Disruption of the Sterol 27-Hydroxylase Gene in Mice Results in Hepatomegaly and Hypertriglyceridemia

REVERSAL BY CHOLIC ACID FEEDING*

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Sterol 27-hydroxylase (CYP27) participates in the conversion of cholesterol to bile acids. We examined lipid metabolism in mice lacking the Cyp27 gene. On normal rodent chow, Cyp27+/− mice have 40% larger livers, 45% larger adrenals, 2-fold higher hepatic and plasma triacylglycerol concentrations, a 70% higher rate of hepatic fatty acid synthesis, and a 70% increase in the ratio of oleic to stearic acid in the liver versus Cyp27+/+ controls. In Cyp27+/− mice, cholesterol 7α-hydroxylase activity is increased 5-fold, but bile acid synthesis and pool size are 47 and 27%, respectively, of those in Cyp27+/+ mice. Intestinal cholesterol absorption decreases from 54 to 4% in knockout mice, while fecal neutral sterol excretion increases 2.5-fold. A compensatory 2.5-fold increase in whole body cholesterol synthesis occurs in Cyp27+/− mice, principally in liver, adrenal, small intestine, lung, and spleen. The mRNA for the cholestero- genic transcription factor sterol regulatory element-binding protein-2 (SREBP-2) and mRNAs for SREBP-2-regulated cholesterol biosynthetic genes are elevated in livers of mutant mice. In addition, the mRNAs encoding the lipogenic transcription factor SREBP-1 and SREBP-1-regulated monounsaturated fatty acid biosynthetic enzymes are also increased. Hepatic synthesis of fatty acids and accumulation of triacylglycerols increases in Cyp27+/− mice and is associated with hypertriglyceridemia. Cholic acid feeding reverses hepatomegaly and hypertriglyceridemia but not adenomegaly in Cyp27+/− mice. These studies confirm the importance of CYP27 in bile acid synthesis and they reveal an unexpected function of the enzyme in triacylglycerol metabolism.

In mammals the conversion of cholesterol to bile acids and their subsequent fecal excretion represents a major route for the elimination of cholesterol from the body (1). There are two main pathways by which cholesterol is converted to bile acids (2–4). The major pathway, also called the classic or neutral pathway, is initiated by cholesterol 7α-hydroxylase (CYP7A1), located in the endoplasmic reticulum of liver cells. The alternate or acidic pathway is initiated by mitochondrial sterol 27-hydroxylase (CYP27), which is present not only in the liver but in extrahepatic organs as well, particularly the lungs (5–7). The synthesis of bile acids via the classic pathway also involves sterol 27-hydroxylase, which facilitates oxidation of the steroid side chain (2, 3). Sterol 27-hydroxylase deficiency in humans results in a marked reduction in total bile acid synthesis, an increase in whole body cholesterol synthesis and the accumulation of cholesterol in tissues (8–10). Cerebrotendinous xanthomatosis, the disease arising from this deficiency, is characterized by several conditions including accelerated atherosclerosis (11). In Cyp27 knockout mice there is a dramatic reduction in total bile acid synthesis, but these animals do not develop cerebrotendinous xanthomatosis (12).

Despite the different pathophysiological consequences of sterol 27-hydroxylase deficiency in humans and mice, further characterization of sterol and lipid metabolism in the Cyp27 knockout mouse is warranted for several reasons. First, there have been several recent discoveries relating to the mechanisms through which feedback regulation of bile acid synthesis is articulated via nuclear receptors (13–16). Therefore, we wanted to study these mechanisms and related aspects of sterol metabolism in an animal model in which the rate of bile acid synthesis was impaired. Second, in our initial evaluation of Cyp27+/− mice, we found that they were hypertriglyceridemic when maintained on a low fat, conventional rodent diet. This observation was potentially important because of earlier findings of an association between hepatic very low density lipoprotein (VLDL)-triacylglycerol secretion and the rate of bile acid synthesis (17–19). Third, the measurement in vivo of the rate of sterol synthesis in the liver and major extrahepatic organs of Cyp27+/− mice, represented a new, more quantitative approach to further investigating the putative role of 27-hydroxysterol as a regulator of cholesterol biosynthesis (20–22). Fourth, our initial investigation of Cyp27+/− mice revealed that they had a significant enlargement of their livers and adrenal glands. Such organomegaly is not seen in mice that lack cholesterol 7α-hydroxylase, but which retain a functional alternate pathway of bile acid synthesis (23). The studies reported here show that sterol 27-hydroxylase plays a more global role in

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The abbreviations used are: CYP, cytochrome P450; ACTH, adrenocorticotropic hormone; SREBP-1 and SREBP-2, sterol regulatory element-binding protein-1 and -2; VLDL, very low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; PAG, polyacrylamide gel electrophoresis.
bodily lipid metabolism than has been documented previously. Surprisingly, the loss of the sterol 27-hydroxylase-initiated pathway of bile acid synthesis and the resultant marked compensatory increase in cholesterol 7α-hydroxylase activity is accompanied by dramatic changes in hepatic fatty acid metabolism and circulating plasma VLDL-triaclyglycerol concentrations.

**EXPERIMENTAL PROCEDURES**

**Animals and Diets**—Sterol 27-hydroxylase-deficient mice were generated as described previously (12). Breeding stock were generously supplied by Dr. Nobuyo Maeda at the University of North Carolina. The mutants (Cyp27α−/−) were maintained in a mixed-strain background (C57BL/6:129Sv) as were matching Cyp27+/+ controls. The litters did not require supplementation with cholic acid or vitamins during the nursing period. The pups were weaned at 4 weeks and thereafter were fed ad libitum a cereal-based rodent diet (Wayne Lab Blox, No. 8604) (Harlan Teklad, Madison, WI), which contained 0.20 mg of cholesterol and 50 mg of total lipid per g of diet. This chow was defined as the basal diet. In all studies the mice were fed the meal form of this diet without any additions except in one case when cholic acid was added (0.1%/w/w). All experiments were carried out in male mice 3–4 months of age that were housed as described (23). Mice were killed at the end of the 12-h dark phase of the lighting cycle and were in the fed state at the time of study. Those that were fasted for 6 h were housed for 6 h in a glass bladder bile. The adrenal glands were excised, cleaned of surrounding tissue, and weighed. Sections of adrenal tissue were stained with hematoxylin and eosin. All experiments were approved by the Institutional Animal Care and Research Advisory Committee.

**Plasma Hormone and Lipoprotein Cholesterol and Triacylglycerol Concentrations**—Mice were exanguinated from the vena cava under ether anesthesia. Blood was anticoagulated with EDTA. Plasma corticosterone, ACTH, and testosterone concentrations were determined by a commercial facility (Endocrine Services Laboratory, Oregon Regional Primate Research Center, Beaverton, OR). Pooled plasma samples were fractionated by fast protein liquid chromatography and the cholesterol and triacylglycerol content of each fraction, and of whole plasma, was determined enzymatically using commercially available kits as was plasma glucose concentration (Roche Molecular Biochemicals and Sigma-Aldrich).

**Tissue Cholesterol and Triacylglycerol Concentrations, Fatty Acid Composition, and Identification of “Non-cholesterol” Sterols in Adrenals and Lungs**—Tissue total cholesterol concentrations and hepatic fatty acid composition were determined by gas-chromatographic methods (24). In the adrenals and lungs of the Cyp27α−/− mice, non-cholesterol sterols were present. These were identified by gas chromatography-mass spectrometry after trimethylsilyl derivatization as described elsewhere (25). Hepatic triacylglycerol concentrations were measured as follows. A 0.3-g aliquot of liver was extracted in chloroform:methanol (2:1, v/v) in a final volume of 100 ml.Duplicate 2-ml aliquots of each extract were washed with 0.5 ml of isotonic saline. After centrifugation, the upper phase was discarded and 20 μl of chloroform:Triton X-114 (1:1, v/v) was added to the lower phase which was then dried completely at 45–50 °C under an air stream. Two ml of Infinity Triglyceride reagent (number 343-25P) (Sigma-Aldrich) was added to the residue and vortexed. After 20 min, the optical densities of the samples were read at 520 nm against standards to which 20 μl of chloroform:Triton X-114 had been added before drying and color development.

**RNA Analysis**—Following exsanguination of the mice under ether anesthesia, aliquots of liver were immediately frozen in liquid nitrogen. Five μg of poly(A)+ RNA prepared from individual livers were subjected to electrophoresis and northern blot analysis using 32P-labeled probes as described previously (26, 27). The amount of radioactivity in each band was quantitated by PhosphorImager and normalized to the signal generated by β-actin. For each mRNA, the fold-change for the Cyp27α−/− mice was expressed relative to matching Cyp27+/+ controls. The litters did not require supplementation with cholic acid or vitamins during the nursing period. The pups were weaned at 4 weeks and thereafter were fed ad libitum a cereal-based rodent diet without added cholesterol. They were studied at 3 to 4 months of age. Values are the mean ± 1 S.E. in the number of animals given in parentheses.

**RESULTS**

**Table I**

| Weight | Cyp27+/+ | Cyp27−/− |
|--------|----------|----------|
| Whole body (g) | 28 ± 1 (20) | 27 ± 1 (22) |
| Liver (g) | 1.44 ± 0.03 (20) | 2.05 ± 0.04 (22) |
| Small intestine (g) | 1.16 ± 0.04 (5) | 1.05 ± 0.04 (5) |
| Kidney (g) | 0.97 ± 0.01 (7) | 0.92 ± 0.01 (10) |
| Lung (g) | 0.16 ± 0.01 (7) | 0.15 ± 0.01 (5) |
| Spleen (g) | 0.08 ± 0.01 (10) | 0.07 ± 0.01 (10) |
| Adrenal (mg) | 4.2 ± 0.1 (14) | 6.1 ± 0.2 (14)* |
| Brain (g) | 0.47 ± 0.01 (11) | 0.44 ± 0.01 (11)* |
| Testis (g) | 0.25 ± 0.01 (10) | 0.25 ± 0.01 (10) |

*Statistically different from value for Cyp27+/+ group (p < 0.05).

The data in Table I show that although there was no difference in body weights of adult male Cyp27−/− and Cyp27+/+ mice, there was a marked increase in the mass of the liver (40%) and adrenal glands (45%) in the Cyp27−/− animals. When maintained on a conventional low fat, low cholesterol rodent diet, the Cyp27−/− mice also manifest a 30% increase in plasma total cholesterol concentration and a 2-fold increase in plasma total triacylglycerol concentration. They maintained normal plasma levels of glucose, corticosterone, ACTH, and testosterone (Table II). In the Cyp27−/− mice the molar ratio of cholesterol in gallbladder bile was double that in the Cyp27+/+ controls. This was due to the fact that the biliary cholesterol concentration in these animals was unchanged, whereas the concentrations of bile acid and phospholipid fell significantly (Table II).

Fractionation of pooled plasma by fast protein liquid chromatography (Fig. 1) showed that the increment in the cholesterol (Fig. 1A) and triacylglycerol (Fig. 1B) concentrations in the Cyp27−/− mice was confined to VLDL. In contrast to the plasma cholesterol level, the concentration of cholesterol in most tissues, and in the whole animal, was the same in both genotypes (Figs. 2, A and B). There were two exceptions. One was the adrenal gland, which in the Cyp27−/− mice had not only a 48% higher concentration of cholesterol (Fig. 2A), but also significant levels of two non-cholesterol sterols, the concentration of which totaled 9.8 ± 0.6 mg/g. These sterols, which were not detected in the adrenals of the Cyp27+/+ mice, were identified by gas chromatography-mass spectrometry as the
Concentrations of lipids and hormones in the plasma and gallbladder bile of Cyp27-/- and Cyp27+/+ mice

These data were derived from many of the same animals used for body and organ weight measurements (Table I). Values are the mean ± 1 S.E. for measurements in the number of animals given in parentheses.

| Parameter                  | Cyp27+/+ | Cyp27-/- |
|---------------------------|---------|---------|
| Plasma cholesterol (mg/dl)| 89.9 ± 3.6 (16) | 116.8 ± 3.0 (18)* |
| Triacylglycerol (mg/dl)   | 132.2 ± 12.1 (11) | 255.6 ± 29.0 (11)* |
| Glucose (mg/dl)           | 211.6 ± 17.7 (5) | 196.5 ± 23.8 (6) |
| Corticosterone (ng/ml)    | 15.3 ± 4.2 (9)   | 24.6 ± 7.6 (8)   |
| ACTH (pg/ml)              | 151.3 ± 20.3 (5) | 110.2 ± 22.4 (6) |
| Testosterone (ng/ml)      | 3.3 ± 2.0 (9)    | 2.2 ± 1.0 (9)    |
| Gallbladder bile concentration |         |         |
| Bile acid (μmol/ml)       | 167.2 ± 14.1 (5) | 62.3 ± 4.8 (6)* |
| Phospholipid (μmol/ml)    | 22.4 ± 3.5 (5)   | 13.4 ± 0.7 (6)* |
| Cholesterol (μmol/ml)     | 2.7 ± 0.4 (5)    | 2.3 ± 0.2 (6)    |
| Gallbladder bile molar ratio |        |         |
| Bile acid (%)             | 87.2 ± 1.1 (5)   | 79.7 ± 0.9 (6)* |
| Phospholipid (%)          | 11.4 ± 1.0 (5)   | 17.4 ± 0.9 (6)* |
| Cholesterol (%)           | 1.4 ± 0.1 (5)    | 2.9 ± 0.1 (6)*   |

* Statistically different from value for Cyp27+/- group (p < 0.05).

FIG. 1. Plasma lipoprotein profiles in Cyp27+/+ and Cyp27-/- mice. Plasma from groups of male mice of both genotypes that had been fed a basal rodent diet without added cholesterol was fractionated by fast protein liquid chromatography, and the cholesterol (A) and triacylglycerol (B) content of each fraction was determined as described under "Experimental Procedures." For each genotype, plasma from 5 mice was combined. LDL, low density lipoprotein; HDL, high density lipoprotein.

FIG. 2. Tissue cholesterol concentration in the liver and extrahepatic organs of Cyp27+/+ and Cyp27-/- mice. Tissue total cholesterol concentrations were measured by gas chromatography in various organs of Cyp27+/+ and Cyp27-/- male mice fed a basal rodent diet without added cholesterol. The concentration values (A) were multiplied by respective organ weights to obtain whole organ cholesterol contents normalized per 100 g of body weight (B). These contents were added to give values for whole animal cholesterol content per 100 g of body weight (inset of B). Values represent the mean ± 1 S.E. of data from at least 9 animals of each genotype (for liver, kidney, spleen, adrenal, and lung) and from 5 animals of each genotype for the other organs. Whole body cholesterol contents were determined in 14 mice of each genotype. *, p < 0.05 compared with value for Cyp27+/- animals.

A major focus of these studies was to characterize the effects of sterol 27-hydroxylase deficiency on an array of proteins in the liver that are involved not only in bile acid and cholesterol metabolism, but also in fatty acid metabolism. This analysis was done by determining the mRNA levels for each of the target proteins in concert with measuring enzyme activities and the rates of various metabolic processes associated with the expression of these proteins. The first set of data relate to various enzymes and receptors involved in regulating bile acid synthesis. As shown in Fig. 3, the Cyp27-/- mice lacked sterol 27-hydroxylase, and manifest an ~10-fold increase in the mRNA for cholesterol 7α-hydroxylase (CYP7A1). The expression of oxysterol 7α-hydroxylase (CYP7B1) in the Cyp27-/- mice was only 40% of that seen in the Cyp27+/+ controls, whereas the expression of sterol 12α-hydroxylase (CYP8B1) was 2.4-fold higher in the Cyp27+/+ animals. The expression of the small heterodimer partner (SHP) in the Cyp27-/- mice was reduced to only 35% of that in the Cyp27+/+ controls, whereas the mRNA levels for both the farnesoid X receptor (FXR) and the liver receptor homologue-1 (LRH-1) showed a slight elevation in the Cyp27+/+ mice.

The enzyme activity data shown in Fig. 4 agree well with the RNA measurements shown in Fig. 3, as exemplified by the 5-fold increase in cholesterol 7α-hydroxylase activity in the Cyp27+/+ mice (Fig. 4A). Total bile acid synthesis in these mice, as measured by the rate of fecal bile acid excretion, averaged 47% of that in the Cyp27+/+ controls (Fig. 4B). In these same Cyp27+/+ mice, bile acid pool size was only 27% of that in the Cyp27+/+ mice (Fig. 4C).
Fig. 3. Relative amount of mRNA for various enzymes and receptors involved in bile acid synthesis in the livers of Cyp27+/+ and Cyp27−/− mice. Five μg of poly(A+) RNA prepared from the livers of mice of each genotype were subjected to electrophoresis and northern blot analysis using the 32P-labeled probes indicated. The amount of radioactivity in each band was quantified by PhosphorImager and normalized to the signal generated by β-actin. For each mRNA, the fold-change for the Cyp27−/− mice was expressed relative to matching Cyp27+/+ mice, which in each case was arbitrarily set at 1.0. The values shown below each blot represent the mean ± S.E. of the fold change found in 6 Cyp27−/− mice relative to that for 6 Cyp27+/+ mice. CYP7A1, cholesterol 7-hydroxylase; CYP27B1, oxysterol 7α hydroxylase; CYP27A1, sterol 12α hydroxylase; FXR, farnesoid X receptor; SHP, small heterodimer partner; LRH-1, liver receptor homologue-1.

Fig. 4. Various parameters of bile acid metabolism and level of intestinal cholesterol absorption in Cyp27+/+ and Cyp27−/− mice. Cholesterol 7α-hydroxylase activity (A), fecal bile acid excretion (B), bile acid pool size (C), and composition (D), intestinal cholesterol absorption (E), and fecal neutral sterol excretion (F) were measured in Cyp27+/+ and Cyp27−/− male mice fed a basal rodent diet without added cholesterol as described under “Experimental Procedures.” In mice of both genotypes, cholic and muricholic together constituted more than 95% of bile acids in the intestinal pool (D). Fecal neutral sterols consisted of cholic acid and its derivatives, coprostanol, epicoprostanol, and cholestane (F). Values represent the mean ± 1 S.E. of data from 5 Cyp27+/+ and 7 Cyp27−/− mice, respectively, for all parameters except fecal bile acid excretion in which case measurements were made in 10 Cyp27+/+ and 12 Cyp27−/− mice, respectively. *, p < 0.05 compared with value for Cyp27+/+ animals.

A cholesterol 7α-hydroxylase activity is shown in the first panel. There was a 50% increase in hepatic cholesterol absorption (from 54 to 4%) (Fig. 4E). This reduction in turn contributed to a 2.5-fold increase in fecal neutral sterol excretion in the Cyp27−/− mice (Fig. 4F). Although the concentration of bile acids in the gallbladder bile of Cyp27−/− mice was only 37% of that in Cyp27+/+ animals (Table II), the species of bile acid were similar in both genotypes with cholic acid predominating in both cases (data not shown).

The marked increase in plasma VLDL-triacylgllycerol concentrations in the Cyp27−/− mice prompted us to investigate the level of expression of several key enzymes involved in hepatic fatty acid metabolism. As shown in the first panel of Fig. 5, there was a 50% increase in the mRNA level of sterol regulatory element-binding protein-1 (SREBP-1), which preferentially activates transcription of genes encoding the enzymes of fatty acid synthesis. Consistent with this change were 1.7- and 2.5-fold increases in the expression of acetyl-CoA carboxylase and fatty acid synthase, respectively. There was also a 3.7-fold increase in the mRNA level for acyl-CoA oxidase, which catalyzes the conversion of stearic to oleic acid. Although the expression of acyl-CoA synthase was unchanged, there was a 50% increase in the mRNA level for acyl-CoA oxidase.

These northern analysis data are consistent with the various metabolic measurements described in Fig. 6. These show that in the Cyp27−/− there was a 2.4-fold increase in hepatic triacylgllycerol concentrations (Fig. 6A), a 70% increase in hepatic fatty acid synthesis (Fig. 6B), and a marked shift in hepatic fatty acid composition characterized by about a 70% increase in the proportion of oleic (18:1) to stearic acid (18:0) (Fig. 6C). Together, the data in Figs. 5 and 6 reveal a major association between sterol 27-hydroxylase and hepatic fatty acid and triacylglycerol metabolism.

The remaining set of mRNA measurement data defines the levels of expression of enzymes involved in hepatic cholesterol synthesis, two of the receptors that facilitate the uptake and intrahepatic handling of lipoprotein cholesterol, and the liver X receptor α, a nuclear receptor that regulates the expression of several lipogenic enzymes (Fig. 7). The first panel shows a 50% increase in the expression of SREBP-2, which activates transcription of genes encoding the enzymes of cholesterol biosynthesis. Consistent with this result was a 2.8- and 2.7-fold increase in the mRNA levels for HMG-CoA synthase and HMG-CoA reductase, respectively. There was also a 50% increase in the expression of the LDL and high density lipoprotein scavenger receptor, class B, type 1 receptors, but little change in the mRNA level for the liver X receptor α.

The rates of cholesterol synthesis in the liver and extrahepatic organs, measured in vivo in matching groups of Cyp27−/−
Cyp27 thesis, and fatty acid composition in described in the legend to Fig. 3. The values shown to that for 6 acyl-CoA oxidase.

manifest in these animals. When fed only the basal diet, each genotype. *, p < 0.05 compared with value for 1/1 animals.

and Cyp27+/+ mice, are shown in Fig. 8. In the Cyp27−/− mouse there were 4-fold increases in cholesterol synthesis in the liver and adrenal gland (Fig. 8A). Significant increases in synthesis also occurred in the lung (2.9-fold), spleen (2.4-fold), and small intestine (1.8-fold). In contrast, there was little or no change in the rates of sterol synthesis in the other extrahepatic organs, including the residual carcass, which comprised mainly skin, muscle, adipose tissue, stomach, and large intestine. There was a trend toward lower rates of sterol synthesis in the kidneys of Cyp27−/− mice. Summation of the rates of synthesis per whole organ (inset Fig. 8B) showed that whole animal sterol synthesis in the Cyp27−/− mice was 2.5-fold greater than in the Cyp27+/+ controls. About 90% of the increase in whole body synthesis was attributable to hepatic synthesis. In contrast to the variable effect of CYP27 deficiency on sterol synthesis in the extrahepatic organs, little or no change in the rates of fatty acid synthesis occurred in tissues such as the adrenal gland, lung, or small intestine (data not shown).

The final study investigated whether feeding cholic acid to the Cyp27−/− mice would ameliorate the metabolic changes manifest in these animals. When fed only the basal diet, Cyp27+/+ and Cyp27−/− mice had 5.2 ± 0.2 (n = 7) and 7.2 ± 0.2 (n = 9) g of liver per 100 g of body weight, respectively. However, in matching Cyp27−/− mice fed a diet supplemented with cholic acid for 10 days, relative liver weight contracted significantly to 5.9 ± 0.1 g/100 g of body weight. In contrast, adrenal gland enlargement was not reversed by cholic acid feeding. Thus, on the basal diet, the Cyp27+/+ and Cyp27−/− mice had 14.4 ± 0.7 (n = 5) and 22.8 ± 1.3 (n = 5) mg of adrenal/100 g of body weight, respectively. In the cholic acid-fed Cyp27−/− mice this value remained at 22.8 ± 1.6 (n = 5) mg/100 g of body weight. The hepatomegaly in the Cyp27−/− mice was also almost fully reversed when chenodeoxycholic acid was fed in place of cholic acid (data not shown).

The metabolic effects of cholic acid supplementation in the Cyp27−/− mice are shown in Fig. 9. This treatment restored intestinal cholesterol absorption to normal levels (Fig. 9A), and suppressed the rate of sterol synthesis in the adrenal gland (Fig. 9B) and liver (Fig. 9C) to values significantly below those seen in Cyp27+/+ mice fed the basal diet alone. Cholic acid supplementation also essentially normalized hepatic fatty acid synthesis (Fig. 9D), and triacylglycerol concentrations in both the liver (Fig. 9E) and plasma (Fig. 9F) of the Cyp27−/− mice.

**FIG. 5.** Relative amount of mRNA for SREBP-1 and various enzymes involved in fatty acid metabolism in the livers of Cyp27+/+ and Cyp27−/− mice. These measurements were made using the same liver RNA that was prepared for the bile acid enzyme mRNA determinations described in the legend to Fig. 3. The values shown below each blot represent the mean ± 1 S.E. of the fold change found in 6 Cyp27−/− mice relative to that for 6 Cyp27−/− mice. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, steryl-CoA desaturase; ACS, acyl-CoA synthase; ACO, acyl-CoA oxidase.

**FIG. 6.** Hepatic triacylglycerol concentration, fatty acid synthesis, and fatty acid composition in Cyp27+/+ and Cyp27−/− mice. Cyp27+/+ and Cyp27−/− male mice that had been fed a basal rodent diet without added cholesterol were used either for the determination of hepatic triacylglycerol concentration (A) and fatty acid composition (C), or for the measurement of the rate of hepatic fatty acid synthesis (B) as described under “Experimental Procedures.” Values for each parameter represent the mean ± 1 S.E. of data from 10 mice of each genotype. *, p < 0.05 compared with value for 1/1 animals.

**FIG. 7.** Relative rate of hepatic bile acid synthesis (A) and accumulation of cholesterol (B) in the liver, plasma, and extrahepatic organs of Cyp27+/+ and Cyp27−/− mice in response to feeding cholic acid. Cyp27+/+ and Cyp27−/− mice had ACO, acyl-CoA oxidase.

**FIG. 8.** Effect of feeding of cholesterol (to +/− mice) or cholic acid (to −/− mice) on cholesterol synthesis (A) and accumulation (B) in the liver, plasma, and extrahepatic organs of Cyp27+/+ and Cyp27−/− mice. Cyp27+/+ and Cyp27−/− mice had 5.2 ± 0.2 (n = 7) and 7.2 ± 0.2 (n = 9) g of liver per 100 g of body weight, respectively. However, in matching Cyp27−/− mice fed a diet supplemented with cholic acid for 10 days, relative liver weight contracted significantly to 5.9 ± 0.1 g/100 g of body weight. In contrast, adrenal gland enlargement was not reversed by cholic acid feeding. Thus, on the basal diet, the Cyp27+/+ and Cyp27−/− mice had 14.4 ± 0.7 (n = 5) and 22.8 ± 1.3 (n = 5) mg of adrenal/100 g of body weight, respectively. In the cholic acid-fed Cyp27−/− mice this value remained at 22.8 ± 1.6 (n = 5) mg/100 g of body weight. The hepatomegaly in the Cyp27−/− mice was also almost fully reversed when chenodeoxycholic acid was fed in place of cholic acid (data not shown).

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**DISCUSSION**

Several major findings from these studies warrant discussion. The first of these relates to bile acid metabolism. In this regard, the present data confirm and extend those of Rosen et al. (12). Thus, we found that adult Cyp27−/− mice fed a basal rodent diet synthesized essentially the same types of bile acids as did Cyp27+/+ controls, but at a markedly reduced rate despite a pronounced up-regulation of cholesterol 7α-hydroxylase. This diminished rate of synthesis was reflected in a marked reduction in biliary bile acid concentration, and a decisive contraction in bile acid pool size. From northern analyses, we showed that the changes in the mRNA levels of several of the major enzymes and related proteins involved in bile acid synthesis agreed well with those seen in the metabolic measurements. In particular, it was clear from the RNA blots that the absence of sterol 27-hydroxylase affected gene regulation by the bile acid nuclear receptor, farnesoid X receptor (13–16). Thus, the mRNA level of small heterodimer partner, a target gene of bile-acid activated farnesoid X receptor (15, 16), was reduced by 65%, and consequently the small heterodimer partner-mediated repression of CYP7A1 (15) was relieved, resulting in a nearly 10-fold increase in CYP7A1 mRNA levels. The northern blots also showed a diminution of oxysterol 7α-hydroxylase expression, and an appreciable rise in the mRNA level for CYP8B1, which fits well with the observed shift toward greater cholic acid enrichment of the intestinal bile acid pool in the Cyp27−/− mice.
of triol 25-hydroxylase. Although this mechanism seems not to occur in cerebrotendinous xanthomatosis patients (29), it may account for the modest rate of bile acid synthesis found here in the sterol 27-hydroxylase-deficient mouse. Two points about the triol 25-hydroxylase pathway are noteworthy. One is that it must be operational at or before birth because Cyp27<sup>−/−</sup> pups, unlike those lacking cholesterol 7α-hydroxylase (30), require no dietary bile acid supplementation. The other point is that although this pathway apparently generates sufficient bile acids to maintain a pool large enough to allow lipid absorption while the Cyp27<sup>−/−</sup> pups are nursing, it nevertheless fails to expand bile acid pool size to normal levels in adulthood. Hence, mature Cyp27<sup>−/−</sup> mice, like those missing cholesterol 7α-hydroxylase (23), absorb very little cholesterol, show marked compensatory increases in hepatic and intestinal cholesterol synthesis, and excrete substantially greater amounts of cholesterol in their feces. These adaptive changes allow whole body cholesterol content to remain normal in both knockout mice, even though there are significant changes in the concentration of cholesterol in the adrenal and lung of the Cyp27<sup>−/−</sup> animals. These mice, unlike those missing cholesterol 7α-hydroxylase, also manifest a significant increase in the relative cholesterol content of the bile. This difference, and the potential for each of these models to spontaneously develop cholesterol gallstones with age, requires additional study.

The second major finding reported here concerns the changes in hepatic fatty acid metabolism and plasma VLDL-triacylglycerol concentrations that accompany sterol 27-hydroxylase deficiency. While these perturbations were fully reversed by cholic acid feeding, the prevailing question is whether they are the product of CYP27 deficiency per se, or ultimately are the result of the pronounced induction of cholesterol 7α-hydroxylase and hepatic cholesterol synthesis. Marked up-regulation of both of these pathways accompanies cholestyramine feeding, a treatment that raises plasma triacylglycerol levels in humans (17–19) and rats (31), but not in mice. One way to possibly address this question would be to make similar measurements to those described here in mice in which CYP27 has been overexpressed.

The third important finding relates to the putative role of 27-hydroxycholesterol as a regulator of cholesterol biosynthesis (20–22). The sterol synthesis data presented here represent the first direct quantitation of the absolute rates of sterol synthesis in mice deficient in sterol 27-hydroxylase. Together, these data do not support the view that 27-hydroxycholesterol plays a physiologically important role in regulating cholesterol biosynthesis in the intact animal. If this were the case, then deletion of the CYP27 gene might be expected to result in an

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2 S. D. Turley and J. M. Dietschy, unpublished data.
marked increase in hepatic and adrenal sterol synthesis was not due to the absence of 27-hydroxycholesterol in these tissues because the restoration of intestinal cholesterol absorption that accompanied dietary cholic acid supplementation resulted in marked suppression of cholesterol synthesis in these two organs to rates that were well below those seen in the Cyp27<sup>−/−</sup> mice fed only the basal diet. This suppression should not have occurred if the increased cholesterol synthesis in these organs under basal dietary conditions was due solely to the absence of 27-hydroxycholesterol. Although it is unclear why sterol 27-hydroxylase deficiency had such a variable effect on cholesterol synthesis in the different organs, in the case of the liver and small intestine, most, if not all, of the stimulation seen in these two organs was likely just a compensatory response to the near zero level of cholesterol absorption. A similar adaptive response is seen in mice lacking cholesterol 7α-hydroxylase, although in those animals there is no up-regulation of sterol synthesis in the adrenal, spleen, and lung (23).

Finally, perhaps the most surprising new finding concerns the effect of sterol 27-hydroxylase deficiency on adrenal mass and cholesterol homeostasis. Presumably, the increase in adrenal size, cholesterol synthesis, and cholesterol content represented a compensatory response needed to maintain plasma corticosterone concentrations within a normal range. Although cholic acid feeding abolished the increase in adrenal steroid synthesis, it did not reverse adrenal gland enlargement, which, by histological analyses was found to reflect cortical cell hyper trophy. While it is not known whether adrenal enlargement occurs in cerebrotendinous xanthomatosis patients, one study reported no evidence of adrenal insufficiency in five out of six such individuals (8).

In summary, these studies have shown that while the loss of sterol 27-hydroxylase results in qualitatively the same adaptive changes in cholesterol balance across the whole body as those previously documented for mice lacking cholesterol 7α-hydroxylase, it also has a significant impact on hepatic fatty acid and triacylglycerol metabolism, and adrenal cholesterol homeostasis. Clearly, further studies in the Cyp27<sup>−/−</sup> mouse are needed to determine exactly how the expression of sterol 27-hydroxylase influences each of these processes. In addition, the deletion of other regulatory proteins involved in sterol metabolism in mice also deficient in sterol 27-hydroxylase should be pursued. For example, deleting the cholesterol 7α-hydroxylase gene in Cyp27<sup>−/−</sup> mice will potentially provide new information regarding other alternative pathways of bile acid synthesis, while removing the LDLR from mice lacking sterol 27-hydroxylase might unmask substantially greater hyperlipidemia than is seen in mice without the CYP27 gene. It will also be important to study both male and female animals in the future, given the marked gender differences in some aspects of cholesterol and bile acid metabolism that have been repeatedly documented in this species (25, 32–34).

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FIG. 9. Various parameters of sterol and triacylglycerol metabolism in Cyp27<sup>−/−</sup> mice fed cholic acid. Intestinal cholesterol absorption (A), adrenal sterol synthesis (B), hepatic sterol synthesis (C), hepatic fatty acid synthesis (D), hepatic triacylglycerol concentration (E), and plasma triacylglycerol concentration (F) in Cyp27<sup>−/−</sup> mice fed the basal diet containing cholic acid (0.1%, w/w) for 10 days compared with matching Cyp27<sup>+/+</sup> and Cyp27<sup>−/−</sup> mice fed the basal diet alone. Values represent the mean ± 1 S.E. of data from 5 to 7 mice in each group for every parameter. * p < 0.05 compared with value for Cyp27<sup>+/−</sup> animals.
Lipid Metabolism in Sterol 27-Hydroxylase-deficient Mice

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