Studies on LXR- and FXR-mediated effects on cholesterol homeostasis in normal and cholic acid-depleted mice

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Abstract **As previously reported by us, mice with targeted disruption of the CYP8B1 gene (CYP8B1$^{-/-}$) fail to produce cholic acid (CA), upregulate their bile acid synthesis, reduce the absorption of dietary cholesterol and, after cholesterol feeding, accumulate less liver cholesterol than wild-type (CYP8B1$^{+/+}$) mice. In the present study, cholesterol-enriched diet (0.5%) or administration of a synthetic liver X receptor (LXR) agonist strongly upregulated CYP7A1 expression in CYP8B1$^{-/-}$ mice, compared to CYP8B1$^{+/+}$ mice. Cholesterol-fed CYP8B1$^{-/-}$ mice also showed a significant rise in HDL cholesterol and increased levels of liver ABCA1 mRNA. A combined CA (0.25%)/cholesterol (0.5%) diet enhanced absorption of intestinal cholesterol in both groups of mice, increased their liver cholesterol content, and reduced their expression of CYP7A1 mRNA. The ABCG5/G8 liver mRNA was increased in both groups of mice, but cholesterol crystals were only observed in bile from the CYP8B1$^{+/+}$ mice. The results demonstrate the cholesterol-sparing effects of CA: enhanced absorption and reduced conversion into bile acids. Farnesoid X receptor (FXR)-mediated suppression of CYP7A1 in mice seems to be a predominant mechanism for regulation of bile acid synthesis under normal conditions and, as confirmed, able to override LXR-mediated mechanisms. Interaction between FXR- and LXR-mediated stimuli might also regulate expression of liver ABCG5/G8.**—Wang, J., C. Einarsson, C. Murphy, P. Parini, I. Björkhem, M. Gåfvels, and G. Eggertsen. **Studies on LXR- and FXR-mediated effects on cholesterol homeostasis in normal and cholic acid-depleted mice.** ***J. Lipid Res.*** **2006.** 47: **421–430.**

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The relationship between cholesterol and atherosclerosis is now well established, but complete understanding of the pathological processes will demand increased knowledge of the overall cholesterol homeostasis as a whole. In humans, a delicate metabolic balance exists between dietary uptake, endogenous synthesis, and biliary excretion. In fact, excess cholesterol is removed via the bile, either after conversion into bile acids or as intact molecules kept in solution by bile acids and phospholipids. Recent progress demonstrates the complexity of this process, which involves multiple transporters, enzyme systems, and nuclear receptors (1).

The major bile acids in mammals are cholic acid (CA), chenodeoxycholic acid (CDCA), and muricholic acids. The latter are more hydrophilic, and are most abundant in rodents. In humans, CA and CDCA are the dominating bile acids, together with the secondary bile acids deoxycholic acid (DCA) and lithocholic acid, which originate in microbiological actions in the large intestine (2). CA is a tricarboxylic acid, mainly produced via the neutral pathway. The enzyme sterol 12α-hydroxylase (CYP8B1) is necessary for its synthesis; otherwise CDCA is produced. In the intestine, CA is a key factor, together with phospholipids, in the formation of micelles to achieve an efficient absorption of cholesterol. In the mouse, it functions as a specific ligand for the nuclear receptor farnesoid X receptor (FXR), which mediates the downregulation of CYP7A1 and CYP8B1 (2). Activated FXR is also reported to modify the expression of bile acid transporters in the liver, which are sodium-taurocholate cotransport polypeptide for bile acid uptake, bile salts export pump (BSEP) for bile acid secretion, and intestine ileal bile acid binding protein (3).

Over the last few years, research efforts in the field of cholesterol and bile acid metabolism have utilized an increasing number of genetically modified mice. A general approach in many studies is to feed high-fat, high-cholesterol diets to such experimental mouse models. Generally, normal mice do not develop atherosclerosis when put on such atherogenic diets, even if their plasma cholesterol levels could vary depending on the strain (4). However, genetically modified strains, for example, LDL receptor-deficient mice, develop advanced arteriosclerotic lesions when fed diets containing cholesterol and CA (5).

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Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; CSI, cholesterol saturation index; DCA, deoxycholic acid; FXR, farnesoid X receptor; LXR, liver X receptor; UDCA, ursodeoxycholic acid.

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Such a combined diet has also been utilized to induce formation of gallstones in susceptible mouse strains. Even if CA is a key element in these pathologic processes, its exact mode of action is not well defined.

To better understand the biological roles for sterol 12α-hydroxylase/CA, we created an experimental mouse model genetically deficient for CYP8B1; these mice do not synthesize CA (2). They have an increased expression of CYP7A1, which causes elevated bile acid synthesis and also biochemical changes, suggesting that the FXR effector system is downregulated. In addition, the animals show a reduced absorption of dietary cholesterol and do not increase their storage of liver cholesterol when fed cholesterol, compared with normal mice (6). The present study characterizes the effects of the administration of cholesterol-enriched and cholesterol-plus-CA diets to CYP8B1$^{-/-}$ mice, with a focus on the cholesterol and bile acid metabolism. Our results indicate that CA per se is an important factor in the regulation of the endogenous cholesterol balance, affecting both degradation and synthesis of cholesterol, as well as its excretion and absorption.

MATERIALS AND METHODS

Chemicals

Sodium cholate (≥99%), cholesterol (>99%), and the liver X receptor (LXR) agonist TO-901317 were purchased from Sigma-Aldrich Corp., St. Louis, MO. The FXR agonist GW4064 was kindly supplied by F. Hoffman-La Roche Ltd, Basel, Switzerland.

Animals and procedures

Mice deficient for the sterol 12α-hydroxylase by targeted disruption of the gene have previously been characterized (2). The corresponding wild-type animals were obtained by cross-breeding of heterozygous individuals. Generally, males aged 2–6 months were used. Mice of both genotypes were housed at 22–24°C at a light cycle of 8 AM to 8 PM in groups of four to six animals and kept on the following diets for 7 days: *i*) standard chow (0.025% cholesterol, w/w); *ii*) 0.5% cholesterol (w/w); *iii*) 0.5% cholesterol plus 0.25% sodium cholate. Controls were kept on standard chow. All diets contained 10% peanut oil (w/w). Both the LXR agonist TO-901317 and the FXR agonist GW4064 were given in gavage for 5 days in doses of 10 mg/kg/day and 50 mg/kg/day, respectively, to groups of CYP8B1$^{+/+}$ and $^{-/-}$ mice. To control animals, 1% methylcellulose was given in the same way. The last dose was delivered 2 h before the animals were euthanized. Water was available ad libitum.

Animals were euthanized by cervical dislocation following CO_2 anesthesia, and the liver and gallbladder were quickly washed in physiological saline and immediately frozen in liquid nitrogen. Blood was collected by heart puncture. Animals used for sampling gallbladder bile were fasted for 12 h, and light microscopy was performed on 5 μl aliquots of bile. Serum total cholesterol was determined by the standard enzymatic procedure. Lipoprotein cholesterol profiles were obtained by fast-protein liquid chromatography. Liver total and free cholesterol were determined by the method described by Bjorkhem (7). To obtain a relative index of hepatic de novo cholesterol synthesis, the concentration of lathosterol was assayed by isotope dilution-mass spectrometry as reported (8), and the ratio of lathosterol to total cholesterol was calculated.

Determination of intestinal cholesterol absorption

Cholesterol absorption was assayed, after feeding the specified diets for 6 days, by the fecal dual-isotope method, essentially as reported by Schwarz et al. (9), except that feces were collected for 24 h after administering the radioactive gavage. Percent cholesterol reabsorbed was estimated by the standard equation. [5,6-^{3}H]β-sitostanol was obtained from American Radiolabel Chemicals, Inc., St. Louis, MO and [$^{4\text{-}12}$C]cholesterol from Amersham Biosciences, Uppsala, Sweden.

Quantitative real-time PCR

Total RNA was isolated from 75–100 mg of liver tissue using the Quick Prep Total RNA Extraction Kit (Amersham Biosciences). Oligo-dT-primed cDNA synthesis was carried out on 3 μg of liver total RNA using Superscript III reverse transcriptase (Invitrogen Life Technologies; Carlsbad, CA). Real-time PCR was carried out on an ABI Prism 7000 Sequence Detection System (Applied Biosystems; Foster City, CA) using either TaqMan probes (labeled with the fluorochromes FAM or VIC and dark quencher or TAMRA) or SYBR Green. The following genes were analyzed: mouse HMG-CoA reductase, CYP7A1, CYP8B1, CYP27, SHP, ABCA1, ABCG5, ABCG8, BSEP, MDR2, apoA-1, LDLR, SREBP1C, and SREBP2. As an internal standard, mouse hypoxanthine phosphoribosyl transferase was utilized. Control of the PCR efficiency was done by plotting standard curves using different dilutions of cDNA. The PCR products were sequenced to confirm that they represented the correct fragments.

Determination of the output of bile acids and neutral sterols in feces

Fecal samples were collected from mice kept in individual cages for 24 h. Analysis of neutral sterols and bile acids by gas-liquid chromatography was performed essentially as reported by Miettinen, Ahrens, and Grundy (10), Miettinen (11), and Grundy, Ahrens, and Miettinen (12).

Analysis of biliary lipids in gallbladder bile

Gallbladder bile samples were examined on slides for cholesterol crystals by polarizing light microscopy. The composition of bile acids in gallbladder bile was analyzed by gas-liquid chromatography, as reported (13). Total bile acid concentration was determined by an enzymatic method (14), as was cholesterol (15). Phospholipids were assayed by the method described by Rouser, Fleischer, and Yamamoto (16). The relative concentrations of biliary lipids were expressed as molar percentages of the total biliary lipids. The cholesterol saturation was calculated according to Carey (17).

Statistical analysis

Data are presented as means ± SEM. Statistical analysis was performed with *STATISTICA* software. The significance of differences was tested by two-way ANOVA, followed by planned comparisons. To stabilize the variances, data were logarithmically transformed when a correlation was found (18).

RESULTS

Effects of cholesterol feeding

Alterations in the metabolism of hepatic cholesterol. Feeding CYP8B1$^{+/+}$ mice a cholesterol diet resulted in a distinct increase in total liver cholesterol content, from a mean

level of 2.3 μg/mg liver tissue to 7.4 μg/mg (**Fig. 1A**). The fraction of cholesteryl esters (CEs) rose from approximately 34% to 74%. CYP8B1$^{-/-}$ animals on chow had lower cholesterol values (1.7 μg/mg) than did the CYP8B1$^{+/+}$ mice, demonstrating only a weak rise to 2.3 μg/mg on the cholesterol diet and no increase in CEs. Hepatic cholesterol synthesis, as evaluated by the ratio of lathosterol to cholesterol, was higher in the CYP8B1$^{-/-}$ mice than in the CYP8B1$^{+/+}$ animals (Fig. 1B). Cholesterol diet induced a reduction in both groups, but the rate of cholesterol synthesis was still more pronounced in the CYP8B1-null mice. The mRNA levels for HMG-CoA reductase and SREBP2 showed a similar pattern, although the CYP8B1-null mice had much higher levels of HMG-CoA reductase mRNA than the CYP8B1$^{+/+}$ animals (Fig. 1C, D).

Alterations in bile acid synthesis and composition of bile. In both CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice, the cholesterol diet induced a rise in bile acid production, as evaluated by the fecal excretion of bile acids (**Fig. 2A**). In the latter group, it was nearly twice as high as in the CYP8B1$^{+/+}$ animals, both when fed normal chow and cholesterol-enriched diet. This is well in agreement with the much higher levels of CYP7A1 mRNA found in the CYP8B1$^{-/-}$ mice, especially after the cholesterol feeding, when the levels were more than 4-fold higher, compared with the CYP8B1$^{+/+}$ animals (Fig. 2B). The levels of CYP27 mRNA did not differ significantly between the different groups of mice. The expression of SHP mRNA, a ubiquitous effector molecule for FXR, was, as expected, approximately 50% lower in the chow-fed CYP8B1$^{-/-}$ mice, compared with the CYP8B1$^{+/+}$ mice. No significant changes were precipitated by the cholesterol diet in the former group, whereas the SHP levels in the CYP8B1$^{+/+}$ mice tended to decrease (data not shown). We also determined the mRNA levels for the bile salt export pump (BSEP, ABCB11) and the multidrug resistance gene 2 (MDR2, ABCB4), the proteins thought to transport bile acids and phospholipids, respectively, from the hepatocytes into the bile canaliculi. No significant differences were observed between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ animals, either on normal or on cholesterol-enriched diet (data not shown).

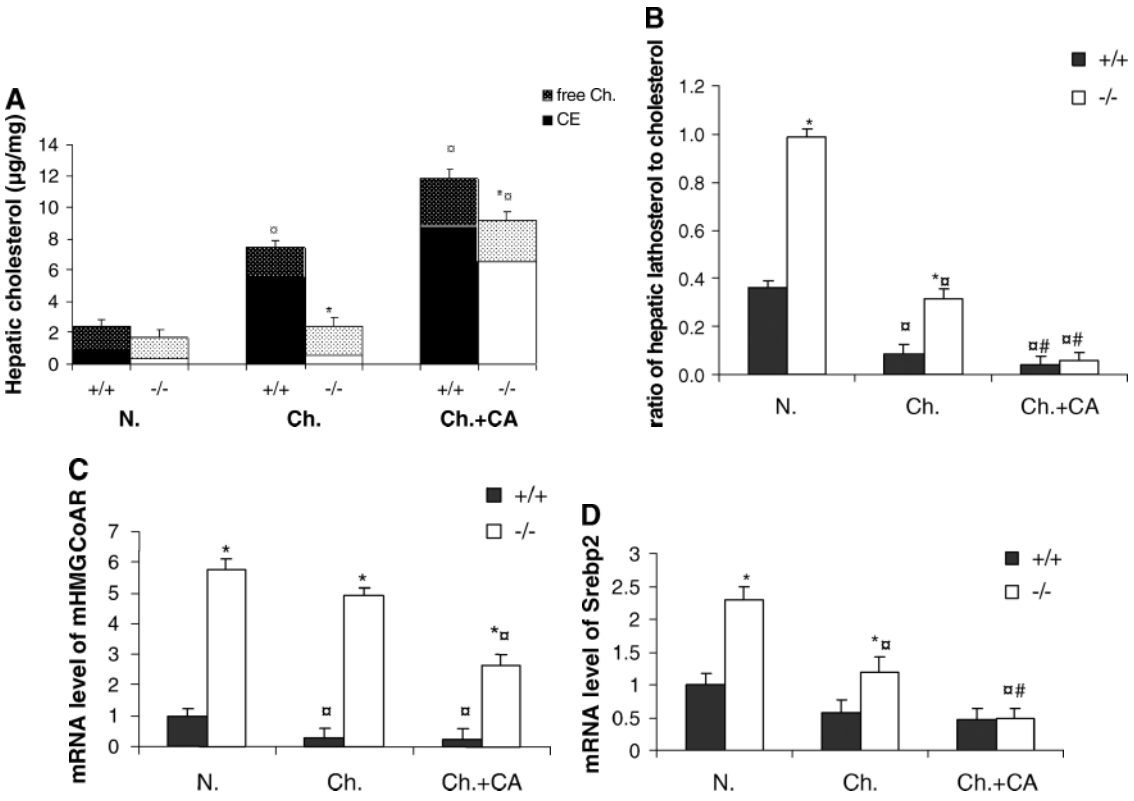

Fig. 1. Alterations of the metabolism of cholesterol in the livers of the CYP8B1$^{+/+}$ (+/+) and CYP8B1$^{-/-}$ (−/−) mice fed normal chow (N.), chow with 0.5% cholesterol (Ch.) or chow with 0.5% cholesterol/0.25% cholic acid (Ch.+CA). A: Hepatic total cholesterol concentration. Upper dotted areas represent free cholesterol and lower esterified cholesterol concentrations. B: Lathosterol-to-cholesterol ratio was used as an indication of the relative level of de novo hepatic cholesterol synthesis. C: Quantitation of the mRNA levels for HMG-CoA reductase, using mouse hypoxanthine phosphoribosyl transferase (HPRT) as an internal control. The value found for the CYP8B1$^{+/+}$ animals on normal chow was set to 1.0. D: Quantitation of the mRNA levels for SREBP2, using HPRT as an internal standard. For both C and D, data are given as fold differences of the value for the CYP8B1$^{+/+}$ mice on normal chow, set to 1.0. *, Significant differences between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice with the same diet or the same treatment; ¤, significant differences between the diet-treated groups and the chow groups, or two agonist groups, compared with vehicle group having the same genotype; #, significant differences between the cholesterol-enriched diet groups and the cholesterol-plus-CA diet groups with the same genotype. Values are expressed as means ± SEM of four to six observations. $P < 0.05$ was considered to be significant.

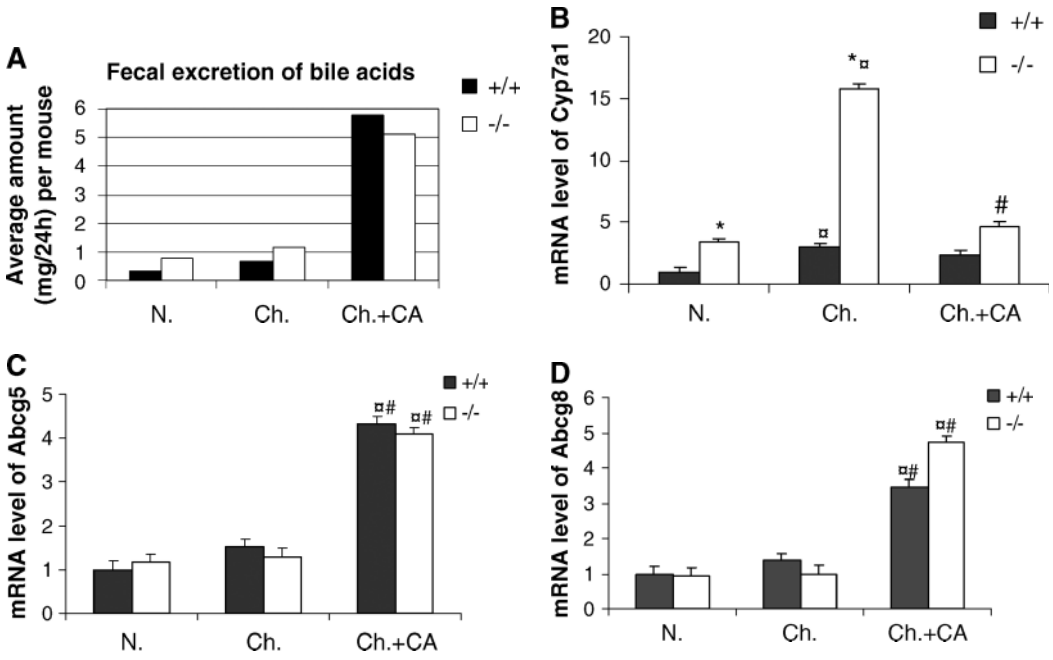

Fig. 2. A: Excretion of fecal bile acids. Feces were collected from individual CYP8B1$^{+/+}$ or CYP8B1$^{-/-}$ animals for 24 h after 6 days of feeding normal chow, chow with 0.5% cholesterol, or chow with 0.5% cholesterol/0.25% CA. B: Quantitation of the hepatic mRNA levels for CYP7A1. C: Quantitation of the hepatic mRNA levels for ABCG5. D: Quantitation of the hepatic mRNA levels for ABCG8. The mRNA quantitations were performed with HPRT as an internal standard. *, Significant differences between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice with the same diet or the same treatment; ¤, significant differences between the diet-treated groups and the chow groups, or two agonist groups, compared with vehicle group having the same genotype. #, significant differences between the cholesterol-enriched diet groups and the cholesterol-plus-CA diet groups with the same genotype. Values are expressed as average amount of bile acids excreted per mouse for 24 h. Levels are expressed as means ± SEM of four to six observations. $P < 0.05$ was considered to be significant.

The gallbladder bile in the CYP8B1$^{-/-}$ mice consisted mainly of muricholic acids with minor quantities of ursodeoxycholic acid (UDCA) and DCA (**Table 1**.). After cholesterol feeding, no major changes occurred in their bile acid proportions, except that in the CYP8B1$^{+/+}$ animals, CA decreased slightly and β-muricholic acid increased. Biliary cholesterol and cholesterol saturation index (CSI %) were higher in the control CYP8B1$^{+/+}$ animals than in the CYP8B1$^{-/-}$ animals, whereas no differences were observed in the phospholipid concentration (**Table 2**). Feeding cholesterol to the CYP8B1$^{+/+}$ mice resulted in an increase of biliary cholesterol and of the CSI %, whereas only modest effects were observed in the CYP8B1$^{-/-}$ mice. Only minor changes were observed in the phospholipid content.

Because ABCG5 and ABCG8 have been reported to enhance the transfer of intact cholesterol into the bile, we decided to measure their expression levels. The mRNA

TABLE 1. The bile acid composition in mouse gallbladder bile

CYP8B1 Genotype	Dietary Treatment	Cholic Acid	β-Muricholic Acid	α-Muricholic Acid + CDCA	DCA	UDCA
+/+	N.	59.0 ± 2.9	30.9 ± 3.8	6.2 ± 2.0	2.2 ± 1.0	1.7 ± 1.1
−/−	N.	2.2 ± 2.2[a]	50.7 ± 2.9[a]	36.8 ± 1.5[a]	Trace	8.6 ± 0.8[a]
+/+	Ch.	45.0 ± 2.2[b]	38.5 ± 3.5	11.1 ± 1.5	2.8 ± 0.9	2.7 ± 0.8
−/−	Ch.	2.1 ± 2.6[a]	52.0 ± 3.4	35.8 ± 1.8[a]	Trace	10.8 ± 0.9[a]
+/+	Ch.+CA	84.6 ± 2.9[b,c]	5.2 ± 3.8[b,c]	1.6 ± 2.0[c]	8.4 ± 1.0[b,c]	0.3 ± 1.1
−/−	Ch.+CA	75.7 ± 2.6[b,c]	13.1 ± 3.4[a,b,c]	3.6 ± 1.8[a,b,c]	5.9 ± 0.9[a,b,c]	1.7 ± 0.9[b,c]

The results are expressed as mean ± SEM. The value represents mol% of total bile acid composition. Before euthanization, the mice were fasted for 10–12 h, and four to six animals were included n each group. CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; N., normal chow; Ch., chow with 0.5% cholesterol; Ch.+CA, chow with 0.5% cholesterol/0.25% CA.

[a] $P < 0.05$. Significant difference between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice fed normal chow, chow with 0.5% cholesterol, and chow with 0.5% cholesterol/0.25% CA, respectively.

[b] $P < 0.05$. Significant difference between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$mice fed chow with 0.5% cholesterol and chow with 0.5% cholesterol/0.25% CA and those who were fed normal chow.

[c] $P < 0.05$. Significant difference between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice fed chow with 0.5% cholesterol/0.25% CA and those fed chow with 0.5% cholesterol.

TABLE 2. The composition of lipids in bile from mouse gallbladder

CYP8B1 Genotype	Dietary Treatment	Cholesterol		Bile Acids		Phospholipids		Total Lipids		Cholesterol Saturation
		μmol/ml	*μmol%*	*μmol/ml*	*μmol%*	*μmol/ml*	*μmol%*	*μmol/ml*	*g/dl*	*CSI*
$^{+/+}$	N.	4.9	4.1	86.3	72.4	28.1	23.6	119.3	6.6	58.7
$^{-/-}$	N.	2.2	2.2	70.1	70.5	28.9	27.4	101.2	5.8	32.2
$^{+/+}$	Ch.	9.8	9.2	70.6	67.7	25.2	23.2	105.6	5.8	140.1
$^{-/-}$	Ch.	3.3	3.8	62.0	71.1	21.4	25.1	86.6	4.8	54.6
$^{+/+}$	Ch.+CA	25.9	13.6	102.2	58.4	52.6	28.0	180.7	10.1	169.4
$^{-/-}$	Ch.+CA	14.7	12.8	68.1	61.9	29.1	25.3	111.8	6.2	175.2

The results were obtained from pooled gallbladder bile from each group containing four to six animals. Before euthanization, mice were fasted for 10–12 h. CSI, cholesterol saturation index.

levels were, however, similar in both groups of animals fed the control diet, and no significant differences were seen after cholesterol feeding (Fig. 2C, D).

Alterations in intestinal cholesterol absorption and excretion. To compensate for possible variations in food intake, fecal cholesterol was expressed as the ratio of neutral sterol levels to plant sterol levels in feces (**Fig. 3A**). Fecal cholesterol excretion increased strongly in animals fed the cholesterol diet, with higher levels in the CYP8B1$^{-/-}$ animals. As expected, the percentage of absorption of cholesterol was significantly lower in the CYP8B1$^{-/-}$ mice than in the CYP8B1$^{+/+}$ mice (42.7% and 68.7%, respectively) and dropped in both groups after cholesterol feeding (14.8% and 31.4%, respectively) (Fig. 3B).

Alterations in serum lipids and lipoproteins. Total cholesterol levels in serum tended to increase slightly in both groups of mice upon cholesterol feeding, although the difference was not significant (**Fig. 4A**). In the CYP8B1$^{+/+}$ mice, no changes in the lipoprotein profile for cholesterol were observed, whereas CYP8B1$^{-/-}$ animals displayed elevated levels of HDL-cholesterol. However, no changes were found in the LDL- and VLDL- fractions (Fig. 4B). Interestingly, cholesterol feeding significantly increased the ABCA1 mRNA levels in CYP8B1$^{-/-}$ mice, compared with the CYP8B1$^{+/+}$ mice (**Fig. 5A**). No differences induced by cholesterol feeding were seen between the control groups. The mRNA levels for apoA-1 did not differ significantly between the CYP8B1$^{-/-}$ and $^{+/+}$ mice, although the values tended to be lower for the cholesterol-fed groups (Fig. 5B). The levels of hepatic LDLR mRNA were similar, both in the two control groups and after cholesterol feeding (data not shown).

Effects of feeding a combined cholesterol/CA diet

Supplementing the cholesterol diet with CA would be expected to give similar effects in both the CYP8B1$^{+/+}$ and $^{-/-}$ animals. Interestingly, however, this was not a general finding.

Alterations in the metabolism of hepatic cholesterol. Hepatic cholesterol levels were strongly increased in both groups, with the highest mean level observed in the CYP8B1$^{+/+}$ animals (11.8 μg/mg, compared with 9.2 μg/mg in the CYP8B1$^{-/-}$ mice), which exceeded the value found in CYP8B1$^{+/+}$ animals receiving cholesterol only (7.4 μg/mg) (Fig. 1A). The fraction of esterified cholesterol was similar in CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice (74.9% and 70.8%, respectively) and close to the value found for the cholesterol-fed CYP8B1$^{+/+}$ animals (74.6%). The liver cholesterol synthesis was further depressed in both groups, as estimated by the lathosterol assay (Fig. 1B). Although decreased, the mRNA quantities for HMG-CoA reductase were markedly

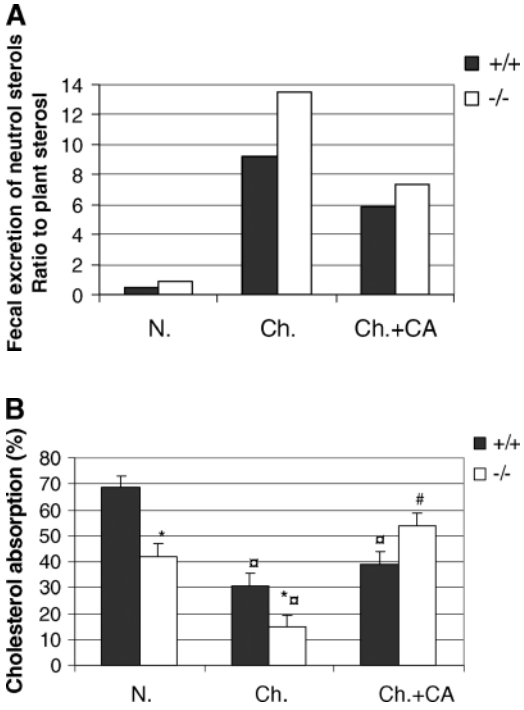

Fig. 3. Alterations in intestinal cholesterol absorption and excretion in CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice fed normal chow, chow with 0.5% cholesterol, or chow with 0.5% cholesterol/0.25% CA. A: Fecal excretion of neutral sterols was calculated as the ratio of neutral sterols to plant sterols to compensate for any variations in food intake. Feces were collected for 24 h after 6 days of feeding the specified food regimes. B: Intestinal cholesterol absorption determined by the dual fecal isotope method. *, Significant differences between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice with the same diet or the same treatment; ¤, significant differences between the diet-treated groups and the chow groups, or two agonist groups, compared with vehicle group having the same genotype; #, significant differences between the cholesterol-enriched diet groups and the cholesterol-plus-CA diet groups with the same genotype. Values are expressed as means ± SEM of four to six observations. $P < 0.05$ was considered to be significant.

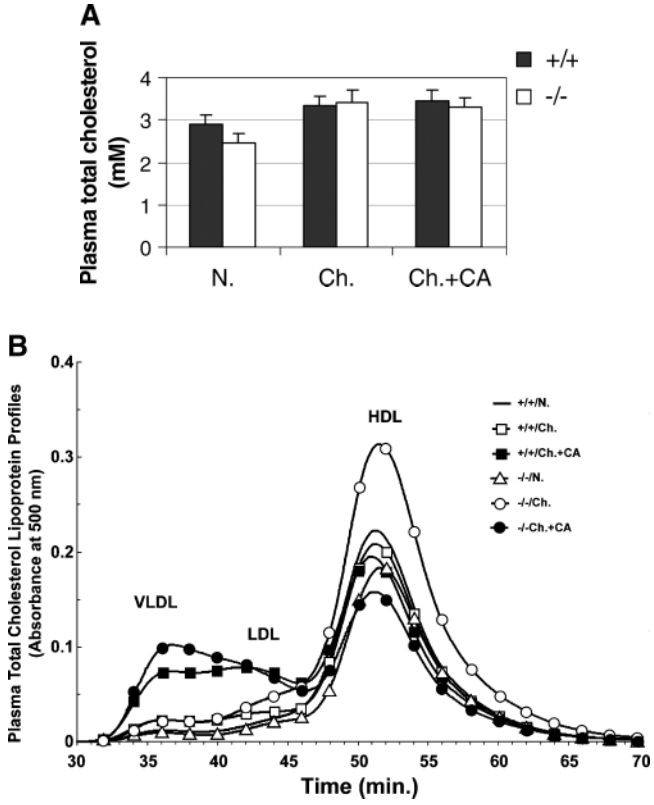

Fig. 4. Alterations in serum cholesterol and lipoproteins in CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice fed normal chow, chow with 0.5% cholesterol, or chow with 0.5% cholesterol/0.25% CA. A: Plasma total cholesterol levels expressed as means ± SEM. There are no significant differences between all the groups. B: Plasma lipoprotein profiles after separation by fast-protein liquid chromatography and determination of the cholesterol concentration. Each mouse group is indicated by an individual symbol. Values on the x-axis denote the elution time of the individual samples.

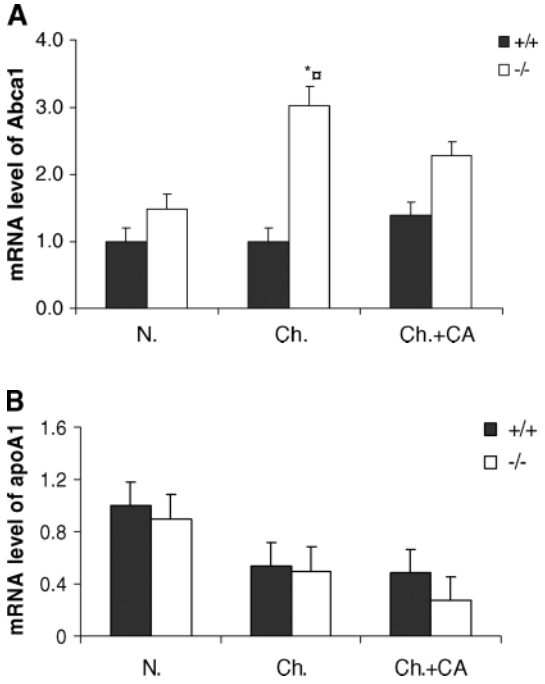

Fig. 5. Quantitation of the hepatic mRNA levels for ABCA1 (A) and apoA-1 (B) in CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice fed normal chow, chow with 0.5% cholesterol, or chow with 0.5% cholesterol/0.25% CA. *, Significant differences between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice with the same diet or the same treatment; ¤, significant differences between the diet-treated groups and the chow groups, or two agonist groups, compared with vehicle group having the same genotype. Values are expressed as means ± SEM of four to six observations. $P < 0.05$ was considered to be significant.

higher in the CYP8B1$^{-/-}$ animals than in the CYP8B1$^{+/+}$ mice, in which no changes were recorded, compared with CYP8B1$^{+/+}$ mice fed cholesterol (Fig. 1C). Although the cholesterol synthesis was depressed to the same level in both CYP8B1$^{+/+}$ and $^{-/-}$ mice, the HMG-CoA reductase mRNA levels were markedly higher in the latter, possibly related to fact that the CYP8B1$^{+/+}$ mice had higher hepatic cholesterol levels than the CYP8B1$^{-/-}$ animals. The SREBP2 mRNA levels were nevertheless nearly equal in both groups, and on levels similar to those of CYP8B1$^{+/+}$ animals receiving cholesterol-enriched diet only (Fig. 1D).

Alterations in bile acid synthesis and composition of bile. The CYP7A1 mRNA levels of the CYP8B1$^{+/+}$ mice did not differ from those of cholesterol-fed CYP8B1$^{+/+}$ animals (Fig. 2B). In CYP8B1$^{-/-}$ mice, the levels tended to be higher than in control mice, but much lower than those seen in the cholesterol-fed CYP8B1$^{-/-}$ mice. In both groups, no significant changes of the CYP27 mRNA were observed, although the levels of SHP mRNA showed a tendency to increase (data not shown). The levels of BSEP and MDR2 did not change significantly, compared with the controls. Interestingly, ABCG5 and ABCG8 mRNA increased 3- to 5-fold in both groups of animals (Fig. 2C, D). In the bile, both the CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice showed similar values for the percentage of total bile acids. However, the fraction of hydrophobic bile acids (cholic acid and deoxycholic acid) was higher in the CYP8B1$^{+/+}$ animals than in the CYP8B1$^{-/-}$ mice, whereas the fraction of more-hydrophilic bile acids (β-muricholic, α-muricholic, and UDCA) was lower (Table 1). No essential differences were found between the two groups in regard to cholesterol, the CSI%, and phospholipids (Table 2), although the first two analytes showed much higher values than those found in the cholesterol-fed CYP8B1$^{-/-}$ mice. Only the concentration of total lipids differed, being lower in the CYP8B1$^{-/-}$mice. In the gallbladder of the CYP8B1$^{+/+}$ animals, plenty of cholesterol crystals were observed, while the bile of the CYP8B1$^{-/-}$ mice was clear and devoid of solid material.

Alterations in intestinal cholesterol absorption and excretion. Fecal cholesterol excretion was similar in both groups, but lower than that found in mice fed 0.5% cholesterol, indicating the enhancing effect of CA on the cholesterol absorption (Fig. 3A). The mean percentage of cholesterol resorption in the CYP8B1$^{-/-}$ mice was 54%, whereas that in the CYP8B1$^{+/+}$ mice was 39% (Fig. 3B).

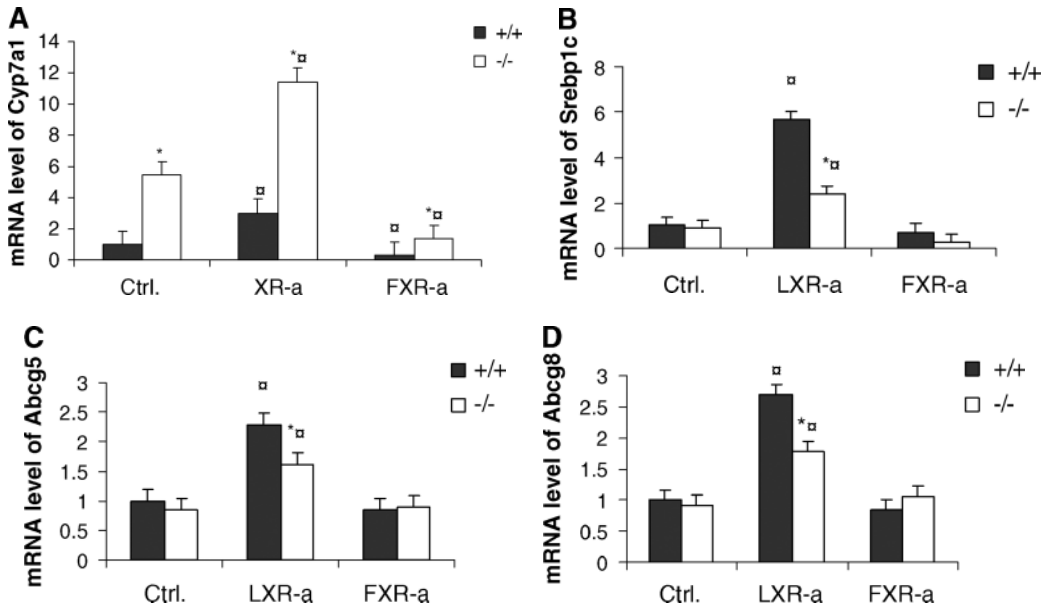

Fig. 6. Quantitation of the hepatic mRNA levels of genes in CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice treated with vehicle (Ctrl.), TO-901317 (LXR-a) and GW4064 (FXR-a). The mRNA quantitations were performed with HPRT as an internal standard. A: Quantitation of the hepatic mRNA levels for CYP7A1. B: Quantitation of the hepatic mRNA levels for SREBP1C. C: Quantitation of the hepatic mRNA levels for ABCG5. D: Quantitation of the hepatic mRNA levels for ABCG8. *, Significant differences between CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice with the same diet or the same treatment; ¤, significant differences between the diet-treated groups and the chow groups, or two agonist groups, compared with vehicle group having the same genotype. Values are expressed as means ± SEM of four observations. $P < 0.05$ was considered to be significant.

Alterations in serum lipids and lipoproteins. Plasma levels of total cholesterol did not change in animals fed the combination of cholesterol and CA (Fig. 4A); the VLDL- and LDL-cholesterol levels increased to the same extent in both groups, whereas the HDL-cholesterol levels were similar to those of the control animals (Fig. 4B). The mRNA levels for ABCA1 (Fig. 5A) and LDLR (data not shown) did not differ between the groups and the values found in animals on normal chow. The mRNA levels of apoA-1 did not show any significant differences, compared with the other mouse groups (Fig. 5B).

Effects of TO-901317 and GW4064 treatment

To better define whether the effects induced by the different diet regimes (cholesterol and cholesterol/CA) were mediated via the nuclear receptors LXR and FXR, mice were treated with specific agonists to each of these receptors.

The results showed that the expression of CYP7A1 was increased significantly by the LXR agonist TO-901317 in both CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice, compared with the controls, although the effect was much more pronounced in the latter group (**Fig. 6A**). Elevated mRNA levels were also found for SREBP1C (Fig. 6B) and ABCG5/G8 (Fig. 6C, D), although no effects were observed for SHP, BSEP, and MDR2 (data not shown). FXR agonist treatment significantly decreased the levels of CYP7A1 mRNA in both groups (Fig. 6A), but no changes were recorded for the mRNA levels of ABCG5/G8 (Fig. 6C, D), SREBP1C (Fig. 6B) and MDR2 (data not shown). SHP showed a tendency to increase in the CYP8B1$^{-/-}$ mice after the GW4064 treatment, whereas no differences were found in the CYP8B1$^{+/+}$ animals (data not shown).

DISCUSSION

The present results show that the CYP8B1$^{-/-}$ mice, despite a preexisting elevated expression of CYP7A1, were able to further upregulate their transcription rate of cholesterol 7α-hydroxylase when fed a cholesterol-enriched diet. The increased conversion of cholesterol into bile acids appears to be one reason these mice accumulate only minor quantities of liver cholesterol, compared with CYP8B1$^{+/+}$ animals. This process appears to occur via the classical pathway as CYP27 expression did not undergo any significant changes. Another contributing factor in the CYP8B1$^{-/-}$ mice may be the reduced influx of cholesterol from the intestine (Fig. 3B).

The rate-limiting enzyme in the bile acid synthesis, CYP7A1, is subjected to a sophisticated regulation in which several nuclear receptors (LXR, FXR, PXR, FTF, and HNF4α) (1) participate. The higher expression level of CYP7A1 in our CYP8B1$^{-/-}$ mice is probably due to elimination of the repressive effects of FXR, for which, in the mouse, CA represents the most important ligand (2). Several other mechanisms have also been described that downregulate CYP7A1 expression, involving PXR, the c-Jun N-terminal kinase (1), and insulin (19), all of which are independent of SHP. It seems likely, however, that

under normal conditions, FXR is the most important repressor of CYP7A1 expression. That the upregulation obtained by cholesterol is mediated by LXR, presumably activated by oxysterols, is supported by our finding that treatment with an LXR agonist induces a pattern similar to the reaction following cholesterol feeding. The difference in CYP7A1 mRNA levels between cholesterol-fed CYP8B1$^{+/+}$ and CYP8B1$^{-/-}$ mice illustrates the repressive power of the FXR-mediated mechanism, with 4-fold higher levels of CYP7A1 mRNA occurring in the CYP8B1$^{-/-}$ mice, although analysis of fecal bile acids indicated a doubling of the production rate. The basal transcription level of CYP7A1 seems to depend more on FXR than on LXRα, because there were no alterations in the mRNA levels for CYP7A1 in LXRα-deficient mice, as opposed to those seen in FXR-null animals (20, 21). The expanded bile acid pool in the CYP8B1$^{-/-}$ mice does not repress the bile acid synthesis, because its composition consists mainly of muricholic acids; these are more hydrophilic than CA and are poor activators of FXR. In addition, they do not promote cholesterol absorption (22). Addition of the FXR agonist GW4064 effectively depressed the transcription of CYP7A1 in both groups of mice to a greater extent than did the combined diet of cholesterol/CA. In the latter case, the CYP7A1 mRNA levels were slightly higher than those observed in the mice fed only chow, representing mixed and counteracting effects by LXR and FXR, whereby the LXR activation was mediated by the increased availability of cholesterol. The FXR effect nevertheless dominates, confirming previous reports (21, 23, 24). These results reflect the dual effect of CA in mice, as a stimulator of cholesterol absorption and as an FXR agonist. SHP mRNA levels were not significantly increased in any of our experimental regimes, but have been reported to rise after treatment using GW4064 at higher doses (100 mg/kg/day) (25) or CA (2).

In normal mice fed cholesterol, the endogenous cholesterol synthesis will be downregulated by increased hepatic cholesterol levels (26). In cholesterol-fed CYP8B1$^{-/-}$ mice, a higher rate of cholesterol synthesis is required to compensate for the amounts converted to bile acids, but even then, the hepatic cholesterol levels are much lower than in the CYP8B1$^{+/+}$ mice. It is likely that fine-tuning of CYP7A1 expression by a careful interplay between FXR and LXR stimuli is aimed at preserving a correct cholesterol economy, a delicate balance that is impaired in CYP8B1$^{-/-}$ mice.

According to current concepts, the transporters ABCG5 and ABCG8 represent the main mechanism for transfer of cholesterol into bile (27, 28). Normal mice fed 2% cholesterol for 1 week increased their hepatic and intestinal ABCG5/G8 expression (primarily considered to be mediated by LXRα) (29, 30) 2- to 3-fold. However, ABCG5/G8 expression nevertheless seems less responsive to cholesterol treatment than is that of CYP7A1, inasmuch as no significant changes were observed for the mRNA levels in the two groups of animals fed 0.5% cholesterol, although the cholesterol content in the bile doubled in the CYP8B1$^{+/+}$ mice. Interestingly, the LXR agonist treatment induced a significant increase of the ABCG5/G8 mRNA, whereas GW4064 did not have any significant effects, compared with the control animals. The prominent increase of ABCG5/G8 mRNA in animals fed the cholesterol/CA diet could include effects mediated by LXR (triggered via the increased hepatic cholesterol) and FXR. CA has previously been reported to enhance the transcription of ABCG5/G8, possibly indirectly via FXR (29, 30). The underlying mechanisms for the regulation of ABCG5/G8 by CA/FXR or LXRα are not known, and no specific responsive elements have so far been identified in the introns or in the short intergenic region between the two genes (31). However, FXR/CA may not be involved in maintaining the basal activity of ABCG5/G8, inasmuch as the CYP8B1$^{-/-}$ mice did not display any significant differences in their ABCG5/G8 mRNA levels, compared with the CYP8B1$^{+/+}$ animals, when fed normal chow. In SHP knock-out mice, slightly increased levels of ABCG5/G8 mRNA were found, but this was ascribed to an enlarged pool of CA (32).

Feeding the combined diet of cholesterol and CA "normalized" most of the effects observed in the CYP8B1$^{-/-}$ mice. Due to improved absorption, fecal cholesterol excretion was reduced to similar levels in both CYP8B1$^{-/-}$ and CYP8B1$^{+/+}$ mice. In the CYP8B1$^{-/-}$ mice, cholesterol accumulated in the liver, and synthesis of cholesterol and bile acids were depressed. Increased quantities of cholesterol shuttled into the bile elevated the CSI % in both animal groups, but cholesterol crystals were only observed in the $^{+/+}$ mice. However, bile from these animals had higher concentrations of total lipids and a more hydrophobic bile acid composition, two factors of importance for rapid cholesterol crystallization (33). Recently, it was shown that gallstone formation in mice could be prevented by GW4064 treatment (25), an effect assumed to depend on upregulation of the hepatic transporters BSEP and MDR2, which would increase the flow of bile acids and phospholipids, respectively, into the bile, thus improving the solubility of cholesterol. However, no significant differences in the mRNA levels of these transporters were observed by us in the CYP8B1$^{-/-}$ and CYP8B1$^{+/+}$ mice fed the CA/cholesterol diet or treated with GW4064, nor did the concentration of phospholipids in the bile differ significantly. With regard to GW4064, the discrepancies might be dependent on the lower doses used in our experiments.

In CYP8B1$^{-/-}$ mice treated with a cholesterol diet, no significant changes were observed in total plasma cholesterol levels, but HDL-cholesterol was increased in parallel with the mRNA levels of the hepatic transporter ABCA1. Absence of CA thus appears to facilitate production of HDL, thereby enhancing efflux of cholesterol from the liver. The human ABCA1 gene contains LXR sites (34), but whether CA directly affects the ABCA1 expression in mice is an open question. Even if supplementation of the diet with cholate reduced the plasma apoA-1 by downregulating its transcription (35), we did not find any significant differences in the mRNA levels of apoA-1 between the CYP8B1$^{+/+}$ and $^{-/-}$ mice kept on normal chow (Fig. 5B), indicating that the basic transcriptional

rate of this gene is not influenced by physiological levels of CA. The HDL-cholesterol levels in cholesterol-fed CYP8B1$^{-/-}$ mice might then depend on the ABCA1 expression, as has been shown in mice overexpressing ABCA1 (36), thereby proposing a novel role for CYP8B1 in the plasma lipoprotein metabolism in mice.

Our results illustrate the key role for CYP8B1 and its effector molecule, CA, in cholesterol homeostasis in mouse. In parallel with the stimulatory effect on cholesterol absorption, CA markedly reduces degradation of cholesterol into bile acids and indirectly affects the rate of cholesterol synthesis and the liver cholesterol content. An optimal cholesterol balance appears to be tightly linked to the CA pool size, which may be one reason why CYP8B1 is subjected to such sophisticated regulation. On the other hand, shortage of CA could protect against effects from excess cholesterol intake. In humans, the situation is somewhat different, inasmuch as CDCA is not converted to muricholic acids, and furthermore, appears to be the most efficient ligand for human FXR (37, 38).

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REFERENCES

1. Russell, D. W. 2003. The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* **72:** 137–174.
2. Li-Hawkins, J. L., M. Gåfvels, M. Olin, E. G. Lund, U. Andersson, G. Schuster, I. Björkhem, D. Russell, and G. Eggertsen. 2002. Cholic acid mediates negative feedback regulation of bile acid synthesis. *J. Clin. Invest.* **110:** 1191–1200.
3. Moore, J. T., B. Goodwin, T. M. Willson, and S. A. Kliewer. 2002. Nuclear receptor regulation of genes involved in bile acid metabolism. *Crit. Rev. Eukaryot. Gene Expr.* **12:** 119–135.
4. Svenson, K., M. Bogue, L. L. Peters. 2003. Identifying new mouse models of cardiovascular disease: a review of high-throughput screens of mutagenized and inbred strains. *J. Appl. Physiol.* **94:** 1650–1659.
5. Breslow, J. L. 1996. Mouse models of atherosclerosis. *Science.* **272:** 685–688.
6. Murphy, C., P. Parini, J. Wang, I. Bjorkhem, G. Eggertsen, and M. Gafvels. 2005. Cholic acid as key regulator of cholesterol synthesis, intestinal absorption and hepatic storage in mice. *Biochim. Biophys. Acta.* **1735:** 167–175.
7. Bjorkhem, I. 1974. Serum cholesterol determination by mass fragmentography. *Clin. Chim. Acta.* **54:** 185–193.
8. Lund, E., L. Sisfontes, E. Reihner, and I. Bjorkhem. 1989. Determination of serum levels of unesterified lathosterol by isotope dilution-mass spectrometry. *Scand. J. Clin. Lab. Invest.* **49:** 165–171.
9. Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. 1998. Marked reduction in bile acid synthesis in cholesterol 7a-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J. Lipid Res.* **39:** 1833–1843.
10. Miettinen, T. A., E. H. Ahrens, and S. M. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
11. Miettinen, T. A. 1982. Gas-liquid chromatographic determination of fecal neutral sterols using a capillary column. *Clin. Chim. Acta.* **124:** 245–248.
12. Grundy, S. M., E. H. Ahrens, and T. A. Miettinen. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
13. Hunt, M., Y-Z. Yang, G. Eggertsen, M. Gåfvels, C. Einarsson, and S. Alexson. 2000. The peroxisome proliferator-activated receptor alpha (PPARα) regulates bile acid biosynthesis. *J. Biol. Chem.* **275:** 28947–28953.
14. Fausa, O., and B. A. Skålhegg. 1974. Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3alpha-hydroxysteroid dehydrogenase. *Scand. J. Gastroenterol.* **9:** 249–254.
15. Roda, A., D. Festi, C. Sama, G. Mazzella, R. Alini, E. Roda, and L. Barbara. 1975. Enzymatic determination of cholesterol in bile. *Clin. Chim. Acta.* **64:** 337–341.
16. Rouser, G., S. Fleischer, and A. Yamamoto. 1970. Two dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorous analysis of spots. *Lipids.* **5:** 494–496.
17. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
18. Parini, P., B. Angelin, A. Stavreus-Evers, B. Freyschuss, H. Eriksson, and M. Rudling. 2000. Biphasic effects of the natural estrogen 17beta-estradiol on hepatic cholesterol metabolism in intact female rats. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1817–1823.
19. Ishida, H., C. Yamashita, Y. Kuruta, Y. Yoshida, and M. Noshiro. 1999. Structure, evolution, and liver-specific expression of sterol 12alpha-hydroxylase P450 (CYP8B). *J. Biochem. (Tokyo).* **126:** 19–25.
20. Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J. M. Lobaccaro, R. E. Hammar, and D. J. Mangelsdorf. 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRa. *Cell.* **93:** 693–704.
21. Sinal, C. J., M. Tohkin, M. Miyata, J. M. Ward, G. Lambert, and F. J. Gonzalez. 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell.* **102:** 731–744.
22. Wang, D. Q-H., S. Tazuma, D. E. Cohen, and M. C. Carey. 2003. Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse. *Am. J. Physiol. Gastrointest. Liver Physiol.* **285:** G494–G502.
23. Xu, G., H. Li, L. X. Pan, Q. Shang, A. Honda, M. Ananthanarayanan, S. K. Erickson, B. L. Shneider, S. Shefer, J. Bollineni, et al. 2003. FXR-mediated down-regulation of CYP7A1 dominates LXRalpha in long-term cholesterol-fed NZW rabbits. *J. Lipid Res.* **44:** 1956–1962.
24. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell.* **6:** 507–515.
25. Moschetta, A., A. L. Bookout, and D. J. Mangelsdorf. 2004. Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. *Nat. Med.* **10:** 1352–1358.
26. Sehayek, E., S. Shefer, L. B. Nguyen, J. G. Ono, M. Merkel, and J. L. Breslow. 2000. Apolipoprotein E regulates dietary cholesterol absorption and biliary cholesterol excretion: studies in C57BL/6 apolipoprotein E knockout mice. *Proc. Natl. Acad. Sci. USA.* **97:** 3433–3437.
27. Yu, L., R. Hammer, J-L. Hawkins, K. von Bergmann, D. Luthjohann, J. C. Cohen, and H. H. Hobbs. 2002. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc. Natl. Acad. Sci. USA.* **99:** 16237–16242.
28. Kosters, A., R. J. J. M. Frijters, F. G. Schaap, E. Vink, T. Plösch, R. Ottenhoff, M. Jirsa, I. M. De Cuyper, F. Kuipers, and A. K. Groen. 2003. Relation between hepatic expression of ATP binding cassette transporters G5 and G8 and biliary cholesterol secretion in mice. *J. Hepatol.* **38:** 710–716.
29. Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Richardson, H. Hobbs, and D. J. Mangelsdorf. 2002. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors α and β. *J. Biol. Chem.* **277:** 18793–18800.
30. Yu, L., S. Gupta, F. Xu, A. D. Liverman, A. Moschetta, D. J. Mangelsdorf, J. J. Repa, H. H. Hobbs, and J. C. Cohen. 2005. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. *J. Biol. Chem.* **280:** 8742–8747.
31. Remaley, A. T., S. Bark, A. D. Walts, L. Freeman, S. Shulenin, T. Annilo, E. Elgin, H. E. Rhodes, C. Joyce, M. Dean, et al. 2002. Comparative genome analysis of potential regulatory elements in the ABCG5-ABCG8 gene cluster. *Biochem. Biophys. Res. Commun.* **295:** 276–282.

32. Kerr, T. A., S. Saeki, M. Schneider, K. Schaefer, S. Berdy, T. Redder, B. Shan, D. W. Russell, and M. Schwarz. 2002. Loss of nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis. *Dev. Cell.* **2:** 713–720.
33. Wang, D. Q-H., F. Lammert, B. Paigen, and M. C. Carey. 1999. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: pathophysiology of biliary lipid secretion. *J. Lipid Res.* **40:** 2066–2079.
34. Singaraja, R. R., V. Bocher, E. James, S. M. Clee, L. H. Zhang, B. R. Leavitt, B. Tan, A. Brooks-Wilson, A. Kwok, N. Bissada, et al. 2001. Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and apoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. *J. Biol. Chem.* **276:** 33969–33979.
35. Srivastava, R. A., N. Srivastava, and M. Averna. 2000. Dietary cholic acid lowers plasma levels of mouse and human apolipoprotein A-I primarily via a transcriptional mechanism. *Eur. J. Biochem.* **267:** 4272–4280.
36. Basso, F., L. Freeman, C. L. Knapper, A. Remaley, J. Stonik, E. B. Neufeld, T. Tansey, M. J. Amar, J. Fruchart-Najib, N. Duverger, et al. 2003. Role of the hepatic ABCA1 transporter in modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations. *J. Lipid Res.* **44:** 296–302.
37. Ellis, E., M. Axelson, A. Abrahamsson, G. Eggertsen, A. Thörne, G. Nowak, B-G. Ericzon, I. Björkhem, and C. Einarsson. 2003. Feedback regulation of bile acid synthesis in primary human hepatocytes. Evidence that CDCA is the strongest inhibitor. *Hepatology.* **38:** 930–938.
38. Lew, J-L., A. Zhao, J. Yu, L. Huang, N. de Pedro, F. Pelaez, S. D. Wright, and J. Cui. 2003. The farnesoid X receptor (FXR) controls gene expression in a ligand- and promoter-selective fashion. *J. Biol. Chem.* **279:** 8856–8861.