Mice Deleted for Fatty Acid Transport Protein 5 Have Defective Bile Acid Conjugation and Are Protected From Obesity

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See CME Quiz on page 1349.

Background & Aims: **Fatty Acid Transport Protein 5 (FATP5) is a liver-specific member of the FATP/Slc27 family, which has been shown to exhibit both fatty acid transport and bile acid-CoA ligase activity in vitro. Here, we investigate its role in bile acid metabolism and body weight homeostasis in vivo by using a novel FATP5 knockout mouse model.** ***Methods:*** **Bile acid composition was analyzed by mass spectroscopy. Body weight, food intake, energy expenditure, and fat absorption were determined in animals fed either a low- or a high-fat diet.** ***Results:*** **Although total bile acid concentrations were unchanged in bile, liver, urine, and feces of FATP5 knockout mice, the majority of gallbladder bile acids was unconjugated, and only a small percentage was conjugated. Primary, but not secondary, bile acids were detected among the remaining conjugated forms in FATP5 deletion mice, suggesting a specific requirement for FATP5 in reconjugation of bile acids during the enterohepatic recirculation. Fat absorption in FATP5 deletion mice was largely normal, and only a small increase in fecal fat was observed on a high-fat diet. Despite normal fat absorption, FATP5 deletion mice failed to gain weight on a high-fat diet because of both decreased food intake and increased energy expenditure.** ***Conclusions:*** **Our findings reveal an important role for FATP5 in bile acid conjugation in vivo and an unexpected function in body weight homeostasis, which will require further analysis. FATP5 deletion mice provide a new model to study the intersection of bile acid metabolism, lipid metabolism, and body weight regulation.**

Fatty acid transport protein 5 (FATP5) (Slc27a5/VLACSR/VLCS-H2) is a liver-specific member of the fatty acid transport protein family.[1–3] Similar to other fatty acid transport protein family members, FATP5 mediates the uptake of long-chain fatty acids (LCFAs) when overexpressed in cultured mammalian cells.[1,4] We recently generated FATP5 knockout mice and showed that primary hepatocytes derived from these mice have a significant reduction in LCFA uptake.[4] We also found that FATP5 knockout mice have decreased intrahepatic triglyceride levels and show a decreased hepatic fatty acid uptake in vivo with redistribution of lipids from the liver to other LCFA metabolizing organs.[4] These data are consistent with an important role for FATP5 in hepatic fatty acid uptake in vivo.

Beside its role in fatty acid uptake, FATP5 has also been suggested to function in bile acid metabolism as a bile acid-CoA ligase (BAL). Purified rat liver BAL was found to be identical to the rat ortholog of FATP5.[5] Recombinant rat FATP5, expressed in insect cells, shows bile acid-CoA ligase activity with kinetic properties similar to rat liver BAL.[5] Furthermore, human FATP5, overexpressed in mammalian cells, confers choloyl-CoA ligase activity.[6,7] These data suggest that FATP5 may indeed be a bile acid–CoA ligase.

By using our recently generated FATP5 knockout mice,[4] we decided to examine whether FATP5 is the major bile acid–CoA ligase in vivo and, if so, what the physiological consequences of its deletion are. Figure 1 shows a simplified diagram of bile acid metabolism.[8–10] The starting point for de novo synthesis of bile acids is acetyl-CoA, which is converted to cholesterol by cholesterol biosynthetic enzymes. Cholesterol is converted to bile acids through the sequential action of bile acid biosynthetic enzymes. Important intermediates in bile

Abbreviations used in this paper: BAL, bile acid–CoA ligase; BAT, bile acid–CoA:amino acid N-acyltransferase; DHCA, 7α,12α-dihydroxy-5β-cholestanoic acid; FATP, fatty acid transport protein; LCFA, long-chain fatty acid; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid.

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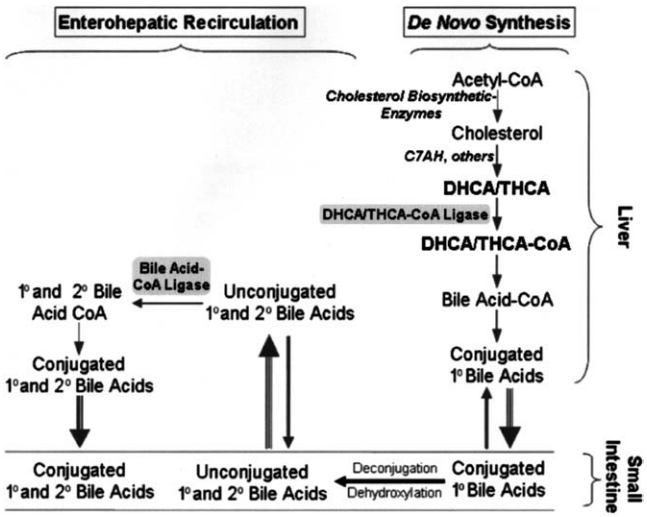

Figure 1. Overview of bile acid metabolism and recycling.

acid synthesis are C_{27} precursors of bile acids, such as 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) and the related molecule 7α,12α-dihydroxy-5β-cholestanoic acid (DHCA). These precursors are activated by addition of CoA to form THCA-CoA and DHCA-CoA, which are subsequently shortened to form the primary bile acids choloyl-CoA and chenodeoxycholoyl-CoA. FATP5 and a related FATP, FATP2, have THCA-CoA ligase activity in vitro and could therefore be involved in de novo bile acid synthesis.[7] CoA derivatives of mature bile acids are conjugated to amino acids, such as taurine, by the bile acid–CoA:amino acid N-acyltransferase (BAT) before secretion into the bile. Bile acids are efficiently reabsorbed from the distal small intestine and reenter the liver to be resecreted into bile, a process that has been termed the enterohepatic recirculation. The recirculation of bile acids is very efficient: under normal conditions, only 5% of bile acids in gall bladder bile is derived directly from de novo synthesis, whereas 95% have been recirculated through the gut at least once.[10] While in the gut, a portion of bile acids is deconjugated and/or dehydroxylated through the action of intestinal bacteria resulting in a mixture of conjugated and unconjugated primary and secondary bile acids, which are then reabsorbed.[8] Purified BAL activates both primary and secondary bile acids[5,11,12] and is thus able to efficiently reconjugate all bile acid species reentering the liver.

What would be the consequences of BAL deletion? It would be expected that conjugated bile acids are synthesized normally via the de novo biosynthetic pathway. However, any bile acid which is deconjugated in the small intestine could not be reconjugated. Over time, because the majority of bile acids in the gall bladder bile have gone through the enterohepatic circulation multiple times, unconjugated bile acids would accumulate. The remaining conjugated bile acids would be mostly primary bile acids, and secondary bile acids would be mostly unconjugated. The physiological consequences of this bile acid conjugation defect might be expected to include hepatic cholestasis and fat malabsorption. In humans, a complete defect in bile acid conjugation, caused by mutation in BAT, leads to increased serum bile acid levels and fat malabsorption.[13] Because unconjugated bile acids are transported poorly by the bile salt export pump (BSEP),[14] BAL deletion has also been predicted to result in a phenotype similar to disruption of BSEP, which in mice causes intrahepatic cholestasis and growth retardation because of nutrient malabsorption.[15]

In addition to BAL activity, FATP5 also has TCHA-CoA ligase activity in vitro.[7] If FATP5 is required for activation of DHCA/THCA, one would expect a complete absence of conjugated bile acids, an absence or reduction of mature C_{24} bile acids, and accumulation of THCA/DHCA. No defects have been described that specifically disrupt DHCA/THCA activation; however, genetic defects in several other bile acid biosynthetic enzymes result in intrahepatic cholestasis, jaundice, and fat malabsorption.[16]

Here we use FATP5 knockout mice to show that FATP5 is required for bile acid reconjugation but not de novo synthesis. Despite a reversal of the ratio of unconjugated to conjugated bile acids, we found no evidence of hypercholonemia or overt fat malabsorption. Interestingly, however, FATP5 knockout mice failed to gain weight on a high-fat diet because of decreased food intake and increased energy expenditure. Although the exact mechanism of this unexpected role for FATP5 in body weight regulation remains to be determined, our data together with the data by Doege et al[4] clearly show that FATP5 functions in both lipid and bile acid metabolism.

Materials and Methods

Animal Experiments

The generation of FATP5 deletion mice has been described.[4] Mice for phenotyping were obtained by brother-sister matings of F2 animals. Genotypes were determined by polymerase chain reaction. Mice were fed a low-fat (Standard chow or Research Diets D12450, New Brunswick, NJ) or high-fat diet (Research Diets D12330 and D12492). All experiments were performed on individually housed male animals. Fat mass was determined by dual-energy x-ray absorptiometry (DEXA) by using a PIXImus mouse densitometer (Lunar Corp, Madison, WI). For glucose-tolerance tests, animals were fasted overnight, 2 g/kg glucose was administered IP, and blood glucose levels were determined by using a

glucometer (Bayer, Elkhard, IN). For tissue and plasma collection, mice were fasted overnight, glucose was measured by using a glucometer, and animals were euthanized by CO_2 asphyxiation. O_2 consumption and CO_2 production were measured in metabolic chambers (Columbus Instruments Inc, Columbus, OH). Mice either had free access to food (first 24 hours) or no access to food (second 24 hours). The respiratory exchange ratio (RER) was calculated as CO_2 produced/O_2 consumed. Activity was measured by using the SmartFrame Activity System (Hamilton/Kinder, Poway, CA) consisting of 12 PC-interfaced horizontal frames fitted with photobeams (8LX4W) spaced 5.5 cm apart. Photobeam breaks (basic movement) were recorded for 70 hours and analyzed in 60-minute blocks by using Motor Monitor Software (Hamilton/Kinder). Statistical significance was determined by using 2-way repeated-measures analysis of variance with genotype and time as factors. Rectal temperature was recorded by using a rectal probe (Thermalert TH-5; PhysiTemp, Clifton, NJ). Animals and procedures were approved by the Millennium and the Palo Alto Medical Foundation Research Institute Institutional Animal Care and Use Committees.

Determination of Bile Acids by Mass Spectroscopy

Bile was collected from gallbladders of ad libitum–fed animals and diluted 1:500 in 50% acetonitrile in water. Ten microliters of the diluent was injected onto an Agilent 1100 capillary HPLC system fitted with a Luna Phenyl-Hexyl 3-μ column (30 × 1 mm; Phenomenex, Torrance, CA) and a single quadrapole mass spectrometer (G1946D SL; Agilent Technologies, Palo Alto, CA). A gradient from 10% to 70% acetonitrile was run at a flow rate of 50 μL/min over 45 minutes (aqueous phase was 10 mmol/L ammonium acetate pH 9.0). Bile acids and bile acid conjugates were monitored in single ion monitoring mode at their respective mass-to-charge ratios. Taurine-conjugated and unconjugated forms of cholate, chenodeoxycholate, α- and β-muricholate, lithocholate, hyodeoxycholate, and hyocholate (all from Steraloids, Newport, RI) were used as standards. Calibration curves for standards were included in all experiments and were linear. The run-to-run error for individual samples was <5%.

Biochemical Assays

Liver microsomes were prepared as described.[17] Cholate-CoA ligase activity was determined radiochemically.[18] Briefly, 40 μg protein was incubated in reaction buffer (100 mmol/L Tris, pH 7.5, 10 mmol/L ATP, 5 mmol/L MgCl, 0.2 mmol/L CoA, 20 μmol/L ^{14}C-cholic acid [American Radiochemical Co., St. Louis, MO]) for 40 minutes at 37°C. EDTA and succinic acid was added, and tubes were extracted twice with water-saturated butanol. To determine fecal fat, feces was dried at 70°C for 30 minutes and then extracted with 1:3.3:4 vol of H_2O:chloroform:methanol for 1 hour. Organic material was dried, weighed, and assigned as total lipid. To determine fecal free fatty acids, the dried pellet was resuspended in 100% ethanol and free fatty acids were determined by using a diagnostic kit (Sigma, St. Louis, MO) with oleate as a standard. Caloric content of feces was determined by using a Parr 1425 calorimeter (Parr Instrument Company, Moline, IL). Briefly, 60 mg dried feces was wrapped with platinum wire, sealed in a calorimetry bomb, and submerged in a water-filled chamber. The temperature difference in the water before and after combustion of the sample was recorded by using a Parr 1672 calorimeter thermometer and used to calculate the total fecal calories. Commercially available kits were used to determine insulin, leptin (both Crystal Chem, Downers Grove, IL), creatinine (BioAssay Systems, Hayward, CA), cholesterol, glycerol, triglycerides, free fatty acids, β-hydroxybutyrate, and total bile acids (all Sigma, St. Louis, MO). Bile acids and cholesterol were extracted from lyophilized liver homogenate or dried feces by incubating with 75% ethanol at 50°C (bile acids) or with 10 mmol/L potassium hydroxide:methanol (1:9) at 66°C (cholesterol) for 2 hours. Bile acid pool size was measured as described.[19] To determine hepatic lipids, 20 mg lyophilized liver homogenate was extracted with 1 mL chloroform:methanol (2:1) at 60°C, filtered over a 0.2-μmol/L nylon membrane spin column (Schleicher & Schuell, Keene, NH), and re-extracted twice with chloroform:methanol. The organic phase was dried, weighed, and assigned as total lipid. Liver glycogen was determined by digesting liver homogenate with amyloglucosidase and then assaying the amount of glucose in the sample by using a diagnostic kit (Sigma).

Analysis of Gene Expression

Gene-profiling data were generated by using the MOE430A chip according to the manufacturer's recommendations (Affymetrix, Santa Clara, CA). Arrays were scanned on an Affymetrix GeneArray scanner and analyzed by using the MAS 5.0 software (Affymetrix). Taqman real-time quantitative PCR was performed by using an ABI Prism 7700 sequence detector (Applied Biosystems, Foster City, CA) with 18S as a control. Gene-specific primers and probes were designed by using the Primer Express software (Applied Biosystems). RNA was digested with DNase A and transcribed to cDNA by using a mixture of random hexamer and oligo dT primers. Relative expression was determined by the C_t method (Applied Biosystems).

Statistical Analysis

Average and standard error of the mean are shown. Unless otherwise indicated, statistical significance was determined by using a Student *t* test.

Results

FATP5 Deletion Mice Have a Reversed Ratio of Conjugated to Unconjugated Bile Acids

Total bile acid concentrations in gall bladder bile, feces, liver, serum, and urine of FATP5 deletion mice

Table 1. Bile Acid Concentrations in FATP5 Knockout Mice

Bile Acids in	−/−	+/+	*P* Value
Liver (*μmol/g*)	0.70 ± 0.06	0.73 ± 0.01	NS
Feces (*μmol/g*)	2.59 ± 0.25	2.22 ± 0.19	NS
Gallbladder (*μmol/dL*)	34.43 ± 1.70	31.94 ± 2.27	NS
Serum (*μmol/dL*)	1.78 ± 0.37	1.95 ± 0.32	NS
Urine (*nmol/mg creatinine*)	30.93 ± 1.31	33.65 ± 1.55	NS
Bile acid pool (*μmol/g tissue*)	5.8 ± 1.31	5.6 ± 0.89	NS

NOTE. Feces and liver were from mice on a high-fat diet for 11 and 20 weeks, respectively. Gallbladder bile, serum, urine, and tissues for determination of bile acid pool size were from 6-month-old mice on a normal diet. n = 3– 5.
NS, not significant.

were similar to controls (Table 1). Furthermore, bile acid pool size was similar in FATP5 deletion and wild-type control mice (Table 1). To determine bile acid composition, we used mass spectroscopy coupled to liquid chromatography (Figure 2). In the bile of wild-type mice, taurine-conjugated trihydroxylated (TriOH) bile acids were most abundant (81% of total: tauro-β-muricholic acid 40%, taurocholic acid 32%, and tauro-α-muricholic acid 9%; Figure 2*D*). Taurine-conjugated dihydroxylated (DiOH) bile acids (taurohyodeoxycholic acid, taurochenodeoxycholic acid, and taurodeoxycholic acid) together constituted 14% of total bile acids (Figure 2*B*), whereas 5% of bile acids was unconjugated cholic acid (Figure 2*C*). We were unable to detect lithocholic acid, glycine-conjugated, or glucuronidated bile acids in our samples, and the levels of sulfated bile acids detected were too low to quantitate (data not shown). In summary, in wild-type mice, 95% of the bile acids in gallbladder bile were conjugated and only 5% were unconjugated (Figure 2*H*). In contrast, in FATP5 knockout mice, 83% of bile acids were unconjugated, whereas only 17% were conjugated (Figure 2*H*). The total amount of mature C_{24} bile acids was similar in FATP5 knockout and wild-type mice (Figure 2*H*) and no masses corresponding to C_{27} precursors were detected in either background (not shown). The majority of bile acids in FATP5 deletion mice were unconjugated trihydroxylated bile acids (65% of total, cholic acid 26%, and multiple species migrating similar to α- and β-muricholic acid 39%, Figure 2*C* and *G*), whereas taurine-conjugated trihydroxylated bile acids accounted for only 14% of bile acids (taurocholic acid 4%, tauro-α-muricholic 4%, and tauro-β-muricholic 4%; Figure 2*D*). This reversal in the abundance of unconjugated versus conjugated bile acid species strongly supports a requirement for FATP5 in bile acid conjugation.

The remaining conjugated bile acids in FATP5 deletion mice could represent newly synthesized bile acids that have not yet undergone deconjugation; alternatively, they could be bile acids that were reconjugated by a bile acid-CoA ligase other than FATP5. To distinguish between these 2 possibilities, we focused our analysis on dihydroxylated taurine-conjugated bile acids (Figure 2*B*). Two bile acids in this group, taurohyodeoxycholate and taurodeoxycholate (peak 1 and 3), cannot be produced by de novo synthesis but are generated from primary bile acids in the small intestine. A third bile acid, taurochenodeoxycholate (peak 2), is produced by de novo bile acid synthesis. Interestingly, conjugated forms of the 2 secondary bile acids are completely absent in the bile of FATP5 knockout mice (Figure 2*B*), suggesting an overall requirement for FATP5 in the generation of these bile acids. In contrast, the conjugated primary bile acid, taurochenodeoxycholate, is present in similar concentrations in FATP5 knockout and wild-type animals (Figure 2*B*). These data support the notion that de novo synthesis of bile acids in FATP5 deletion mice is largely intact and that the increase in unconjugated bile acids in FATP5 knockout mice is because of a defect in bile acid reconjugation. Surprisingly, unconjugated secondary dihydroxylated bile acids were not increased in FATP5 deletion mice (Figure 2*A*). It is possible that unconjugated bile acids are rehydroxylated more readily than conjugated bile acids or are taken up less efficiently by the liver.

Interestingly, FATP5 deletion mice had significant amounts of conjugated (2%) and unconjugated (13%) tetrahydroxylated bile acids (Figure 2*E* and *F*). Tetrahydroxylated (TetraOH) bile acids are not present in normal bile but are found in the bile of BSEP knockout mice[15] and in the urine of humans with cholestasis.[20] The increase in tetrahydroxylated bile acids in FATP5 knockout mice may allow these mice to more efficiently export unconjugated bile acids, resulting in normal levels of intrahepatic bile acids.

In summary, our data show that bile acid reconjugation is a major function of FATP5, whereas de novo synthesis of bile acids appears to be intact. To verify the role of FATP5 in bile acid reconjugation, we measured BAL activity in microsomes from wild-type and knockout livers by using ^{14}C-cholic acid as a substrate. BAL activity was significantly decreased in knockout compared with wild-type microsomes (3.4 ± 0.68 pmol/min/mg vs. 37.7 ± 19.5 pmol/min/mg), consistent with FATP5 being the major bile acid–CoA ligase.

FATP5 Deletion Mice Have Normal Fat Absorption

FATP5 deletion mice survived to adulthood at mendelian ratios, and their body weight and length were similar to controls at 8 weeks of age (data not

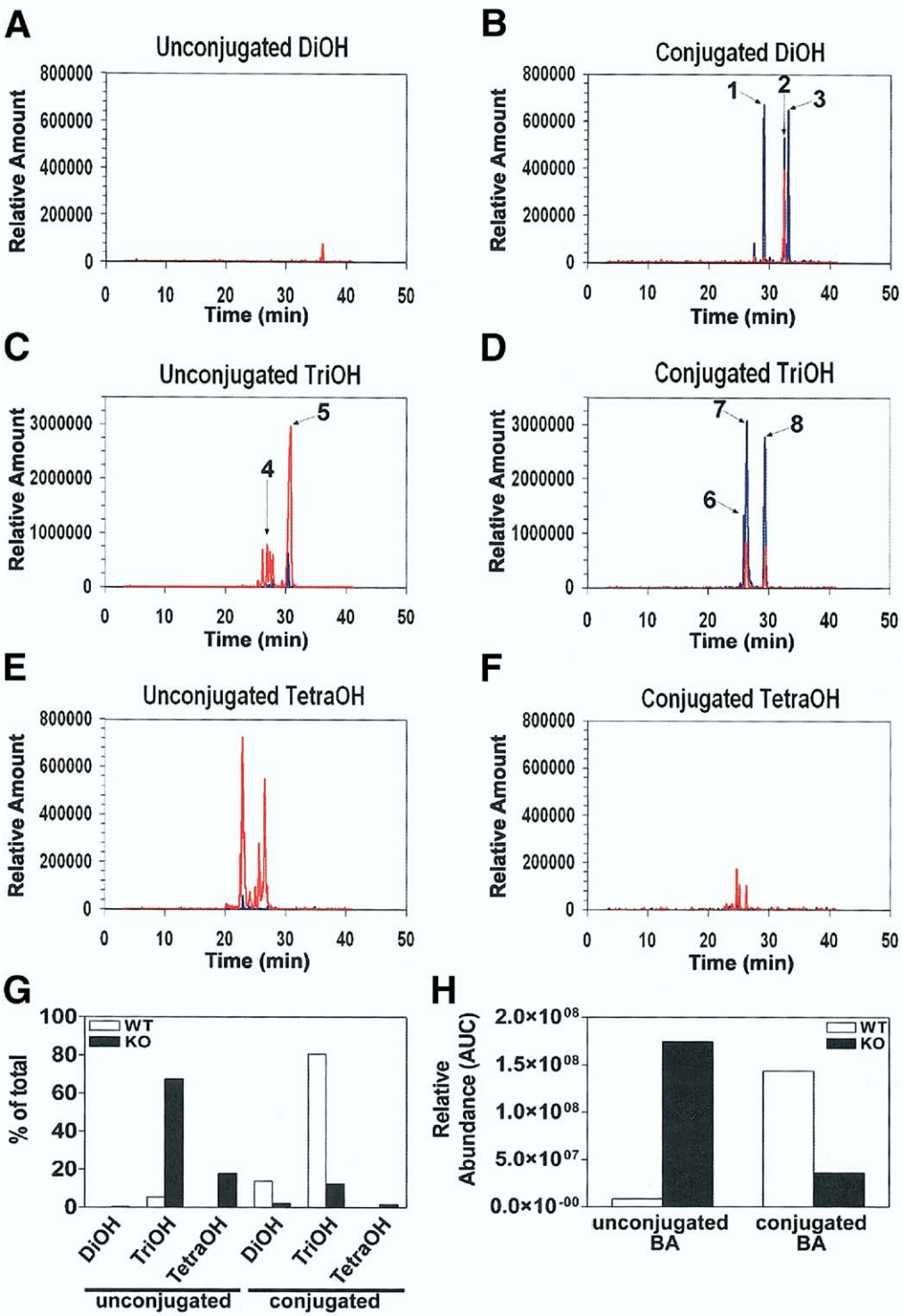

Figure 2. Bile acid composition of gallbladder bile of male FATP5 knockout (KO) and wild-type (WT) mice. (*A–F*) Ion chromatograms from mass spectroscopy coupled to liquid chromatography analysis. Red indicates KO; blue indicates WT. Traces represent the averages of 3 individual mice; variation between mice was <15%. Numbers indicate the migration of standards: *1*, taurohyodeoxycholate; *2*, taurochenodeoxycholate; *3*, taurodeoxycholate; *4*, α and β muricholate; *5*, cholate; *6*, tauro-α-muricholate; *7*, tauro-β-muricholate; and *8*, taurocholate. Unconjugated dihydroxylated (DiOH, *m/z* 391, *A*), trihydroxylated (TriOH, *m/z* 407, *C*), and tetrahydroxylated (TetraOH, *m/z* 423, *E*) bile acids and taurine-conjugated dihydroxylated (DiOH, *m/z* 498, *B*), trihydroxylated (TriOH, *m/z* 514, *D*), and tetrahydroxylated (TetraOH, *m/z* 530, *F*) bile acids are shown. (*G*) The area under the curve (AUC) of individual peaks in *A–F* was added and expressed as % of total detectable bile acids. (*H*) The sum of all detectable unconjugated and conjugated bile acid peaks expressed as AUC. (*A–G*) n = 3.

shown), suggesting normal fat absorption during postnatal development. To examine fat absorption during adulthood, we determined fecal fat and caloric content after feeding a high-fat diet. FATP5 deletion mice showed a subtle increase in the percentage of fat in the feces (Figure 3*A*); however, the magnitude of this increase was very small (1.14-fold). Fecal caloric content and fecal free fatty acids were not significantly altered in FATP5 deletion mice (Figure 3*B* and *C*). The amount of cholesterol in the feces of wild-type and FATP5 knockout mice was also similar (10.04 ± 1.32 mg/g and 9.89 ± 1.03 mg/g), and similar amounts of stool were produced by wild-type and knockout mice (data not shown). Thus, although

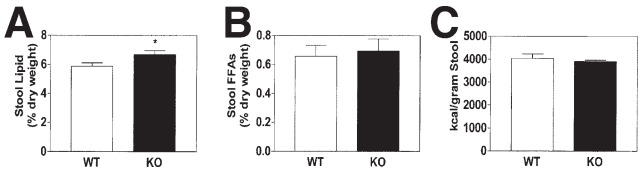

Figure 3. Lack of overt fat malabsorption in FATP5 deletion mice. (*A*) Stool lipid content and (*B*) free fatty acid content of feces collected from mice after 11 weeks on the high-fat diet. (*C*) Caloric content of feces of mice after 5 days on a high-fat diet. $*P < 0.05$.

FATP5 deletion mice show a very subtle increase in fecal fat, their fat absorption appears to be largely normal even when high-fat feeding.

FATP5 Knockout Mice Are Resistant to Diet-Induced Obesity Because of Decreased Food Intake and Increased Energy Expenditure on a High-Fat Diet

Although FATP5 deletion mice appeared indistinguishable from controls on a chow diet, we noticed that knockout mice appeared lighter on a high-fat diet. We therefore placed knockout and wild-type mice on a high-fat diet for 13 weeks and monitored body weight and food intake weekly. At the end of the diet, glucose clearance, adiposity, and oxygen consumption were measured; animals were then sacrificed, and organ weights and plasma parameters were determined. Two separate experiments were performed, and results were similar in both studies. As can be seen in Figure 4*A* and Table 2, FATP5 deletion mice gained significantly less weight on a high-fat diet compared with control mice (Figure 4*A*). DEXA (Figure 4*B*) and determination of fat pad weights (Table 2) showed that the difference in body weight between wild-type and FATP5 knockout mice was accompanied by a difference in adipose mass. Lean mass, as assessed by DEXA scanning, was unchanged between wild-type and knockout mice on a high-fat diet (not shown). Similar to our findings on a low fat-diet,[4] we observed a trend toward increased liver weight in FATP5 deletion mice on a high-fat diet (Table 2). As might be expected from the decrease in body weight, FATP5 knockout mice on a high-fat diet also showed an improved glucose tolerance profile (Figure 4*C* and *D*) and a trend toward decreased plasma insulin (Table 2). Glucose-tolerance profiles on a low-fat diet were similar between wild-type and FATP5 knockout mice (not shown).

Because the caloric content of the feces of FATP5 deletion mice was not altered even on a high-fat diet (Figure 3*C*), nutrient malabsorption cannot account for the lack of body weight gain on the high-fat diet. Analysis of food intake shows that FATP5 knockout mice consumed significantly fewer calories on the high-fat but not the low-fat diet (Figure 5*A* and Table 2). Caloric calculations show that this difference in food intake is sufficient to account for all of the difference in body weight gain. Thus, a major reason why FATP5 knockout mice are leaner on a high-fat diet is their failure to consume additional calories.

We also examined energy expenditure in FATP5 deletion mice fed a high-fat diet by monitoring O_2 consumption and CO_2 generation in a metabolic chamber (Figure 5*B* and *C*). O_2 consumption (Figure 5*B*) and CO_2 production (not shown) of FATP5 deletion mice was significantly higher compared with the wild-type controls in the fed state but not in the fasted state, suggesting an increase in diet-induced thermogenesis. This increased thermogenesis is not because of a selective increase in fatty acid oxidation because the RER was not altered in FATP5 knockout mice during either the fasted or the fed state (Figure 5*C*). Activity in FATP5 knockout mice was similar to controls and showed normal circadian rhythmicity (Figure 5*D*). We also observed an increase in body temperature, which was significant under fed conditions (Figure 5*E*), consistent with increased energy expenditure in FATP5 deletion mice. Interestingly, this increase in body temperature was observed in mice fed a low-fat diet, suggesting that, unlike the

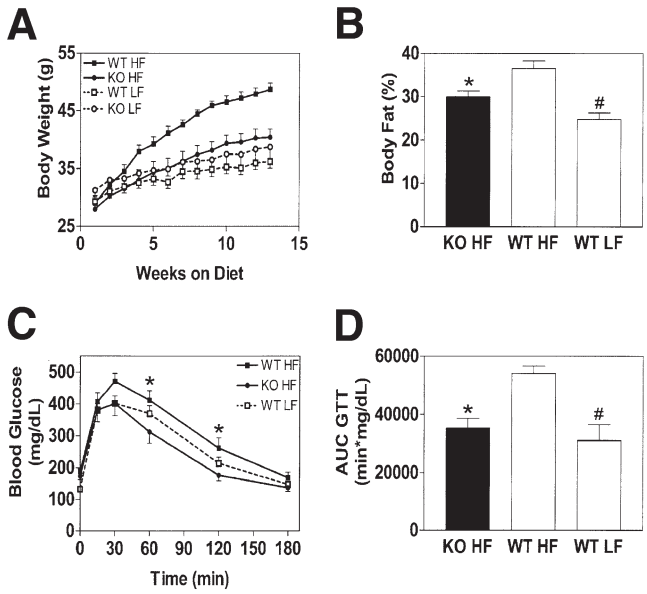

Figure 4. FATP5 deletion mice are protected from diet-induced obesity and insulin resistance. (*A*) Body weights of knockout (KO) and wild-type (WT) mice fed a low-fat (LF) or high-fat (HF) diet (n = 6). Differences between WT HF and KO HF or WT LF were significant ($P < 0.05$) at all time points between 4 and 13 weeks. (*B*) Body composition and (*C*) glucose tolerance test of KO and WT mice after (*B*) 17 and (*C*) 13 weeks on the high-fat diet (n = 12–14). (*D*) Area under the curve for the glucose excursion shown in *C*. $*P < 0.05$, KO HF versus WT HF. $^{\#}P < 0.05$, WT HF versus WT LF.

Table 2. Organ Weights and Biochemical Parameters on a High-Fat Diet

Genotype Diet	−/− High Fat	+/+ High Fat	+/+ Low Fat
Body weight (*g*)	38.0 ± 1.1[a]	43.2 ± 1.6	38.4 ± 0.9[b]
Food intake (*kcal/13 weeks*)	1114 ± 33[a]	1216 ± 29	ND
Epididymal fat (*g*)	2.00 ± 0.16[a]	2.69 ± 0.31	2.34 ± 0.20
Retroperitoneal fat (*g*)	0.72 ± 0.09[a]	1.10 ± 0.13	0.68 ± 0.07[b]
Liver (*g*)	1.54 ± 0.11	1.32 ± 0.09	1.32 ± 0.06
Liver lipid (*% w/w*)	14.57 ± 0.47[a]	16.55 ± 0.47	16.05 ± 0.37
Liver cholesterol (*mg/g*)	14.96 ± 1.63	14.94 ± 1.49	ND
Liver glycogen (*mg/g*)	11.82 ± 1.74	11.23 ± 1.3	10.67 ± 1.18
Blood glucose (*mg/dL*)	121.2 ± 9.1	113.3 ± 9.6	92.1 ± 8.4
Plasma insulin (*ng/mL*)	1.19 ± 0.21	2.06 ± 0.4	1.50 ± 0.29
Plasma triglyceride (*mg/dL*)	158.9 ± 15.6	201 ± 25	180.4 ± 14.2
Plasma free fatty acids (*mmol/L*)	1.38 ± 0.08	1.65 ± 0.10	1.68 ± 0.10
Plasma glycerol (*mg/dL*)	46.99 ± 0.83	58.25 ± 0.96	51.24 ± 0.50
Plasma cholesterol (*mg/dL*)	304.9 ± 30.9	279.5 ± 22.9	200.5 ± 21.3[b]
Plasma-OH-butyrate (*mg/dL*)	6.86 ± 1.34	9.38 ± 1.11	9.67 ± 1.20
Plasma leptin (*ng/mL*)	28.21 ± 4.13[a]	51.73 ± 8.79	28.77 ± 4.07[b]

NOTE. Body weight and food intake: fed mice, 13 weeks on the diet. All other data: fasted mice, 20 weeks on the diet.
[a]$P < 0.05$ knockout versus wild-type mice (high-fat diet).
[b]$P < 0.05$ high-fat versus low-fat fed wild-type mice. n = 5–6 for liver lipid, cholesterol, and glycogen, n = 11–14 for all other measurements.

decreased food intake, increased thermogenesis may not be dependent on the high-fat diet. In summary, our data suggest that FATP5 deletion mice are resistant to diet-induced obesity due to both a decrease in food intake and an increase in energy expenditure.

Gene Expression Changes in the Livers of FATP5 Deletion Mice

As a first step toward investigating the molecular changes associated with FATP5 deletion, we examined gene expression in the livers of high-fat–fed FATP5 knockout and wild-type control mice by using transcriptional profiling on Affymetrix chips. The expression of 78 and 55 genes, respectively, was significantly ($P < 0.05$ by a Student *t* test) and strongly (at least 2-fold difference in mean expression) up- and down-regulated between the 2 groups (Supplementary Table 1; see supplemental material online at http://www.pamf.org/research/stahl/resources/gastro2692). We noticed an up-regulation (either significantly or only as a trend) of many cholesterol biosynthetic enzymes as well as a significant up-regulation of cholesterol-7 α-hydroxylase (Cyp7a1), the rate-limiting enzyme in bile acid biosynthesis (Supplementary Table 2; see supplemental material online at http://www.pamf.org/research/stahl/resources/gastro2692). Taqman real-time reverse-transcription polymerase chain reaction confirmed an up-regulation of Cyp7a1 in FATP5 deletion mice (Table 3). Expression of the small heterodimer partner SHP, which mediates the inhibition of Cyp7A1 expression in response to high bile acid concentrations,[21,22] was decreased in FATP5 knockout mice, although this regulation did not reach statistical significance (Table 3). These data raise the

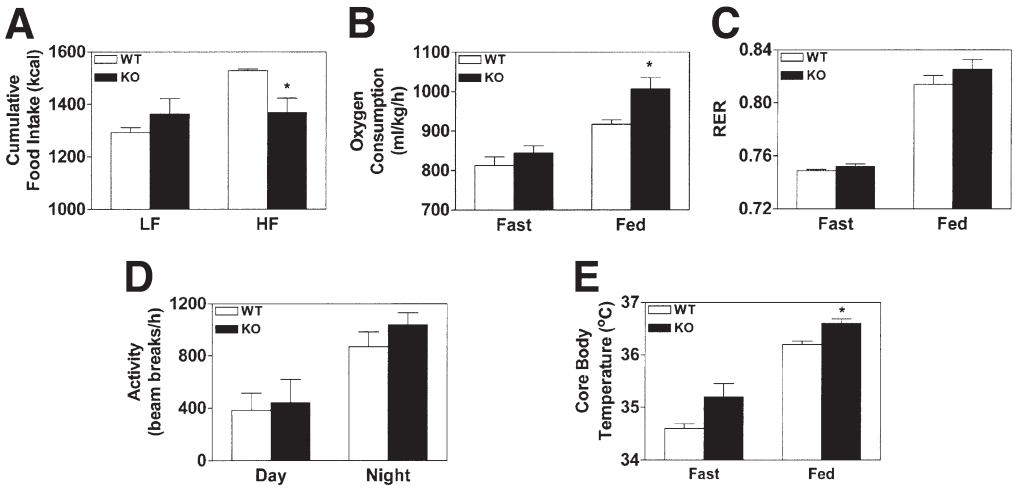

Figure 5. Food intake and energy expenditure of FATP5 knockout (KO) and wild-type (WT) mice. (*A*) Cumulative food intake on a low-fat (LF) or high-fat (HF) diet (n = 6). (*B*) Oxygen consumption and (*C*) respiratory exchange ratio (RER) after 18 weeks on a high-fat diet (n = 4). (*D*) Basic movements of fed animals during the inactive (day) or active (night) period (n = 6). (*E*) Rectal temperature of mice on a chow diet (n = 5). *$P <$ 0.05, KO HF versus WT HF. #P < 0.05, WT HF versus WT LF.

Table 3. Gene Regulation in the Liver of FATP5 KO Mice on a High-Fat Diet

Gene Name	−/−	+/+	*P* Value
Cholesterol-7α-hydroxylase (Cyp7a1)	19.8 ± 4.7	4.8 ± 1.8	.04
Small heterodimer partner (Shp)	13.7 ± 3.8	18.9 ± 2.9	.34
Na-taurocholate cotransporter (Ntcp)	8.3 ± 1.8	2.8 ± 0.9	.05
Bile salt export pump (Bsep)	8.5 ± 1.7	4.1 ± 0.2	.06

NOTE. mRNA expression in the liver of fasted animals (n = 3) after 20 weeks on the high-fat diet analyzed by Taqman Q-PCR. All values are relative expression.

possibility that the altered bile acid composition in FATP5 deletion mice results in a feedback regulation of bile acid biosynthesis, which in turn may increase expression of cholesterol biosynthesic enzymes. However, it is important to note that the observed gene expression changes are not sufficient to result in measurable increases in bile acid pool size or fecal bile acid content (Table 1). We also examined the expression of the bile acid transporters BSEP and the Na-taurocholate cotransporter NTCP. Both BSEP and NTCP expression were up-regulated in FATP5 deletion mice (Table 3), possibly to allow increased transport of the remaining conjugated bile acids. As might be expected from the demonstrated role of FATP5 in lipid metabolism,[4] several genes involved in fatty acid synthesis and uptake were also up-regulated (Supplementary Table 2). In summary, the observed alterations in gene expression are consistent with a role for FATP5 in lipid and bile acid metabolism.

Discussion

To date, the function of FATP5 in bile acid metabolism has been studied only in vitro. Here we show for the first time that FATP5 deletion mice have a specific defect in bile acid conjugation in vivo. These findings are consistent with a requirement for FATP5 in bile acid reconjugation and recycling but not in de novo synthesis of bile acids. Our in vivo data agree well with a recently proposed model that suggests that FATP5 is the major enzyme responsible for bile acid reactivation and recycling, whereas another enzyme, possibly FATP2, is responsible for activating bile acid precursors during de novo synthesis.[7] We found a severe conjugation defect in FATP5 knockout mice: in contrast to wild-type mice, where 95% of bile acids in bile are conjugated, only 17% of biliary bile acids are conjugated in FATP5 deletion mice. This is consistent with a requirement for FATP5 during most, if not all, reconjugation during the enterohepatic recirculation. Although FATP5 can activate THCA-CoA in vitro,[7] we did not find any evidence of defective conjugation during de novo synthesis in FATP5 knockout mice. The levels of mature C_{24} bile acids in the bile of FATP5 knockout and wild-type mice were similar, and no C_{27} bile acid precursors were detectable in the bile of FATP5 knockout mice. Conjugated primary, but not secondary, bile acids were detected in FATP5 deletion mice, showing that the remaining conjugation events in FATP5 knockout mice occur primarily during de novo bile acid synthesis. Interestingly, FATP2, a candidate for the THCA/DHCA-CoA ligase, is not up-regulated in FATP5 knockout mice.[4] Thus, compensation by FATP2 cannot mask a requirement for FATP5 in de novo synthesis.

Given the severity of the bile acid conjugation defect, we were surprised to observe no intrahepatic cholestasis and only minor effects on fat absorption in FATP5 deletion mice. Humans that are unable to conjugate bile acids because of a mutation in BAT show hypercholonemia and fat malabsorption,[13] which has been attributed to an inability of the bile salt export pump BSEP to transport unconjugated bile acids.[14] In contrast to the human mutation, however, significant amounts of conjugated bile acids remain in the FATP5 deletion mice, possibly accounting for the milder phenotype. Mutations in BAT, but not FATP5, would also be expected to result in hepatic accumulation of bile acid–CoA derivatives, which may be poor substrates for export and could interfere with the export of mature bile acids. A recent study showed transport of cholic acid by rat BSEP,[23] suggesting that at least in rodents some BSEP-mediated transport of unconjugated bile acids can occur. In addition, significant amounts of BSEP-independent bile acid transport occurs in rodents,[15] and unconjugated bile acids have been shown to enter cells without a bile acid transporter.[24] Our observation that unconjugated bile acids can be secreted efficiently into bile is consistent with previous studies that showed significant amounts of unconjugated bile acids in the bile of mice with liver-specific deletion of HNF4α (H4LivKO mice)[25] or mice that have been fed cholic acid.[26] FATP5 deletion mice also have increased expression of BSEP and show increased levels of tetrahydroxylated bile acids in the bile, 2 mechanisms that may allow for more efficient export of bile acids. It is likely that a combination of the above factors contribute to the apparently normal bile acid secretion in FATP5 deletion mice. The absence of a hepatic bile acid export defect together with the normal bile acid pool size likely explain the lack of fat malabsorption observed in FATP5 knockout mice.

It is interesting to compare FATP5 deletion mice to H4LivKO mice.[25,27,28] These mice have increased levels of unconjugated bile acids because of decreased expression of both FATP5 and BAT. Unlike FATP5 deletion mice, however, H4LivKO mice have increased serum bile acid concentrations and show increased levels of glycine-conjugated bile acids. Comparison of the gene expression changes in H4LivKO mice[27,28] with FATP5 deletion mice (Supplemental Tables 1 and 2) shows numerous differences that may explain the divergences between the 2 models. In particular, although H4LivKO mice have decreased expression of the bile acid transporters NTCP and OATP1, FATP5 deletion mice have increased NTCP mRNA levels (Table 3) and no change in OATP1 levels (profiling dataset, data not shown), possibly explaining the absence of hypercholonemia in FATP5 deletion mice. The increase in glycine-conjugated bile acids in H4LivKO mice has been suggested to be because of up-regulation of a novel BAT homolog (GeneBank accession number NM_145368; Gene ID 209186). Interestingly, this gene is down-regulated in FATP5 deletion mice (Supplementary Table 1), consistent with the lack of appearance of glycine-conjugated bile acids. It is likely that the involvement of HNF4α in the regulation of numerous bile acid metabolic enzymes results in a more complex and severe phenotype compared with deletion of FATP5 alone.

The most pronounced phenotype of FATP5 knockout mice at the whole-body level is their poor weight gain when placed on a high-fat diet. This is not caused by fat malabsorption because the caloric content of stool is similar in wild-type and FATP5 deletion mice even on a high-fat diet. Furthermore, even though we observe a subtle increase in fecal fat, caloric calculations show that this increase can account for no more than 5% of the observed difference in body weight. The failure of FATP5 deletion mice, compared with the wild-type littermates, to gain weight on a high-fat diet, however, can be fully explained by their inability to increase caloric intake like the wild-type animals on this diet. An interesting question is whether this altered food intake is caused by the alteration in bile acid metabolism as described in this study, the altered lipid metabolism demonstrated by Doege et al[4], or a combination thereof. Alterations in hepatic lipid metabolism have been shown to affect food intake through vagal afferent fibers[29]; however, in these studies, a decrease in fatty acid oxidation led to an increase in food intake. Similar to what was observed on a low-fat diet,[4] we find decreased intrahepatic lipids and a trend toward decreased ketone bodies in FATP5 deletion mice on a high-fat diet, suggesting decreased fatty acid oxidation. An alternative hypothesis is that the redistribution of fatty acids from the liver to adipose tissue and muscle in FATP5 knockout mice[4] results in secondary metabolic changes that contribute to the decreased food intake. Leptin levels, when adjusted for adipose mass, are not altered in FATP5 deletion mice (Table 2), suggesting that FATP5 deletion does not cause dysregulation of leptin secretion. It is important to note that the decrease in food intake in FATP5 deletion mice is only observed on a high-fat diet, suggesting that high-fat diet-specific alterations are responsible for this effect. A small increase in fecal fat was observed in FATP5 deletion mice. Because both bile acids[30] and fatty acids[31] can modulate secretion of the intestinal satiety peptide Glp-1, it is possible that the altered bile acid composition combined with the small increase in fecal fat detectable on a high-fat diet affects the levels of intestinal neuropeptides. Finally, although the role of bile acids in regulating food intake is poorly understood, bile acid feeding has been shown to decrease food intake in rodents specifically on a high-fat diet.[32] Clearly, additional studies are required to determine the mechanism underlying the alterations in food intake in FATP5 deletion mice.

A second alteration in whole-body metabolism in FATP5 deletion mice is increased energy expenditure. Unlike the alterations in food intake, increased energy expenditure is observed even on a low-fat diet as evidenced by the increase in body temperature of FATP5 deletion mice fed a normal chow diet. This increase in energy expenditure is not because of a selective increase in fatty acid oxidation because we did not observe a change in the respiratory quotient under either fasting or fed conditions. Interestingly, increased energy expenditure is detectable primarily under fed conditions. One hypothesis is that this increase is because of the redistribution of dietary fatty acids into peripheral organs other than the liver.[4] It will be interesting to assess whether FATP5 deletion mice have increased brown adipose tissue thermogenesis because of increased fatty acid availability to this tissue.

Although studies on a low-fat diet show increased serum glucose and insulin levels in FATP5 deletion mice,[4] in our high-fat diet studies, we observed no change in serum glucose levels and a trend toward decreased serum insulin levels in FATP5 knockout mice on a high-fat diet. Furthermore, glucose clearance was improved in high-fat–fed FATP5 knockout mice, consistent with improved insulin sensitivity. We currently do not understand the reason for the apparent differences in glucose homeostasis between low- and high-fat diet. However, because the insulin resistance observed when high-fat feeding is driven by the increase in caloric intake, a decrease in food intake would be expected to abolish the development of diet-induced insulin resistance. It is possible that the magnitude of this effect

may mask any underlying defects in glucose homeostasis in FATP5 deletion mice.

In conclusion, together with the accompanying paper by Doege et al[4], we have shown that FATP5 is a protein with multiple activities in vivo. Our data show that FATP5 is indeed the major bile acid–CoA ligase in vivo. Interestingly, decreased bile acid conjugation in these mice did not result in overt cholestasis or fat malabsorption, showing that unconjugated bile acids can mediate largely normal fat absorption at least in mice. Unexpectedly, we found that FATP5 deletion mice fail to gain weight on a high-fat diet because of a combination of decreased food intake and increased energy expenditure. Future studies will be required to determine whether the changes in body-weight homeostasis in FATP5 deletion mice reflect the role of FATP5 in bile acid conjugation or lipid metabolism or a combination of both. However, it is clear that FATP5 deletion mice represent an interesting new model to examine both the role of bile acid conjugation in normal physiology and to dissect the contributions of bile acid and lipid metabolism to body-weight regulation.

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