Marked reduction in bile acid synthesis in cholesterol 7α-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia

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Abstract **These studies used mice that were deficient in cholesterol 7α-hydroxylase to determine the effects of reduced bile acid synthesis on cholesterol homeostasis. In mice lacking this enzyme, bile acid synthesis was reduced from 8.3 to 3.4 μmol/day per 100 g body weight, the intestinal bile acid pool was decreased from 62.5 to 13.2 μmol/100 g bw, and the proportion of hyodeoxycholate, relative to cholate, in this pool was significantly increased. Associated with these changes, intestinal cholesterol absorption decreased from 37% to <1% while triacylglycerol absorption and animal weight gain remained essentially unaffected. The very low rate of cholesterol absorption could be corrected by feeding the mutant mice cholate, but not hyodeoxycholate. The reduction in sterol uptake across the intestine was associated with a 2-fold increase in cholesterol synthesis in the small bowel and liver and an increase in fecal neutral sterol excretion from 15.2 to 35.7 μmol/day per 100 g bw. The size of the cholesterol pools in the plasma, various organs and whole animal remained constant. Thus, under circumstances where the excretion of sterol as bile acids was markedly reduced, total cholesterol turnover actually increased from 164 to 239 mg/day per kg bw. This study demonstrates the complex interactions between bile acid and cholesterol metabolism and the dramatic effects of eliminating a single gene product; however, even though a major catabolic pathway was deleted, cholesterol balance across the animal was maintained.—**Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. **Marked reduction in bile acid synthesis in cholesterol 7α-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J. Lipid Res.* 1998.** 39: **1833–1843.**

Supplementary key words cholesterol 7α-hydroxylase • cholesterol absorption • cholesterol synthesis • bile acid pool size • liver • small intestine

Control of the biosynthetic pathways that lead to bile acid formation is of critical importance in maintaining overall sterol balance in the animal, in regulating the circulating levels of cholesterol carried in low density lipoprotein (LDL-C), and in facilitating the intestinal absorption of a variety of dietary lipids, fat soluble vitamins, and cholesterol (1–5). Feeding exogenous bile acid, for example, can partially suppress endogenous bile acid synthesis, enhance intestinal cholesterol absorption, and raise circulating plasma LDL-C concentrations. Enhancing bile acid loss from the body, on the other hand, can lead to an increase in the rate of endogenous bile acid synthesis, suppression of intestinal cholesterol absorption, and a decrease in the circulating levels of plasma sterol (6, 7).

It is now recognized that there are at least two pathways for the conversion of cholesterol to bile acid, and these two pathways involve two distinct 7α-hydroxylase enzymes. The first of these is initiated in the endoplasmic reticulum by cholesterol 7α-hydroxylase (*Cyp7a* in mice), a well-characterized cytochrome P450 enzyme that catalyzes the formation of 7α-hydroxycholesterol (8). The second pathway is initiated by the hydroxylation of a carbon atom in the side chain of cholesterol with the formation of oxysterols such as 27-hydroxycholesterol which, in turn, are hydroxylated in the 7α position by a distinct oxysterol 7α-hydroxylase (*Cyp7b1* in the mouse) (9–11). It should be emphasized that *Cyp7a* cannot hydroxylate 27-hydroxycholesterol and *Cyp7b1* cannot hydroxylate unmodified cholesterol. These two pathways, both of which lead to the formation of 7α-hydroxylated bile acids, are regulated differently (11). Output from the cholesterol 7α-hydroxylase pathway is controlled by the return of bile acids to the liver via the enterohepatic circulation. Expansion of this pool leads to decreased transcription of the cholesterol 7α-hydroxylase gene, while depletion of this pool increases transcription of the gene (12, 13). In contrast, manipulating the size of the bile acid pool has a much smaller effect on the flow of cholesterol through the oxysterol 7α-hydroxylase pathway

Abbreviations: LDL-C, low density lipoprotein-cholesterol; LDLR, LDL receptor; HDL, high density lipoprotein; DPS, digitonin-precipitable sterols; IBAT, ileal bile acid transporter.

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[2]The cholesterol 7α-hydroxylase knockout mouse is available from The Jackson Laboratory (Bar Harbor, ME) where it is listed as *Cyp7a1* in the Mouse Genome Database.

(11). The relative contribution of the two 7α-hydroxylase pathways to overall bile acid synthesis is currently being investigated. In humans, the oxysterol 7α-hydroxylase pathway may account for up to 50% of total bile acid synthesis under certain metabolic conditions (10) and, in the rat, this pathway is apparently up-regulated when the cholesterol 7α-hydroxylase pathway is suppressed (14).

A genetic demonstration of the quantitative contribution of the oxysterol 7α-hydroxylase pathway comes from mice in which the cholesterol 7α-hydroxylase pathway was eliminated by disruption of the *Cyp7a* gene (15, 16). Approximately 90% of homozygous pups died in the first 3 wk of life as a result of fat and vitamin malabsorption. Death could be prevented in the neonatal period, however, by supplementing the diet of the nursing mothers with cholic acid and fat-soluble vitamins. In the absence of supplementation, survival of the homozygous offspring was correlated with the induction of the oxysterol 7α-hydroxylase pathway that takes place at about 3 wk of age (16). Once these animals reached adulthood, the phenotype of the mice appeared normal and the concentration of cholesterol and triacylglycerol in the plasma, and the distribution of these lipids in various lipoprotein fractions, was essentially the same as in the control animals (16).

The potential importance of these biosynthetic pathways in the regulation of plasma LDL-C levels and, hence, the severity of atherosclerosis is now partially understood. In all species that have been investigated, including the mouse and primate, >90% of the LDL receptor (LDLR) activity manifested in vivo is found in the liver (5, 17, 18). The level of hepatic LDLR activity in the steady state, in turn, is largely determined by the rate of net cholesterol delivery to the liver relative to the rate of net sterol excretion as either neutral or acidic sterols (3, 5). There are only two sources for net hepatic cholesterol acquisition. These include sterol that is newly synthesized in the extrahepatic organs and delivered to the liver in high density lipoprotein (HDL) and dietary cholesterol that reaches the liver carried in chylomicron remnants. Similarly, there are only two major processes for the removal of cholesterol from the liver. These include the net excretion of sterol across the canalicular membrane into bile either as cholesterol itself or as bile acid.

The balance between these rates of net hepatic sterol acquisition and excretion largely determines whether an individual animal (or human) responds to an increase of the level of dietary cholesterol with only a modest rise in the LDL-C level, i.e., the hyporesponder phenotype, or with a more marked increase, i.e., the hyperresponder (19). The metabolic difference between the hyperresponder and hyporesponder is remarkably subtle. In the cynomolgus monkey, for example, a difference in the net delivery of cholesterol to the liver of only 2.1 mg/d per kg bw is sufficient to produce the hyperresponder phenotype. Under circumstances where total cholesterol turnover equals about 16 mg/d per kg bw and where de novo cholesterol synthesis in the extrahepatic organs is the same, the hyperresponder monkey must either have absorbed an additional 2.1 mg/d per kg bw of cholesterol from the diet or, alternatively, must have converted 2.1 mg/d per kg bw less cholesterol to bile acids. Thus, the genetic reason for the hyperresponder phenotype in these animals must reside in one of the proteins that dictate either the rate of cholesterol absorption across the intestine or the rate of synthesis of bile acids.

Unfortunately, it has been impossible to directly test the role of bile acid synthesis in the regulation of cholesterol balance as animal models have not been available that are genetically identical except for their rates of bile acid synthesis. With the successful deletion of the *Cyp7a* gene in the mouse such a model is now available. These studies, therefore, were undertaken to define the effect of the loss of the cholesterol 7α-hydroxylase pathway on rates of bile acid synthesis and intestinal pool size, on rates of intestinal cholesterol absorption, and on overall sterol balance in the animal. They revealed that, contrary to expectations, net cholesterol turnover actually increased in animals with reduced bile acid synthesis.

MATERIALS AND METHODS

Animals and diets

Cholesterol 7α-hydroxylase-deficient mice were generated by gene disruption in a 129Sv-derived ES cell line (15). The mutation (*Cyp7a*−/−) was maintained in a mixed strain background (C57BL/6J/129Sv) by crossing homozygous carriers (15). C57BL/6J/129Sv hybrids served as control (*Cyp7a*+/+) animals.[2] To increase the survival rate of −/− pups, the diet of nursing females was routinely supplemented with a vitamin mixture and 1.0% (wt/wt) cholic acid until the fourth postnatal week (15). This supplementation did not alter any aspect of cholesterol metabolism that was subsequently measured at 3 months of age. Rates of cholesterol absorption, for example, were the same at 3 months in mutant animals regardless of whether or not their mothers received the supplemented diet during the 4-wk period of nursing. After the nursing period, and during all studies, −/− and +/+ animals 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. In some experiments, the meal form of this basal diet was supplemented with cholic acid, hyodeoxycholic acid (Sigma Chemical Co., St. Louis, MO), or hydrogenated coconut oil (ICN Pharmaceuticals, Costa Mesa, CA). All studies were carried out in male mice 3 months of age. They were housed in plastic colony cages containing wood shavings in a temperature-controlled room (22°C) with light cycling. All experiments were performed towards the end of the 12-h dark phase of the cycle and all mice were in the fed state at the time of study unless otherwise stated. Experiments were approved by the Institutional Animal Care and Research Advisory Committee.

Intestinal cholesterol and lipid absorption

Cholesterol absorption was measured by a fecal dual-isotope ratio method (20). Mice were dosed i.g. with a mixture of 2 μCi [5,6-^{3}H]sitostanol (American Radiolabeled Chemicals, Inc., St. Louis, MO) and 1 μCi [4-^{14}C]cholesterol (New England Nuclear, Boston, MA). They were then housed individually in fresh cages and stools were collected over the following 3 d. Aliquots of stool and the dosing mixture were extracted, and the ratio of ^{14}C to ^{3}H in each was determined. The percent cholesterol absorption was calculated from these data as described (20). To determine the

level of total lipid absorption, the lipid content of the diet and stools was determined gravimetrically. These data, together with the amount of diet consumed and stool excreted (both expressed as g/d per 100 g bw), were used to calculate the fraction of lipid consumed that was absorbed. In the study in which mice were fed diets containing increasing amounts of coconut oil, the data are presented as the total lipid content of the stools rather than as total lipid absorption. This calculation was done to allow a direct comparison between fecal lipid content in adult mice to that in suckling neonatal animals from an earlier study (16).

Bile acid pool size and composition

Pool size was determined as the total bile acid content of the small intestine, gallbladder, and liver, which were extracted together in ethanol with [24-^{14}C]taurocholic acid (New England Nuclear) added as an internal standard. The extract was filtered and brought to a final volume of 100 ml, of which a 20-ml aliquot was taken to dryness. The residue was dissolved in 1 ml of methanol and centrifuged at 14000 *g*, and the supernatant was passed through a filter (Acrodisc LC13 PVDF, 0.45 um) (Gelman Sciences, Ann Arbor, MI). A 100-μl aliquot of the filtrate was dried down and used for determination of recovery of the internal standard by scintillation counting. The remaining filtrate was subjected to HPLC using a C_{18} column (4.6 × 250 mm No. 054275, Waters Corp., Milford, MA) and a mobile phase consisting of methanol–water–glacial acetic acid 21:9:1 (v/v/v), pH 5.0, at a flow rate of 1.0 ml/min. Bile acids were detected by measurement of the refractive index using a model 410 differential refractometer (Waters Corp.) and identified by comparison of their respective retention times to those of authentic standards. For quantitation, peak areas were integrated using software (ValueChrom) from Bio-Rad Laboratories (Hercules, CA). Together with the recovery of the radiolabeled internal standard these data were used to calculate bile acid pool size, expressed as μmol/100 g bw, and the percentage of each bile acid in the pool.

Fecal bile acid and neutral sterol excretion

Stools collected from individually housed mice over 3 d were dried, weighed, and ground. A 1-g aliquot of this material was used to determine total bile acid content by an enzymatic method previously described (21). A second 1-g aliquot was subjected to alkaline hydrolysis at 120–130°C for 12 h. The sample was then dried and 10 ml of water and 10 ml of ethanol were added. The sample was extracted in 15 ml of petroleum ether to which had been added 1.0 mg of 5-cholestene (Sigma) as an internal standard. The amount of cholesterol, coprostanol, epicoprostanol, and cholestanone in the extracts was quantitated by gas chromatography (22). The daily excretion rates of both bile acid and neutral sterol were then calculated and expressed as μmol/d per 100 g bw.

Biliary lipid composition

Gallbladder bile was harvested from mice that had been fasted for 4 h. The absolute concentrations of bile acid, phospholipid, and cholesterol were measured as previously described (23) and were expressed as a molar percent of the total lipid content of the sample.

Sterol synthesis in liver and extrahepatic organs

The rate of sterol synthesis in all major organs was measured in vivo as described (18, 24, 25). Mice were given an i.p. injection of 40 mCi of [^{3}H]water (New England Nuclear) and after 1 h were anesthetized and exsanguinated. Aliquots of liver and various extrahepatic organs and the remaining carcass were saponified and their content of radiolabeled digitonin-precipitable sterols (DPS) was measured (24, 25). The rate of sterol synthesis in each organ was expressed as the nmol of [^{3}H]water incorporated into DPS per hour per g of tissue, while whole animal synthesis was calculated as μmol of [^{3}H]water incorporated per hour per 100 g bw. The rate of incorporation of [^{3}H]water into sterols by the whole body was converted to an equivalent mg quantity assuming 0.69 ^{3}H atoms were incorporated into the sterol molecule per carbon atom entering the biosynthetic pathway as acetyl CoA (26). After the labeled sterols were extracted, the saponified liver and small bowel samples were acidified and extracted with hexane. Aliquots of the organic phase were taken for measurement of [^{3}H-labeled] fatty acid content.

Cholesterol 7α-hydroxylase and oxysterol 7α-hydroxylase activity

These enzymes were assayed as described previously, using [4-^{14}C]cholesterol and 25-[26,27-^{3}H]hydroxycholesterol (New England Nuclear) as substrates, respectively (15, 16).

Preparation of brush border membranes for measurement of the ileal bile acid transporter protein

Small intestines were rinsed, cut into five sections of equal length, and homogenized. The membranes were prepared as described (27) and analyzed by SDS-PAGE and immunoblotting (28). The anti-IBAT polyclonal antibody used was a gift from P. A. Dawson, Bowman Gray School of Medicine.

Plasma, tissue, and dietary cholesterol levels

Plasma total cholesterol concentrations were measured using a reagent mixture (No. 1127771) supplied by Boehringer Mannheim (Indianapolis, IN). Aliquots of liver, extrahepatic tissues, and the residual carcass (consisting principally of skeleton, muscle, skin, and adipose tissue) were saponified and extracted, and their cholesterol concentrations were measured by GC using stigmastanol (Sigma) as an internal standard (22). The total cholesterol concentration in each of the tissues (mg/g) was multiplied by the respective organ weight, and whole organ cholesterol contents were then summed to give whole animal cholesterol content, which was expressed as mg/100 g bw. Dietary cholesterol levels were also determined by GC using 5-cholestene as an internal standard (22).

Analysis of data

All data are reported as the mean ± 1 SEM in the specified number of individual animals. Differences between mean values were tested for statistical significance ($P < 0.05$) by the two-tailed Student's *t*-test assuming equal variance.

RESULTS

Tissue cholesterol concentrations and whole animal cholesterol contents

Initial experiments were undertaken to measure the cholesterol pools in the various tissues and whole animal in the two genotypes. The adult, 3-month-old +/+ and −/− male mice fed the low cholesterol basal diet to steady state did not differ with respect to food intake (20.4 ± 2.2 and 21.9 ± 3.5 g/d per 100 g bw, respectively), and had similar body weights at the time of study (27 ± 1 g and 26 ± 1 g, respectively). There was also no difference in the distribution of body mass among the various organs in mice of the two genotypes. Plasma total cholesterol concentrations averaged 93 ± 3 mg/dl in +/+ mice and 96 ± 7

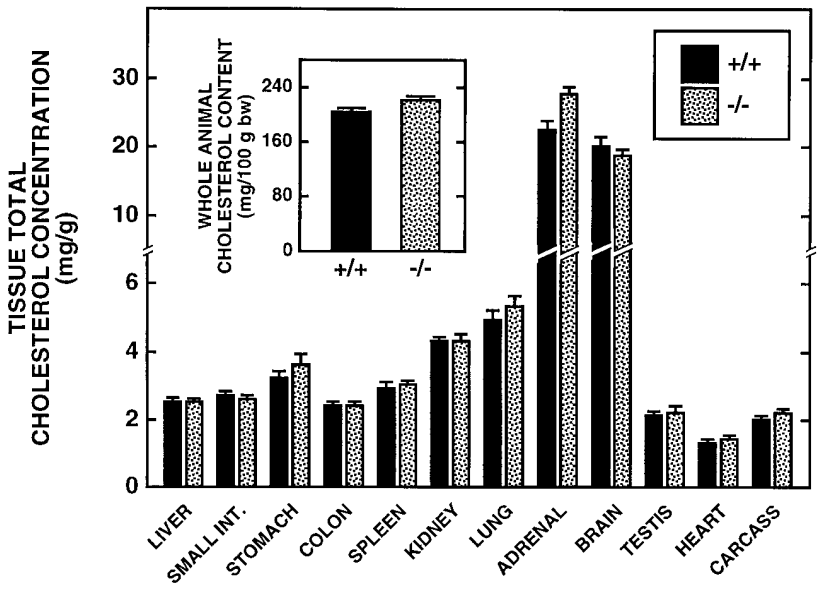

Fig. 1. Tissue cholesterol concentrations in cholesterol 7α-hydroxylase +/+ and −/− mice. Male mice 3 months of age were fed ad libitum a basal cereal-based rodent diet without added cholesterol. In the case of the liver, small intestine, adrenal, and whole animal (shown in inset), the values represent the mean ± 1 SEM of data from nine to twelve animals. For all other tissues, values represent data from six +/+ and five −/− animals. In no individual organ was the difference in mean values statistically significant.

mg/dl in −/− animals, and the level of apolipoprotein B in the plasma was similar in both genotypes as judged by immunoblotting analysis (29). Most importantly, as shown in **Fig. 1**, there was no significant difference in the concentration of cholesterol in any organ so that the pool of sterol in the whole animal equalled approximately 210 mg/100 g bw in both the +/+ and −/− genotypes (insert). Thus, in contrast to expectations, deletion of cholesterol 7α-hydroxylase activity in the mouse did not alter the steady-state cholesterol pool in the plasma, liver, small intestine, other extrahepatic organs, or whole animal.

Bile acid metabolism

Given the fact that 7α-hydroxylase deletion did not alter cholesterol pools in the mouse, it was of crucial importance to establish that there was, in fact, a significant quantitative change in bile acid metabolism in the −/− animals. As summarized in **Table 1**, while no cholesterol 7α-hydroxylase activity was detected in the livers of the −/− mice, there was no adaptive increase in the alternative biosynthetic pathway, as manifested by the level of oxysterol 7α-hydroxylase in these adult animals. As a consequence, the absolute rate of bile acid synthesis, as mirrored by fecal bile acid excretion, equalled only 3.4 μmol/d per 100 g bw in the −/− mice compared to a rate of 8.3 μmol/d per 100 g bw in the control animals, and the steady-state pool of bile acids in the small intestine and biliary tract was reduced from 62.5 μmol/100 g bw to only 13.2 μmol/100 g bw (Table 1). Nevertheless, the molar ratio of cholesterol in the bile was the same (1.7%) in the control and mutant animals. Strikingly, however, while deletion of cholesterol 7α-hydroxylase significantly reduced the rate of bile acid synthesis and excretion in −/− mice, there was a parallel 2.3-fold increase in fecal cholesterol excretion, from 15.2 to 35.7 μmol/d per 100 g bw in these animals (Table 1).

We next investigated whether there was a correlation between bile acid pool size and the level of expression of the ileal sodium-dependent bile acid transporter (IBAT), which mediates the return of bile acids to the liver (30).

TABLE 1. Parameters of sterol metabolism in cholesterol 7α-hydroxylase +/+ and −/− mice

Parameter	Genotype +/+	Genotype −/−
Cholesterol 7α-hydroxylase (pmol/min per mg protein)	50.0 ± 6.0 (3)	0 (3)[a]
Oxysterol 7α-hydroxylase (pmol/min per mg protein)	6.7 ± 1.3 (5)	4.7 ± 2.0 (5)
Fecal bile acid excretion (μmol/d per 100 g bw)	8.3 ± 1.1 (6)	3.4 ± 0.6 (6)[a]
Bile acid pool size (μmol/100 g bw)	62.5 ± 4.2 (4)	13.2 ± 3.2 (4)[a]
Cholesterol in gallbladder bile (molar ratio)	1.7 ± 0.1 (4)	1.7 ± 0.2 (4)
Fecal cholesterol excretion (μmol/d per 100 g bw)	15.2 ± 1.0 (10)	35.7 ± 1.6 (10)[a]
Total fecal sterol excretion (μmol/d per 100 g bw)	23.5 ± 1.5 (16)	39.1 ± 1.7 (16)[a]

Three-month-old male mice were housed individually and fed ad libitum the basal rodent diet without added cholesterol. Different groups of animals were used for the sterol excretion, pool size, and enzyme activity measurements. Stool was collected over a period of 72 h and the contents of bile acids and neutral sterols (cholesterol and its derivatives coprostanol, epicoprostanol, and cholestanone) were determined. Bile acid pool size represents the total bile acid content of the small intestine, gallbladder, and liver combined. Values represent the mean ± 1 SEM of data from the number of animals given in parentheses.

[a] The value for the −/− animals is significantly different from that for the +/+ mice ($P < 0.05$).

Immunoblotting experiments with brush border membrane preparations from different intestinal sections of +/+ and −/− mice established that IBAT expression was confined to the terminal sections of the small intestine in both genotypes, as shown in **Fig. 2**. More importantly, the level of transporter expression in the +/+ and −/− mice appeared to be identical. Thus, the maximal transport velocity of bile acid absorption across the ileum presumably was unchanged in the −/− mice. Histological examination of sections from the duodenum, jejunum, and ileum of both types of mice revealed morphologically intact microvilli and epithelial cell layers of normal thickness. Similar analyses of the liver in these animals also showed normal morphology with well-defined portal triads and no signs of cholestasis.

Taken together, these initial studies demonstrated that bile acid synthesis in the mutant animals was markedly reduced, and this presumably accounted for the similar reduction in the size of the functional bile acid pool in the enterohepatic circulation. However, while deletion of cholesterol 7α-hydroxylase significantly reduced fecal acidic sterol excretion, total fecal sterol output markedly increased, from 23.5 μmol/d per 100 g bw in the control mice to 39.1 μmol/d per 100 g bw in the mutant animals (Table 1).

Intestinal cholesterol and total lipid absorption

The reduction in bile acid pool size in the −/− animals, combined with the earlier observation that these mice deliver significant amounts of hyodeoxycholic acid into this pool (15, 16), raised the possibility that cholesterol and total lipid absorption might also be impaired in this model. The data in **Fig. 3** show that while the level of intestinal cholesterol absorption averaged 37 ± 4% in +/+ mice, there was, indeed, no detectable absorption of sterol in the knockout animals (A). In contrast, these mice still absorbed about 78% of their total daily lipid intake (B). To investigate whether this slight impairment of fat absorption would become more pronounced with an increasing dietary lipid load, control and knockout animals were challenged with diets containing increasing amounts of triacylglycerol. As seen in **Fig. 4A**, the stool fat content was markedly higher in suckling −/− neonates. In contrast, adult animals of either genotype exhibited little steatorrhea, even when the lipid content of the diet was raised to 35%.

The data presented in Figs. 3 and 4 established that −/− mice failed to absorb dietary cholesterol but had essentially normal levels of triacylglycerol absorption. Studies were next undertaken to determine whether this major suppression of cholesterol absorption was a result of events within the intestinal lumen, i.e., reduction in bile acid pool size and hydrophobicity, or was the result of an

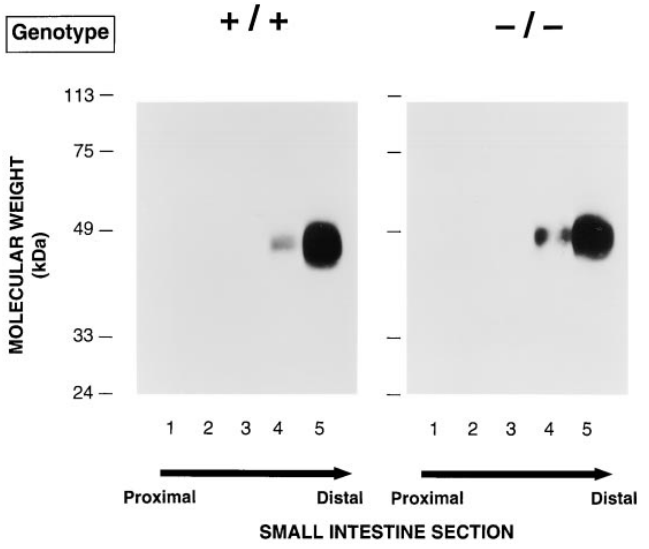

Fig. 2. Expression of the bile acid transporter in the small intestine of cholesterol 7α-hydroxylase +/+ and −/− mice fed the basal diet. Small intestines were cut into five sections of equal length and homogenized. Brush border membranes were prepared by the calcium precipitation method (27). Membrane proteins were separated on a denaturing 10% polyacrylamide gel, transferred to a polyvinylfluoride membrane, and incubated with anti-IBAT polyclonal antibody as described (28).

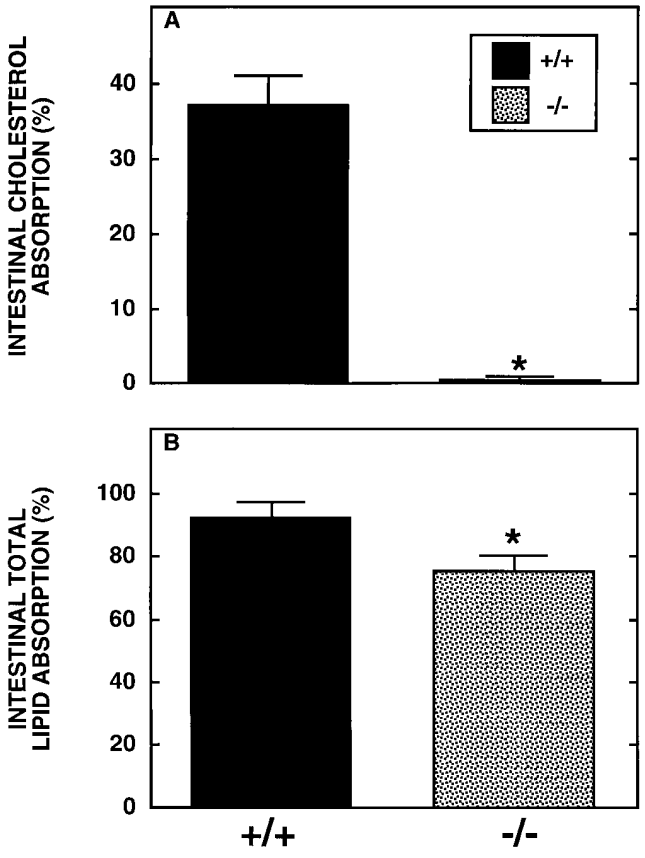

Fig. 3. Levels of intestinal cholesterol and total lipid absorption in cholesterol 7α-hydroxylase +/+ and −/− mice. Male mice 3 months of age were individually housed and fed the basal rodent diet. Cholesterol absorption (A) was measured by a fecal dual-isotope ratio method (20). Total lipid absorption (B) was determined from measurements of dietary lipid intake and fecal lipid excretion. Values are the mean ± 1 SEM of data from four animals of each genotype. An asterisk denotes that the value for the −/− animals is significantly different from that for the +/+ mice ($P < 0.05$).

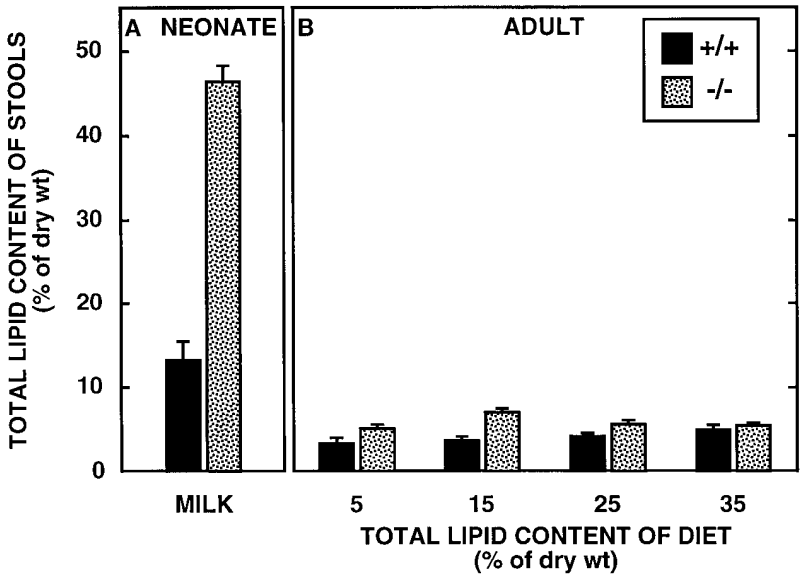

Fig. 4. Stool lipid content in cholesterol 7α-hydroxylase +/+ and −/− mice. The stool of neonatal animals was collected from suckling +/+ and −/− mice. The lipid content of dam's milk was approximately 10% (wt/wt). Three-month-old adult animals were individually housed and initially fed the basal diet that had a total lipid content of approximately 5%. Stools were collected over a 48-h period after which mice were switched to coconut oil-enriched diets. The values for the neonatal animals were reported earlier (16) and represent the mean ± 1 SEM of data from five +/+ and −/− pups. Adult values represent the mean ± 1 SEM of data from three +/+ and eight −/− animals, respectively.

unexpected defect in the trans-epithelial movement of sterol in the −/− animals. Groups of animals were fed diets supplemented with cholic acid or hyodeoxycholic acid. After a feeding period of 11 d, intestinal cholesterol absorption was determined in mice of both genotypes. A recovery period of 3 wk then followed during which time all mice were fed the basal diet. After this interval, intestinal cholesterol absorption in both the knockout and control mice had returned to original levels (data not shown). After a further recovery period of 3 wk, the bile acid feeding experiment was repeated with the same groups of mice and the bile acid pool size was determined. The results of both experiments are summarized in **Fig. 5**. Supplementation of the diet with 0.2% cholic acid expanded the pool in the +/+ mice from 63 to 116 μmol/100 g bw and in −/− animals from 13 to 142 μmol/100 g bw. In both genotypes, cholic acid constituted >80% of the pool. At the same time, cholesterol absorption increased from 37 to 62% in the +/+ mice and from 0 to 42% in the −/− animals. In contrast, when the diet was supplemented with 0.2% hyodeoxycholic acid, the bile acid pool size decreased 37% in the +/+ animals but increased 3.6-fold in

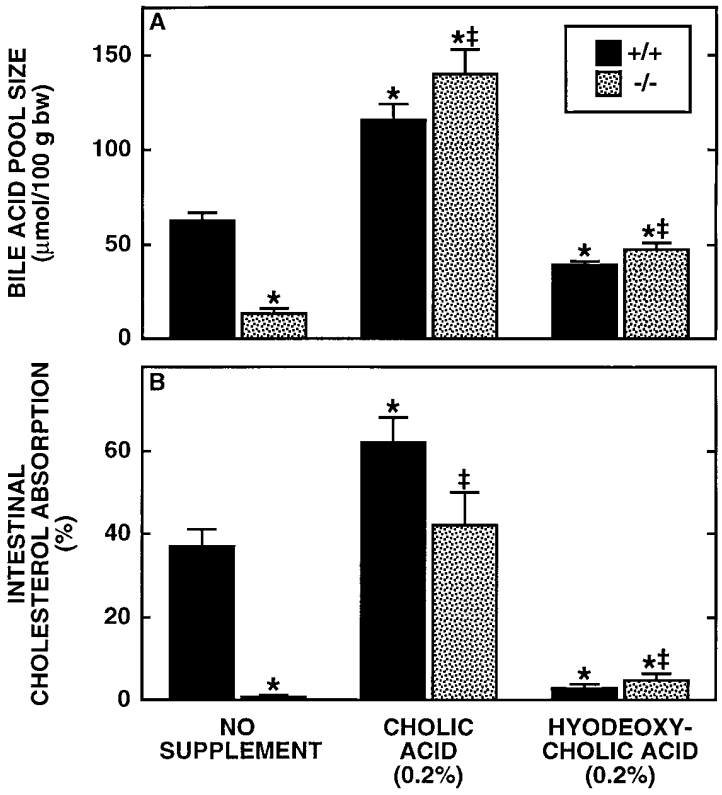

Fig. 5. Bile acid pool size and level of intestinal cholesterol absorption in cholesterol 7α-hydroxylase +/+ and −/− mice. Male mice of both genotypes were individually housed and fed ad libitum either a basal rodent diet or the same diet containing either cholic acid (0.2% wt/wt) or hyodeoxycholic acid (0.2%) for 11 days. The same group of animals was used for the measurement of bile acid pool size (A) and cholesterol absorption (B). Pool size represents the total bile acid content of the small intestine, gallbladder, and liver. Values equal the mean ± 1 SEM of data from four animals in each group. An asterisk denotes that the value for the −/− animals given the plain diet alone, or for either the +/+ or −/− mice in the groups given cholic or hyodeoxycholic acid, is significantly different from that for the +/+ animals fed the plain diet ($P < 0.05$). The ‡ denotes that the value for the −/− animals given either cholic or hyodeoxycholic acid is significantly different from that for −/− mice given the plain diet ($P < 0.05$).

the −/− mice. In both genotypes, therefore, the bile acid pool size achieved similar values, >80% of these pools was hyodeoxycholic acid and cholesterol absorption was reduced to <5%. Thus, the inability of the −/− mice to absorb dietary cholesterol resulted from a defect in luminal micellar solubilization that was a consequence of the small pool of hydrophilic bile acids normally present in these animals and not from a defect in epithelial cell sterol transport.

Rates of cholesterol synthesis in liver and extrahepatic organs

The finding that −/− mice maintained on the low cholesterol basal diet had normal plasma and tissue cholesterol levels despite absorbing negligible amounts of dietary cholesterol suggested that the small bowel or some other organ must compensate for the absence of exogenous cholesterol by raising its rate of de novo sterol synthesis. To test this hypothesis, [^{3}H]water was used as a precursor for the sterol biosynthetic pathway to first measure the rate of cholesterol synthesis in vivo along the length of the small intestine. The profile of synthetic activity found for both genotypes is shown in **Fig. 6**. In most sections of the intestine, sterol synthetic activity was 2- to 3-fold greater in the −/− animals than in +/+ controls. A comprehensive study of sterol synthetic activity in the other organs of the +/+ and −/− mice was next performed. As shown in **Fig. 7A**, the rate of cholesterol synthesis per g of tissue was increased 2-fold in the small intestine and liver of the −/− mice. A smaller, but significant, increase in synthesis was also seen in the residual tissues of the carcass. There was, however, no change in the rate of fatty acid synthesis in the liver or small intestine of the −/− animals (data not shown), and there was no significant alteration in the rate of cholesterol synthesis in any of the other organs of the body. The data in Fig. 7A were used to calculate whole organ sterol synthesis (B), and from these calculations, whole animal sterol synthesis (B, inset). All values in Fig. 7B, including those in the inset, represent the μmol of [^{3}H]water incorporated into sterols and adjusted to a constant animal weight of 100 g. The data show that in −/− mice, whole body sterol synthesis was nearly 60% higher than in +/+ mice. Furthermore, 76% of this additional synthetic activity was accounted for in the small intestine and liver, the two organs in the body principally responsible for the absorption and processing of dietary cholesterol. These findings confirm the earlier observations on external sterol balance (Table 1) that in the absence of cholesterol 7α-hydroxylase there is increased, not reduced, cholesterol turnover in the whole animal.

DISCUSSION

These studies illustrate that disruption of the 7α-hydroxylase gene does not, as expected, reduce cholesterol turnover in the mouse but, rather, this manipulation initiates a series of more complex events that dramatically affect cholesterol balance in the whole animal. Cholesterol absorption is reduced to undetectable levels, and the source of sterol is replaced by a compensatory increase in sterol synthesis in the intestine and liver of the mutant animals. Thus, despite marked reduction of bile acid synthesis and intestinal pool size, tissue and plasma cholesterol levels remain unchanged. These findings illustrate the wide range of adaptations possible in the opposing pathways of cholesterol supply and breakdown, and provide detailed information on how variation in the output from one of these pathways is balanced to maintain cholesterol homeostasis in the whole organism.

In all species that have been studied, including the primate, liver plays the central role of maintaining net cho-

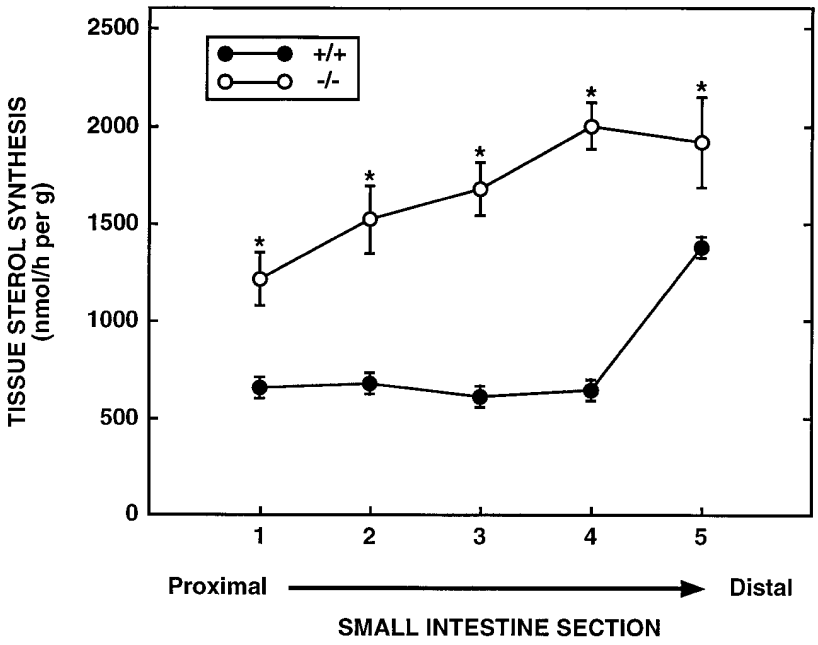

Fig. 6. Rates of sterol synthesis in different regions of the small intestine in cholesterol 7α-hydroxylase +/+ and −/− mice. Male mice of both genotypes were fed ad libitum the basal rodent diet. Animals were administered a bolus of [^{3}H]water i.p. and killed 1 h later. The rate of sterol synthesis is expressed as the nmol of [^{3}H]water incorporated into digitonin-precipitable sterols per hour per gram tissue. Values are the mean ± 1 SEM of data from six +/+ and five −/− animals, respectively. An asterisk denotes that the value for that particular section of intestine in the −/− animals is significantly different from the value for that same section in +/+ mice ($P < 0.05$).

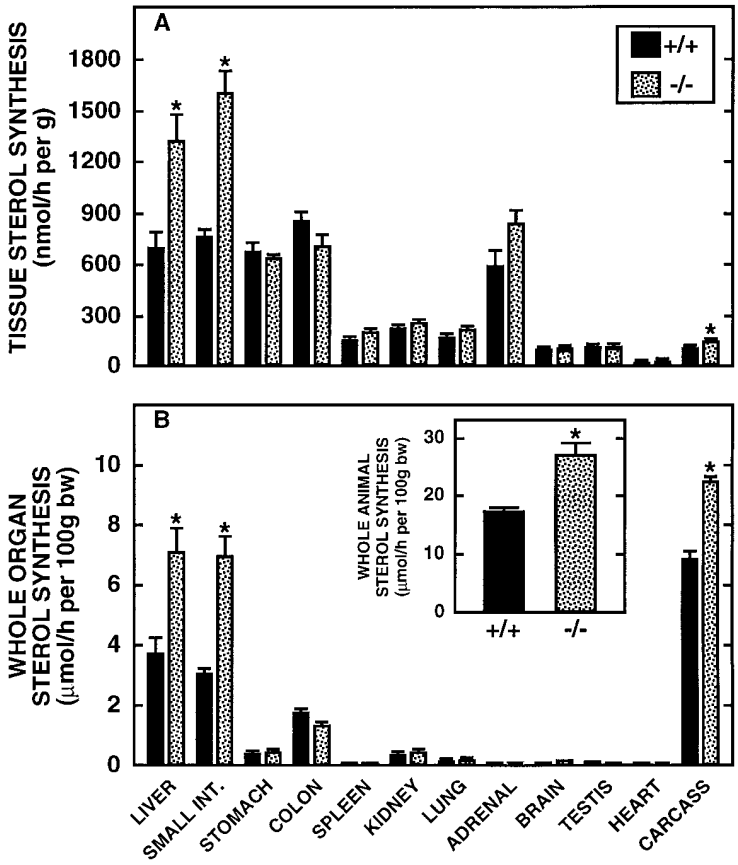

Fig. 7. Rates of sterol synthesis in the liver and extrahepatic organs of cholesterol 7α-hydroxylase +/+ and −/− mice. Mice of both genotypes were fed ad libitum the basal rodent diet. They were administered a bolus of [^{3}H]water i.p. and after 1 h were anesthetized and exsanguinated. The rates of sterol synthesis in each organ (A) are expressed as the nmol of [^{3}H]water incorporated into [^{3}H]DPS per hour per gram tissue. These rates were used to calculate the rates of whole organ sterol synthesis, which were then normalized to 100 g body weight (B). Whole animal sterol synthesis was calculated as the sum of the rates in all the organs. Values represent the mean ± 1 SEM of data from six +/+ and five −/− animals, respectively. An asterisk denotes that the value for the −/− animals is significantly different from that for the +/+ mice ($P < 0.05$).

lesterol balance across the whole animal and of regulating the levels of circulating lipoprotein-cholesterol. Clearly, these two processes are interrelated. In any animal on a relatively low cholesterol diet, most of the net input of sterol to the liver comes from de novo synthesis in the extrahepatic organs while a lesser amount is derived from the diet (5, 17). In the steady state, this rate of net input must be balanced by an equal rate of net sterol output from the liver as cholesterol itself or as bile acid. While small amounts of cholesterol can be excreted through the skin and as steroid hormones, the majority is disposed of through the gastrointestinal tract as either neutral or acidic fecal sterols (3). To a limited degree the liver can compensate for a change in these rates of net sterol flux. For example, hepatic cholesterol synthesis may be reduced under circumstances where there is an increase in sterol absorption from the diet or diminished bile acid synthesis. However, once hepatic synthesis approaches zero, further increases in net cholesterol delivery to the liver are associated with an expansion of the cholesteryl ester pool, suppression of LDLR activity, an increase in the LDL-C production rate, and a rise in the circulating LDL-C concentration (6, 7, 31).

Within a population, there are individuals who respond to dietary cholesterol challenge with either a smaller or larger change in the concentration of plasma cholesterol. Individuals in whom a smaller increase is realized are referred to as hyporesponders whereas those who experience a larger increase are termed hyperresponders (32). These phenotypes are heritable and can arise from polymorphisms that affect the number or functional affinity of proteins such as the LDLR (33, 34) or polymorphisms that result in a change in net delivery of cholesterol to the liver. In theory, for example, the hyperresponder phenotype could be the result of a polymorphism in one of the proteins that leads to cholesterol overproduction in the peripheral organs, increased sterol absorption across the intestine, or diminished bile acid synthesis. While peripheral cholesterol overproduction as a cause of this phenotype has been ruled out, at least in one primate (19), increased cholesterol absorption has been described in several species (35–39). However, the role of diminished bile acid synthesis has yet to be evaluated in quantitative terms. The current studies, therefore, took advantage of the newly described cholesterol 7α-hydroxylase knockout animal to obtain detailed quantitative data on the effect of diminished bile acid synthesis on the many processes that determine net sterol balance in the mouse.

To facilitate direct comparison of the various net flux rates, the measurements made in this study have been converted to mg of cholesterol flowing through each pathway per d per kg bw, and these are summarized in **Fig. 8**. In control mice, the net input of cholesterol into the body equalled 164 mg/d per kg bw, 41 mg of which came from intake of the diet while 123 mg was newly synthesized (Fig. 7). Furthermore, of the sterol ingested in the diet, at least 15 mg/d per kg bw was absorbed across the small bowel (Fig. 3) and entered the cellular pools of the intes-

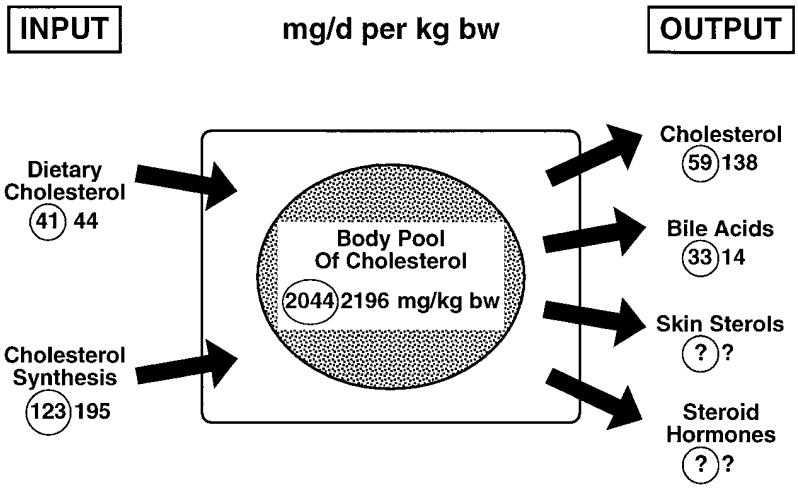

Fig. 8. Summary of the pathways involved in whole body cholesterol flux in cholesterol 7α-hydroxylase +/+ and −/− mice fed a low cholesterol diet. The values shown for cholesterol input (diet plus synthesis) and output (fecal excretion of neutral sterols and bile acids) were obtained directly from measurements made in the present study. The value for cholesterol output includes three other neutral sterols: coprostanol, epicoprostanol, and cholestanone. All parameters are expressed in terms of mg cholesterol per day per kg bw except for those for body cholesterol pool size which represent mg/kg bw. The values in the circles are for the +/+ animals, while the numbers adjacent to the circles are for the −/− mice.

tine and liver. Of the total 164 mg of cholesterol fluxing through the system, 92 mg/d per kg bw was excreted as either fecal neutral sterols (59 mg) or as acidic (33 mg) sterols (Fig. 8). Output as skin sterols and steroid hormones was not quantitated in these experiments although, in contrast to the primate, these pathways are known to be relatively large in small rodents (40). In the cholesterol 7α-hydroxylase-deficient mice, the excretion of cholesterol as bile acid was reduced to only 14 mg/d per kg bw (Fig. 8). However, because the absorption of cholesterol in these animals was also essentially reduced to zero (Fig. 3), the rates of cholesterol synthesis in the intestine and liver doubled (Fig. 7), and this was reflected in a marked increase in fecal cholesterol output from 59 to 138 mg/d per kg bw (Fig. 8). Thus, paradoxically, while deletion of the 7α-hydroxylase gene reduced the net excretion of cholesterol as bile acid by 19 mg/d per kg bw, net sterol flux through the whole animal increased from 164 mg/d per kg bw in the +/+ mice to 239 mg/d per kg bw in the −/− animals. Despite this difference, however, the steady-state levels of cholesterol in all of the organs (Fig. 1), as well as the plasma and the whole animal (Figs. 1, 8), remained essentially unchanged.

In the mutant mice, a significant reduction in the intestinal bile acid pool and hydrophobicity had only a minimal effect on total lipid absorption but essentially eliminated cholesterol absorption (Figs. 3, 4). These findings are fully explained by the known effects of bile acid pool size and hydrophobicity on unesterified cholesterol movement across the intestinal brush border (41). There is currently no convincing evidence that uptake of free fatty acid, monoacylglycerol, or cholesterol is receptor dependent. Rather, uptake appears to be passive and is made unidirectional through the immediate esterification of these molecules in the epithelial cell by the monoacylglycerol and α-glycerol phosphate pathways in the case of fatty acid and monoacylglycerol, and the acyl-coenzyme A:cholesterol acyltransferase pathway in the case of cholesterol (42). The rate-limiting step in these processes resides at the interface of the bulk luminal phase and the brush border membrane. The rate of uptake of these lipids is equal to the product of their maximal concentration at this aqueous/lipid interface and their respective passive permeability coefficients (43, 44). In the presence of significant diffusion barriers (45) this maximal concentration term, in turn, is critically dependent upon the presence of bile acid micelles in the bulk phase.

In the presence of an adequate concentration of hydrophobic bile acids, the product of these two constants is more than 100-fold larger for fatty acid and monoacylglycerol than for cholesterol, and this finding is apparently one factor that accounts for the 100-fold greater absolute rate of dietary triacylglycerol absorption (100–150 g/d in humans) than of cholesterol uptake (<1 g/d) in most species. Furthermore, this model also predicts that the intestinal uptake of very hydrophobic molecules like cholesterol is much more dependent upon micellar solubilization than is the uptake of fatty acid and monoacylglycerol. This has been found experimentally to be the case both here and in humans. In the absence of bile acids in the intestinal lumen in humans, for example, fatty acid absorption is decreased only 15–25% while cholesterol uptake is essentially abolished (41, 46, 47).

The current studies provide important insights into the quantitative importance of the oxysterol 7α-hydroxylase pathway for overall bile acid synthesis in the mouse. With deletion of the cholesterol 7α-hydroxylase pathway, bile acid synthesis decreased from 33 mg/d per kg bw to only 14 mg/d per kg bw (Fig. 8). As there was apparently no compensatory increase in the velocity of the oxysterol 7α-hydroxylase pathway in the −/− mice, at least as judged by the levels of enzyme activity (Table 1), this finding suggests that normally about 40% of bile acid synthesis in the mouse occurs through this pathway. Quantitatively, this value is similar to that reported in humans (10). Furthermore, as there was also no change in the ileal bile acid transporter level (Fig. 2), the marked reduction in pool size (Table 1) was presumably due solely to the observed reduction in bile acid synthesis in the mutant animals.

This reduction in the size and hydrophobicity of the bile acid pool, and the attendant reduction of cholesterol absorption, increased the rate of sterol synthesis along the

small intestine. In the normal animal, including the human and other primates, cholesterol synthesis is partially suppressed in the proximal and midintestine by the dietary and biliary sterol that is being continuously absorbed across the epithelial cells of this region (1, 48, 49). Only in the terminal ileum, where luminal bile acid concentration decreases to very low levels and cholesterol uptake ceases, is the rate of cholesterol synthesis in the intestinal epithelium relatively high (Fig. 6). When the bile acid concentration in the intestinal lumen is markedly reduced, the rate of sterol synthesis increases at all levels of the small intestine although, as seen in the −/− mice, the greatest relative increase is always in the mid-intestinal segments (Fig. 6) (48, 49).

Thus, taken together, these studies demonstrate the role of bile acid synthesis in maintaining sterol balance in the whole animal and how this process may contribute physiologically to the genesis of the hyporesponder and hyperresponder phenotypes. This role is complex, however, as changes in rates of synthesis affect the size and composition of the intestinal pool of bile acid which, in turn, alters dietary cholesterol entry into the body. Three examples of this complexity can be cited. First, increased excretion of cholesterol as bile acid might, in theory, lead to enhanced net sterol loss from the liver and the hyporesponder phenotype. This could arise from a genetically or pharmacologically dictated increase in the velocity of either the cholesterol 7α-hydroxylase or oxysterol 7α-hydroxylase pathway, or both. However, such an increase might well expand the intestinal pool of bile acid and increase the rate of dietary cholesterol absorption. Depending upon whether the change in cholesterol absorption was greater than or less than the increase in the rate of bile acid synthesis, the individual could exhibit the hyperresponder or hyporesponder phenotype, respectively. Second, reduced excretion of cholesterol as bile acid might, in theory, lead to the hyperresponder phenotype. However, as illustrated by the present studies, this reduction in bile acid synthesis could be offset by reduced dietary cholesterol absorption and, depending upon the relative magnitude of these two processes, could lead to either of the phenotypes. Finally, any change in the two bile acid pathways that alters the mean hydrophobicity of the intestinal pool could also profoundly change the rate of cholesterol absorption and, hence, the phenotype of the animal without altering the absolute rates of bile acid synthesis. These possible outcomes emphasize the importance of obtaining quantitative data regarding the other interlocking sterol pathways before attempting to interpret genetic abnormalities in these pathways or designing pharmaceutical agents to modulate the rates of bile acid synthesis.

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REFERENCES

1. Stange, E. F., and J. M. Dietschy. 1985. Cholesterol absorption and metabolism by the intestinal epithelium. *In* Sterols and Bile Acids. H. Danielsson, and J. Sjövall, editors. Elsevier Science Publishers, New York. 121–149.
2. Westergaard, H., and J. M. Dietschy. 1987. The uptake of lipids into the intestinal mucosa. *In* Membrane Transport Processes in Organized Systems. T. E. Andreoli, J. F. Hoffman, D. D. Fanestil, and S. G. Schultz, editors. Plenum Medical Book Company, New York. 213–224.
3. Turley, S. D., and J. M. Dietschy. 1988. The metabolism and excretion of cholesterol by the liver. *In* The Liver: Biology and Pathobiology. I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 617–641.
4. Carey, M. C., and O. Hernell. 1992. Digestion and absorption of fat. *Sem. Gastrointest. Dis.* **3:** 189–208.
5. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34:** 1637–1659.
6. Spady, D. K., E. F. Stange, L. E. Bilhartz, and J. M. Dietschy. 1986. Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver. *Proc. Natl. Acad. Sci. USA.* **83:** 1916–1920.
7. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1997. Regulation of fecal bile acid excretion in male golden Syrian hamsters fed a cereal based diet with and without added cholesterol. *Hepatology.* **25:** 797–803.
8. Russell, D. W., and K. D. R. Setchell. 1992. Bile acid biosynthesis. *Biochemistry.* **31:** 4737–4749.
9. Wachtel, N., S. Emerman, and N. B. Javitt. 1968. Metabolism of cholest-5-ene-3β, 26-diol in the rat and hamster. *J. Biol. Chem.* **243:** 5207–5212.
10. Axelson, M., and J. Sjövall. 1990. Potential bile acid precursors in plasma—possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J. Steroid Biochem.* **36:** 631–640.
11. Schwarz, M., E. G. Lund, R. Lathe, I. Björkhem, and D. W. Russell. 1997. Identification and characterization of a mouse oxysterol 7α-hydroxylase cDNA. *J. Biol. Chem.* **272:** 23995–24001.
12. Pandak, W. M., Y. C. Li, J. Y. L. Chiang, E. J. Studer, E. C. Gurley, D. M. Heuman, Z. R. Vlahcevic, and P. B. Hylemon. 1991. Regulation of cholesterol 7α-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266:** 3416–3421.
13. Spady, D. K., J. A. Cuthbert, M. N. Willard, and R. S. Meidell. 1996. Feedback regulation of hepatic 7α-hydroxylase expression by bile salts in the hamster. *J. Biol. Chem.* **271:** 18623–18631.
14. Vlahcevic, Z. R., R. T. Stravitz, D. M. Heuman, P. B. Hylemon, and W. M. Pandak. 1997. Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat. *Gastroenterology.* **113:** 1949–1957.
15. Ishibashi, S., M. Schwarz, P. K. Frykman, J. Herz, and D. W. Russell. 1996. Disruption of cholesterol 7α-hydroxylase gene in mice: I. Postnatal lethality reversed by bile acid and vitamin supplementation. *J. Biol. Chem.* **271:** 18017–18023.
16. Schwarz, M., E. G. Lund, K. D. R. Setchell, H. J. Kayden, J. E. Zerwekh, I. Björkhem, J. Herz, and D. W. Russell. 1996. Disruption of cholesterol 7α-hydroxylase gene in mice: II. Bile acid deficiency overcome by induction of oxysterol 7α-hydroxylase. *J. Biol. Chem.* **271:** 18024–18031.
17. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1995. Role of liver in the synthesis of cholesterol and the clearance of low density lipoproteins in the cynomolgus monkey. *J. Lipid Res.* **36:** 67–79.
18. Osono, Y., L. A. Woollett, J. Herz, and J. M. Dietschy. 1995. Role of the low density lipoprotein receptor in the flux of cholesterol through the plasma and across the tissues of the mouse. *J. Clin. Invest.* **95:** 1124–1132.

19. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1997. Identification of a metabolic difference accounting for the hyper- and hyporesponder phenotypes of cynomolgus monkey. *J. Lipid Res.* **38:** 1598–1611.
20. Turley, S. D., B. P. Daggy, and J. M. Dietschy. 1994. Psyllium augments the cholesterol-lowering action of cholestyramine in hamsters by enhancing sterol loss from the liver. *Gastroenterology.* **107:** 444–452.
21. Turley, S. D., B. P. Daggy, and J. M. Dietschy. 1996. Effect of feeding psyllium and cholestyramine in combination on low density lipoprotein metabolism and fecal bile acid excretion in hamsters with dietary-induced hypercholesterolemia. *J. Cardiovasc. Pharmacol.* **27:** 71–79.
22. Turley, S. D., M. W. Herndon, and J. M. Dietschy. 1994. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J. Lipid Res.* **35:** 328–339.
23. Turley, S. D., B. P. Daggy, and J. M. Dietschy. 1991. Cholesterol-lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. *Metabolism.* **40:** 1063–1073.
24. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H]water. *J. Lipid Res.* **21:** 364–376.
25. Turley, S. D., J. M. Andersen, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
26. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
27. Kessler, M., O. Acuto, C. Storelli, H. Murer, M. Müller, and G. Semenza. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. *Biochim. Biophys. Acta.* **506:** 136–154.
28. Thigpen, A. E., R. I. Silver, J. M. Guileyardo, M. L. Casey, J. D. McConnell, and D. W. Russell. 1993. Tissue distribution and ontogeny of steriod 5α-reductase isozyme expression. *J. Clin. Invest.* **92:** 903–910.
29. Young, S. G., C. M. Cham, R. E. Pitas, B. J. Burri, A. Connolly, L. Flynn, A. S. Pappu, J. S. Wong, R. L. Hamilton, and R. V. Farese, Jr. 1995. A genetic model for absent chylomicron formation: mice producing apolipoprotein B in the liver, but not in the intestine. *J. Clin. Invest.* **96:** 2932–2946.
30. Wong, M. H., P. Oelkers, A. L. Craddock, and P. A. Dawson. 1994. Expression cloning and characterization of the hamster ileal sodium-dependent bile acid transporter. *J. Biol. Chem.* **269:** 1340–1347.
31. Spady, D. K., and J. M. Dietschy. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J. Clin. Invest.* **81:** 300–309.
32. Beynen, A. C., M. B. Katan, and L. F. M. Van Zutphen. 1987. Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Adv. Lipid Res.* **22:** 115–171.
33. Woollett, L. A., Y. Osono, J. Herz, and J. M. Dietschy. 1995. Apolipoprotein E competitively inhibits receptor-dependent low density lipoprotein uptake by the liver but has no effect on cholesterol absorption or synthesis in the mouse. *Proc. Natl. Acad. Sci. USA.* **92:** 12500–12504.
34. Goldstein, J. L., and M. E. Brown. 1989. Familial Hypercholesterolemia. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1215–1250.
35. Bhattacharyya, A. K., and D. A. Eggen. 1980. Cholesterol absorption and turnover in rhesus monkeys as measured by two methods. *J. Lipid Res.* **21:** 518–524.
36. St. Clair, R. W., L. L. Wood, and T. B. Clarkson. 1981. Effect of sucrose polyester on plasma lipids and cholesterol absorption in African green monkeys with variable hypercholesterolemic response to dietary cholesterol. *Metabolism.* **30:** 176–183.
37. Kesäniemi, Y. A., and T. A. Miettinen. 1987. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur. J. Clin. Invest.* **17:** 391–395.
38. Beynen, A. C., G. W. Meijer, A. G. Lemmens, J. F. C. Glatz, A. Versluis, M. B. Katan, and L. F. M. Van Zutphen. 1989. Sterol balance and cholesterol absorption in inbred strains of rabbits hypo- or hyperresponsive to dietary cholesterol. *Atherosclerosis.* **77:** 151–157.
39. Overturf, M. L., S. A. Smith, A. M. Gotto, Jr., J. D. Morrisett, T. Tewson, J. Poorman, and D. S. Loose-Mitchell. 1990. Dietary cholesterol absorption, and sterol and bile acid excretion in hypercholesterolemia-resistant white rabbits. *J. Lipid Res.* **31:** 2019–2027.
40. Miettinen, T. A., A. Proia, and D. J. McNamara. 1981. Origins of fecal neutral steroids in rats. *J. Lipid Res.* **22:** 485–495.
41. Dietschy, J. M. 1978. The uptake of lipids into the intestinal mucosa. *In* Physiology of Membrane Disorders. T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil, editors. Plenum Publishing Corporation, New York. 577–592.
42. Heider, J. G., C. E. Pickens, and L. A. Kelly. 1983. Role of acyl CoA:cholesterol acyltransferase in cholesterol absorption and its inhibition by 57-118 in the rabbit. *J. Lipid Res.* **24:** 1127–1134.
43. Westergaard, H., and J. M. Dietschy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **58:** 97–108.
44. Dietschy, J. M. 1978. General principles governing movement of lipids across biological membranes. *In* Disturbances in Lipid and Lipoprotein Metabolism. A. M. Gotto, and J. A. Ontko, editors. Waverly Press, Inc., Baltimore. 1–28.
45. Thomson, A. B. R., and J. M. Dietschy. 1977. Derivation of the equations that describe the effects of unstirred water layers on the kinetic parameters of active transport processes in the intestine. *J. Theor. Biol.* **64:** 277–294.
46. Wilson, F. A., and J. M. Dietschy. 1971. Differential diagnostic approach to clinical problems of absorption. *Gastroenterology.* **61:** 911–931.
47. Wilson, F. A., and J. M. Dietschy. 1972. Approach to the malabsorption syndromes associated with disordered bile acid metabolism. *Arch. Intern. Med.* **130:** 584–594.
48. Dietschy, J. M., and J. D. Wilson. 1968. Cholesterol synthesis in the squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. *J. Clin. Invest.* **47:** 166–174.
49. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47:** 286–300.