Selective sterol accumulation in ABCG5/ABCG8-deficient mice

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Abstract The ATP binding cassette (ABC) transporters ABCG5 and ABCG8 limit intestinal absorption and promote biliary secretion of neutral sterols. Mutations in either gene cause sitosterolemia, a rare recessive disease in which plasma and tissue levels of several neutral sterols are increased to varying degrees. To determine why patients with sitosterolemia preferentially accumulate noncholesterol sterols, levels of cholesterol and the major plant sterols were compared in plasma, liver, bile, and brain of wild-type and ABCG5/ABCG8-deficient (G5G8−/−) mice. The total sterol content of liver and plasma was similar in G5G8−/− mice and wild-type animals despite an ~30-fold increase in noncholesterol sterol levels in the knockout animals. The relative enrichment of each sterol in the plasma and liver of G5G8−/− mice (stigmasterol > sitosterol = cholestanol > baysicapsterol > campesterol > cholesterol) reflected its relative enrichment in the bile of wild-type mice. These results indicate that 24-alkylated, Δ22, and 5α-reduced sterols are preferentially secreted into bile and that preferential biliary secretion of noncholesterol sterols by ABCG5 and ABCG8 prevents the accumulation of these sterols in normal animals. The mRNA levels for 13 enzymes in the biosynthetic pathway were reduced in the livers of the G5G8−/− mice. The elucidation of the molecular basis of sitosterolemia, a rare autosomal recessive disorder of sterol metabolism, revealed a crucial component of the cellular machinery that limits the accumulation of noncholesterol sterols. Patients with sitosterolemia have increased fractional absorption and impaired biliary secretion of neutral sterols, resulting in the accumulation of these sterols in the blood and tissues (6–9). The disorder is caused by mutations in either of two genes that encode the ATP binding cassette (ABC) half-transporters, ABCG5 and ABCG8 (10, 11). ABCG5 and ABCG8 are expressed almost exclusively in hepatocytes and enterocytes and form a heterodimer that resides on the apical plasma membrane of these cells (12, 13). Transgenic mice expressing high levels of human ABCG5 and ABCG8 have reduced fractional absorption of dietary sterols, increased biliary secretion of cholesterol, and reduced plasma levels of noncholesterol sterols (14). Conversely, mice lacking ABCG5 and ABCG8 exhibit many features of sitosterolemic patients, including a striking accumulation of sitosterol and campesterol in the blood and liver, increased fractional absorption of noncholesterol sterols, and mark-

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edly reduced levels of biliary cholesterol (15). Taken together, these data indicate that the physiological role of ABCG5 and ABCG8 is to limit sterol accumulation by opposing the absorption of sterols in the intestine and promoting the secretion of sterols from the liver.

The accumulation of noncholesterol sterols in sitosterolemia and in ABCG5/ABCG8-deficient (G5G8−/−) mice indicates that ABCG5 and ABCG8 are required to maintain cholesterol as virtually the exclusive sterol in cell membranes. The mechanism by which ABCG5/ABCG8 specifically excludes noncholesterol sterols from cell membranes remains to be elucidated. Although there is a generalized increase in the fractional absorption of neutral sterols in sitosterolemic patients, the rank order of the efficiency of absorption (cholesterol > campesterol > sitosterol) is maintained in this disorder (8). The same rank order of efficiency of sterol absorption is observed in G5G8−/− mice (15). Thus, factors independent of ABCG5 and ABCG8 must contribute to the selectivity of the intestine for the absorption of different sterols.

ABCG5 and ABCG8 appear to mediate the preferential secretion of noncholesterol sterols from hepatocytes into bile. In normal individuals, sitosterol and other noncholesterol sterols are more concentrated in bile than in plasma, whereas in sitosterolemic patients, the ability to preferentially concentrate noncholesterol sterols in bile is lost (16, 17). These data are consistent with the preferential secretion of noncholesterol sterols into bile by ABCG5 and ABCG8, but the relationship between hepatic and biliary sterol concentrations has not been directly determined. The development of mice lacking ABCG5 and ABCG8 provides a model system in which the role of these two proteins in the enterohepatic transport of different sterols can be more directly assessed. In the current study, we compare levels of neutral sterols in the bile, plasma, and selected tissues of wild-type and G5G8−/− mice to examine the relative role of ABCG5 and ABCG8 in the trafficking of the different species of neutral sterols and oxysterols. The data indicate a direct relationship between the efficiency of biliary sterol secretion and the level of accumulation of the different sterols in the liver and plasma.

METHODS

Materials

The synthetic liver x receptor (LXR) agonist T0901317 was purchased from Cayman Chemical Co. (Ann Arbor, MI). Sterols were obtained either from Steraloids, Inc. (Newport, RI) or Sigma-Aldrich (St. Louis, MO).

Animals and diets

Mice homozygous for disrupted Abg5 and Abg8 alleles (G5G8−/−) were generated as described (15). The mice used in these studies were offspring of G5G8−/− mice of mixed genetic background (129S6Ev × C57BL/6) and were housed in plastic cages in a temperature-controlled room (22°C) with a daylight cycle from 6 AM to 6 PM. The mice were fed ad libitum a cereal-based rodent chow diet (Diet 7001; Harlan Teklad, Madison, WI) containing 0.02% cholesterol and 4% fat. Bile was collected from the gallbladders of anesthetized mice using a 30-gauge needle. Livers and brains were excised, rinsed, and frozen in liquid nitrogen. All animal procedures were performed with the approval of the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

T0901317 treatment

Diets containing 0.025% (w/w) T0901317 (T-diet) were made by mixing powdered chow diet (Diet 7001) with T0901317 and stored in aluminum foil-covered containers at 4°C for no more than 3 days before use. Mice were housed individually for 1 week before initiation of the T-diet and then fed for 1 week with either the T-diet or the chow diet dispensed from a feeder jar.

Lipid chemistries

Sterol levels in plasma, bile, liver, and brain were measured by gas chromatography-mass spectrometry (GC-MS) as described previously (18, 19). In addition, stigmasterol was monitored at m/z 484 and brassicasterol was monitored at m/z 470. Plasma and tissues were saponified in 90% ethanol sodium hydroxide (1N) at 66°C for 1 h after addition of 5a-cholesterol and epicoprostanol as internal standards. Lpidids were extracted using cyclohexane and dried under nitrogen. The residual lipids were redissolved in trimethylsilyl (TMS) reagent (pyridine-hexamethyldisilasane-chlorotrimethylsilane, 9:3:1, v/v/v) for analysis by GC-MS. Aliquots of plasma and brain were subjected to thin-layer chromatography on silica plates (20 × 20 cm; Kieselgel 60; Merck, Darmstadt, Germany) to separate free and esterified sterols. A mixture of toluene-ethyl acetate (9:1) was used as mobile phase, and retardation factor values for free cholesterol and unesterified plant sterols were determined from authentic compounds. The spots corresponding to free and esterified sterols were removed from the plate and transferred to 2 ml cartridges. The sterols were eluted from the silica material by 6 ml of cyclohexane. The organic solvent was evaporated, and the residues were dissolved in 50 µl of n-decane, silylated by the addition of 20 µl of TMS reagent, and analyzed by GC-MS as described previously (18, 19).

DNA microarrays

mRNA levels of genes in the cholesterol biosynthetic pathway were analyzed using oligonucleotide microarrays (Affymetrix). Total RNA was extracted from female wild-type and G5G8−/− mice aged 3–4 months using RNA STAT-60 (Tel-Test, Friendswood, TX). Equal aliquots of total RNA from each of five mouse livers in each group were pooled and labeled as described in the Affymetrix technical bulletin (www.affymetrix.com). Pooled samples were hybridized to Affymetrix GeneChips® Murine Genome MU74 version 2 arrays as described (20). Three independent experiments were performed with five wild-type and five G5G8−/− mice in each experiment. The microarray data were processed with Affymetrix Microarray Suite 5.0 software.

Statistical analysis

Mean sterol values and bile-liver sterol ratios were compared using unpaired t-tests. P-values were adjusted for multiple testing using Bonferroni’s correction. P = α/n, where P is the adjusted P value, α is the probability of falsely rejecting the null hypothesis, and n is the number of tests.

RESULTS

Total sterol content of liver and plasma is maintained in G5G8−/− mice

The concentration of cholesterol was significantly lower and the levels of the major plant sterols, sitosterol and
campesterol, were markedly higher in the plasma and livers of chow-fed G5G8−/− mice compared with their wild-type littermates (Table 1), which is similar to previous findings in these animals (15). The levels of stigmasterol and brassicasterol, the Δ22 derivatives of sitosterol and campesterol, respectively, were also increased in the tissues of the knockout animals (Table 1). The hepatic and plasma cholesterol levels in the G5G8−/− mice were reduced by ~50% compared with those of the wild-type animals, but the total sterol content (cholesterol, sitosterol, campesterol, stigmasterol, and brassicasterol) did not differ between the two strains of mice (Table 1, Fig. 1).

**Differential accumulation of neutral sterols in plasma reflects selective sterol secretion into bile**

The plasma levels of the sterols differed markedly between the wild-type and G5G8−/− mice (Table 1). The relative enrichment of sterols in the plasma of knockout animals ranged from an ~50% reduction in cholesterol to an ~300-fold increase in stigmasterol (Fig. 2A). The relative accumulation of each sterol in the plasma of G5G8−/− mice paralleled the efficiency with which that sterol was secreted into bile in the wild-type animals (Fig. 2B).

**Preferential secretion of noncholesterol sterols into bile requires ABCG5 and ABCG8**

The biliary cholesterol concentration was ~15-fold lower in the G5G8−/− mice than in control mice (Table 1, Fig. 1). In contrast to cholesterol, the levels of the other neutral sterols were similar or only modestly decreased in the bile of the G5G8−/− mice (Table 1) despite the increased levels of these sterols in the livers of these animals (Table 1, Fig. 1). Thus, the ratios of biliary sterols to liver sterols were uniformly much greater in the wild-type animals than in the G5G8−/− mice (Fig. 2B). In wild-type mice, the ratio of biliary sterol to liver sterol increased progressively with alkylation and unsaturation of the side chain. The highest ratio was observed for stigmasterol, which has both an ethyl group at C24 and a double bond.

**Fig. 1.** Sterol concentrations in plasma, liver, and bile of wild-type (WT) and ABCG5/ABCG8-deficient (G5G8−/−) [knockout (KO)] mice. Seventeen-week-old female wild-type (n = 9) and G5G8−/− (n = 7) mice consuming powdered chow diets (Diet 7001; Harlan Teklad, Madison, WI) were killed after a 4 h fast during the daylight cycle. Blood samples were drawn into tubes containing EDTA, and the plasma was isolated by centrifugation and maintained at ~80°C until sterols were measured. Bile was aspirated from the gallbladder using a 30 gauge needle. The livers were removed, carefully rinsed with saline solution, and frozen in liquid nitrogen. Sterol concentrations were measured by gas chromatography-mass spectrometry (GC-MS) as described in Methods.

**Fig. 2.** A: Relative accumulation of plasma neutral sterols in G5G8−/− mice. Plasma sterol concentrations were assayed by GC-MS. For each sterol, the mean concentration in G5G8−/− mice was divided by the corresponding value in wild-type mice. B: Efficiency of biliary sterol secretion in wild-type and G5G8−/− mice. Neutral sterols in liver, plasma, and bile samples from 17-week-old female wild-type (n = 9) and G5G8−/− (n = 7) mice were assayed using GC-MS. The ratio of biliary sterol to liver sterol was calculated for each animal. Bars indicate means ± SEM. C: Structures of cholesterol and noncholesterol sterols.

**TABLE 1.** Sterol concentrations in G5G8−/− mice and their wild-type littermates

| Sterols          | Plasma | Bile | Liver | Brain |
|------------------|--------|------|-------|-------|
|                  | Wild Type | G5G8−/− | Wild Type | G5G8−/− | Wild Type | G5G8−/− |
| Cholesterol      | 115 ± 7 | 58 ± 4* | 94 ± 11 | 6 ± 1* | 2,989 ± 99 | 1,239 ± 52* |
| Campesterol      | 0.9 ± 0.1 | 12.6 ± 1.1* | 2.2 ± 0.4 | 1.1 ± 0.2 | 19 ± 3 | 322 ± 14* |
| Sitosterol       | 0.4 ± 0.04 | 38.4 ± 4* | 1.3 ± 0.2 | 1.4 ± 0.2 | 6 ± 0.6 | 686 ± 39* |
| Brassicasterol   | 0.008 ± 0.0003 | 0.15 ± 0.01* | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.26 ± 0.01 | 9 ± 0.6* |
| Stigmasterol     | 0.003 ± 0.0003 | 0.87 ± 0.08* | 0.08 ± 0.01 | 0.1 ± 0.02 | 0.14 ± 0.01 | 35 ± 1.9* |
| Cholesterol      | 0.28 ± 0.03 | 0.57 ± 0.06 | 1.3 ± 0.1 | 0.19 ± 0.03* | 6.7 ± 0.5 | 9.7 ± 1.1 |
| Total sterol     | 116.6 ± 7.4 | 105.9 ± 8.9 | 99.3 ± 11.8 | 8.7 ± 1.5* | 2,432 ± 101 | 2,315 ± 65 |

Values are means ± SEM for nine wild-type and seven ABCG5/ABCG8-deficient (G5G8−/−) mice. *P values were determined using unpaired t-tests and adjusted for multiple testing using the Bonferroni correction (*α/5). **P < 0.005.

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the 5α-reduced derivative of cholesterol, was also significantly higher than that of cholesterol in the wild-type animals (2 ± 0.2 versus 0.4 ± 0.05). Disruption of Abcg5 and Abcg8 abolished the differential enrichment of sterols in bile; the ratio of biliary sterols to liver sterols was ~0.05 for all of the sterols in the G5G8−/− mice (Fig. 2B).

In contrast to the biliary-liver sterol ratios, the ratio of the levels of plasma to liver sterols was similar for all sterols examined and was not significantly affected by disruption of Abcg5 and Abcg8 (Fig. 3). Thus, the noncholesterol sterols appear to be incorporated into lipoproteins in proportion to their relative concentration in the liver.

**LXR agonist increases the efficiency of biliary sterol secretion**

Treatment with the LXR agonist T0901317 increased the levels of Abcg5 and Abcg8 mRNA (21) and protein (Fig. 4A) in both liver and jejunum, resulting in decreased concentrations of the major neutral sterols (cholesterol, campesterol, and sitosterol) in the plasma and liver and increased bile-liver ratios of these sterols in wild-type mice (Fig. 4B). The bile-liver ratios for these sterols were increased, indicating that the increase in the expression of Abcg5 and Abcg8 increased the efficiency with which they were secreted into bile (Fig. 4B). In contrast to the wild-type mice, LXR agonist treatment did not increase the bile-liver ratio of any of the neutral sterols in the G5G8−/− mice (data not shown). Treatment with the LXR agonist also decreased the hepatic levels of the minor sterols brassicasterol (from 0.26 ± 0.01 to 0.14 ± 0.01 µg/g) and stigmasterol (from 0.14 ± 0.01 to 0.07 ± 0.01 µg/g) in the wild-type mice but did not increase the bile-liver ratios in these animals (Fig. 4B). Presumably, the transport of these low-abundance sterols into bile is limited by the rate at which they gain access to the transporter rather than by the amount of transporter available in wild-type animals.

**Oxysterols do not accumulate in G5G8−/− mice**

Oxysterols are oxygenated derivatives of cholesterol that traverse cell membranes more readily than cholesterol (22). The three major oxysterols in the circulation are formed from cholesterol by three sterol hydroxylases that catalyze the addition of a single hydroxyl group to C24, C25, or C27 to form 24-hydroxycholesterol, 25-hydroxycholesterol, or 27-hydroxycholesterol, respectively (23). The level of 27-hydroxycholesterol was significantly lower in the bile, liver, and plasma of the G5G8−/− mice (Fig. 5A). The decrease in 27-hydroxycholesterol may reflect an inhibition of sterol 27-hydroxylase activity by noncholesterol sterols, as has been reported in liver specimens from patients with sitosterolemia (24). Alternatively, the reduction in 27-hydroxycholesterol may be attributable to reduced levels of cholesterol, the substrate of sterol 27-hydroxylase, or to reduced access of substrate to the enzyme in these animals. The levels of 24-hydroxycholesterol, the major oxysterol in brain, were similar in the brains (117 ± 10 versus 85 ± 6, P = 0.4) and plasma (Fig. 5A) of G5G8−/− and wild-type mice but were reduced in the liver and bile of the knockout animals (Fig. 5A). The ratios of biliary oxysterol to liver oxysterol did not change with inactivation of Abcg5 and Abcg8 (Fig. 5B). Essentially identical results

![Fig. 3. Plasma-liver sterol ratios in wild-type and G5G8−/− mice. Plasma and liver sterol concentrations from 17-week-old female wild-type (n = 9) and G5G8−/− (n = 7) mice were assayed by GC-MS. The ratio of plasma sterol to liver sterol was calculated for each animal. Bars indicate means ± SEM.](image)

![Fig. 4. Liver x receptor (LXR) agonist promotes biliary sterol secretion. A: Immunoblot analysis of ABCG5 from liver and jejunum of wild-type and G5G8−/− mice. A total of 50 µg of pooled membrane protein (n = 5 mice in each group) was fractionated by SDS-PAGE and immunoblotted with rabbit polyclonal antibodies raised against mouse ABCG5 (15) and receptor-associated protein (RAP). The filters were exposed to Kodak X-Omat Blue films for 5–30 s at room temperature. B: Bile-liver sterol ratios in wild-type mice treated with T0901317 (T). Seventeen-week-old female wild-type (n = 9) and G5G8−/− (n = 7) mice were fed a powdered chow diet (Diet 7001) with or without 0.025% of the synthetic LXR agonist T0901317 for 7 days. Mice were killed after a 4 h fast during the daylight cycle, liver and gallbladder bile samples were collected, and sterol levels were determined as described in the legend to Fig. 1. The ratio of bile sterol to liver sterol was calculated for each animal. Bars indicate means ± SEM. P values were adjusted for multiple testing using the Bonferroni correction. * P < 0.05, chow diet versus T0901317-treated.](image)
Expression of genes encoding cholesterol biosynthetic enzymes is suppressed in G5G8−/− mice

Oligonucleotide expression arrays indicated that mRNA levels of 13 genes in the cholesterol biosynthetic pathway were obtained in a second set of five wild-type and five G5/ G8−/− mice (data not shown). Accordingly, these results were assessed at the nominal P values rather than the Bonferroni corrected P values.

Table 2. Expression of genes encoding cholesterol biosynthetic enzymes in G5G8−/− mice

| Gene Name                      | Experiment 1 | Experiment 2 | Experiment 3 |
|-------------------------------|--------------|--------------|--------------|
| HMG-CoA synthase              | 0.5          | 0.5          | 0.71         |
| HMG-CoA reductase             | 0.62         | 0.62         | 0.62         |
| Mevalonate kinase             | NA           | NA           | NA           |
| Phosphomevalonate kinase      | 0.66         | 0.62         |              |
| Diphosphomevalonate decarboxylase | NA       | NA           | NA           |
| Farnesyl diprophosphate synthase | 0.44   | 0.50         | 0.47         |
| Squalene synthase             | 0.54         | 0.66         | 0.57         |
| Squalene epoxidase            | 0.35         | 0.35         | 0.35         |
| Lanosterol synthase           | 0.54         | 0.50         | 0.57         |
| Lanosterol                    | 0.41         | 0.38         | 0.50         |
| 14α-demethylase               | 0.66         | 0.66         | 0.66         |
| NADPH sterol                  | 0.54         | 0.41         | 0.66         |
| Delta5,Delta8-isomerase       | 0.57         | 0.38         | 0.71         |
| 3β-Hydroxysterol              | 0.87         | 0.93         | 0.93         |
| Lathosterol oxidasre           | 0.76         | 0.62         | 1.32         |
| 7-Dehydrocholesterol reductase | 0.87   | 0.76         | 0.57         |
| Desmosterol reductase         | ND           | ND           | ND           |

Values are relative hepatic mRNA levels of G5G8−/− mice compared with wild-type mice. Each experiment compared one pooled RNA sample from five wild-type mice and five G5G8−/− mice. RNA samples were hybridized to oligonucleotide arrays as described in Methods. NA, data quality not acceptable; ND, gene not represented on the expression array.

Increased noncholesterol sterols in brains of G5G8−/− mice

The cholesterol content of the brain was slightly lower and the content of noncholesterol sterols was significantly higher in G5G8−/− mice than in wild-type animals (Table 1). Noncholesterol sterols constituted a much smaller fraction (~1.5%) of total sterols in the brains of G5G8−/− mice than in either the plasma or the liver (Fig. 6A). To determine if the increase in the noncholesterol sterol content of the brain was attributable to blood contamination, the proportion of free and esterified sterol was measured in the plasma and in the brain. Essentially all of the sitosterol and campesterol in the brain was present as the free sterol (Fig. 6B). In contrast to that in the brain, only 30–40% of the plant sterols in the blood circulated in the free form (Fig. 6B), presumably reflecting the action of LCAT, which esterifies noncholesterol sterols (25, 26).

Discussion

The results of this study demonstrate that the pattern of sterol accumulation in G5G8−/− mice reflects the loss of preferential sterol secretion in bile. The relative enrichment of the noncholesterol neutral sterols in the circulation of the knockout animals reflected the relative enrichment of these sterols in the bile of wild-type animals. The preferential secretion of noncholesterol sterols was de-
pended on the expression of ABCG5 and ABCG8, as indicated by the lack of biliary enrichment for noncholesterol sterols in the G5G8−/− mice. Increasing the expression of ABCG5 and ABCG8 by treatment with an LXR agonist further increased the bile-liver ratios of the major sterols in wild-type mice but had no effect on biliary sterol excretion in the knockout animals. These data indicate that ABCG5/ABCG8-mediated secretion of noncholesterol sterols into bile plays a crucial role in preventing their accumulation in the body.

The preferential secretion of noncholesterol neutral sterols into bile has been inferred from previous studies in humans, in which plasma sterol levels were compared with the levels in duodenal bile (5, 9, 27). By simultaneously measuring neutral sterol concentrations from bile and liver samples, the present study provides direct evidence that ABCG5 and ABCG8 are required for the efficient biliary secretion of these sterols. In mice expressing ABCG5 and ABCG8, the ratio of biliary sterol to liver sterol is determined by the chemical structure of the sterol and by the level of expression of ABCG5 and ABCG8. At least three distinct structural modifications of cholesterol affect the transport of sterol into bile by ABCG5 and ABCG8. Alkylation of C24, as occurs in campesterol and sitosterol, resulted in a 3- to 5-fold biliary enrichment of these sterols compared with cholesterol. Biliary enrichment was even greater (∼15-fold) for stigmasterol, the Δ22 derivative of sitosterol. The bile-liver ratio of cholestanol, the 5α-reduced derivative of cholesterol, was ∼5-fold greater than that of cholesterol. In contrast to these changes, the bile-liver ratios of sterols hydroxylated at either C24 or C27 were similar in wild-type and G5G8−/− mice, suggesting that these sterols are not substrates for ABCG5 and ABCG8 (Fig. 5). Therefore, relatively minor modifications of the cholesterol nucleus or side chain have a profound effect on the relative efficiency of sterol trafficking by ABCG5 and ABCG8. In the absence of ABCG5 and ABCG8, the bile-liver sterol ratios were far lower than the corresponding values in wild-type animals, and the biliary concentration of each sterol was directly related to the concentration of that sterol in liver. These data (Fig. 2) indicate that ABCG5 and ABCG8 are required for the efficient secretion of neutral sterols and for the preferential secretion of noncholesterol sterols into bile.

The mechanism(s) responsible for the differential excretion of different sterols by ABCG5 and ABCG8 remain(s) unclear. Differential sterol secretion may reflect differences in the rates at which the various sterols gain access to the transporter. The observation that overexpression of ABCG5 and ABCG8 in transgenic mice leads to a proportional increase in biliary cholesterol concentration suggests that the delivery of cholesterol to the transporter is not limited within the physiological range of ABCG5/ABCG8 activity, but we cannot exclude the possibility that noncholesterol sterols are preferentially delivered to the transporter. In contrast to cholesterol, noncholesterol sterols are poorly esterified by ACAT (28) and cannot be efficiently sequestered in intracellular lipid droplets. The inability to esterify these sterols may result in their preferential translocation to the canalicular membrane. An alternative possibility is that the ABCG5/ABCG8 transporter may have different affinities for the various neutral sterols. ABC transporters such as MRP1 (ABCC1) transport a variety of substrates with widely differing efficiency (29). Differential sterol secretion may also occur as a consequence of differences in the rate at which sterols are taken up by mixed micelles in the bile. Small (30) has proposed that ABCG5 and ABCG8 increase the off rate of sterols from the canalicular membrane into the bile by partially displacing the sterol from the plane of the membrane so that it is more accessible to mixed bile-salt phospholipid micelles in bile. Biochemical assays of ABCG5/ABCG8 function in reconstituted systems will be required to distinguish among these possibilities.

Whereas preferential sterol secretion by the liver is abolished in G5G8−/− mice, differential absorption of sterols in the intestine is maintained in these animals. Disruption of ABCG5 and ABCG8 is associated with increased fractional absorption of noncholesterol neutral sterols, but cholesterol remains the most efficiently absorbed sterol in these animals, with a fractional absorption rate almost 2-fold greater than that of campesterol and 4-fold greater than that of sitosterol (15). Thus, the rank order for the fractional absorption of dietary sterols in the intestine of the G5G8−/− mice matches that of wild-type animals, indicating that factors independent of ABCG5 and ABCG8, such as differential micellar solubilization, recognition by cell surface transporters, intracellular sterol movement, or sterol esterification by ACAT, contribute to preferential sterol absorption in the intestine.

Studies in sitosterolemia patients indicate that noncholesterol neutral sterols accumulate in tissues in proportion to their accumulation in plasma but that the brain is shielded from sterol accumulation, presumably by the blood-brain barrier (7). Noncholesterol sterols were increased in the brains of the G5G8−/− animals, although the extent of the accumulation was far lower in this organ than in the liver and plasma. The noncholesterol sterols in the brain were almost entirely in the free form, whereas the major fraction of noncholesterol sterols in plasma was esterified. Therefore, the high levels of noncholesterol sterols in the brain of the G5G8−/− mice do not reflect contamination from the circulation.

The total neutral sterol pool size in the liver and plasma was essentially identical in G5G8−/− and wild-type mice, despite the markedly reduced hepatic sterol secretion and increased noncholesterol sterol levels of the knockout animals. Thus, ABCG5 and ABCG8 are required to maintain the composition, but not the total concentration, of plasma and liver sterols in chow-fed animals. In the absence of ABCG5 and ABCG8, the accumulation of noncholesterol sterols in the liver was balanced by a decrease in cholesterol levels. The mRNA levels of 13 enzymes in the cholesterol biosynthetic pathway were decreased in the G5G8−/− animals, indicating that the molecular apparatus that maintains cholesterol homeostasis in the liver responds to the accumulation of noncholesterol sterols by decreasing cholesterol synthesis, thereby preserving the
total neutral sterol concentration in the livers of these animals.\textsuperscript{14}

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