The Farnesoid X-receptor Is an Essential Regulator of Cholesterol Homeostasis*

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To address the importance of the farnesoid X-receptor (FXR; NR1H4) for normal cholesterol homeostasis, we evaluated the major pathways of cholesterol metabolism in the FXR-deficient (−/−) mouse model. Compared with wild-type, FXR(−/−) mice have increased plasma high density lipoprotein (HDL) cholesterol and a markedly reduced rate of plasma HDL cholesterol ester clearance. Concomitantly, FXR(−/−) mice exhibit reduced expression of hepatic genes involved in reverse cholesterol transport, most notably, that for scavenger receptor BI. FXR(−/−) mice also have increased: (i) plasma non-HDL cholesterol and triglyceride levels, (ii) apolipoprotein B-containing lipoprotein synthesis, and (iii) intestinal cholesterol absorption. Surprisingly, biliary cholesterol elimination was increased in FXR(−/−) mice, despite decreased expression of hepatic genes thought to be involved in this process. These data demonstrate that FXR is a critical regulator of normal cholesterol metabolism and that genetic changes affecting FXR function have the potential to be pro-atherogenic.

The nuclear receptor superfamily represents a large group of ligand-activated transcription factors that are involved in a variety of physiological, developmental, and toxicological processes (1). This group is characterized by highly conserved DNA and ligand binding domains and includes the functionally diverse estrogen, progesterone, peroxisome proliferator-activated, constitutive androstane, retinoic acid, vitamin D, and thyroid hormone receptors. A growing body of evidence indicates that an important function fulfilled by some members of the nuclear receptor superfamily is to contribute to the homeostatic control of lipid metabolism. In particular, LXR (NR1H3),1 the liver oxysterol-activated receptor (2), and FXR (NR1H4), the bile acid (BA)-activated farnesoid X-receptor (3–5), regulate the hepatic biosynthesis of BAs from cholesterol, a quantitatively important route for the elimination of excess cholesterol. Cross-talk between LXR feed-forward and FXR feedback regulation, particularly of the cytochrome P450 (CYP) gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of the classic BA biosynthetic pathway, permits fine-tuning of hepatic cholesterol homeostasis (6, 7). LXR and FXR also modulate the expression of several genes involved in lipoprotein metabolism and thus, contribute to the maintenance of proper plasma cholesterol levels. LXR positively regulates the expression of the genes encoding lipoprotein lipase (LPL) (8), cholesterol ester transfer protein (9), and the ATP-binding cassette (ABC) A1 transporter (10, 11). FXR has been shown to be a positive regulator of the apolipoprotein (apo) C-II (12) and the phospholipid transfer protein (PLTP) (13) genes, and a negative regulator of apoA-I gene expression in cultured cells (14).

The role of LXR as a sterol sensor coordinating the expression of genes involved in various aspects of sterol homeostasis has been firmly established by the use of a synthetic LXR agonist (T0901317) and LXRα/β-knockout mice. T0901317 induces the intestinal expression of ABCA1 and concomitantly decreases the absorption of cholesterol in wild-type but not in LXRα/β knockout mice (15). T0901317 also regulates the expression of two additional ABC transporters, ABCG5 and ABCG8, responsible for the exclusion of dietary sterols other than cholesterol from absorption by the enterocyte (16). Characterization of FXR knockout mice has firmly established the critical role for this receptor in bile acid homeostasis by virtue of its role as an intracellular bile acid sensor (17). This function is achieved in large measure through the regulation of genes involved in BA transport and biosynthesis. These include CYP7A1, the liver canalicular, the bile salt export pump (BSEP-ABCB11), the ileal bile acid binding protein (I-ABBP), the hepatic basolateral sodium taurocholate co-transporter protein (NTCP), and the sterol 12α-hydroxylase (CYP51B1), which controls the ratio of cholic acid to chenodeoxycholic acid in the bile (18). Thus, although FXR is a key regulator of BA homeostasis, its physiological role in the overall metabolism of cholesterol remains to be established. The purpose of the present study was to elucidate the role of FXR as a regulator of cholesterol homeostasis and to gain mechanistic insight into the means by which this is achieved.

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1 The abbreviations used are: LXR, liver oxysterol receptor; ABC, ATP-binding cassette; apo, apolipoprotein; BA, bile acid; BSEP, bile salt export protein; CE, cholesterol ester; CEs, cholesterol ester hydrolyase; CETP, cholesteryl palmitoyl transfer; CYP, cytochrome P450; FC, free cholesterol; FFA, free fatty acid; FXR, farnesoid X-receptor; HDL, high density lipoprotein; HL, hepatic lipase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzymeA; LCAT, lecithin cholesterol acyl transferase; LDL, low density lipoprotein; LDLr, low density lipoprotein receptor; LpB, apolipoprotein B-containing lipoprotein; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein; FXR, farnesoid X-receptor; SCP, sterol carrier protein; SRBI, scavenger receptor BI; TG, triglycerides; VLDL, very low density lipoprotein; FCR, fractional catabolic rate; MOPS, 4-morpholinoethane sulfonic acid.
C57BL mice were labeled with [3H]cholesterylpalmityl ether (CEt) or exposed to phosphorimaging screens and were visualized using a Storm trichillation counter and that of 125I/131I was measured using a Cobra II liquid scintillation counter. The radioactivity of 3H was quantitated in a Tri-Carb 2500 TR liquid scintillation counter. Mouse apoA-I and apoA-II as previously described (23). The apoB was measured by enzyme-linked immunosorbent assay (ELISA). High density lipoprotein (HDL) subclass and VLDL were resolved by two-dimensional gel electrophoresis (19). Biosciences, Piscataway, NJ). High density lipoprotein (HDL) subclasses were resolved by two-dimensional gel electrophoresis (19). Plasma apoB was measured by enzyme-linked immunosorbent assay (20).

EXPERIMENTAL PROCEDURES

Animals and Diets—FXR(-/-) mice (17) were backcrossed to strain C57BL/6 for at least five generations. Mice were housed in a pathogen-free animal facility under a standard 12-h light/12-h dark cycle and were fed a standard rodent chow (AIN-93G) and water ad libitum. For some experiments, mice were fed a standard chow diet supplemented with 0.4% (w/w) cholic acid (BioServ, Frenchtown, NJ). For tissue collection, mice were euthanized by carbon dioxide asphyxiation. The livers and ilea were quickly removed, snap-frozen in liquid N2, and stored at −80 °C until use. All protocols and procedures were reviewed and approved by the National Institutes of Health animal care and use committee. Plasma Lipids and Lipoproteins—Plasma total cholesterol and triglycerides (TG) (Sigma, St. Louis, MO), as well as free cholesterol (FC) and phospholipids (Wako, Osaka, Japan) were measured in a 12.5 μl aliquot of plasma using commercial kits and the Hitachi 911 automated diagnostic kit. VLDL/TG was measured in plasma aliquots and ascer-

RESULTS

Previously, we reported that FXR(-/-) mice maintained on a regular chow diet exhibit profound hyperlipidemia (17). To further elucidate the role of FXR in cholesterol and lipoprotein homeostasis, plasma lipid concentrations were determined and the lipoprotein profile of non-fasting wild-type and FXR(-/-) mice was compared using gel filtration (FPLC) analysis. Compared with wild-type mice, FXR(-/-) mice had 2-fold higher plasma total cholesterol, cholesterol ester (CE) and FC concentrations (Table I). Based upon this FPLC profile, it was evident that these increases in plasma cholesterol were primarily due to increased quantities of VLDL, low density lipoprotein (LDL), as well as HDL cholesterol levels (Fig. 1a).

HDL Metabolism and Reverse Cholesterol Transport Are Impaired in FXR(-/-) Mice—In FXR(-/-) mice, the increase in plasma HDL cholesterol was associated with an increased HDL particle size, as indicated by the presence of large HDL2 particles before the main HDL2apoA-I peak (Fig. 1a). Separation of plasma lipoproteins by two-dimensional native gel electrophoresis and immunoblotting against mouse apoA-I (Fig. 1b), confirmed the increase of HDL size concomitant with a relative dimension of the proportion of small HDL2apoA-I particles and an increase in the proportion of large HDL1 particles in FXR(-/-) mice (Fig. 1b). Northern blot analysis indicated that the hepatic and intestinal mRNA expression levels of the two major protein components of HDL, apoA-I, and apoA-II, were similar in FXR(-/-) and wild-type mice (Fig. 2). The catabolism of HDL 125I-apoA-I (fractional catabolic rate (FCR) = 2.3 ± 0.2

| TC  | TG  | PL  | FC   | HDL cholesterol | Non-HDL cholesterol | ApoB |
|-----|-----|-----|------|-----------------|---------------------|------|
| mg/dl| mg/dl| mg/dl| mg/dl| mg/dl| mg/dl| mg/dl|
| Wild-type | 108 ± 8 | 99 ± 18 | 163 ± 14 | 25 ± 2 | 80 ± 6 | 25 ± 4 | 24 ± 1 |
| FXR(-/-) | 202 ± 5 | 161 ± 29 | 285 ± 8 | 49 ± 1 | 137 ± 12 | 66 ± 9 | 39 ± 4 |

*p < 0.05, FXR(-/-) versus wild-type.
versus 2.4 ± 0.2 day⁻¹) and that of HDL 131I-apoA-II (FCR = 2.6 ± 0.5 versus 2.7 ± 0.2 d⁻¹) were also similar (n = 6; p > 0.5, all) in FXR(−/−) and wild-type animals, respectively (Fig. 3). The catabolism of HDL [3H]CEt, a non-degradable analogue of CE, was significantly delayed in FXR(−/−) compared with wild-type mice (FCR = 3.2 ± 0.2 versus 4.1 ± 0.3 d⁻¹, respectively, n = 8, p < 0.02) (Fig. 3) due to impaired hepatic uptake of HDL [3H]CEt (64 ± 2% versus 78 ± 1% of injected dose, respectively, n = 8, p < 0.01). The uptake of HDL [3H]CEt by other organs (i.e. adrenals, testis, heart, lungs, kidneys, and spleen) was not significantly different between both groups (data not shown). All together, these data demonstrate that the selective uptake of HDL-CE by the liver is impaired in FXR(−/−) mice.

To determine the molecular mechanisms responsible for delayed HDL-CE hepatic uptake in FXR(−/−) livers, mRNA levels of the major hepatic genes involved in HDL metabolism were measured in this animal model. The expression of LCAT, HL, and of SRBI, the HDL receptor, which promotes the selective uptake (29) of HDL-CE, were decreased, whereas that of ABCA1 and PLTP were not changed in the livers of FXR(−/−) mice compared with wild-type (Figs. 2 and 4a). However, the plasma LCAT activity (20 ± 2 versus 18 ± 1 nmol of CE/ml/h, n = 8, p > 0.5), as well as the post-heparin HL activity (67 ± 23 versus 58 ± 10 nmol of free fatty acid (FFA)/ml/h, respectively, n = 8, p > 0.5) was similar in both mouse models. In agreement with the mRNA data, Western blotting revealed that the hepatic expression of SRBI was significantly reduced (−63%) in FXR(−/−) (Fig. 4, b and c) versus wild-type mice, consistent with the HDL kinetic analysis data (Fig. 3). When the mice were placed on a diet containing 0.4% of the FXR agonist cholic
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FXR affects the expression of several genes involved in HDL and intracellular cholesterol metabolism. A, Northern blot analysis of mRNAs from wild-type (+/+) and FXR (−/−) livers. B, Northern blot analysis of hepatic lipoprotein receptors mRNAs from wild-type (+/+) and FXR (−/−) mice fed regular chow or a 0.4% cholic acid (Ch. Acid) diet for 5 days. C, Western blot analysis of hepatic lipoprotein receptors from wild-type (+/+) and FXR (−/−) mice fed regular chow or a 0.4% cholic acid (Ch. Acid) diet for 5 days. Purified liver membrane proteins (10 µg) were separated by electrophoresis, transferred onto Immobilon membranes, and probed with polyclonal anti-mouse-LDLr and -SRBI antibodies. Protein expression was measured in four animals of each genotype and each diet and expressed as fold-change relative to wild-types on chow. Two representative animals of each group are displayed.

Synthesis of Apolipoprotein B-containing Lipoprotein (LpB) Is Increased in FXR (−/−) Mice—In agreement with our previous studies, plasma apoB and non-HDL cholesterol levels were increased 1.6- and 2.6-fold in FXR (−/−) compared with wild-type mice, respectively (Table I). By FPLC, both VLDL and LDL cholesterol levels were increased in FXR (−/−) mice (Fig. 1a). FXR (−/−) mice also had a 1.7-fold increased plasma phospholipids (PL) and TG levels, compared with controls. Whereas the distribution of PL among the various classes of lipoproteins was similar to that of cholesterol (not shown), more than 90% of the TG in FXR (−/−) plasma was associated with VLDL-sized particles (Fig. 1a). To examine the mechanism underlying the increased plasma LpB levels in FXR (−/−) mice, we measured the expression of the LDL receptor (LDLR), the major receptor for LpB uptake by Northern and Western (Fig. 4, b and c) blots. The expression of the LDLr was similar in FXR (−/−) and wild-type livers, ruling out delayed hepatic catabolism to significantly account for elevated LpB in FXR (−/−) mice. Particularly vexing were the parallel increases in plasma TG levels and post-heparin LPL activity (306 ± 49 versus 179 ± 18 nmol of FFA/mg/h, n = 8, p < 0.05) observed in FXR (−/−) compared with wild-type mice.

We reasoned that if the catabolism of LpB cholesterol and TG was not significantly impaired in FXR (−/−) mice, the production of LpB would be increased in this animal model. By Northern blot analysis, the hepatic and ileal expression of apoB, apoE, apoC-II, and apoC-III, the major proteins of LpB, were similar in FXR (−/−) and wild-type mice (Fig. 2). The expression of the microsomal triglyceride transfer protein (MTP), which plays a major role in LpB assembly, was not increased in FXR (−/−) mice (Fig. 2). However, despite reduced hepatic MTP and HMG-CoA reductase expression, the accumulation of plasma TG was dramatically increased in FXR (−/−) mice compared with wild-type mice (Fig. 5a) after tyloxapol injection, which blocks the hydrolysis of TG by LPL, demonstrating that the production of LpB is increased upon FXR deletion.

Furthermore, the expression of apoA-IV, an important protein component of newly synthesized LpB, particularly chylomicrons (32), was increased 5.1-fold in the liver and 4.5-fold in the ileum of FXR (−/−) compared with wild-type animals (Fig. 2). Next, we measured the absorption of dietary cholesterol in

Fig. 4 FXR affects the expression of several genes involved in HDL and intracellular cholesterol metabolism. a, Northern blot analysis of mRNAs from wild-type (+/+) and FXR (−/−) livers. b, Northern blot analysis of hepatic lipoprotein receptors mRNAs from wild-type (+/+) and FXR (−/−) mice fed regular chow or a 0.4% cholic acid (Ch. Acid) diet for 5 days. Bands were quantitated and are expressed as fold-change after correction for β-actin level, relative to wild-types (n = 3; *; p < 0.05). c, Western blot analysis of hepatic lipoprotein receptors from wild-type (+/+) and FXR (−/−) mice fed regular chow or a 0.4% cholic acid (Ch. Acid) diet for 5 days. Purified liver membrane proteins (10 µg) were separated by electrophoresis, transferred onto Immobilon membranes, and probed with polyclonal anti-mouse-LDLr and -SRBI antibodies. Protein expression was measured in four animals of each genotype and each diet and expressed as fold-change relative to wild-types on chow. (*, p < 0.05). Two representative animals of each group are displayed.

Fig. 5 FXR deletion increases VLDL/chylomicron TG production and affects biliary bile acid and lipid secretion. a, VLDL/chylomicron-TG clearance in mice was inhibited by tyloxapol injection. Kinetics of newly synthesized VLDL/chylomicron-TG was measured in plasma aliquots 30, 90, 150, and 330 min after injection in wild-type mice. Values are expressed as mean ± S.E. b, Wild-type and FXR (−/−) mice were fed a control diet or a diet supplemented with 0.4% cholic acid for 3 (0.4% CA-d3) or 5 (0.4% CA-d5) days. Hepatic bile was collected by gravity. Biliary cholesterol, PL, and BA flows were calculated as nanomoles/h corrected for the weight of each animal. (a, p < 0.05 versus wild-type fed chow; b, p < 0.05 versus wild-type after 5 days on the 0.4% cholic acid diet).
FXR(−/−) and wild-type mice by the dual isotope plasma ratio method (28). Control mice absorbed only 62 ± 4% of the ingested cholesterol dose, whereas 97 ± 2% was absorbed by the intestine of FXR(−/−) mice (n = 6, p < 0.003). Taken together, these results demonstrate that FXR deletion in mouse results in increased plasma non-HDL cholesterol and TG levels caused by an increase in LpB synthesis associated with an elevation in intestinal cholesterol absorption. Thus, FXR is a negative regulator of dietary cholesterol absorption and of chylomicrons and/or VLDL secretion.

FXR Regulates the Enterohepatic Circulation of Cholesterol—To elucidate the molecular mechanism responsible for increased dietary cholesterol absorption in FXR(−/−) mice, we measured the ileal mRNA levels of ABC transporters proposed to be involved in sterol absorption. Surprisingly, the expression of ABCA1, a transporter that is thought to reduce cholesterol absorption in the enterocyte (6), was increased 3-fold in the ileum of FXR(−/−) compared with wild-type (Fig. 2). In contrast, the expression of one of the two ABCG transporters cooperatively involved in the efflux of steroids from the enterocyte into the intestinal lumen (16) was sharply decreased in the ileum of FXR(−/−) mice compared with wild-types (Fig. 2). Hence, ABCG8 expression was reduced (−82%) in FXR(−/−) mice, but that of ABCG5 was not significantly altered.

Because FXR plays a critical role in BA homeostasis, a function achieved through the regulation of genes involved in BA transport and biosynthesis (17), we also measured the mRNA expression of a series of genes proposed to play a role in biliary cholesterol secretion in FXR(−/−) and wild-type mice. In the liver, ABCG5/8 have been proposed to play a key role in the biliary excretion of steroids (7, 33). Both ABCG5 (−76%) and ABCG8 (−66%) were down-regulated in the liver of FXR(−/−) compared with wild-type mice. The expression of the cholesterol ester hydrolase (CEH), an enzyme responsible for the conversion (34) of intra-cellular CE into FC and of the sterol carrier protein (SCP), a protein thought to be involved in the hepatocellular trafficking of FC to the canalicular membrane (35, 36) were also reduced in the liver of FXR(−/−) mice (Fig. 4a). Together, these data suggest that CEH-mediated mobilization, SCP-mediated intracellular transport, and ABCG5/8-mediated biliary secretion of FC may be decreased in FXR(−/−) mice.

However, very surprisingly, the flow of biliary cholesterol, but also the flow of biliary PL and bile salts, measured after cannulation of the common biliary duct (5), were increased in FXR(−/−) mice compared with wild-types (Fig. 5b). In separate experiments the mice were challenged for 3 or 5 days with a diet containing 0.4% cholic acid to saturate their hepatic biliary secretion ability, and their flow of biliary PL, FC, and BAs was measured. As anticipated, the biliary flow of BAs increased in wild-type mice upon cholic acid feeding (Fig. 5b). Conversely, there was a non-significant trend toward a decrease in the biliary flow of BAs in FXR(−/−) mice maintained 3 and 5 days on a 0.4% cholic acid diet, indicating that the hepatic BAs secretion ability of FXR(−/−) mice was saturated under these experimental conditions (Fig. 5b). The biliary flow of PL and FC were sharply decreased in FXR(−/−) compared with wild-type mice maintained for 5 days on 0.4% cholic acid. Together, these data indicate that FXR deletion in mouse results in an apparent increase in bile flow, despite the down-regulation of a series of genes involved in hepatic mobilization, transport, and canalicular secretion of biliary acids and lipids. However, upon cholic acid feeding, which saturates canalicular BA secretion in FXR(−/−), the biliary cholesterol and PL flow was reduced dramatically, whereas it increased in wild-type mice.

**DISCUSSION**

In the present report, we address the physiological role of the nuclear hormone receptor FXR on cholesterol homeostasis in vivo. We have shown that FXR(−/−) mice have increased plasma HDL cholesterol due to defective HDL-CE removal associated with decreased hepatic SRBI expression. FXR(−/−) mice also had increased LpB cholesterol and TG plasma levels caused by an increase in LpB synthesis and intestinal cholesterol absorption. Very surprisingly, the biliary flow was increased in FXR(−/−) mice, despite decreased expression of genes involved in the mobilization, intracellular transport, and canalicular secretion of cholesterol and bile acids, a phenomenon reversed upon cholic acid feeding. A schematic representation of the integrated role of FXR in bile acid and cholesterol metabolism is presented in Fig. 6. This model is based upon our current and previous (17) studies, as well as contributions from a number of independent laboratories (15, 16, 18).

Our study establishes that the increase in plasma HDL levels in FXR(−/−) mice does not result from altered mRNA expression of apoA-I and apoA-II, or hepatic ABCA1, a major contributor to plasma HDL levels (20, 37), indicating that the production of HDL is not altered in FXR(−/−) mice. Consistent with our data, Fuchs et al. (38) demonstrated that gallstone-susceptible C57L/J or gallstone-resistant AKR mice had similar apoA-I mRNA and protein levels when placed either on chow or on 0.5% cholic acid diet. In contrast, Claudel et al. (14) reported that administration of the FXR agonist taurocholate repressed apoA-I expression in human hepatoma HepG2 cells as well as in human apoA-I transgenic mice. The differences between murine and human apoA-I promoters may account for the apparent discrepancy between these studies. FXR(−/−) mice exhibited reduced mRNA expression of LCAT and HL, but their plasma LCAT and HL enzymatic activities were similar to those of wild-types. FXR(−/−) mice exhibited reduced
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mRNA expression of LCAT and HL. Hepatocyte nuclear factor (HNF)-1α-deficient mice also present with decreased hepatic LCAT and HL mRNA levels, accompanied by a concomitant reduction in FXR expression (39), which suggests that FXR may be a modulator of LCAT and HL expression in vivo. However, FXR(−/−) and wild-type mice had similar plasma LCAT and HL enzymatic activities. Thus, the definitive role of FXR on the hepatic production, secretion, and/or plasma activation of these two enzymes remains to be established.

After ruling out differential HDL production or plasma enzymatic remodeling to account for elevated plasma HDL levels in FXR(−/−) mice, we showed that there was delayed selective uptake of HDL-CE by FXR(−/−) livers, a mechanism mediated primarily by the HDL receptor (29) SRBI. A similar increase in the proportion of large HDL particles is also observed with partial (40) or total (41) SRBI deficiency. Consistent with our finding, SRBI expression is induced in the liver of C57L/J mice upon cholic acid feeding (38). In the present report, we also showed that cholic acid positively regulates SRBI mRNA and protein expression in the liver of wild-type but not in that of FXR(−/−) mice. These combined findings establish for the first time that FXR is a physiological modulator of SRBI expression; thereby enhancing HDL-mediated reverse cholesterol transport.

Next, we assessed the major aspects of LpB metabolism in FXR(−/−) and wild-type mice. Plasma LpB levels were sharply increased in FXR(−/−) mice. The degradation of LpB cholesterol and TG did not appear to be significantly impaired in FXR(−/−) mice, because neither the hepatic LDLr protein expression nor the plasma LPL activity were reduced in this animal model. Despite no change in the expression of the major LpB apolipoproteins, we observed a dramatic increase of LpB production in FXR(−/−) mice. Likewise, CYP7A1 overexpression (42) results in increased secretion of LpB, FXR(−/−) mice also present with increased hepatic CYP7A1, due to the lack of repression (17), but unlike CYP7A1 transgenic mice, FXR(−/−) animals do not have increased hepatic lipogenic enzymes (data not shown) and furthermore have decreased hepatic HMG-CoA reductase and MTP expression, suggesting that, unlike the CYP7A1 transgenic mouse model, the liver is not responsible for increased LpB production in FXR(−/−) mice. These results are intriguing, because, despite a large reduction in hepatic MTP expression, LpB production increases. The intestine may account for an significant proportion of increased LpB production in FXR(−/−) mice, particularly in the post-prandial state, as discussed below. We cannot rule out that FXR(−/−) livers secrete dysfunctional VLDL (e.g. rich in apoA-IV and/or apoC-III or poor in apoC-II), resistant to vascular and circulating lipases catabolism and/or uptake. Indeed the expression of apoA-IV was increased in the liver as well as in the ileum of FXR(−/−) mice. Because, of the various apolipoproteins, only apoA-IV synthesis and secretion is stimulated by fat absorption (32), we measured the absorption of dietary cholesterol in FXR(−/−) and wild-type animals. The intestinal absorption of cholesterol was dramatically increased upon FXR deficiency, consistent with increased plasma apoB48/apoB100 ratio (17), indicating increased chylomicron production in FXR(−/−) mice. Of note, the intestinal expression of HMG-CoA reductase and MTP was not decreased in FXR(−/−) ileum. Taken together, these results demonstrate that FXR deletion in mouse results in increased plasma non-HDL cholesterol and triglyceride levels, increased LpB (mostly chylomicrons) synthesis, and an elevation in intestinal cholesterol absorption. We conclude that FXR is a negative regulator of dietary cholesterol absorption and of the subsequent intestinal LpB secretion.

The expression of ABCG8 but not of ABCG5, two transporters cooperatively involved in the efflux of sterols from the enterocyte into the intestinal lumen (16), was sharply decreased in the ileum of FXR(−/−) mice. These data indicate that the down-regulation of at least one of these two cooperative ABCG transporters may account for a diminution in dietary sterol exclusion, and thus for an increase in intestinal cholesterol absorption by FXR(−/−) mice. However, feeding the FXR agonist chenodeoxycholic acid does not induce ABCG5 and ABCG8 expression in the jejunum of mice (33). The differential regulation pattern of ABCG5/8 expression along the cephalo-caudal axis of the intestine may explain this discrepant finding. More likely, because the ileum is the portion of the intestine where BAs are preferentially absorbed (49) and where FXR expression levels are the greatest (44), FXR, and consequently its target genes, are not efficiently activated in the upper intestine. Surprisingly, the expression of ABCA1, also proposed to reduce cholesterol absorption in the enterocyte (33, 45), was increased in the ileum of FXR(−/−) mice. The physiological significance of this result is difficult to establish, because ABCA1 is primarily expressed in the duodenum and jejunum (6) and because the exact role played by ABCA1 in intestinal cholesterol absorption remains controversial (33, 46).

Finally, we have shown that FXR(−/−) mice have decreased hepatic mRNA expression of several genes encoding enzymes (CEH, SCP2) and transporters (ABCG5, ABCG8) involved in the biliary secretion of cholesterol (Fig. 6). This is consistent with recent data indicating that BA feeding of wild-type mice results in increased hepatic ABCG5/8 mRNA levels and that both synthetic and natural FXR ligands increase the expression of ABCG5/8 in hepatoma cells (33). Surprisingly, the biliary flow of cholesterol, PL, and BAs was increased in FXR(−/−) mice, despite decreased expression of the BSEP-ABCB11. With respect to the biliary output of cholesterol and PL, this finding is consistent with the phenotype of BSEP-ABCB11 knockout mice (47). Recent studies have established that, upon abnormal physiological conditions where BSEP-ABCB11 is impaired, elevated intracellular concentrations of toxic BAs may activate another nuclear hormone receptor, the pregnane X-receptor (PXR; NR1I2). A number of target genes encoding proteins with relevance to BA metabolism are regulated by PXR. For example, the gene encoding CYP3A, an enzyme capable of the metabolism and detoxification of certain BAs (48, 49), is regulated in a positive manner by PXR. The gene encoding multiple drug resistance-associated protein-2, which mediates the efflux of bile acids and lipids by epithelial cells of the gallbladder (43). When FXR(−/−) mice were fed cholic acid to saturate this alternate pathway, their ability to excrete biliary cholesterol and phospholipids was impaired. Studies are currently underway in PXR/FXR double-knockout mice to definitively establish the physiological importance of the PXR-regulated pathway for backup biliary secretion. Nevertheless, since on chow diet (i) the net output of fecal BA is decreased in FXR(−/−) mice (17) and (ii) 97% of intestinal cholesterol is absorbed by FXR(−/−) enterocytes, compared with only 62% in wild-types, our data indicate that FXR plays a major role in the enterohepatic circulation of sterols and thereby is a positive regulator of excess cholesterol disposal out of the body (Fig. 6).

Considering the established role of FXR as the primary regulator of bile acid homeostasis and the intimate linkage between bile acid biosynthesis and cholesterol elimination, targeted disruption of the FXR gene might be expected to promote
hepatic cholesterol elimination due to a lack of feedback repression of CYP7A1 and CYP27B1 expression. Indeed, a recent study (51) demonstrated that administration of guggulsterone, a traditional medicine in India used to treat hyperlipidemia, blocked hepatic cholesterol accumulation in cholesterol-fed wild-type but not FXR(−/−) mice. The authors also demonstrated that guggulsterone is an effective antagonist of FXR in transient transfection assays, a finding that has been independently confirmed (52). Thus, the observation that FXR gene deletion results in hepatic cholesterol accumulation (17) and hypercholesterolemia is intriguing. A number of possibilities may account for the apparent discrepancy of these results. For example, the developmental and lifelong absence of FXR may result in abnormal metabolic effects that are quite different from those caused by acute, transient antagonism of this receptor. An alternative explanation is that one or more of the sites of pharmacological action of guggulsterone do not include all of the tissues in which FXR is functional. That is, although FXR expression is uniformly absent in all tissues of the FXR(−/−) mouse model, guggulsterone may antagonize FXR only within a subset of these sites. This is further complicated by expression of multiple splice variants of FXR in mouse (53) and human and hamster (54). Developmental and tissue-specific patterns of expression of these isoforms may provide for cell-specific FXR-dependent functions and response to ligands.

The current absence of in vivo data regarding the modulation of FXR target gene expression by guggulsterone (51) makes comparisons between our data and the actions of this antagonist very difficult. Added to this is the recent demonstration that the FXR agonist taurocholate reduced serum HDL and apoA-I in vivo by 50% (54, 55). Thus, it is clear that modulation of FXR function, either genetically or pharmacologically, has a profound impact on cholesterol homeostasis.
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