Intracellular cholesterol transport proteins enhance hydrolysis of HDL-CEs and facilitate elimination of cholesterol into bile

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Abstract **While HDL-associated unesterified or free cholesterol (FC) is thought to be rapidly secreted into the bile, the fate of HDL-associated cholesteryl esters (HDL-CEs) that represent >80% of HDL-cholesterol, is only beginning to be understood. In the present study, we examined the hypothesis that intracellular cholesterol transport proteins [sterol carrier protein 2 (SCP2) and fatty acid binding protein-1 (FABP1)] not only facilitate CE hydrolase-mediated hydrolysis of HDL-CEs, but also enhance elimination of cholesterol into bile. Adenovirus-mediated overexpression of FABP1 or SCP2 in primary hepatocytes significantly increased hydrolysis of HDL-[^{3}H]CE, reduced resecretion of HDL-CE-derived FC as nascent HDL, and increased its secretion as bile acids. Consistently, the flux of [^{3}H]cholesterol from HDL-[^{3}H]CE to biliary bile acids was increased by overexpression of SCP2 or FABP1 in vivo and reduced in SCP2$^{-/-}$ mice. Increased flux of HDL-[^{3}H]CE to biliary FC was noted with FABP1 overexpression and in SCP2$^{-/-}$ mice that have increased FABP1 expression. Lack of a significant decrease in the flux of HDL-[^{3}H]CE to biliary FC or bile acids in FABP1$^{-/-}$ mice indicates the likely compensation of its function by an as yet unidentified mechanism. Taken together, these studies demonstrate that FABP1 and SCP2 facilitate the preferential movement of HDL-CEs to bile for final elimination.—**Wang, J., J. Bie, and S. Ghosh. **Intracellular cholesterol transport proteins enhance hydrolysis of HDL-CEs and facilitate elimination of cholesterol into bile. *J. Lipid Res.* 2016.** 57: **1712–1719.**

Supplementary key words cholesterol elimination • hepatocyte • reverse cholesterol transport • bile acid secretion • high density lipoprotein • cholesteryl esters • sterol carrier protein 2 • fatty acid binding protein-1

Due to the lack of enzymes required to degrade the steroid nucleus, balance between synthesis and elimination of cholesterol is crucial to the maintenance of whole body cholesterol homeostasis in mammals, including humans. Liver is the central organ for regulating the rate of cholesterol biosynthesis as well as coordinating the elimination of cholesterol via direct secretion into bile or by conversion to more water soluble bile acids. While dietary cholesterol fluxes through the liver in the form of chylomicron remnants taken up via the VLDL or LDL receptor, endogenously synthesized cholesterol is secreted by the liver as part of VLDL and returns to the liver either as IDL or LDL. However, excess cholesterol from nonhepatic peripheral tissues, including artery wall-associated macrophage foam cells, returns to the liver as part of HDL where about 80% of cholesterol is present as cholesteryl esters (CEs) and unesterified or free cholesterol (FC) represents less than 20% of the total HDL cholesterol. HDL-FC is thought to be rapidly and directly secreted into bile without entering the hepatic FC pools (1, 2) and the fate of HDL-associated CEs (HDL-CEs) within a hepatocyte is now beginning to be defined. We identified the neutral CE hydrolase (CEH; gene symbol CES1 in humans and Ces1d in mice) as the enzyme facilitating the intrahepatic hydrolysis of HDL-CE (3) and demonstrated the requirement of SR-BI for this process (3, 4). Gain-of-function (5) and loss-of-function (6) studies further established the role of hepatic CEH in regulating the flux of HDL-CEs to bile, preferentially as bile acids.

HDL-derived cholesterol (FC or FC generated after CEH-mediated hydrolysis of HDL-CE) can potentially have multiple fates within the hepatocyte. It can either be resecreted as part of nascent HDL or VLDL following esterification; the two processes that will not facilitate the final elimination of cholesterol from the body. Alternatively, FC can either be directly secreted into bile or converted into bile acids prior to biliary secretion; the two pathways resulting in final elimination of cholesterol from the body. While selective uptake of HDL-CE/FC occurs via SR-BI at the

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Abbreviations: CE, cholesteryl ester; CEH, cholesteryl ester hydrolase; FABP1, fatty acid binding protein-1; FC, free cholesterol; HDL-CE, HDL-associated cholesteryl ester; SCP2, sterol carrier protein 2.

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plasma membrane, CE hydrolysis occurs at the surface of cytoplasmic lipid droplets and FC esterification occurs in the endoplasmic reticulum by ACAT2. The rate limiting step of bile acid synthesis by the classical pathway catalyzed by cholesterol-7α hydroxylase (CYP7A1) occurs in the endoplasmic reticulum and that for the acidic pathway by cholesterol-27α hydroxylase (CYP27A1) occurs in the mitochondria. Thus, the fate of HDL-derived cholesterol is likely to be determined by the intracellular processes that regulate the "delivery" of cholesterol to the appropriate subcellular organelle for the respective fates.

Intracellular translocation of hydrophobic cholesterol in the aqueous cytoplasmic milieu is challenging and is facilitated by intracellular cholesterol carrier/transport proteins. To date several sterol carrier/transport proteins have been identified, including the two high affinity cholesterol binding proteins present in hepatocyte cytosol, namely, sterol carrier protein 2 (SCP2) (7) and fatty acid binding protein-1 (FABP1) (8–11). SCP2 is thought to be involved in the delivery of FC to the endoplasmic reticulum for ACAT-dependent esterification (12) and in facilitating the transport of HDL-derived FC to the canalicular membrane for secretion into bile (10). FABP1, shown to bind FC as well as bile acids with weak affinity (13), is thought to be involved in the secretion of FC into bile (11). However, the role of these two proteins in determining the fate of HDL-FC as well as FC generated following hydrolysis of HDL-CE remains undefined.

The objective of the present study was to examine the role of these currently known hepatic cholesterol transport proteins, namely SCP2 and FABP1, in the metabolism of HDL-CE. The data show that SCP2 and FABP1 not only enhance the intracellular hydrolysis of HDL-CE but also enhance the flux of cholesterol from HDL-CE toward biliary secretion either as FC or as bile acids. Taken together with the observed decrease in the resecretion of FC derived from HDL-CE from hepatocytes overexpressing SCP2 and FABP1, these two proteins are potentially anti-atherogenic.

MATERIALS AND METHODS

Animals

C57BL/6, ScarB1$^{-/-}$ (SR-BI$^{-/-}$), and FABP1$^{-/-}$ mice were obtained from Jackson Laboratory. It should be noted that we obtained the first generation of FABP1$^{-/-}$ mice after this strain was deposited at the Jackson Laboratory by Dr. Freidhem Schroeder. We also obtained the first generation of SCP2/SCPx$^{-/-}$ mice (designated as SCP2$^{-/-}$ throughout this manuscript) from the Mutant Mouse Regional Resource Center, Chapel Hill, NC, after this strain was deposited at this consortium by Dr. Schroeder's laboratory. All mice were maintained in a helicobacter-free barrier facility at Virginia Commonwealth University and all procedures were approved by the Virginia Commonwealth University institutional animal care and use committee. Mice of both genders were included in all in vivo studies.

Intracellular HDL-CE hydrolysis

Primary hepatocytes were isolated as described earlier (14) and plated in collagen-coated 6-well plates (0.7×10^6 per well) and the medium was replaced after 3 h. After 24 h, cells were transduced with Ad-SCP2 or Ad-FABP1 (from SignaGen® Laboratories expressing human SCP2 or FABP1 under the control of cytomegalovirus promoter); cells transduced with Ad-LacZ were used as controls (multiplicity of infection = 10 for all viruses). After 24 h, the medium was replaced with fresh medium containing HDL-[^{3}H]CE. Purified human HDL was purchased from Intracel and labeled with cholesteryl oleate [cholesteryl 1,2-^{3}H(N)] (Perkin Elmer) using recombinant CETP (Roar Biomedical, Inc.) as described earlier (15). Medium was replaced with fresh growth medium after 4 h and cells incubated for an additional 24 or 48 h. Total cellular lipids were extracted and neutral lipid separated by TLC using hexane:diethyl ether:acetic acid (90:10:1 v/v) as the solvent system. Spots corresponding to FC and CE were scraped and associated radioactivity determined by liquid scintillation counting. CE hydrolysis was assessed by determining the radiolabel associated with [^{3}H]FC released and normalized to total cellular protein.

Flux of HDL-[^{3}H]CE to VLDL, HDL, and bile acids

Primary mouse hepatocytes were transduced with the indicated adenoviruses. After 48 h, the medium was replaced with fresh medium containing HDL-[^{3}H]CE. Except when resecretion of HDL-derived [^{3}H]FC as VLDL was being monitored, ACAT inhibitor (CP 113-818, 1.25 μg/ml) was included in the culture medium. After 4 h, the medium was replaced with fresh growth medium and conditioned medium was collected after an additional 24 h. The conditioned medium was either used to extract bile acids, as described earlier (6), or concentrated using Millipore ultracentrifuge filters with a 100 kDa cutoff, supplemented with 100 μl of mouse plasma and lipoproteins separated by FPLC (using a Superose-6 column). Radioactivity associated with all fractions was determined. The presence of ApoA1 in the fractions was determined by Western blot analysis (using rabbit polyclonal antibody to ApoA1 from Santa Cruz Biotechnology) to confirm the presence of HDL in the corresponding fractions.

Flux of HDL-[^{3}H]CE to bile in vivo

Mice maintained on chow diet were injected (iv) with HDL-[^{3}H]CE and after 48 h, the mice were euthanized and blood and liver as well as gallbladder bile were collected. Radioactivity associated with plasma and liver was monitored. Biliary cholesterol as well as bile acids were extracted from the gall bladder bile and associated radioactivity was determined as described (16).

Analytical procedures

Following transduction with respective adenoviruses, expression of human CEH, SCP2, or FABP1 was monitored by real-time RT-PCR using total RNA from hepatocytes/liver and species-specific TaqMan assays. Total lipids were extracted by the procedure described by Bligh and Dyer (17). Neutral lipids were separated by TLC using hexane:diethyl ether:acetic acid (90:10:1 v/v) as the solvent system. Separated lipids were identified by staining with iodine, marked, and silica-gel scraped to determine the associated radioactivity. Gallbladder bile was used to extract biliary bile acids and cholesterol as described (16). Biliary phospholipids were determined by using the Phospholipids C kit from Wako (Richmond, VA). It should be noted that this kit only measures choline-containing phospholipids. Protein levels were determined using the BCA reagent according to the manufacturer's instructions.

Statistical analyses

All data were analyzed using GraphPad Prism software. Statistical significance of the difference between two groups was determined

by one-way ANOVA and two-way ANOVA was used to compare multiple groups. $P < 0.05$ was considered statistically significant. Individual P values are included in the text or figure legends.

RESULTS

Adenovirus-mediated overexpression of SCP2 or FABP1 increases the hydrolysis of HDL-CE in primary mouse hepatocytes

We have earlier established that hepatic CEH characterized in our laboratory (15) associates with CE delivered to the hepatocytes by HDL and catalyzes the hydrolysis of HDL-CEs (3). Furthermore, expression of SR-BI was critical for this process and CEH-dependent increase in hydrolysis of HDL-CE was attenuated in SR-BI$^{-/-}$ mice or hepatocytes (3). Effective removal of the product, namely unesterified or FC, is required for continual hydrolysis as CEH activity is inhibited by product accumulation (18). We hypothesized that intracellular FC binding proteins might facilitate the removal of FC and thereby increase CEH-mediated hydrolysis of HDL-CE. Consistently, adenovirus-mediated overexpression of human SCP2 or FABP1 enhanced the hydrolysis of HDL-CE in primary mouse hepatocytes (**Fig. 1**). It should be noted that fold increase in expression of SCP2 or FABP1 could not be calculated because species-specific TaqMan assays were used and no Ct value was obtained in control Ad-LacZ-transduced mouse hepatocytes compared with Ct values ranging from 23 to 25 for hepatocytes transduced with human Ad-SCP2 or Ad-FABP1 viruses, indicating increased expression.

SCP2 and FABP1 attenuate the resecretion of HDL-CE

Delivery of HDL-CE represents the critical step in the return of cholesterol from the peripheral tissues back to the liver. However, intracellular processes involved in the movement of FC between subcellular organelles are likely to determine whether cholesterol derived from HDL is resecreted back into the circulation either as VLDL (after esterification in the endoplasmic reticulum) or associated with nascent HDL particles (following efflux via ABCA1 to secreted ApoA1). To determine the role of SCP2 or FABP1 in regulating the fate of HDL-derived cholesterol, appearance of [^{3}H]cholesterol delivered as HDL-[^{3}H]CE to hepatocytes in VLDL and HDL was monitored. As shown in **Fig. 2A, C**, adenovirus-mediated overexpression of either SCP2 or FABP1 significantly reduced the secretion of cholesterol as HDL (fractions containing HDL confirmed by the presence of ApoA1 in those fractions, Fig. 2B); no significant difference was noted in the secretion of cholesterol associated with VLDL particles (Fig. 2D). These data demonstrate that SCP2 and FABP1 attenuate the return of HDL-derived cholesterol back to the circulation. It needs to be emphasized that monitoring the appearance of radiolabel from HDL-[^{3}H]CE into secreted HDL does not measure the total nascent HDL secretion from hepatocytes.

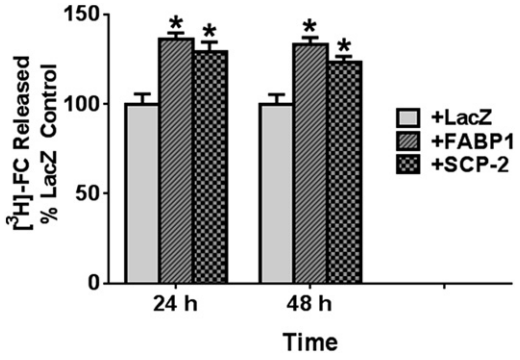

Fig. 1. Overexpression of FABP1 and SCP2 increases intracellular hydrolysis of HDL-CE. Primary hepatocytes were isolated from C57BL/6 mice, transduced with the indicated adenoviruses, and incubated with HDL-[^{3}H]CE as described in the Materials and Methods. Hydrolysis of HDL-CE was assessed by monitoring the levels of [^{3}H]FC in total lipid extracts and expressed as percent LacZ control. Data are expressed as mean ± SD, n = 3, *$P < 0.05$ with respect to LacZ control.

SCP2 and FABP1 enhance the secretion of HDL-CE-derived cholesterol as bile acids

In addition to resecretion as VLDL or HDL, HDL-CE also provides FC to serve as the substrate for bile acid synthesis. It is well-established that HDL-delivered FC is rapidly secreted into bile (1, 2), but the fate of HDL-CE is not as clear. We demonstrated earlier that CEH-mediated hydrolysis of HDL-CE enhanced secretion of the resulting FC as bile acids (3). Based on the observed increase in CE hydrolysis by SCP2 and FABP1, we postulated that these two intracellular FC binding proteins would modulate the secretion of HDL-CE as bile acids. Adenovirus-mediated overexpression of SCP2 or FABP1 in hepatocytes significantly increased the appearance of [^{3}H]cholesterol delivered as HDL-[^{3}H]CE into bile acids secreted into the medium (**Fig. 3**). StARD1 was overexpressed as a positive control and, consistent with its role in increasing bile acid synthesis (19), increased secretion of HDL-[^{3}H]CE as bile acids was noted in cells overexpressing StARD1. Taken together with the data presented in Fig. 2, these data indicate that SCP2 and FABP1 regulate the flux of cholesterol returning to the liver as HDL-CE within a hepatocyte by decreasing the resecretion as HDL and enhancing the secretion as bile acids.

To further establish the role of SCP2 and FABP1 in regulating cholesterol flux from HDL to bile, in vivo reverse cholesterol transport studies were performed and flux of HDL-[^{3}H]CE-derived [^{3}H]cholesterol to biliary bile acids or biliary FC was monitored. Adenovirus-mediated overexpression of SCP2 and FABP1 significantly increased the appearance of [^{3}H]cholesterol in biliary bile acids (**Fig. 4A**). A significant increase in biliary FC was also seen when FABP1 was overexpressed, but only a small nonsignificant increase was seen in cells overexpressing SCP2 (Fig. 4B). These data are not only consistent with the in vitro studies described above, but are also consistent with SCP2- and FABP1-dependent enhancement of HDL-delivered CE hydrolysis (Fig. 1) and attenuated flux of released FC toward resecretion (Fig. 2).

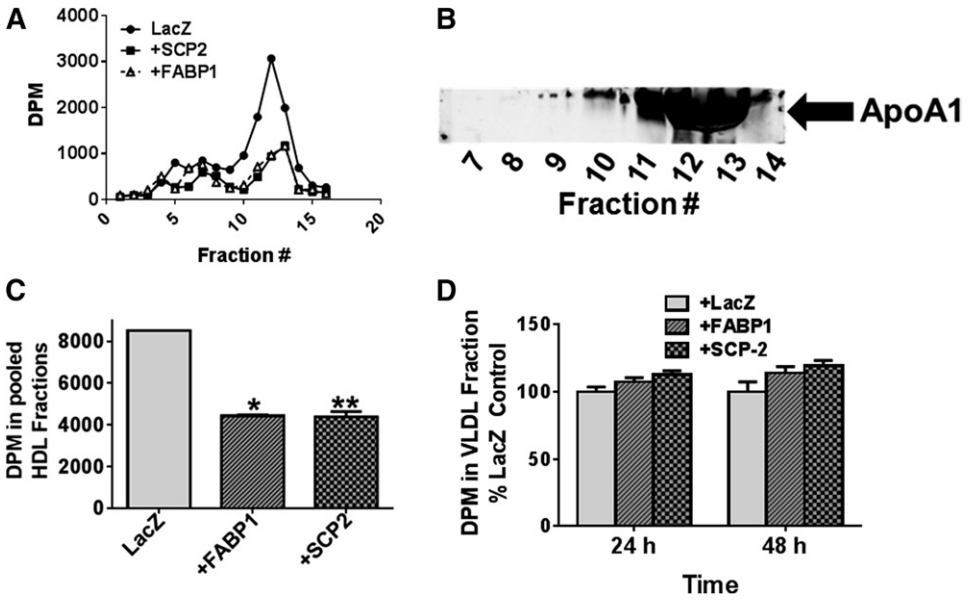

Fig. 2. Overexpression of SCP2 or FABP1 decreases the resecretion of FC from HDL-CE as nascent HDL. Primary hepatocytes were isolated from C57BL/6 mice, transduced with the indicated adenoviruses, and incubated with HDL-[^{3}H]CE as described in the Materials and Methods. Resecretion of FC from HDL-CE into the conditioned medium was assessed by measuring the [^{3}H]label associated with HDL or VLDL fractions. A: Representative FPLC chromatogram showing the radioactivity associated with each fraction. B: Presence of ApoA1 in FPLC fractions was monitored by Western blot analysis to establish the elution of HDL in fractions 10–14; no immunoreactive ApoA1 band was seen in fractions 7–9. C: Total disintegrations per minute (DPM) associated with HDL-containing fractions. D: Total DPM associated with the VLDL fraction expressed as percent LacZ control, mean ± SD, n = 4, *P = 0.0001; **P = 0.003 with respect to LacZ control.

Flux of HDL-CE in vivo using SCP2$^{-/-}$ and FABP1$^{-/-}$ mice

To further establish the role of SCP2 and FABP1 in the flux of HDL-CE to bile, in vivo reverse cholesterol transport studies were conducted in SCP2$^{-/-}$ and FABP1$^{-/-}$ mice. Deficiency of SCP2 or FABP1 did not affect the levels of [^{3}H]cholesterol in plasma or liver (**Fig. 5A, B**), indicating that deficiency of these proteins does not affect the uptake of HDL by the liver. Radioactivity associated with hepatic FC was significantly reduced in SCP2$^{-/-}$ mice and significantly increased in FABP1$^{-/-}$ mice (Fig. 5C). Secretion of HDL-derived [^{3}H]cholesterol into bile was significantly increased in SCP2$^{-/-}$ mice and no difference was noted in FABP1$^{-/-}$ mice (Fig. 5D). Deficiency of SCP2 resulted in a decrease in hepatic as well as biliary bile acids, while FABP1 deficiency led to a nonsignificant increasing trend in hepatic bile acids and a significant increase in biliary bile acids (Fig. 5E, F). This observed decrease in the flux of HDL-CE to hepatic and biliary bile acid in SCP2$^{-/-}$ mice suggests that SCP2 might be involved in regulating the delivery of FC for bile acid synthesis.

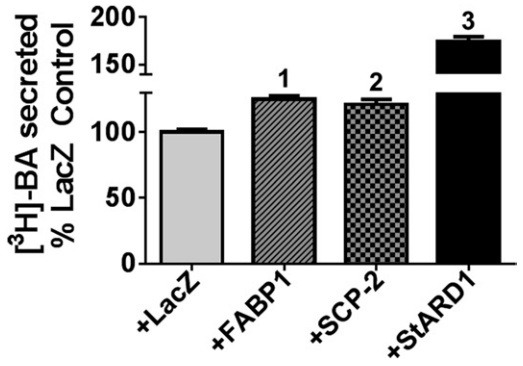

Fig. 3. Overexpression of SCP2 or FABP1 increases the appearance of HDL-CE-derived FC as bile acids. Primary hepatocytes were isolated from C57BL/6 mice, transduced with the indicated adenoviruses, and incubated with HDL-[^{3}H]CE as described in the Materials and Methods. Secretion of bile acids derived from FC generated by hydrolysis of HDL-CE was assessed by monitoring the [^{3}H] associated with bile acids in the culture medium. Data are expressed as percent LacZ control (cells transduced with Ad-LacZ), mean ± SD, n = 4 with each measurement performed in duplicate. 1, P = 0.009; 2, P = 0.008; 3, P = 0.0007 with respect to LacZ control.

SCP2 is required for CEH-dependent increase in HDL-CE hydrolysis

We have earlier demonstrated that hepatic overexpression of CEH enhances the hydrolysis of HDL-CE and the flux of the resulting FC toward bile acids (3). Based on the observed effects of SCP2 deficiency on the flux of HDL-CE to bile acids, we examined the effects of CEH overexpression on the flux of HDL-CE in SCP2$^{-/-}$ mice. Transient adenovirus-mediated overexpression of CEH in WT or SCP2$^{-/-}$ mice did not affect the levels of HDL-derived [^{3}H]cholesterol in plasma or liver (**Fig. 6A, B**). Significantly higher hepatic hydrolysis of HDL-CE was observed in WT mice and it was further enhanced by adenovirus-mediated CEH overexpression (Fig. 6C) and this effect was attenuated in SCP2$^{-/-}$ mice. Two-way ANOVA shows

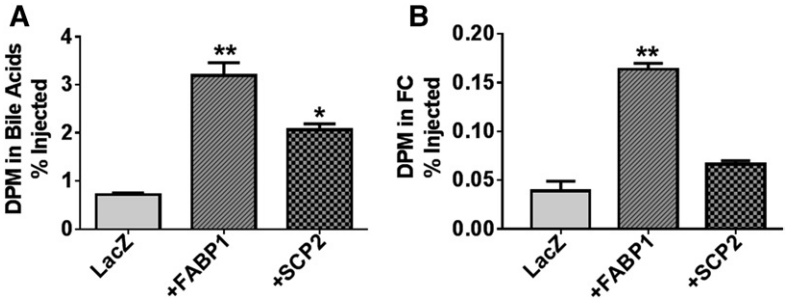

Fig. 4. Overexpression of SCP2 or FABP1 in vivo increases the flux of HDL-CE to biliary bile acids. C57BL/6 mice were injected (iv) with the indicated adenoviruses followed by HDL-[^{3}H]CE as described in the Materials and Methods. Mice were euthanized after 48 h and gallbladder bile was collected and used for bile acid extraction. [^{3}H] associated with the bile acids (A) and biliary FC (B) was determined. Data are expressed as percent of the injected HDL-[^{3}H]CE-associated disintegrations per minute (DPM). Data are expressed as mean ± SD, n = 3; *P = 0.01, **P = 0.05 with respect to LacZ control.

$P < 0.0001$ for the effects of genotype and $P = 0.0091$ for the effect of adenovirus-mediated CEH overexpression. These data establish the role of SCP2 in CEH-dependent hydrolysis of HDL-CE and are consistent with the observed increase in HDL-CE hydrolysis by overexpression of SCP2, as shown in Fig. 1. Furthermore, similar to the dependence of CEH-mediated increase in HDL-CE hydrolysis on SR-BI expression (3), SCP2-mediated increase in HDL-CE hydrolysis was also abolished in SR-BI$^{-/-}$ mice (Fig. 6D, $P = 0.0009$ for genotype effects and $P = 0.002$ for the effects of adenovirus-mediated SCP2 overexpression). When the flux of HDL-[^{3}H]CE to bile acids was examined, a significantly attenuated appearance of HDL-CE-derived [^{3}H]cholesterol in hepatic and biliary bile acids was observed in SCP2$^{-/-}$ mice ($P < 0.0001$ for genotype). While the effect of CEH overexpression was significant for the flux of HDL-CE-derived [^{3}H]cholesterol into biliary bile acids ($P = 0.022$ for the adenovirus used), this increasing trend was not statistically significant for the hepatic bile acid levels. Collectively, these data indicate that SR-BI, CEH, and SCP2 function together to facilitate the hydrolysis of HDL-derived CE and enhance the flux of the resulting FC toward bile acid synthesis/secretion.

DISCUSSION

Although the fate of HDL-FC is well-characterized and it is thought to be directly secreted based on its rapid appearance in the bile, the fate of HDL-CE (which represents >80%

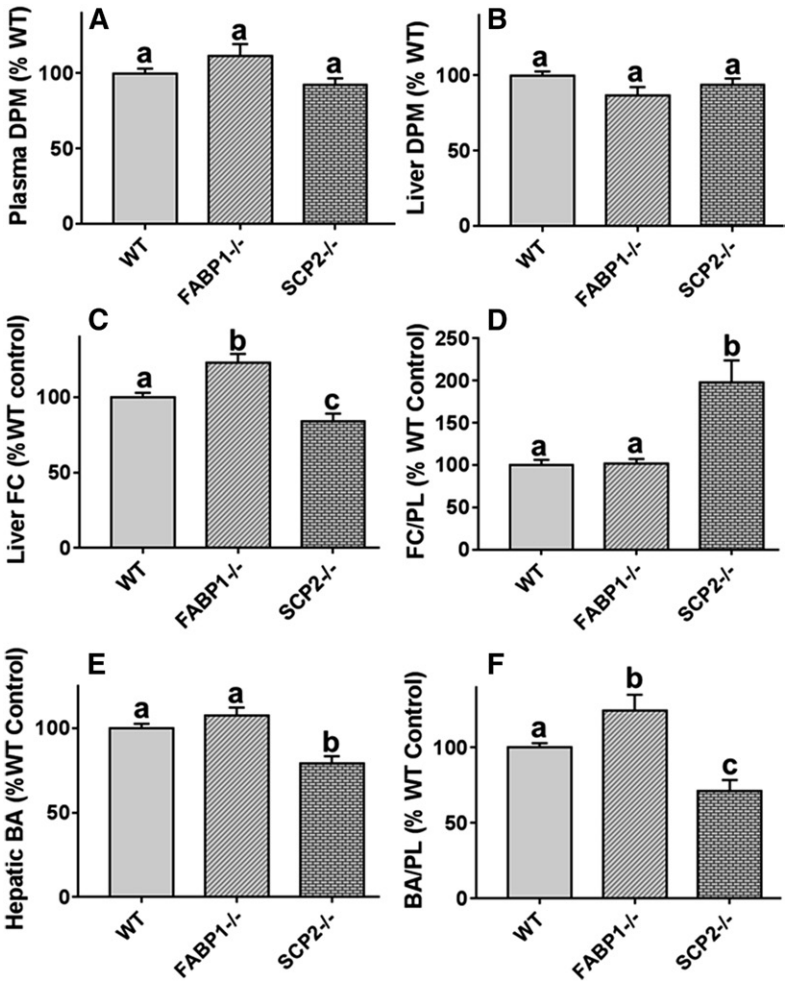

Fig. 5. Flux of HDL-[^{3}H]CE to biliary bile acids or FC in SCP2$^{-/-}$ or FABP1$^{-/-}$ mice. HDL-[^{3}H]CE was injected (iv) into C57BL/6 (WT) or SCP2$^{-/-}$ or FABP1$^{-/-}$ mice and mice were euthanized after 48 h and blood, liver, and gallbladder were collected and processed as described in the Materials and Methods. A: The disintegrations per minute (DPM) associated with plasma, expressed as percent WT. B: The DPM associated with liver. C: Total hepatic lipids were extracted and neutral lipids were separated by TLC. HDL-CE hydrolysis was assessed by monitoring the levels of [^{3}H]FC generated. D: The [^{3}H] label associated with FC in gallbladder bile. E: The [^{3}H]label associated with hepatic bile acids (BA) normalized to the total liver weight and expressed as percent WT control. F: The [^{3}H]label associated with biliary bile acids, normalized to total phospholipids in bile and expressed as percent WT control. All data are mean ± SD, n = 6, both males and females. Dissimilar letters indicate $P < 0.05$.

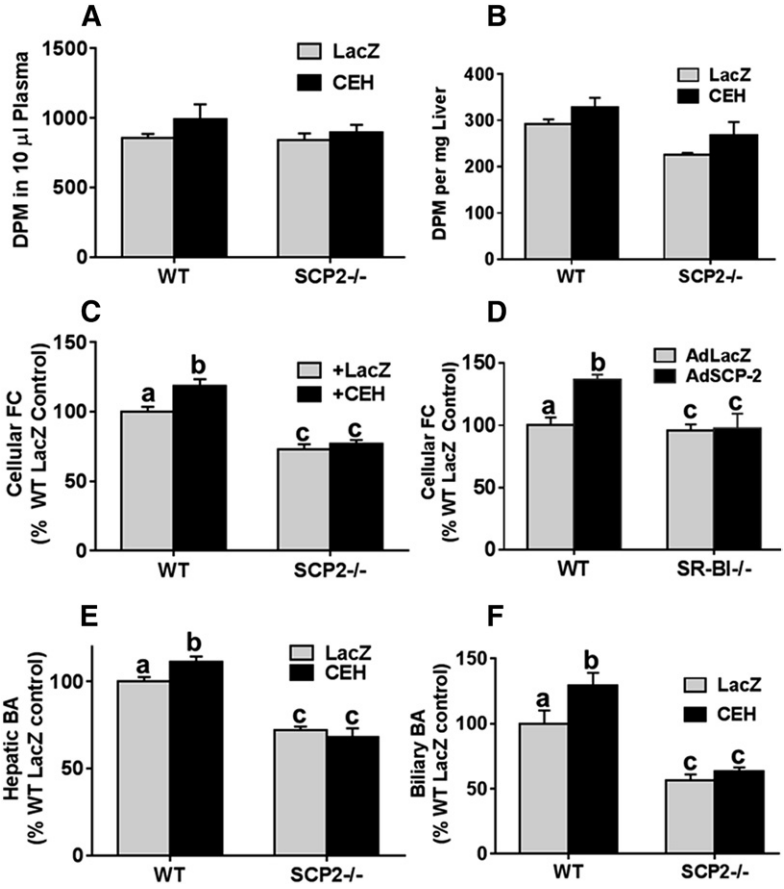

Fig. 6. Effects of CEH overexpression on the flux of HDL-[^{3}H]CE in SCP2$^{-/-}$ mice. The indicated adenoviruses were injected (iv) into C57BL/6 (WT) or SCP2$^{-/-}$ mice. After 3 days, these mice were also injected (iv) with HDL-[^{3}H]CE and the mice were euthanized after an additional 48 h. A: The disintegrations per minute (DPM) associated with plasma. B: The DPM associated with liver. C: Total hepatic lipids were extracted and neutral lipids were separated by TLC. HDL-CE hydrolysis was assessed by monitoring the levels of [^{3}H]FC generated ($P < 0.0001$ for the effects of genotype and $P = 0.0091$ for the effect of adenovirus-mediated CEH overexpression). D: Hydrolysis of HDL-[^{3}H]CE monitored as [^{3}H]FC accumulation in WT and SR-BI$^{-/-}$ mice following adenovirus-mediated overexpression of SCP2 ($P = 0.0009$ for genotype effects and $P = 0.002$ for the effects of adenovirus-mediated SCP2 overexpression). E: The [^{3}H]label associated with hepatic bile acids (BA) normalized to the total liver weight and expressed as percent LacZ control ($P < 0.0001$ for genotype). F: The [^{3}H]label associated with biliary bile acids, normalized to total phospholipids in bile and expressed as percent LacZ control ($P < 0.0001$ for genotype and $P = 0.022$ for adenovirus effects). All data are mean ± SD, n = 3, both males and females, significance of the observed differences between groups was determined by two-way ANOVA. Dissimilar letters indicate $P < 0.05$.

of HDL-associated cholesterol) is only beginning to be understood. Earlier studies from our laboratory demonstrated the role of hepatic CEH in the intracellular hydrolysis of HDL-CE. This study demonstrates for the first time that intracellular sterol carrier proteins, namely SCP2 and FABP1, enhance the hydrolysis of HDL-CE and facilitate the transport of the resulting FC toward secretion into bile either as bile acids or as FC. It is noteworthy that the major source of biliary cholesterol or bile acids is not de novo synthesized cholesterol but lipoprotein-derived cholesterol, specifically HDL-associated cholesterol. Furthermore, SCP2 or FABP1 also reduce the resecretion of FC derived from HDL-CE as nascent HDL. These studies, therefore, extend our current understanding of hepatic clearance of cholesterol returned to the liver as HDL-CE and also establish the role of SCP2 and FABP1 in this process. The importance of defining the role of SCP2 or FABP1 in regulating the flux of HDL-CE is underscored by the fact that human genetic variations in SCP2 inhibit cholesterol metabolism (20), a phenotype shared with SCP2$^{-/-}$ mice (21) and human liver fatty acid binding protein (L-FABP) T94A polymorphism that occurs with a frequency of 32–37% in Caucasians results in hepatic cholesterol accumulation (22). It is noteworthy that deficiency of either SCP2 or FABP1 does not affect the levels of plasma triglyceride, cholesterol, or nonesterified fatty acids in mice (21, 23, 24).

Conversion of FC to bile acids occurs via a tightly regulated complex pathway involving several subcellular compartments, including endoplasmic reticulum, mitochondria, peroxisomes, and cytoplasm. The mechanism(s) of transport of cholesterol and relatively hydrophobic intermediates in hepatic bile acid synthesis between these compartments and finally to the bile canaliculus for secretion is largely unknown. Liver bile acid binding protein (L-BABP) is thought to be involved in facilitating such transport in nonmammalian vertebrates (25), but L-BABP is not expressed in humans or other mammals. Liver fatty acid binding protein (L-FABP or FABP1) is expressed in mammalian liver that, in addition to binding FC, can also bind bile acids (13). SCP2, on the other hand, has been shown to increase the activity of CYP7A1 in in vitro assay systems and its overexpression increases bile acid synthesis (26). The data presented here are consistent with this role of SCP2 and further demonstrate that SCP2 regulates the flux of HDL-CE toward bile acid synthesis and secretion into bile, both by increasing the intracellular hydrolysis of HDL-CE and by likely increasing the transport of the resulting FC to the endoplasmic reticulum to serve as the substrate for CYP7A1. The mechanism(s) by which SCP2 facilitates such a specific transfer remains to be established. SCP2 is thought to be present in sufficiently close enough proximity for direct interaction with SR-BI (10) and our earlier results have demonstrated the interaction as well as hydrolysis of SR-BI-delivered HDL-CE by CEH (3). Consistently, SCP2 deficiency failed to increase CEH-mediated hydrolysis of HDL-CE and overexpression of SCP2 in SR-BI$^{-/-}$ mice also failed to increase hydrolysis

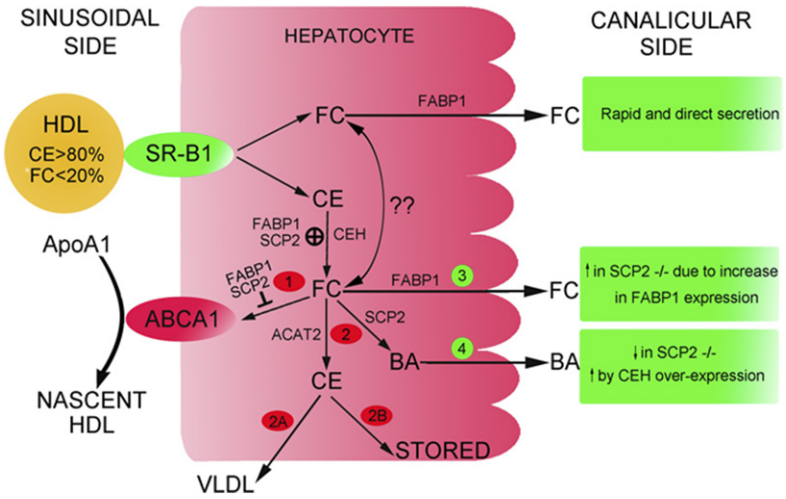

Fig. 7. Proposed model for the flux of HDL-CE-derived FC to bile. At this moment, it is not established whether the pools of HDL-FC and FC generated by CEH-mediated hydrolysis of HDL-CE equilibrate within the hepatocyte (indicated by "??"). FABP1 and SCP2 increase hydrolysis of HDL-CE (Fig. 1) and FC released following hydrolysis of from HDL-CE can have multiple fates (numbered 1–4) where fates 1 and 2 will result in resecretion of HDL-derived cholesterol either as nascent HDL (number 1) or following esterification as VLDL (number 2A); alternatively, CE generated as a result of ACAT2-mediated esterification can also be stored within the hepatocyte (number 2B). Fates 3 (direct secretion of FC) and 4 [secretion of FC after conversion to bile acids (BA)] will lead to final elimination of HDL-derived cholesterol in bile. Data presented here (Fig. 2) suggest that FABP1 as well as SCP2 attenuate the resecretion of FC from HDL-CE into nascent HDL. SCP2, known to transport FC to the endoplasmic reticulum and increase bile acid synthesis, regulates the flux of HDL-CE to biliary bile acids as shown in Figs. 3, 4, and 6. FABP1, on the other hand, modulates the secretion of HDL-CE-derived FC into bile.

of HDL-CE (Fig. 6) suggesting a likely concerted role of SR-BI-CEH-SCP2 in facilitating/increasing the hydrolysis of HDL-CE.

SCP2 deficiency leads to a compensatory increase in FABP1 (normal intracellular concentration of FABP1 in hepatocytes/liver itself is >10 times that of SCP2) and increased appearance of HDL-[^{3}H]CE-derived [^{3}H]FC in bile suggests that under conditions of SCP2 deficiency, the increased levels of FABP1 are likely responsible for facilitating the transport of FC into bile. However, loss of FABP1 did not affect the level of HDL-CE-derived FC in the bile in vivo. These data are consistent with the earlier reports demonstrating unaltered biliary cholesterol secretion as well as cholesterol saturation index in mice deficient in FABP1 (27, 28), although the flux of cholesterol from HDL-CE to biliary FC was not specifically monitored in those studies. It is also noteworthy that in FABP1$^{-/-}$ mice, increased biliary cholesterol secretion and cholesterol saturation index is observed in response to cholesterol-rich or lithogenic diets (27, 28). Unlike the reported increase in FABP1 associated with SCP2 deficiency, the changes in the expression of other sterol binding proteins in FABP1$^{-/-}$ mice is currently not known. Furthermore, as true for any single physiological process modulated by multiple gene products, it is also likely that an as yet unidentified sterol carrier protein compensates for the function of FABP1 in FABP1$^{-/-}$ mice. Future studies characterizing the complete repertoire of intracellular sterol carrier proteins will be needed to identify the protein(s) underlying this observed effect.

Secretion of FC into bile needs to be balanced with an appropriate secretion of bile acids to maintain the cholesterol saturation index. While overexpression of both SCP2 and FABP1 increased the flux of HDL-[^{3}H]CE to [^{3}H] bile acids, significant reduction in this parameter was only noted in SCP2$^{-/-}$ mice (Fig. 5), indicating a more important role for SCP2 in modulating bile acid synthesis/secretion. This is consistent with earlier studies demonstrating SCP2-mediated increase in CYP7A1 activity and bile acid synthesis in vitro (24). SCP2 is also thought to be involved in transport of FC to the endoplasmic reticulum and it is highly likely that by making FC available at the endoplasmic reticulum, SCP2 also increases CYP7A1 activity in vivo and stimulates bile acid synthesis. Targeted future studies will be needed to confirm this hypothesis. Deficiency of FABP1, on the other hand, increased the flux of HDL-[^{3}H]CE to biliary bile acids. FABP1 binds bile acids (13) and it is likely that its deficiency leads to an increase of unbound bile acids available for biliary secretion.

The model depicted in **Fig. 7** summarizes the data obtained in this study in the context of earlier observations. HDL-FC is rapidly transported to bile and this process is facilitated by FABP1. HDL-CE that represents >80% of HDL-associated cholesterol, is hydrolyzed intracellularly by CEH and both SCP2 and FABP1 enhance this process, as shown in Fig. 1. At this moment, it is not established whether the pools of HDL-FC and FC generated by CEH-mediated hydrolysis of HDL-CE equilibrate within the hepatocyte. FC released following hydrolysis of HDL-CE can have multiple fates (numbered 1–4), where fates 1 and 2 will result in resecretion of HDL-derived cholesterol and fates 3 and 4 will lead to final elimination of HDL-derived cholesterol in bile. Data presented here (Fig. 2) suggests that FABP1 as well as SCP2 attenuate the resecretion of FC from HDL-CE into nascent HDL. SCP2, known to transport FC to the endoplasmic reticulum and increase bile acid synthesis, regulates the flux of HDL-CE to biliary bile acids, as shown in Figs. 3, 4, and 6. FABP1, on the other hand, modulates the secretion of HDL-CE-derived FC into bile. In summary, by appropriately transporting HDL-CE-derived cholesterol, SCP2 and FABP1 facilitate final elimination of cholesterol into bile as FC or bile acids and are, therefore, likely to be anti-atherogenic.

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