Transgenic expression of CYP7A1 in LDL receptor-deficient mice blocks diet-induced hypercholesterolemia[S]

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Abstract Constitutive expression of a cholesterol-7α-hydroxylase (CYP7A1) transgene in LDL receptor-deficient mice blocked the ability of a cholesterol-enriched diet to increase plasma levels of apolipoprotein B-containing lipoproteins. LDL receptor-deficient mice expressing the CYP7A1 transgene exhibited complete resistance to diet-induced hypercholesterolemia and to the accumulation of cholesterol in the liver. Hepatic mRNA expression of liver X receptor-inducible ABCG5 and ABCG8 was decreased in CYP7A1 transgenic, LDL receptor-deficient mice fed a cholesterol-enriched diet. Thus, increased biliary cholesterol excretion could not account for the maintenance of cholesterol homeostasis. CYP7A1 transgenic, LDL receptor-deficient mice fed the cholesterol-enriched diet exhibited decreased jejunal Niemann-Pick C1-Like 1 protein (NPC1L1) mRNA expression, an important mediator of intestinal cholesterol absorption. A taurocholate-enriched diet also decreased NPC1L1 mRNA expression in a farnesoid X receptor-independent manner. Reduced expression of NPC1L1 mRNA was associated with decreased cholesterol absorption ($\sim$20%; $P <$ 0.05) exhibited by CYP7A1 transgenic LDL receptor-deficient mice fed the cholesterol-enriched diet.**JLR** The combined data show that enhanced expression of CYP7A1 is an effective means to prevent the accumulation of cholesterol in the liver and of atherogenic apolipoprotein B-containing lipoproteins in plasma.—Ratliff, E. P., A. Gutierrez, and R. A. Davis. Transgenic expression of CYP7A1 in LDL receptor-deficient mice blocks diet-induced hypercholesterolemia. *J. Lipid Res.* 2006. **47:** 1513–1520.

Hepatic expression of cholesterol-7α-hydroxylase (CYP7A1), the enzyme regulating the conversion of cholesterol to bile acids, plays a crucial role in eliminating excess cholesterol from the body (reviewed in 1). Studies in rats show that biliary excretion of bile acids and cholesterol accounts for ~85% of the cholesterol eliminated from the body (2). The ability of the cholesterol-to-bile acid pathway to vary in parallel with changes in the endogenous cholesterol pool plays an important role in maintaining whole body cholesterol homeostasis (3, 4). In rodents such as mice and rats, sterol-mediated activation of the nuclear liver X receptor (LXR) increases CYP7A1 gene expression and, thus, provides a mechanism to maintain cholesterol homeostasis (5, 6). In contrast to the rat and mouse CYP7A1 genes, the human CYP7A1 gene does not display LXR-mediated enhancement of transcription (7, 8).

By altering hepatic expression of sterol-regulatory element binding protein (SREBP)-mediated genes, CYP7A1 has a marked influence on many processes that control lipoprotein production as well as LDL receptor-dependent removal from plasma (9, 10). Hepatic expression of CYP7A1 indirectly controls plasma LDL levels by influencing the expression of LDL receptors (11). Studies showing that the expression of CYP7A1 in nonhepatic CHO cells enhanced the expression of LDL receptors further indicate the critical role for cholesterol catabolism in maintaining cholesterol homeostasis (9). Transgenic expression of CYP7A1 in hepatoma cells (10), CHO cells (12), and macrophages (13) also activated SREBP-mediated LDL receptor gene expression. These findings led to the proposal that transgenic expression of CYP7A1 in mice would reduce diet-induced hypercholesterolemia by increasing SREBP-mediated transcription of the LDL receptor and, thus, increasing hepatic LDL uptake. This hypothesis was tested in mice that expressed a CYP7A1 transgene whose liver-specific expression was controlled by the human apolipoprotein E enhancer element (14). The data showed that constitutive expression of the CYP7A1 transgene in mice resulted in increased SREBP-mediated expression of the LDL receptor and reduced susceptibility to diet-induced hypercholesterolemia (15).

In this study, we examined how expression of the CYP7A1 transgene affects diet-induced hypercholesterolemia in mice lacking functional LDL receptors. Our results show that expression of the CYP7A1 transgene in LDL receptor-deficient mice protected the mice from diet-induced hypercholesterolemia via a mechanism linked to increased conversion of cholesterol to bile acids and expansion of the bile acid pool size, which, in turn, was associated with decreased intestinal expression of Niemann-Pick C1-Like 1 protein (NPC1L1) and reduced cholesterol absorption.

MATERIALS AND METHODS

Mice and diets

All mice used were between 6 and 8 weeks old at the beginning of the experiment. C57BL/6 and LDL receptor-deficient mice were originally purchased from Jackson Laboratory, and colonies were maintained at San Diego State University. The generation of CYP7A1 transgenic mice has been described previously (14). CYP7A1-transgenic, LDL receptor-deficient mice and nontransgenic littermates exhibited similar weight gain and appeared normal for the duration of the experiments (data not shown). Farnesoid X receptor (FXR)-deficient mice were a generous gift from Dr. Frank Gonzalez (National Cancer Institute). Mice were maintained on a 12 h light/12 h dark cycle (light cycle from 6:00 AM to 6:00 PM). Mice were fed a standard chow diet (Harlan Teklad number 5001) before the beginning of the experiment. During the 2 weeks of experimental feeding, mice were fed a diet consisting of 1.25% cholesterol (Harlan Teklad number TD 96335). In studies examining NPC1L1 mRNA expression, mice were fed a diet consisting of chow, 20% olive oil, 2% cholesterol, and 0.5% taurocholate. All procedures were approved and performed in accordance with the Institutional Animal Care and Use Committee at San Diego State University.

Plasma lipids, fast-protein liquid chromatography analysis, and hepatic cholesterol levels

After an overnight fasting period, the mice were anesthetized with isoflurane at mid-light and blood was collected via retro-orbital puncture. Blood was centrifuged for 15 min at 4°C, and the resulting plasma samples were assayed using the commercially available Infinity Cholesterol Reagent Kit (Thermo Electron Corp.) and Wako Triglycerides Kit (Wako Chemicals USA). HDL levels were determined after treating samples with non-HDL cholesterol precipitant (Boehringer Mannheim). Plasma was injected onto a Superdex 200 column (Amersham Pharmacia Biotech) with a mobile-phase buffer consisting of 10 mM Tris, 1 mM EDTA, and 150 mM NaCl. Fractions (0.5 ml) were collected at a flow rate of 0.5 ml/min. Individual fractions were assayed for cholesterol as described above. Hepatic cholesterol levels were determined after the homogenization of the liver samples (∼50 mg) and subsequent chloroform-methanol (2:1) extraction. Stigmasterol was added before extraction and used as an internal control. Separation of cholesterol and stigmasterol was performed using a TR-50MS column (Thermo Electron Corp.) on a Trace gas chromatograph (Thermo Electron Corp.).

Western blot analysis

Plasma samples were diluted with 1 M Tris (pH 6.7) and water followed by the addition of SDS sample buffer (pH 8.0). Hepatic samples were obtained after homogenization of tissue (∼200 mg) in buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% sodium lauryl sulfate (SDS), 1% Triton X-100, and 1% deoxycholate. Crude extracts were centrifuged at 13,000 rpm for 20 min. Protein concentrations from the resulting supernatant were determined using the Bio-Rad Protein Assay Kit. Thirty micrograms of protein from each sample was added to SDS sample buffer (pH 6.7). Samples were separated using a 4–12% Tris-glycine gradient gel (Invitrogen) and then electroblotted onto polyvinylidene difluoride membranes (Amersham Pharmacia Biotech). Nonspecific binding sites were blocked using 10% nonfat dry milk/Tris Buffered Saline with Tween 20. Immunodetection was performed after incubation with the appropriate primary and secondary antibodies (Kirkengaard and Perry Lab) using ECL Plus Western Blotting Detection Reagents (Amersham Pharmacia Biotech). Blots were exposed to Hyperfilm ECL (Amersham Pharmacia Biotech). Antibodies to murine LDL receptor were a generous gift from Dr. Joachim Herz (University of Texas Southwestern Medical Center). Immunodetection of albumin (plasma) and tubulin (liver lysate) was performed as a loading control.

Generation of proprotein convertase subtilisin/kexin type 9 antibody

The murine proprotein convertase subtilisin/kexin type 9 (PCSK9) amino acid sequence (SwissProt accession number Q80W65) was analyzed using the Invitrogen peptideselect design tool (https://peptideselect.invitrogen.com/peptide/). The segment corresponding to amino acids 460–477 was submitted to BioSource (Quality Controlled Biochemicals), where the peptide was synthesized, conjugated to Keyholelimpet hemocyanin, and injected into two rabbits. Antiserum was affinity-purified and recognized two bands corresponding to the precursor and cleaved forms of PCSK9, 76 and 62 kDa, respectively.

Liver and jejunum RNA isolation and cDNA synthesis

After the experimental feeding period, the mice were anesthetized with isoflurane and euthanized at mid-light after an overnight fast. In studies examining NPC1L1 mRNA expression, mice were euthanized at mid-dark under nonfasting conditions. Livers were excised and immediately flash-frozen in liquid nitrogen. Intestines were removed and flushed twice with ice-cold PBS. The first 5 cm from the cecum was considered to be the ileum, and the segment corresponding to 5–15 cm from the cecum was considered to be the jejunum. The segments corresponding to the ileum and the jejunum were excised and flash-frozen in liquid nitrogen. RNA was isolated from frozen tissue using the Versagene RNA Tissue Kit and DNase-treated using the Versagene DNase Kit (Gentra Systems) according to the manufacturer's protocol for 80 mg of tissue. RNA was reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad), which consists of a mixture of random primers, oligo(dT), and iScript reverse transcriptase.

Quantitative PCR analysis

Quantitative PCR, using SYBR Green detection chemistry, was performed on an IQ-cycler (Bio-Rad). Primer sequences are shown in supplementary **Fig. I**. Melt curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were determined using the $\Delta\Delta$ Ct method, and expression of GAPDH was used as the internal control.

Cholesterol absorption

Mice were fed the 1.25% cholesterol diet for 2 weeks before the cholesterol absorption studies. After a 4 h fast, mice were given an oral gavage of 4 μCi of [³H]sitostanol (American Radiochemicals, Inc.) and 4 μCi of [¹⁴C]cholesterol (New England

Nuclear) dissolved in olive oil. Mice were returned to their cages and allowed free access to the cholesterol-enriched diet. After 72 h, feces were collected, homogenized, and extracted using chloroform-methanol (2:1). The organic layer was dried down using N_2, 10 ml of Ecolite was added (ICN Radiochemicals), and the ratio of [^{14}C]cholesterol to [^{3}H]sitostanol was measured using a β-scintillation counter (Beckman Instruments).

Statistical analysis

Statistical analyses between groups of mice were performed using Student's *t*-test (two-tailed, unpaired). All values are reported as means ± SD.

RESULTS

Expression of the CYP7A1 transgene blocks diet-induced hypercholesterolemia via a mechanism independent of the LDL receptor

The presence of the CYP7A1 transgene in LDL receptor-deficient mice did not affect plasma total cholesterol and HDL cholesterol levels when mice were fed a standard chow diet (**Fig. 1A**). However, when challenged with a cholesterol-enriched diet, LDL receptor-deficient mice expressing the CYP7A1 transgene displayed a marked resistance to diet-induced hypercholesterolemia (Fig. 1A). In contrast to the modest ~20% increase in plasma total cholesterol levels observed in LDL receptor-deficient mice expressing the CYP7A1 transgene, nontransgenic LDL receptor-deficient mice displayed an ~6-fold increase in plasma total cholesterol levels in response to the cholesterol-enriched diet (Fig. 1A). The accumulation of cholesterol in the plasma of nontransgenic LDL receptor-deficient mice was attributed to an increase in VLDL, intermediate density lipoprotein, and LDL (Fig. 1B) and was associated with increased plasma levels of both apolipoprotein B-100 and apolipoprotein B-48 (Fig. 1C).

Although the presence of the CYP7A1 transgene in LDL receptor-deficient mice did not affect hepatic cholesterol levels when mice were fed a chow diet, it completely blocked the accumulation of both free and esterified cholesterol caused by the cholesterol-enriched diet (Fig. 1D). These data indicate that the expression of the CYP7A1 transgene blocks diet-induced hypercholesterolemia via a mechanism independent of the LDL receptor.
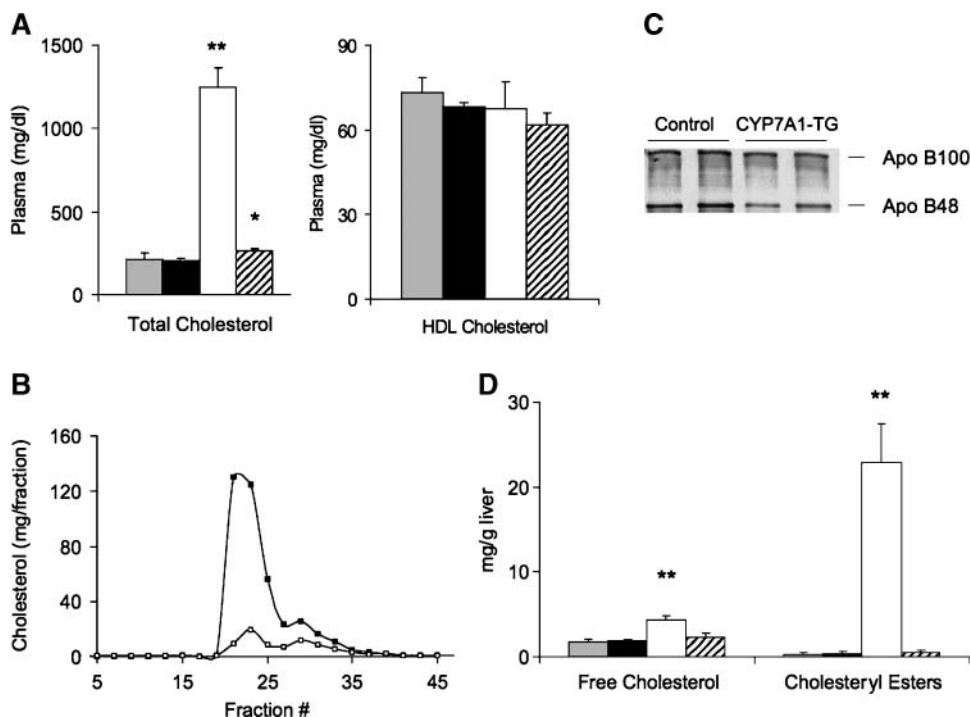

Fig. 1. Transgenic expression of cholesterol-7α-hydroxylase (CYP7A1) prevents diet-induced hypercholesterolemia in the absence of the LDL receptor. A: LDL receptor-deficient mice were fed either a chow diet (shaded bars) or a 1.25% cholesterol diet (open bars), and CYP7A1 transgenic, LDL receptor-deficient mice were fed either a chow diet (closed bars) or a 1.25% cholesterol diet (hatched bars). After an overnight fast (~18 h), mice were euthanized and livers and plasma were obtained. Plasma total cholesterol and HDL cholesterol levels were measured using commercially available enzyme assays. B: Profiles of lipoproteins fractionated by fast-protein liquid chromatography of pooled plasma samples from LDL receptor-deficient and CYP7A1 transgenic, LDL receptor-deficient mice (n = 5) fed the 1.25% cholesterol diet for 16 weeks. Closed squares denote LDLR$^{-/-}$ mice and open squares denote the CYP7A1-TG, LDLR$^{-/-}$ mice. C: Plasma apolipoprotein B protein levels were determined by Western blot. Apolipoprotein B-100 (Apo B100) and apolipoprotein B-48 (Apo B48) bands are indicated. A representative blot from multiple experiments is shown. TG, transgene. D: Hepatic free cholesterol and cholesteryl ester contents were determined by flame ionization detection after gas chromatography. Mean values ± SD (n = 3–6) are shown. * $P < 0.05$, ** $P < 0.0005$ for differences between chow and 1.25% cholesterol diets within a group.

The CYP7A1 transgene blocks diet-induced hypercholesterolemia via a mechanism not dependent upon increased mRNA expression of hepatic lipoprotein receptors and LXRα-regulated ABC transporters

The presence of the CYP7A1 transgene did not increase the hepatic mRNA expression of low-density lipoprotein receptor-related protein(LRP)α, scavenger receptor A, scavenger receptor class B type I, or the VLDL receptor (**Fig. 2A**). The presence of the CYP7A1 transgene actually reduced mRNA expression of scavenger receptor A and VLDL receptor. These data suggest that increased expression of these hepatic lipoprotein receptors is not responsible for the resistance to diet-induced hypercholesterolemia caused by the expression of the CYP7A1 transgene in LDL receptor-deficient mice.

The ABC transporter family members ABCA1 (16), ABCG5/ABCG8 heterodimers (17), and ABCG1 (18) are transcriptionally regulated by LXRα and have been shown to promote cholesterol excretion from cells. The expression of the CYP7A1 transgene in LDL receptor-deficient mice was associated with decreased hepatic mRNA expression of LXRα as well as ABCG5, ABCG8, and ABCG1 (Fig. 2B). Thus, enhanced cholesterol excretion mediated by the expression of these ABC transporters could not account for the resistance to diet-induced hypercholesterolemia afforded by expression of the CYP7A1 transgene in LDL receptor-deficient mice.
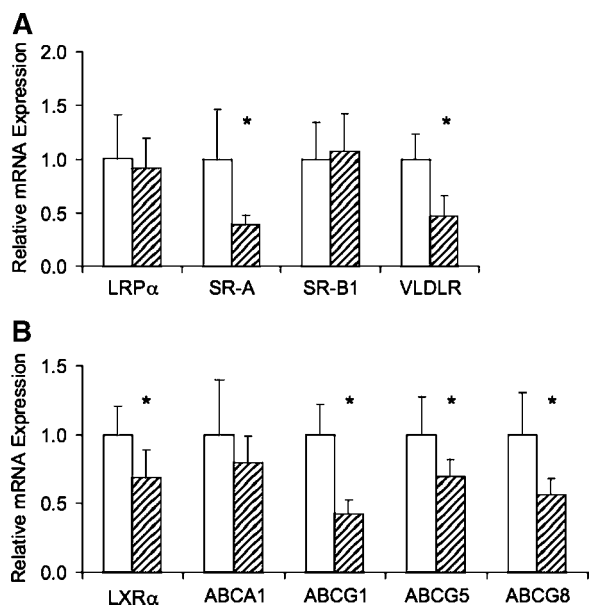
Fig. 2. The protective effect of the CYP7A1 transgene is independent of increased mRNA expression of hepatic lipoprotein receptors and liver X receptor α (LXRα)-mediated cholesterol excretion. LDL receptor-deficient mice (open bars) and CYP7A1 transgenic, LDL receptor-deficient mice (hatched bars) were fed a 1.25% cholesterol-containing diet for 2 weeks. Hepatic mRNA expression is shown for the cholesterol receptors LRPα, scavenger receptor A (SR-A), scavenger receptor class B type I (SR-B1), and VLDL receptor (VLDLR) (A) and for LXRα and the LXRα target genes ABCA1, ABCG1, ABCG5, and ABCG8 (B). mRNA expression was normalized with LDL receptor-deficient expression equal to 1. Mean values ± SD (n = 5) are shown. * $P < 0.05$.

The CYP7A1 transgene induces the expression of SREBP-2 and PCSK9

Expression of the CYP7A1 transgene in LDL receptor-deficient mice fed the cholesterol-enriched diet resulted in increased expression of SREBP-2 mRNA as well as the expression of many SREBP-mediated gene targets controlling hepatic lipogenesis (**Fig. 3A**). These findings are similar to those obtained by expressing a CYP7A1 transgene in cultured hepatoma cells (10) and in C57BL/6 mice (14, 15).

Of all of the mRNAs quantitated, the SREBP-2 target, PCSK9 (19, 20), exhibited the greatest induction (i.e., $\sim$60-fold) in response to the expression of the CYP7A1 transgene in LDL receptor-deficient mice (Fig. 3A). PCSK9 has been identified as a proteinase K-like subtilase (21). Adenoviral overexpression of PCSK9 in mice caused hypercholesterolemia as a result of reduced LDL uptake attributable to enhanced degradation of hepatic LDL receptors (22, 23). Conversely, ablation of PCSK9 gene expression in mice led to decreased plasma LDL cholesterol attributable to increased expression of hepatic LDL receptors (24). Because PCSK9 appears to be a negative regulator of hepatic LDL receptor protein expression, the $\sim$60-fold increase in PCSK9 mRNA expression in LDL receptor-deficient mice caused by the CYP7A1 transgene (Fig. 3A) could not account for their resistance to diet-induced hypercholesterolemia.

Factors in addition to PCSK9 determine its ability to degrade hepatic LDL receptors

Because transgenic expression of CYP7A1 increased the expression of PCSK9 mRNA by $\sim$16-fold in wild-type C57BL/6 mice (Fig. 3B), we examined how this affected hepatic expression of LDL receptor protein. The 2-fold increased expression of LDL receptor mRNA displayed by livers from CYP7A1 transgenic mice (Fig. 3B) corresponded to a similar $\sim$2-fold increase in LDL receptor protein (Fig. 3C). Thus, the CYP7A1 transgene did not decrease the expression of LDL receptor protein relative to expression of its mRNA. Although expression of the CYP7A1 transgene did not affect the relative level of the 76 kDa precursor form of PCSK9, it increased by $\sim$3-fold the relative amount of the mature 62 kDa form (Fig. 3D). Because autocatalytic processing of PCSK9 to its mature 62 kDa form reflects its enzyme specificity and activity (21), livers of CYP7A1 transgenic mice express greater PCSK9 enzyme activity. These data suggest that expression of the CYP7A1 transgene increased the expression of PCSK9 mRNA, PCSK9 protein, and its activity, as determined by its ability to process the 76 kDa precursor form to the 62 kDa form (Fig. 3). The lack of a decrease in LDL receptor protein in wild-type mice expressing the CYP7A1 transgene suggests that factors in addition to the presence of the mature 62 kDa PCSK9 determine its ability to degrade hepatic LDL receptors.

The CYP7A1 transgene blocks diet-induced hypercholesterolemia in part by decreasing intestinal expression of NPC1L1 and cholesterol absorption

NPC1L1 is a transmembrane protein expressed in the jejunal segment of the intestine and plays an important role in regulating cholesterol absorption (25–28). In mice
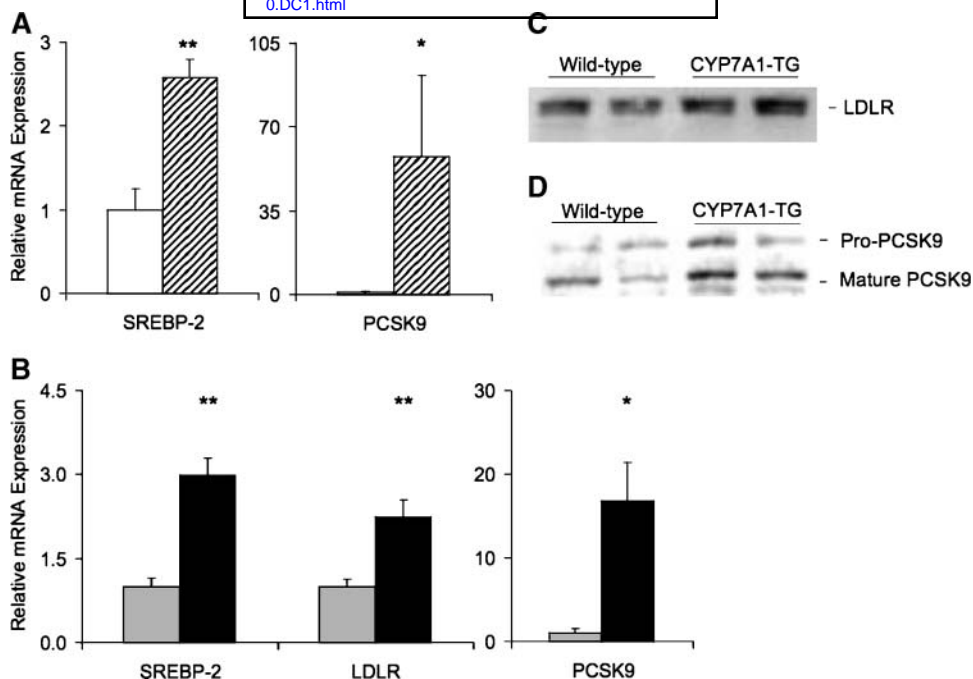
Fig. 3. Examination of proprotein convertase subtilisin/kexin type 9 (PCSK9) in CYP7A1 transgenic (TG) mice. A: LDL receptor-deficient mice (open bars) and CYP7A1 transgenic, LDL receptor-deficient mice (hatched bars) were fed a 1.25% cholesterol-containing diet for 2 weeks. B–D: Wild-type mice (shaded bars) and CYP7A1 transgenic (closed bars) mice were sustained on a chow diet. After an overnight fast (~18 h), mice were euthanized and livers and plasma were obtained. Hepatic mRNA expression of sterol-regulatory element binding protein-2 (SREBP-2), LDL receptor (LDLR), and PCSK9 was determined by quantitative real-time PCR and normalized with LDL receptor-deficient or wild-type expression equal to 1 (A, B). Hepatic PCSK9 (C) and LDL receptor (D) protein levels were determined by Western blot. Mean values ± SD (n = 4–5) are shown. * $P < 0.01$, ** $P < 0.005$.

fed the cholesterol-enriched diet, jejunal expression of NPC1L1 mRNA was reduced by >40% ($P < 0.005$) in LDL receptor-deficient mice expressing the CYP7A1 transgene (**Fig. 4A**). These data suggest that reduced cholesterol ab-
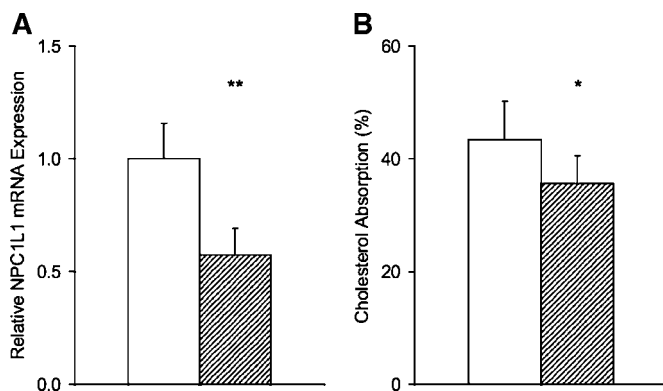

Fig. 4. Transgenic expression of CYP7A1 reduces jejunal Niemann-Pick C1-Like 1 protein (NPC1L1) mRNA expression and cholesterol absorption. LDL receptor-deficient mice (open bars) and CYP7A1 transgenic, LDL receptor-deficient mice (hatched bars) were fed a 1.25% cholesterol-containing diet for 2 weeks. A: Jejunal mRNA expression of NPC1L1. B: Percentage cholesterol absorption as determined by the fecal dual isotope method described in Materials and Methods. mRNA expression was normalized with LDL receptor-deficient expression equal to 1. Mean values ± SD (n = 5–6) are shown. * $P < 0.05$, ** $P < 0.005$.

sorption may contribute to the resistance of LDL receptor-deficient mice expressing the CYP7A1 transgene to diet–induced hypercholesterolemia. Indeed, expression of the CYP7A1 transgene in LDL receptor-deficient mice fed the cholesterol-enriched diet resulted in an ~20% reduction in cholesterol absorption ($P < 0.05$) (Fig. 4B).

The CYP7A1 transgene reduces intestinal expression of NPC1L1 independent of its activation of FXR

The bile acid pool of mice expressing the CYP7A1 transgene is increased by 2-fold (15). Bile acids bind to and activate the nuclear receptor FXR (29). Increased bile acid pool size in mice expressing the CYP7A1 transgene may explain why mRNA levels of the hepatic FXR-inducible gene bile salt export pump are increased ~2-fold in the livers of CYP7A1 transgenic LDL receptor-deficient mice when fed the cholesterol-enriched diet (**Fig. 5A**). Similarly, intestinal mRNA expression of the FXR-inducible gene ileal bile acid binding protein was also increased by ~2-fold in LDL receptor-deficient mice that expressed the CYP7A1 transgene (Fig. 5B).

To examine whether bile acid induction of FXR was responsible for the decreased expression of NPC1L1 mRNA in LDL receptor-deficient mice expressing the CYP7A1 transgene (Fig. 4A), C57BL/6 mice (expressing the LDL receptor) were fed a diet containing 0.5% taurocholate. The taurocholate-containing diet decreased the expres-
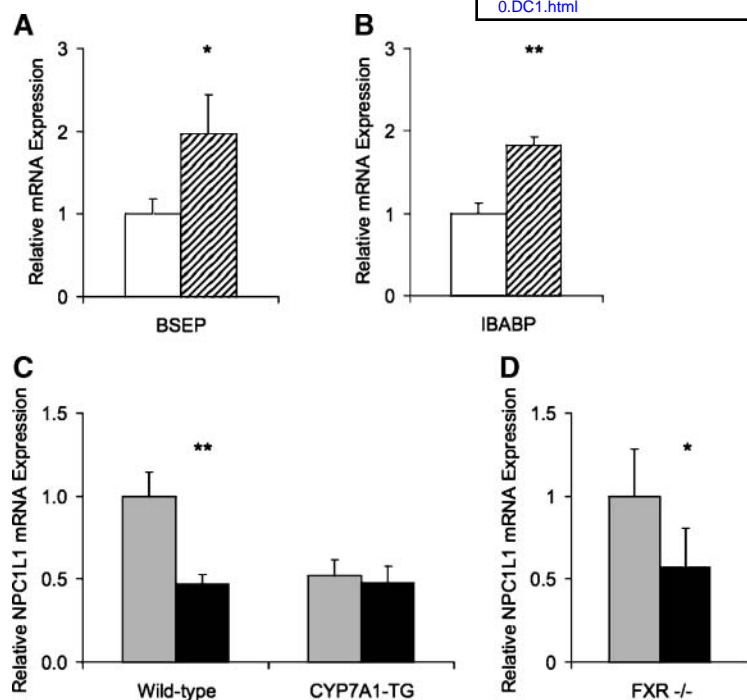
Fig. 5. Bile acid-mediated reduction of NPC1L1 is independent of farnesoid X receptor (FXR). A, B: LDL receptor-deficient mice (open bars) and CYP7A1 transgenic, LDL receptor-deficient mice (hatched bars) were fed a 1.25% cholesterol-containing diet for 2 weeks. Hepatic mRNA expression of bile salt export pump (A) and ileal mRNA expression of ileal bile acid binding protein (B) are shown. C, D: Wild-type, CYP7A1 transgenic (TG), and FXR-deficient (FXR$^{-/-}$) mice were sustained on a chow diet (shaded bars) or fed a taurocholate-containing diet (closed bars). Jejunal mRNA expression of NPC1L1 (C) and whole intestine mRNA expression of NPC1L1 (D) are shown. mRNA expression was normalized with LDL receptor-deficient or wild-type and FXR-deficient expression on chow equal to 1. Mean values ± SD (n = 5) are shown. * $P < 0.05$, ** $P < 0.005$.

sion of NPC1L1 mRNA to a similar extent as that observed in mice expressing the CYP7A1 transgene (Fig. 5C). Feeding the taurocholate-containing diet to mice expressing the CYP7A1 transgene did not further reduce NPC1L1 mRNA expression (Fig. 5C). We have shown that feeding the taurocholate-containing diet to wild-type C57BL/6 mice expressing the CYP7A1 transgene does not increase the bile acid pool size beyond the already 2-fold increase associated with expression of the CYP7A1 transgene (15). These data suggest that increased bile acid pool size is responsible for the decreased expression of NPC1L1 mRNA. Because feeding the taurocholate-containing diet to FXR-deficient mice caused an ~50% reduction in NPC1L1 mRNA (Fig. 5D), which is similar to the reduction observed in the wild type (Fig. 5C), bile acids reduce NPC1L1 mRNA expression via a mechanism independent of FXR.

DISCUSSION

The results of these studies demonstrate that expression of a CYP7A1 transgene in LDL receptor-deficient mice completely prevents diet-induced hypercholesterolemia. Further analysis indicates that CYP7A1-mediated conversion of cholesterol to bile acids evokes several complementary mechanisms that facilitate the maintenance of cholesterol homeostasis even in the absence of functional LDL receptors. Studies in CYP7A1 knockout mice demonstrated that a minimal amount of bile acids is necessary for the intestinal absorption of cholesterol and other essential fat-soluble nutrients (30). Our findings indicate that expansion of the endogenous bile acid pool via expression of the CYP7A1 transgene actually reduced cholesterol absorption by decreasing jejunal expression of NPC1L1 mRNA. Enhanced conversion of cholesterol to

bile acids in combination with reduced cholesterol absorption acts to eliminate cholesterol from the body and maintain cholesterol homeostasis.

Analysis of hepatic mRNA expression indicated that the CYP7A1 transgene did not enhance the expression of genes responsible for receptor-mediated lipoprotein removal from plasma (LRP1, SR-A, SR-B1, VLDL receptor) (Fig. 2A) or biliary cholesterol excretion (ABCG5, ABCG8) (Fig. 2B). The finding that expression of the CYP7A1 transgene in C57BL/6 mice (expressing LDL receptors) reduced biliary cholesterol excretion (15) further supports this conclusion. Because ABCG5 and ABCG8 play important roles in mediating biliary cholesterol excretion, the lack of an induction in their mRNA expression suggests that the CYP7A1 transgene prevented hepatic cholesterol accumulation by converting it to bile acids, not by enhancing biliary cholesterol excretion.

The CYP7A1 transgene is expressed exclusively in the liver; hepatic microsomes obtained from mice expressing this CYP7A1 transgene exhibit an ~6-fold increase in CYP7A1 enzyme activity and a 2-fold increase in bile acid pool size (14). Normally, CYP7A1 is expressed in only a "few" parenchymal cells located in the pericentral zone of the liver acinus (31). Expression of CYP7A1 mRNA changes rapidly (half-life of mRNA < 0.5 min (32)] and varies widely in response to diet, diurnal cycle, and changes in the size of the bile acid pool (reviewed in 1). Because the CYP7A1 transgene is driven by the constitutive and highly active liver-specific enhancer element from the human apolipoprotein E gene (14, 33), its functional importance is magnified with regard to the mass conversion of hepatic cholesterol into bile acids. Thus, the most parsimonious interpretation of the combined data is that increased conversion of hepatic cholesterol to bile acids is the primary mechanism responsible for blocking diet-induced hyper-

cholesterolemia in LDL receptor-deficient mice expressing the CYP7A1 transgene. Our studies also revealed secondary intestinal responses to the doubling of the endogenous bile acid pool size in CYP7A1 transgenic mice (15). These include decreased jejunal mRNA expression of NPC1L1 mRNA (Fig. 4A), which can account for the ~20% decrease in cholesterol absorption exhibited by LDL receptor-deficient mice expressing the CYP7A1 transgene (Fig. 4B) (27). Our findings showing that a taurocholate-containing diet reduced the expression of NPC1L1 mRNA in wild-type mice, but not in mice expressing the CYP7A1 transgene, indicate that bile acids negatively affect the expression of NPC1L1 mRNA. Additional studies showing that FXR-deficient mice also decreased the expression of NPC1L1 mRNA in response to the taurocholate-containing diet (Fig. 5D) indicate that bile acids repress NPC1L1 expression via a mechanism independent of FXR activation.

Our findings demonstrate the important influence that biliary bile acids have on intestinal function and gene expression. The findings showing that bile acids reduce the mRNA expression of NPC1L1, whose gene product regulates cholesterol absorption, are unexpected because is has been generally assumed that bile acids promote cholesterol absorption (30). Bile acids are essential to emulsify cholesterol and allow it to be absorbed in the proximal intestine (34). A plethora of FXR-dependent (29) and FXR-independent mechanisms (35–38) are evoked to prevent the accumulation of potentially cytotoxic bile acids. These mechanisms limiting the expansion of the circulating bile acid pool are graphically illustrated in CYP7A1 transgenic mice, whose bile acid pool is doubled (14) but cannot be expanded further by feeding a taurocholate-containing diet (15). Because of saturation of the processes responsible for returning intestinal bile acids to the enterohepatic circulation, bile acids are lost via fecal excretion. Decreased expression of NPC1L1 mRNA (Fig. 4A) and the corresponding ~20% reduction in cholesterol absorption (Fig. 4B) also contribute to the elimination of cholesterol via fecal excretion.

Enhanced conversion of cholesterol to bile acids, biliary bile acid excretion, and fecal bile acid loss provide the most direct mechanism to prevent the accumulation of excess cholesterol in liver and plasma. Clearly, CYP7A1 is an effective therapeutic target in preventing diet-induced hypercholesterolemia.∎

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