < 0.001). Plasma triglycerides were unchanged in wild-type mice receiving AdhEL (77 ± 6 versus 68 ± 9 mg/dl, n.s.) and decreased significantly in SR-BI knock-out mice receiving AdhEL compared with AdNull-treated SR-BI knock-out controls (130 ± 14 versus 71 ± 6 mg/dl, p = 0.01).

Fast protein liquid chromatography analysis indicated that EL differentially affected the size distribution of HDL in wild-type mice compared with SR-BI knock-out mice. Although in C57BL/6 mice EL resulted preferentially in the removal of larger sized HDL (Fig. 2B), in SR-BI knock-out mice preferentially larger sized HDL particles remained (Fig. 2C).

To further confirm these results, HDL was analyzed by agarose gel electrophoresis. Staining for apoA-I revealed that in wild-type mice preferentially α-migrating particles were decreased by EL overexpression (Fig. 2D), and the apparent α to pre-β size distribution was reversed by EL overexpression. However, in SR-BI knock-out mice, a large portion of α-migrating particles was not remodeled, and the α to pre-β HDL size distribution indicated that still the majority of the particles exhibited an α migration pattern (Fig. 2D). Staining for apoE indicated that in wild-type mice apoE-containing α HDL particles were almost completely removed by EL expression (Fig. 2E), whereas in SR-BI knock-out mice overexpressing EL, a substantial part of apoE-containing HDL remained, indicating that SR-BI might be required for remodeling of these particles.
EL overexpression differentially affects HDL size distribution in wild-type and SR-BI knock-out mice. The samples were taken at day 5 following injection of either the control adenovirus AdNull or the EL expressing adenovirus AdhEL in C57BL/6 control mice and SR-BI knock-out mice. A, plasma total cholesterol levels (n = 6–8 mice/group). The data are presented as the means ± S.E. * indicates statistically significant differences compared with the respective AdNull-injected controls (p < 0.01) as assessed by Mann-Whitney U test. Fast protein liquid chromatography profiles of C57BL/6 (B) and SR-BI knock-out mice (C) injected with the respective adenoviruses. Pooled plasma samples were subjected to gel filtration using a Superose 6 column, and cholesterol levels in each fraction were measured as described under “Experimental Procedures.” Relative elution positions of different lipoprotein subclasses are indicated. Agarose gel electrophoresis followed by absorption blotting for the distribution of apoA-I (D) and apoE (E) within HDL performed as described under “Experimental Procedures.” wt, wild type.

|                       | C57BL/6 | SR-BI knock-out |
|-----------------------|---------|-----------------|
|                       | AdNull  | AdhEL           | AdNull  | AdhEL           |
| Cholesterol ester     | 16 ± 2  | 8 ± 1*          | 13 ± 2  | 11 ± 2          |
| Free cholesterol      | 3 ± 1   | 3 ± 1           | 10 ± 2  | 15 ± 2*         |
| Phospholipids         | 20 ± 2  | 9 ± 1*          | 22 ± 2  | 19 ± 2          |
| Triglycerides         | 3 ± 1   | 10 ± 1*         | 3 ± 1   | 5 ± 1*          |
| Protein               | 58 ± 3  | 70 ± 4*         | 52 ± 3  | 50 ± 4          |

* At least p < 0.05.

(p < 0.001; Table 1) increased as a result of EL modification of the HDL particle in this model.

EL Overexpression Significantly Increases the Rate of HDL Cholesteryl Ester Degradation as a Function of SR-BI Expression—The above data indicated that the effects of EL overexpression on HDL metabolism exhibit substantial differences in mice lacking SR-BI receptors compared with wild-type controls. To further elucidate the underlying metabolic basis of these findings, HDL kinetic studies were performed. In AdNull-injected wild-type mice, the FCR of HDL cholesteryl ester was almost twice as high as the FCR of HDL protein, indicating the presence of functional in vivo selective uptake (0.083 ± 0.008 versus 0.158 ± 0.02 pools/h, p < 0.001; Fig. 3A). In contrast, although having comparable FCRs for HDL protein catabolism, HDL cholesteryl ester catabolism in SR-BI knock-out mice administered AdNull was not increased, confirming the absence of selective uptake in this model (0.088 ± 0.01 versus 0.077 ± 0.01 pools/h, n.s.; Fig. 3B). EL overexpression in wild-type mice resulted in a 64% increase in the HDL protein FCR (0.136 ± 0.03 pools/h, p <
The apparent whole body selective uptake from EL-modified HDL calculated as the difference between the HDL CE and the HDL protein FCRs was therefore almost 3-fold higher than the uptake from control HDL. These data confirm in vivo the results of the in vitro experiments described above that EL modification of HDL increases SR-BI-mediated selective uptake.

EL overexpression in SR-BI knock-out mice caused a similar increase in the HDL protein FCR by 64% (0.144 ± 0.026 pools/h, p < 0.001; Fig. 3B). In contrast, the HDL cholesteryl ester FCR was increased, however, comparably with the HDL protein FCR (0.138 ± 0.031, p < 0.001; Fig. 3B). Therefore, in the absence of SR-BI whole body selective uptake from EL-modified HDL is not increased. These data also indicate that besides stimulating selective uptake, EL modification of HDL results in increased whole body HDL holoparticle uptake.

EL Overexpression Significantly Alters the Tissue Catabolism of HDL Dependent on Functional SR-BI Expression—The liver is a main organ in HDL catabolism. In AdNull-injected wild-type mice, 17 ± 2% of the injected protein dose and 41 ± 5% of the injected cholesteryl ester dose were taken up by the liver (Fig. 4A), resulting in an apparent selective uptake of 24 ± 4% (Fig. 4C). This selective uptake is essentially due to the expression of SR-BI, because in AdNull-injected SR-BI knock-out mice, the hepatic HDL protein uptake (19 ± 2%; Fig. 4B) did not differ from the HDL cholesteryl ester uptake (20 ± 3%; Fig. 4B), resulting in no appreciable net selective uptake (2 ± 1%; Fig. 4C). EL overexpression in wild-type mice increased HDL protein as well as HDL cholesteryl ester uptake significantly (38 ± 6 and 78 ± 10%, respectively, p < 0.01; Fig. 4A), resulting in increased EL-mediated hepatic selective uptake (40 ± 7%, p < 0.01; Fig. 4C). In contrast, in SR-BI knock-out mice EL stimulated hepatic HDL protein catabolism and HDL cholesteryl ester catabolism to a similar extent (48 ± 4 and 47 ± 8%, respectively, n.s.; Fig. 4B) translating into absent selective uptake (−2 ± 1%; Fig. 4C). These data indicate that the increase in hepatic selective uptake mediated by EL in wild-type mice was due to functional SR-BI expression. However, as evidenced in the SR-BI knock-out model, EL also has the potential to mediate increased holoparticle uptake into the liver.

The kidneys are mainly responsible for the catabolism of small poorly lipidated apoA-I molecules. This is underlined in AdNull administered wild-type controls by an apparent uptake of HDL protein (5.1 ± 0.4%; Fig. 4D), whereas uptake of HDL cholesteryl ester is almost absent (0.5 ± 0.1%; Fig. 4D). In this process SR-BI does not play a direct role, resulting in renal uptake of HDL protein (5.4 ± 0.7%; Fig. 4E) and HDL cholesteryl ester (0.4 ± 0.2%; Fig. 4E) in AdNull-injected SR-BI knock-out mice comparable with the results obtained in wild-type mice. Therefore, negative selective uptake into the kidneys (relatively more protein catabolism over cholesteryl ester catabolism) was similar in AdNull-injected wild-type and SR-BI knock-out mice (−4.6 ± 0.5 versus −5.0 ± 0.7%, respectively; Fig. 4E). EL overexpression in wild-type mice resulted in a dramatic increase in HDL protein uptake into the kidneys (16 ± 2%, p < 0.001; Fig. 4D), whereas HDL cholesteryl ester uptake was almost unchanged (0.6 ± 0.2%, n.s.; Fig. 4D). Therefore, renal negative selective uptake was increased more than 3-fold in response to EL overexpression in wild-type mice (−15.4 ± 1.9%, p < 0.001; Fig. 4F). On the other hand, catabolism of HDL protein by the kidneys in response to EL overexpression was also increased in SR-BI knock-out mice (10.5 ± 1.5%, p < 0.001; Fig. 4E), whereas HDL cholesteryl ester uptake was not different compared with AdNull-injected SR-BI knock-outs (0.6 ± 0.2%, n.s.; Fig. 4E). However, net negative selective uptake stimulated by EL overexpression was less than 2-fold (−9.9 ± 1.2%, p < 0.001; Fig. 4F) and therefore clearly less (p < 0.01) than the increase observed in wild-type mice injected with AdhEL.

The Hepatic Expression Pattern of Genes Involved in HDL Remodeling Does Not Change in Response to EL Overexpression in Wild-type and SR-BI Knock-out Mice—To investigate potential EL-induced alterations in gene expression levels, we determined hepatic mRNA levels of a number of other proteins involved in HDL remodeling by real time quantitative PCR. As shown in Table 2, EL did not exhibit a significant effect on the mRNA expression of apoA-I, apoE, low density lipoprotein receptor, and hepatic lipase in either wild-type mice or SR-BI knock-out mice. SR-BI expression in wild-type mice did not change in response to EL expression. However, there was a significant increase in the expression of hepatic ABCA1 (1.00 ± 0.06 versus 1.31 ± 0.07, p < 0.05; Table 2) as well as low density lipoprotein receptor-related protein (LRP) (1.00 ± 0.09 versus 2.31 ± 0.07, p < 0.01; Table 2) in wild-type mice overexpressing EL, but these changes in gene expression levels did not occur in SR-BI knock-out mice. These results indicate that other factors involved in HDL remodeling are unlikely to interfere with the results obtained in our study.

DISCUSSION

The results of this study demonstrate that SR-BI expression and SR-BI-mediated selective uptake are required for the remodeling of large HDL particles by endothelial lipase in vivo. Our data indicate that cholesteryl ester within EL-modified HDL are more susceptible toward selective uptake by SR-BI in...
In vitro and in vivo. In addition, we show that in wild-type mice EL preferentially generates small particles, whereas in the absence of SR-BI, larger HDL particles predominate that are not sufficiently remodeled. Our data point toward a functional interplay between EL and SR-BI and are consistent with a model that (i) after initial hydrolysis of HDL phospholipids by EL, the HDL particle is destabilized, which results (ii) in the shedding of poorly lipidated apoA-I that are cleared by the kidneys, followed by (iii) increased SR-BI-mediated depletion of the HDL particle in cholesteryl ester, which is an essential prerequisite for remodeling to continue.

EL has been characterized as a member of the triacylglycerol lipase gene family that has predominantly A1-phospholipase activity; however, in contrast to the other members of this gene family only very little TG hydrolyzing activity (6). In vitro, HDL phospholipids and thereby HDL particles have been demonstrated to be a preferred substrate for EL (6, 7). Consequently, EL overexpression has been shown to decrease HDL cholesterol plasma levels (8), whereas inhibition of EL or a decrease in its expression levels resulted in increased plasma HDL cholesterol (9–11). We demonstrated previously that EL overexpression resulted in a dose-dependent increase in the catabolic rate of
HDL apolipoproteins in wild-type C57BL/6 mice (8). However, the impact of EL on the catabolism of HDL cholesteryl ester as well as mechanisms of EL-mediated HDL remodeling and the role of selective uptake by SR-BI herein have never been addressed experimentally in vivo thus far.

In our present study we show that an intermediate dose of EL results in increased HDL protein clearance as well as HDL cholesteryl ester catabolism. Importantly, the EL-mediated increase in the HDL cholesteryl ester FCR was almost two times higher than the increase in the HDL apolipoprotein FCR. These data indicate that EL increases total body selective uptake from HDL consistent with the in vitro data that were obtained in this study using a stably transfected cell line. Consistent with previous results (14, 15, 18, 19), SR-BI knock-out mice lack whole body selective uptake indicated by similar FCRs for HDL protein and HDL cholesteryl ester catabolism. In this model, EL was able to increase HDL protein catabolism to an extent similar to that observed in wild-type mice, however, because of the lack of selective uptake, the HDL CE FCR did not increase.

The liver represents an important organ for HDL catabolism equipped for HDL holoparticle uptake as well as selective uptake of CE from HDL (25). Our data indicate that in wild-type mice, EL modification of HDL increases both HDL holoparticle uptake as well as HDL CE selective uptake. In the absence of SR-BI, however, only increased HDL holoparticle uptake occurs in response to EL overexpression. The underlying mechanisms and receptor(s) for HDL holoparticle catabolism in liver are presently not sufficiently defined and require further investigation. Thus far, it has been shown that holoparticle uptake of HDL is independent of SR-BI and can be mediated by low as well as high affinity binding sites on hepatocytes (27). Although HDL particles enriched in triglycerides only bind to low affinity sites, HDL3, apoA-I, and remnant HDL2 depleted in TG by the action of hepatic lipase bind to both high and low affinity sites. As one high affinity binding site for HDL a complex containing the ectopic β-chain of ATP synthase has been defined (26). Data currently available suggest that apoA-I binding to ATP synthase stimulates extracellular ATP hydrolysis to generate ADP. ADP then activates the nucleotide receptor P2Y13, resulting in clathrin-dependent HDL holoparticle endocytosis via as yet unidentified low affinity binding sites (27). However, also LRP has been proposed to participate in HDL catabolism (28). In this respect it is interesting to note that hepatic LRP expression in wild-type mice is increased ~2-fold in response to EL overexpression and that LRP is increased in the livers of SR-BI knock-out mice. However, in SR-BI knock-outs there was no further increase of LRP expression in response to EL overexpression, making a causal contribution of LRP in our experimental setting less likely. On the other hand, the kidneys play a role in the catabolism of poorly lipidated apoA-I molecules. Consistent with our working model, EL hydrolysis of HDL results in an about 3-fold increase in the renal catabolism of these in wild-type mice. In SR-BI knock-out mice there was still significantly increased HDL protein catabolism by the kidneys upon EL-mediated HDL phospholipid hydrolysis but to a lesser extent than in wild-type controls. These data underline our finding that remodeling of large HDL particles subsequently leading to the generation of lipid-poor apoA-I is hampered in the absence of SR-BI.

Our data presented here for EL are in a certain analogy to HDL phospholipid hydrolysis by the type IIA secretory phospholipase A2 (sPLA2) (21–24, 29, 30); however, EL appears to be more potent in this respect. Also sPLA2 is able to hydrolyze HDL phospholipids resulting in increased HDL catabolic rates and subsequently lower HDL cholesterol plasma levels (22, 24). In addition, in vitro (23) and apparently also in vivo (23) selective uptake by SR-BI is increased following sPLA2 modification of HDL. However, although sPLA2 is only expressed at very low levels within the vascular wall in the absence of inflammation (29–31), EL is even under steady-state noninflamed conditions a major modifier of HDL cholesterol plasma levels.

The large HDL particles present in SR-BI knock-out mice have been shown to be enriched in apoE content (14). Therefore, there is a certain possibility that apoE content of HDL renders these particles a less efficient substrate for EL-mediated hydrolysis. However, recent data have demonstrated that in vitro assays apoE content as well as the different apoE isoforms have no impact on the rate of HDL phospholipid hydrolysis by EL (32). In addition, as indicated by the Western blots for apoE within HDL performed in our study, apoE-containing particles are rapidly remodeled in wild-type mice. We therefore consider the apoE content of HDL not as a major impacting factor on EL-mediated remodeling.

In summary, this study demonstrates that functional SR-BI expression is required for a sufficient remodeling preferentially of large HDL particles by EL. Our data are consistent with a model that EL-mediated phospholipid hydrolysis of HDL destabilizes the particle resulting in (i) the shedding of poorly lipidated apoA-I that are preferentially cleared by the kidneys and (ii) increased susceptibility of cholesteryl ester within the HDL particle toward SR-BI-mediated selective uptake. Selective uptake is apparently necessary for remodeling to continue. These data therefore indicate a functional interplay of EL and SR-BI in the remodeling of HDL particles in vivo.

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