Cooperation between hepatic cholesteryl ester hydrolase and scavenger receptor BI for hydrolysis of HDL-CE

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Abstract  Liver is the sole organ responsible for the final elimination of cholesterol from the body either as biliary cholesterol or bile acids. High density lipoprotein (HDL)-derived cholesterol is the major source of biliary sterol and represents a mechanism for the removal of cholesterol from peripheral tissues including artery wall-associated macrophage foam cells. Via selective uptake through scavenger receptor BI (SR-BI), HDL-cholesterol is thought to be directly secreted into bile, and HDL cholesteryl esters (HDL-CEs) enter the hepatic metabolic pool and need to be hydrolyzed prior to conversion to bile acids. However, the identity of hepatic CE hydrolase (CEH) as well as the role of SR-BI in bile acid synthesis remains elusive. In this study we examined the role of human hepatic CE hydrolase (CEH) in facilitating hydrolysis of SR-BI-delivered HDL-CEs. Over-expression of CEH led to increased hydrolysis of HDL-[3H]CE in primary hepatocytes and SR-BI expression was required for this process. Intracellular CEH associated with BODIPY-CE delivered by selective uptake via SR-BI, CEH and SR-BI expression enhanced the movement of [3H]label from HDL-[3H]CE to bile acids in vitro and in vivo. Taken together, these studies demonstrate that SR-BI-delivered HDL-CEs are hydrolyzed by hepatic CEH and utilized for bile acid synthesis. —Yuan, Q., J. Bie, J. Wang, S. S. Ghosh, and S. Ghosh. Cooperation between hepatic cholesteryl ester hydrolase and scavenger receptor BI for hydrolysis of HDL-CE, J. Lipid Res. 2013. 54: 3078–3084.

Supplementary key words  HDL-cholesteryl ester • bile acid synthesis • cholesteryl ester

Multiple pathways are involved in maintaining whole-body cholesterol homeostasis in adult organisms for which intestinal cholesterol absorption and endogenous cholesterol biosynthesis must be balanced with biliary cholesterol and bile acid secretion. Endogenous synthesis of cholesterol, which occurs predominantly in the liver, is tightly regulated by cholesterol levels. Failure to appropriately eliminate excess cholesterol, dietary or endogenously synthesized, results in its accumulation within the body leading to pathological consequences. Under homeostatic conditions, excess cholesterol accumulated in the peripheral tissues, e.g., arterial wall-associated macrophages, is returned back to the liver via high density lipoprotein (HDL). Liver plays a central role in the regulation of plasma cholesterol concentrations, as it is not only the major site for cholesterol synthesis and secretion of lipoproteins into blood, but it is also the sole organ able to remove cholesterol from the body. In species that do or do not express cholesteryl ester transfer protein (CETP), scavenger receptor BI (SR-BI)-mediated HDL uptake by liver represents the major mechanism for clearance of HDL-cholesterol (HDL-C) and cholesteryl esters (CEs).

Hepatic SR-BI facilitates selective cholesterol uptake from HDL particles in which HDL-C as well as CEs are directly transferred to the cells without internalization and hydrolytic disassembly of the HDL particles. Free or unesterified cholesterol (FC) from HDL delivered via SR-BI is thought to be directly secreted into bile without entering the hepatic metabolic pool (1, 2). Hepatic SR-BI thus regulates plasma HDL-C levels as well as the utilization of this cholesterol for biliary secretion (3). Pieters et al. (4) demonstrated that selective uptake of HDL-cholesteryl esters (HDL-CEs) is efficiently coupled to bile acid synthesis. However, based on unchanged bile acid pool size and composition as well as fecal bile acid excretion in SR-BI−/− mice, Mardones et al. (5) proposed a limited role for SR-BI in hepatic bile acid metabolism and suggested the presence of as yet unrecognized compensatory mechanisms in SR-BI−/− mice. Nonetheless, at present the role of SR-BI in bile acid metabolism remains unclear and no direct studies have been conducted to evaluate the effects of SR-BI on bile acid synthesis from HDL-derived FC or CE.

Abbreviations: AdCEH, cholesteryl ester hydrolase expressing adenovirus; AdLacZ, β galactosidase expressing adenovirus; AdSR-BI, scavenger receptor BI expressing adenovirus; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free or unesterified cholesterol; HDL-C, HDL-cholesterol; HDL-CE, HDL cholesteryl ester; MOI, multiplicity of infection; NPC1, Niemann-Pick C1; RCT, reverse cholesterol transport; SR-BI, scavenger receptor BI.

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This work was supported in part by National Institutes of Health Grant HL-097346 to S.G.

Manuscript received 6 June 2013 and in revised form 26 August 2013. Published, JLR Papers in Press, August 20, 2013

DOI 10.1194/jlr.M040998

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Greater than 80% of cholesterol associated with HDL is present as CEs and intracellular hydrolysis is required to make FC available for either secretion into the bile or for the synthesis of bile acids. Furthermore, Sampson et al. showed that FC entering the hepatocytes from HDL also undergoes esterification (6), and CEs thus generated are hydrolyzed in a nonacidic compartment (7). Despite the obvious “need” for intracellular, and probably neutral, hydrolysis of HDL-derived CEs, the identity of the enzyme responsible for this hydrolysis remains elusive. Connelly et al. (8) suggested that this neutral CE hydrolysis of SR-BI-delivered HDL-CEs occurs by a cell-type-specific neutral CE hydrolase (CEH), and 70–80% of HDL-CEs delivered via SR-BI are rapidly hydrolyzed to facilitate FC trafficking and utilization within the cell. While hormone sensitive lipase is thought to hydrolyze SR-BI-delivered HDL-CEs in adrenals, the enzyme involved in other tissues, including liver, is not yet characterized (9). We have earlier identified and cloned human liver CEH (gene symbol CES1) and demonstrated its role in enhancing bile acid synthesis (10). In addition, while adenovirus-mediated over-expression of CEH enhanced macrophage-to-eces reverse cholesterol transport (RCT) in wild-type C57BL/6 mice, this increased fecal bile acid excretion was significantly attenuated in SR-BI-deficient mice, indicating that CEH hydrolyzes SR-BI-delivered CE making FC available for bile acid synthesis (11).

HDL is the preferred lipoprotein source of plasma cholesterol for biliary steroid secretion in humans, and 50–60% of cholesterol metabolized per day is transformed into bile acids with about 30% secreted as biliary cholesterol (12) underscoring the importance of evaluating the intracellular pathways/enzymes involved in transformation of HDL-CE into bile acids. In the present study we focused on two main questions: Is SR-BI-mediated HDL-CE utilized for bile acid synthesis and does CEH hydrolyze SR-BI-delivered HDL-CE in the liver? The data presented here demonstrates that HDL-CE is utilized for bile acid synthesis and hepatic CEH associates with SR-BI-delivered HDL-CE and facilitates its hydrolysis.

**EXPERIMENTAL PROCEDURES**

**Animals**

C57BL/6 and SR-BI knockout mice (Scarb1tm1Kri/J) were originally purchased from Jackson Laboratory and a colony was maintained in the laboratory. Mice were fed ad libitum with a standard rodent chow and 10-week-old mice were used for the studies. All procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

**Labeling of HDL**

Purified human HDL was purchased from Intracel and labeled with [3H]cholesterol oleate using recombinant CETP (13). In brief, 4 mg of HDL protein and 50 μg of CETP were added to dried [3H]cholesterol oleate (100 μCi) and the volume was brought to 1.5 ml with 10 mM potassium phosphate buffer (pH 7.2) containing 150 mM NaCl. The mixture was incubated for 5 h at 37°C with constant stirring. Unincorporated label was removed by filtration using Amicon Ultra with a 50 K cutoff. Labeled HDL (HDL-[3H]CE) was concentrated and protein content determined. An aliquot was used to extract lipids, free cholesterol (FC), and CEs separated by TLC and distribution of radioactivity was determined. Greater than 85% of radioactivity was associated with CEs. Labeled HDL was stored at 4°C and used for in vitro as well as in vivo studies. The same protocol was used to label HDL with BODIPY-CE.

**Intracellular hydrolysis of HDL-CE**

Primary hepatocytes were prepared from C57BL/6 and SR-BI−/− mice as described earlier (14). Cells (0.7 × 10⁷/well) were plated in collagen-coated 6-well plates and the medium (William’s E medium without phenol red and containing hepatocyte thawing and plating supplement from Invitrogen) was changed after 3 h. ACAT inhibitor (CP118, 1.25 μg/ml) was added to the medium and was included in the growth medium for the entire study. After 24 h, the medium was replaced and cells were transduced with β galactosidase expressing adenovirus (AdLacZ) [multiplicity of infection (MOI) 20], CEH expressing adenovirus (AdCEH) + AdLacZ (MOI 18+2), AdCEH + scavenger receptor BI expressing adenovirus (AdSR-BI) (MOI 18+2). After an additional 24 h, the medium was replaced with fresh medium containing labeled HDL (HDL-[3H]CE) and cells were incubated for 48 h. At the end of incubation, cells were washed twice and total lipids were extracted and separated by TLC (hexane:diethyl ether:acetic acid, 90:10:1). Intracellular hydrolysis of HDL-delivered [3H]CE was monitored by determining the radioactivity associated with FC as described earlier (15).

**Colocalization of CEH and SR-BI-delivered HDL-CE**

Primary hepatocytes were prepared from SR-BI−/− mice and plated in two-chamber glass slides. Medium was changed after 3 h and cells were transduced with AdSR-BI. After 24 h, medium was replaced with fresh medium containing BODIPY-CE-labeled HDL and cells incubated for 2 h to facilitate HDL-CE uptake. Cells were washed twice and then fixed in 4% buffered formalin for 30 min. Endogenous CEH (mice Ces3) was stained using rabbit polyclonal CES1 antibody, goat anti-rabbit biotinylated secondary antibody, and Neutravidin-conjugated Alexa549 as the fluorescent detection reagent. It should be noted that due to a high degree of homology between human CEH (CES1) and mouse Ces3, at this time there is no specific antibody available to distinguish between these two proteins. Images were acquired using a Zeiss inverted microscope fitted with a digital camera in a multi-acquisition mode using pseudocoloring.

**Measurement of cholesterol movement from HDL to bile in vivo**

C57BL/6 and SR-BI−/− mice maintained on chow diet were injected (iv) with AdLacZ (2.2 × 10⁸ pfu), AdCEH + AdLacZ, or AdCEH + AdSR-BI (2.0 × 10⁸ pfu + 0.2 × 10⁸ pfu) on day 1. The final virus titer was kept constant at 2.2 × 10⁸ pfu. On day 4, mice were injected (iv) with labeled HDL (HDL-[3H]CE), and after 48 h mice were euthanized and gall bladder bile was collected. Radioactivity associated with biliary cholesterol as well as bile acids were determined as described (16). Biliary phospholipid content was measured using the Phospholipid C kit from Wako (Richmond, VA).

**Bile acid synthesis in primary hepatocytes**

Primary hepatocytes were prepared from SR-BI−/− mice and plated as described above. After 24 h, medium was replaced and cells were transduced with AdLacZ, AdCEH + AdLacZ, or AdCEH + AdSR-BI (final MOI 20; 18+2). After 24 h, medium was replaced
with fresh medium containing labeled HDL (HDL-[\textsuperscript{3}H]CE) and cells were incubated for an additional 48 h. At the end of the incubation period, medium was harvested and incorporation of [\textsuperscript{3}H]cholesterol into bile acids secreted into the medium was determined following extraction (10).

**Real-time PCR**

Expression of CEH and SR-BI following transduction with adenoviruses was determined by real-time PCR. Total RNA was extracted using the RNeasy kit (Qiagen). cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems). Optimized probe/primer set and TaqMan Universal PCR Master Mix from Applied Biosystems were used: human CEH (Hs00275607_m1), mouse ABCG5 (Mm00446249_m1), mouse ABCG8 (Mm00445970_m1), and mouse NPC1 (Mm00435300_m1). SR-BI mRNA was measured using Power SYBR Green PCR Master Mix from Applied Biosystems with 5'-CTGCCTGTTTGCTGCTGGTC as the forward primer and 5'-TCCCGGACTACTGGCTTCT as the reverse primer.

**RESULTS**

**CEH hydrolyzes SR-BI-delivered HDL-CE**

We earlier demonstrated that CEH-dependent increase in bile acid secretion was abolished in SR-BI\textsuperscript{−/−} mice indicating cooperation between hepatic CEH and SR-BI (11). Greater than 80% of cholesterol carried by HDL is in the form of CE, and intracellular hydrolysis of SR-BI-delivered HDL-CE is obligatory for the availability of FC for bile acid synthesis. Consistently, Goodman and Lequire (17) demonstrated rapid hydrolysis of internalized CE. To directly determine whether SR-BI expression affects CEH-mediated hydrolysis of HDL-CE, primary hepatocytes from wild-type C57BL/6 as well as SR-BI\textsuperscript{−/−} mice were used and expression of CEH and SR-BI was manipulated by transduction with adenoviruses expressing CEH, SR-BI, or both. Initial optimization using varying MOI (5, 10, or 20) and plated cell densities (0.5 or 1.0 \times 10^6 cells/well) showed a comparable increase in CEH mRNA expression at the two plating densities with increasing MOI (Fig. 1A). Cells (0.7 \times 10^6 cells/well) were, therefore, transduced with AdCEH (MOI, 20) and then incubated with HDL-[\textsuperscript{3}H]CE. HDL-CE hydrolysis was assessed by monitoring the increase in intracellular accumulation of [\textsuperscript{3}H]FC. Consistent with its hydrolytic activity, CEH over-expression increased the hydrolysis of HDL-CE in C57BL/6 hepatocytes, and over-expression of SR-BI significantly enhanced HDL-CE hydrolysis indicating that CEH hydrolyzes SR-BI-delivered HDL-CE (Fig. 1B). It should be noted that over-expression of SR-BI led to an expected increase in cellular CE (151% increase) due to increased uptake. However, overexpression of CEH led to increased hydrolysis and intracellular FC levels were significantly higher (369% increase, \( P = 0.0014 \)). The CEH-mediated increase in intracellular hydrolysis of HDL-CE was not observed in SR-BI-deficient hepatocytes, and over-expression of SR-BI completely reversed this effect; comparable levels of intracellular FC were observed in C57BL/6 or SR-BI\textsuperscript{−/−} hepatocytes with SR-BI over-expression. These data demonstrate that SR-BI-delivered HDL-CEs are hydrolyzed by CEH.

**CEH associates with SR-BI-delivered HDL-CE**

Intracellular CEs are present as cytoplasmic lipid droplets, and for efficient hydrolysis CEH needs to be associated with these droplets. We have earlier demonstrated redistribution of CEH upon lipid loading where CEH was found associated with cytoplasmic lipid droplets in lipid-loaded cells (18). To evaluate whether CEH associates with SR-BI-delivered HDL-CE, primary hepatocytes from SR-BI\textsuperscript{−/−} mice were transduced with AdSR-BI and incubated with BODIPY-labeled HDL. Following immunostaining for CEH, colocalization of BODIPY-labeled CE and
CEH was monitored. Cells that were not incubated with HDL and not immune-stained for CEH (no primary antibody) did not show the presence of either the green CE droplets or red CEH staining (Fig. 2A). BODIPY-labeled CE droplets were visible within the cells incubated with labeled HDL (Fig. 2B, green). Cells were also uniformly stained for CEH (Fig. 2C, red). In cells incubated with HDL and immune-stained for CEH, colocalization of CEH and BODIPY-CE droplets was observed (Fig. 2D, yellow droplets) confirming the association of CEH with SR-BI-delivered HDL-CE. Taken together with the observed increase in cellular FC in hepatocytes transduced with AdCEH, these results demonstrates that CEH associates with SR-BI-delivered HDL-CE and facilitates the hydrolysis of HDL-CE.

Hepatic SR-BI is required to convert HDL-C to bile acids

It is well established that HDL-associated FC is directly secreted into bile and expression of hepatic SR-BI is required for this process. Furthermore, Mardones et al. (5) reported a decrease in biliary cholesterol with no change in bile acid or phospholipid secretion in SR-BI−/− mice. Because conversion of cholesterol to bile acids is a major route for the final elimination of cholesterol from the body, we directly examined whether SR-BI was required for the conversion of HDL-C to bile acids. Primary hepatocytes from SR-BI−/− mice were incubated with labeled HDL (HDL-[3H]CE) and appearance of [3H]label in bile acids secreted in the culture medium was monitored. Adenovirus-mediated over-expression of SR-BI significantly increased the appearance of radiolabel in secreted bile acids (Fig. 3) providing evidence that SR-BI facilitates the conversion of HDL-associated cholesterol to bile acids by hepatocytes.

Coexpression of CEH with SR-BI significantly increases movement of cholesterol from HDL-[3H]CE to bile

We have earlier demonstrated that in the absence of SR-BI, adenovirus-mediated over-expression of CEH failed to enhance in vivo RCT (11). To obtain direct proof of the concept that SR-BI and CEH cooperate in vivo for removal of HDL-C as FC or bile acids, C57BL/6 or SR-BI−/− mice were transduced with AdSR-BI with or without AdCEH. At the viral titer used, while SR-BI protein expression was enhanced in wild-type mice injected with AdSR-BI as reported earlier (19), in SR-BI−/− mice the levels were increased but were still lower than seen in C57BL/6 wild-type mice (data not shown). Following intravenous injection of HDL-[3H]CE, appearance of radiolabel in biliary cholesterol or bile acids was monitored. No significant difference was seen in the distribution of radiolabel in plasma, liver, or adrenal glands with expression of SR-BI alone or in combination with CEH (Fig. 4), indicating that adenovirus-mediated CEH expression does not affect the uptake of HDL-[3H]CE by tissues. However, over-expression of CEH significantly increased the appearance of radiolabel in bile acids as well as biliary FC in C57BL/6 mice (Fig. 5). Similarly, CEH expression also increased the appearance of radiolabel in bile acids in SR-BI−/− mice; no significant difference was noted in biliary FC. Taken together with in vitro data shown above, these results demonstrate that CEH and SR-BI cooperate to mobilize HDL-CE for elimination into bile, both as FC and bile acids.

While Niemann-Pick C1 (NPC1) is a key component for the intracellular distribution of cholesterol originating from lipoprotein endocytosis and plays a role in biliary cholesterol secretion (20), ABCG5/G8 directly facilitates cholesterol secretion into the bile. However, the role of ABCG5/G8 in SR-BI-mediated enhancement of biliary cholesterol secretion is currently controversial (19, 21). The effect of CEH and SR-BI on expression of NPC1 and ABCG5/G8 was monitored and as shown in Fig. 6: a significant increase in mRNA expression of ABCG5 and ABCG8, as well as NPC1, was only noted when both SR-BI and CEH were coexpressed, further supporting the observed cooperation between SR-BI and CEH.
CEH catalyzes the hydrolysis of SR-BI-delivered HDL-CEs to bile acids (BA). This process occurs within the hepatocytes/liver, increasing bile acid synthesis, likely by increasing the availability of the FC substrate. Furthermore, CEH-mediated hydrolysis of HDL-CE by hepatocytes in vitro or by liver in vivo and movement of tracer from HDL-CE to bile acids is dependent on the expression of SR-BI. This is consistent with our earlier data showing significant attenuation of CEH over-expression-dependent increase in bile acid excretion in SR-BI−/− mice (11).

**DISCUSSION**

HDL delivers cholesterol to the cells by selective uptake of HDL-CE or HDL-C, a process mediated by SR-BI. SR-BI is highly expressed in the liver where it controls the levels of plasma HDL-C and its transport into bile. While HDL-derived FC is believed to be directly secreted into bile, intracellular hydrolysis of HDL-CE is required to generate FC that is either directly secreted or converted to bile acids. The data presented here demonstrate that human liver cells express CEH, which catalyzes the hydrolysis of SR-BI-delivered HDL-CEs to bile acids, thereby increasing bile acid synthesis, likely by increasing the availability of the FC substrate. Furthermore, CEH-mediated hydrolysis of HDL-CE by hepatocytes in vitro or by liver in vivo and movement of tracer from HDL-CE to bile acids is dependent on the expression of SR-BI. This is consistent with our earlier data showing significant attenuation of CEH over-expression-dependent increase in bile acid excretion in SR-BI−/− mice (11).

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**Fig. 3.** SR-BI expression rescues conversion of HDL-CEs to bile acids (BA). Primary hepatocytes were prepared from SR-BI−/− mice and transduced with either AdLacZ or AdSR-BI. Twenty-four hour posttransduction, medium was replaced with normal growth medium containing labeled HDL (HDL-[3H]CE) and incubation was continued for an additional 48 h. Culture medium was collected and radioactivity associated with secreted bile acids determined. Data (mean ± SD, n = 6) are presented as percent LacZ transduced controls. *P < 0.05.

**Fig. 4.** Tissue uptake of [3H]CE from intravenously injected HDL-[3H]CE. C57BL/6 or SR-BI−/− mice were injected (iv) with AdLacZ, AdCEH and subsequently (72 h later) given labeled HDL (HDL-[3H]CE). Mice were euthanized after an additional 48 h and radioactivity associated with indicated tissues was determined. Data (mean ± SD, n = 6) are presented as percent radioactivity injected. *P < 0.05.

**Fig. 5.** Increased elimination of HDL-CEs as bile acids (BA) by SR-BI and CEH over-expression. C57BL/6 or SR-BI−/− mice were injected (iv) with AdSR-BI with AdLacZ or AdCEH and subsequently (72 h later) given labeled HDL (HDL-[3H]CE). Mice were euthanized after an additional 48 h and gall bladder bile was collected. Total phospholipid (PL) content and radioactivity associated with bile acids and FC was determined. Data (mean ± SD, n = 6) are presented as percent radioactivity injected. *P < 0.05.

**Fig. 6.** Increased expression of genes involved in cholesterol trafficking by SR-BI and CEH coexpression. C57BL/6 mice were injected (iv) with AdLacZ, AdCEH, AdSR-BI, or AdSR-BI + AdCEH and mRNA levels of indicated genes determined by real-time RT-PCR using total liver RNA. Data (mean ± SD, n = 3) are presented as fold increase over AdLacZ (for CEH) or SR-BI (for SR-BI + CEH). *P < 0.05.
Although selective uptake of HDL-CE via SR-BI is well established, the fate of HDL-CE once incorporated into the cell is not completely defined. Connely et al. (8) showed that HDL-CEs are delivered to a compartment distinct from LDL-CEs and are rapidly hydrolyzed by a neutral CEH, and concluded that SR-BI directs HDL-CEs more efficiently than LDL-CEs for hydrolysis. The activity of neutral CE hydrolytic activity varies among tissues, and multiple enzymes are thought to be responsible for this catalysis. In addition, the tissues with high CE hydrolytic activity, such as liver, adrenals, and gonads, are also the tissues with high SR-BI expression suggesting that a specific CEH in these tissues may be responsible for hydrolysis of HDL-CEs delivered by SR-BI (8). While hormone-sensitive lipase catalyzes the hydrolysis of HDL-CEs in adrenals, the enzyme responsible for this hydrolysis in liver has not been identified (9). We had earlier demonstrated that human liver CEH-mediated increase in bile acid synthesis was significantly attenuated in SR-BI−/− mice, indicating that SR-BI-delivered HDL-CEs serve as substrates for CEH in the liver. The data presented herein directly demonstrate the hydrolysis of HDL-CEs in hepatocytes by CEH and the absolute requirement of SR-BI for this process (Fig. 1B). Furthermore, the endogenous CEH in mouse hepatocytes (Ces3) associates with SR-BI-delivered HDL-CE (Fig. 2). Consistently, in vivo adenovirus-mediated over-expression of CEH also enhances the movement of radiolabel from HDL-[3H]CE to bile. Taken together, these studies demonstrate that human liver CEH (gene symbol CES1) facilitates hydrolysis of SR-BI-delivered HDL-CEs and thereby provides the FC substrate for subsequent bile acid synthesis. CES1 is ubiquitously expressed in all tissues (10), and it remains to be seen whether it plays a role in HDL-CE hydrolysis in other tissues, especially adrenals and gonads where SR-BI-delivered HDL is the primary lipoprotein source of CEs for steroidogenesis (22, 23).

It is noteworthy that CEH belongs to the carboxylesterase family of enzymes with broad substrate specificities, and the data presented here does not preclude the role of other members of this family in the hydrolysis of SR-BI-delivered HDL-CEs. While selective pharmacological inhibitors for individual carboxylesterases are not currently available, induction of compensatory mechanisms limits the use of single gene ablation as a strategy to identify the role of a single gene product (24). It is likely that, similar to CE hydrolysis in macrophages, hepatic CE hydrolysis is also a multi-enzyme process. Consistently, liver-specific deficiency of Ces3 led to attenuation but not complete ablation of hepatic CE hydrolytic activity, resulting in reduced elimination of cholesterol and bile acids in feces (15). It needs to be emphasized that because an increase in CE hydrolytic activity is required to enhance cholesterol elimination from the body and macrophage-specific transgenic expression of CES1 led to significant reduction in diet-induced atherosclerosis (16), over-expression strategy was used in the current study to demonstrate that human CES1 (ortholog of mouse Ces3) facilitates the hydrolysis of HDL-CE in cooperation with SR-BI and increases cholesterol elimination.

Liver is the sole organ responsible for the final elimination of cholesterol from the body, either as biliary cholesterol or bile acids. De novo-synthesized cholesterol represents only a small fraction (5–20%) of biliary cholesterol and the remainder is supplied by the hepatic uptake of lipoproteins (25, 26). Robins and Fasulo (27, 28) demonstrated that HDL, but not other lipoproteins provide a vehicle for sterol transport into bile. Compared with HDL-FC-associated tracer, the appearance of HDL-CE-associated tracer into bile is delayed, suggesting that although HDL-FCs may be secreted directly into the bile, HDL-CEs enter the hepatic metabolic pool, delaying the appearance into bile (2). Sampson et al. (6) showed that FC entering the hepatocytes from HDL also undergoes esterification and that subsequent hydrolysis is required for its secretion into the bile or conversion into bile acids. Pieters et al. (4) confirmed that selective uptake of CE from HDL is efficiently coupled to bile acid synthesis. Because HDL-CEs are delivered to the liver by selective uptake via SR-BI, requirement of SR-BI for conversion of HDL-CEs to bile acids is obligatory. The data presented here provide direct evidence for the requirement of SR-BI for hepatic bile acid synthesis; not only did adenovirus-mediated expression of SR-BI in SR-BI-deficient hepatocytes increase bile acid secretion (Fig. 3), but decreased appearance of HDL-CE-associated tracer into bile acids was also noted in SR-BI−/− mice (Fig. 5). Although the majority of bile acids secreted into the bile are returned to the liver via the enterohepatic circulation, bile acids are secreted in such large amounts that even a fractional removal in feces still represents a significant portion of the total cholesterol elimination in humans (29). Therefore via cooperation, SR-BI and CEH increase the flux of cholesterol from HDL to bile and function as a positive regulator of cholesterol elimination from the body.

In conclusion, human hepatic CEH catalyzes the hydrolysis of SR-BI-delivered HDL-CE, and collectively SR-BI and CEH enhance movement of HDL-CE into bile. Taken together with our earlier studies demonstrating the role of macrophage CEH in enhancing macrophage-to-feces RCT and attenuating diet-induced atherosclerosis and liver-specific Ces3 deficiency leading to increased atherosclerosis, these studies establish the anti-atherosclerotic role of hepatic CEH.  

The authors acknowledge the technical assistance of Ms. Reem Hassan in acquiring the fluorescent images.

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