Cholesterol 7α-hydroxylase-deficient mice are protected from high-fat/high-cholesterol diet-induced metabolic disorders

Jessica M. Ferrell,* Shannon Boehme,* Feng Li,† and John Y. L. Chiang1,∗

Department of Integrative Medical Sciences,*† Northeast Ohio Medical University, Rootstown, OH; and Department of Molecular and Cellular Biology,1 Baylor College of Medicine, Houston, TX

Abstract Cholesterol 7α-hydroxylase (CYP7A1) is the first and rate-limiting enzyme in the conversion of cholesterol to bile acids in the liver. In addition to absorption and digestion of nutrients, bile acids play a critical role in the regulation of lipid, glucose, and energy homeostasis. We have backcrossed Cyp7a1−/− mice in a mixed B6/129Sv genetic background to C57BL/6J mice to generate Cyp7a1−/− mice in a near-pure C57BL/6J background. These mice survive well and have normal growth and a bile acid pool size ∼60% of WT mice. The expression of the genes in the alternative bile acid synthesis pathway are upregulated, resulting in a more hydrophilic bile acid composition with reduced cholic acid (CA). Surprisingly, Cyp7a1−/− mice have improved glucose sensitivity with reduced liver triglycerides and fecal bile acid excretion, but increased fecal fatty acid excretion and respiratory exchange ratio (RER) when fed a high-fat/high-cholesterol diet. Supplementing chow and Western diets with CA restored bile acid composition, reversed the glucose tolerant phenotype, and reduced the RER. Our current study points to a critical role of bile acid composition, rather than bile acid pool size, in regulation of glucose, lipid, and energy metabolism to improve glucose and insulin tolerance, maintain metabolic homeostasis, and prevent high-fat diet-induced metabolic disorders.—Ferrell, J. M., S. Boehme, F. Li, and J. Y. L. Chiang. Cholesterol 7α-hydroxylase-deficient mice are protected from high-fat/high-cholesterol diet-induced metabolic disorders. J. Lipid Res. 2016, 57: 1144–1154.

Supplementary key words bile acids and salt/metabolism • cholesterol/diet • liver • lipids

Bile acids are amphipathic molecules that aid in the absorption of dietary fats, nutrients, and vitamins. Bile acids are also endogenous ligands that activate the nuclear receptor, farnesoid X receptor (FXR), and the membrane G protein-coupled bile acid receptor, Gpbar-1 (aka TGR5). Bile acids are synthesized from cholesterol exclusively in the liver by cholesterol 7α-hydroxylase (CYP7A1), the first and rate-limiting enzyme in the classic bile acid biosynthetic pathway. Sterol 12α-hydroxylase (CYP8B1) catalyzes the synthesis of cholic acid (CA) and determines the ratio of CA to chenodeoxycholic acid (CDCA) and its derivatives, and thus, the hydrophobicity of the bile acid pool. In mouse liver, CDCA is converted to α- and β-muricholic acid (MCA), which are highly soluble and are FXR antagonists. Bile acids can also be synthesized via the alternative pathway, which is initiated by mitochondrial sterol 27-hydroxylase (CYP27A1) and oxysterol 7α-hydroxylase (CYP7B1) to synthesize mainly CDCA in the liver. The alternative pathway contributes to ∼18% of the total bile acids in humans and as much as 50% in rodents. Bile acids are conjugated to the amino acids taurine or glycine in humans, but predominantly taurine in mice, and are secreted into bile and stored in the gallbladder until they are released into the small intestine after the ingestion of food. Within the intestinal track, bile acids emulsify dietary fats and form mixed micelles with fatty acids and 2-monoacylglycerols, which are absorbed into the enterocyte. Here, bacterial bile salt hydrolase deconjugates the

Abbreviations: ASBT, apical sodium-dependent bile acid transporter; CA, cholic acid; CDCA, chenodeoxycholic acid; CLAMS, comprehensive lab animal monitoring system; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; DCA, deoxycholic acid; FGF15, fibroblast growth factor 15; FGF19, fibroblast growth factor receptor 4; FXR, farnesoid X receptor; LDLr, LDL receptor; MCA, muricholic acid; NEOMED, Northeast Ohio Medical University; Pekv9, propoprotein convertase subtilisin/kexin type 9; QTOFMS, quadrupole TOFMS; RER, respiratory exchange ratio; SHP, small heterodimer partner; Sirt1, sirtuin; Sdrd4, steroidogenic acute regulatory protein-related lipid transfer domain containing 4; TCA, tauro-cholic acid; TCDCA, taurochenodeoxycholic acid; TDECA, taurodeoxycholic acid; Tauro-α-MCA, tauro-α-muricholic acid; Tβ-MCA, tauro-β-muricholic acid; UHPLC, ultra HPLC.

To whom correspondence should be addressed.

email: jchiang@neomed.edu

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gene expression was upregulated in phenotype (19, 20). These studies also reported that Cyp8b1 even low dose bile acid feeding could rescue the knockout diabetic patients after bariatric surgery and in animal models of diabetes and obesity and in nonalcoholic steatohepatitis patients (11–13). Bile acids also have been implicated in rapidly improving insulin resistance in type 2 diabetic patients (18), though females in this colony a milder phenotype (18), though females in this colony were resistant to high-fat/high-cholesterol diet-induced disease, we generated Cyp7a1−/− mice in a C57BL/6J genetic background to test to determine whether Cyp7a1−/− mice develop symptoms of metabolic syndrome expected from high-fat/high-cholesterol diet feeding. To our surprise, the Cyp7a1−/− mice we generated had improved glucose tolerance; these mice also had a larger bile acid pool size than Cyp7a1−/− (B6/129Sv) mice and had altered bile acid composition consistent with induction of the genes in the alternative bile acid synthesis pathway, which may play a critical role in maintaining liver metabolic homeostasis and preventing diet-induced obesity and diabetes.

**Materials and Methods**

**Animals**

Cyp7a1−/− mice originally obtained from Jackson Laboratory (Bar Harbor, ME) (Cyp7a1−/−; C57BL/6J) were backcrossed with C57BL/6J mice for seven generations to produce founder Cyp7a1−/− mice in an approximately 99.6% pure C57BL/6J background. Founder mice were pair-bred in the Comparative Medicine Unit at Northeast Ohio Medical University (NEOMED). Male WT C57BL/6J mice (originally obtained from Jackson Laboratory) were bred at NEOMED to serve as controls. All animal protocols were approved by the Institutional Animal Care and Use Committee of NEOMED.

Six-week-old male WT C57BL/6J and Cyp7a1−/− mice (hereafter referred to as C57BL/6J genetic background) were maintained on a standard chow diet (LabDiet #5008; St. Louis, MO) with water ad libitum in a temperature-controlled facility with a 12 h light/12 h dark schedule (6:00 PM – lights off). Additional age-matched cohorts of male C57BL/6J and Cyp7a1−/− mice were maintained on a high-fat/high-cholesterol Western diet (42% kcal from fat, 0.2% cholesterol; TD.88137; Harlan Laboratories, Indianapolis, IN) for 18 weeks (n = 6). To determine whether bile acid supplementation could reverse the knockout phenotype, 8-week-old C57BL/6J and Cyp7a1−/− male mice were maintained on a standard chow diet or a Western diet supplemented with 0.03% CA (19) (w/w; Sigma-Aldrich, St. Louis, MO) for 10 weeks.

**Metabolic Analysis**

At 5–6 months of age, metabolic analysis of male WT and Cyp7a1−/− mice fed chow or Western diet with or without CA was performed using a comprehensive lab animal monitoring system (CLAMS) (Columbus Instruments, Columbus, OH). The CLAMS utilizes indirect calorimetry to determine metabolic performance continuously. Briefly, mice were housed individually in sealed clear Plexiglas cages through which fresh room air was continuously passed at 0.5 l/min. Via O2 and CO2 sensors, exhaust air was sampled in each cage in succession at 2 min intervals over 24 h. O2 consumption (V02), CO2 production (VCO2), respiratory exchange ratio [RER (VCO2/V02), an estimate of fuel usage], and energy (heat) production were calculated and recorded electronically over 24 h for each mouse (following a 48 h acclimation period). Total locomotor activity (measured by conjugated bile acids and 7α-dehydroxylase converts CA and CDCA to deoxycholic acid (DCA) and lithocholic acid, respectively. These secondary bile acids are toxic and excreted into feces. Approximately 95% of bile acids, consisting of mainly CA, DCA, and CDCA in humans and CA and α/β-MCA in mice, are reabsorbed in the ileum and transported back to the liver via portal circulation. This enterohepatic circulation occurs multiple times per day to regulate bile acid synthesis and aid in distribution of nutrients, drugs, and xenobiotics to the liver. The small amount of bile acids lost in feces is replenished by de novo synthesis in the liver [reviewed in (5)]. Bile acids inhibit bile acid synthesis by two mechanisms. In the liver, bile acids activate FXR, which induces the inhibitory nuclear receptor, small heterodimer partner (SHP), to inhibit Cyp7a1 gene transcription (6). In the intestine, FXR induces fibroblast growth factor 4 (FGF15; or human ortholog FGF19), which is circulated to hepatocytes to activate fibroblast growth factor receptor 4 (FGFR4) signaling to suppress Cyp7a1 gene expression via activation of mitogen-activated protein kinases, including the extracellular signal-related kinase and cJun (7).

Bile acid synthesis represents the main catabolic pathway for disposal of cholesterol and maintenance of whole body cholesterol homeostasis. More recently studies have unveiled critical roles of bile acids in the regulation of lipid, glucose, and energy metabolism (8–10). Manipulations of the bile acid pool size by bile acid supplementation, bile acid sequestrants, or pharmacological activation of FXR and TGR5 signaling have been shown to improve insulin sensitivity and glucose tolerance in mouse models of diabetes and obesity and in nonalcoholic steatohepatitis patients (11–13). Bile acids also have been implicated in rapidly improving insulin resistance in type 2 diabetic patients after bariatric surgery and in animal models (14–16).

Genetic ablation of the Cyp7a1 gene in mice (Cyp7a1−/−) resulted in high postnatal mortality and malnutrition phenotypes that were prevented by bile acid and vitamin supplementation (17). The original adult Cyp7a1−/− mice in the mixed genetic background (B6/129Sv) had a reduced bile acid pool (~30% of WT mice) and a normal lipid profile, while a later study of the same Cyp7a1−/− mice (B6/129Sv) in a different colony demonstrated improved survival and a milder phenotype (18), though females in this colony were hypercholesterolemic. Two recent studies using the original Cyp7a1−/− mice (B6/129Sv) demonstrated that even low dose bile acid feeding could rescue the knockout phenotype (19, 20). These studies also reported that Cyp8b1 gene expression was upregulated in Cyp7a1−/− mice (B6/129Sv), while genes involved in the alternative bile acid synthesis pathway were not, despite increased oxidized sterol content. We reported recently that transgenic overexpression of CYP7A1 in C57BL/6J mice resulted in increased bile acid pool with altered bile acid composition consisting of reduced CA and increased CDGA, resulting in reduced fat absorption and activation of FXR signaling to improve glucose and insulin tolerance. Interestingly, these mice were resistant to high-fat/high-cholesterol diet-induced insulin resistance and obesity (21). In Cyp7a1−/−transgenic mice, liver de novo cholesterol synthesis was stimulated but fatty acid synthesis was decreased, coupled with increased biliary bile acid and cholesterol secretion to maintain bile acid and cholesterol homeostasis (22). To further study bile acid synthesis in liver metabolism and disease, we generated Cyp7a1−/− mice in a C57BL/6J genetic background to test to determine whether Cyp7a1−/− mice develop symptoms of metabolic syndrome expected from high-fat/high-cholesterol diet feeding. To our surprise, the Cyp7a1−/− mice we generated had improved glucose tolerance; these mice also had a larger bile acid pool size than Cyp7a1−/− (B6/129Sv) mice and had altered bile acid composition consistent with induction of the genes in the alternative bile acid synthesis pathway, which may play a critical role in maintaining liver metabolic homeostasis and preventing diet-induced obesity and diabetes.
x, y, and z axis infrared beam breaks) and diet consumption were also recorded electronically for each mouse. Weekly body weight was recorded in all mice. An EchoMRI 3-in-1 body composition analyzer (EchoMRI; Houston TX) was used to record measurements of fat and lean tissue mass in live mice before metabolic analysis. After completion of recording, mice were fasted overnight before euthanization and tissues were then collected for analysis.

**Glucose, insulin, and fat tolerance tests**

Glucose tolerance tests were performed in separate cohorts of 5-month-old male WT and Cyp7a1−/− mice maintained on chow or Western diet with or without 0.03% CA supplementation (n = 5–7). Mice were ip injected with D-glucose dosed at 2 g/kg body weight following a 16 h fast. Insulin tolerance was tested by ip injection of insulin (Humulin R; Eli Lilly, Indianapolis, IN) dosed at 0.75 U/kg body weight following a 5 h fast. Blood samples were collected via tail vein and blood glucose was measured using a OneTouch Ultra Mini glucometer (LifeScan, Milpitas, CA). Oral fat tolerance was determined in chow-fed male WT and Cyp7a1−/− mice. Mice were gavaged with 15 ml/kg corn oil (Sigma-Aldrich) and blood was collected hourly via the tail vein for serum analysis of triglycerides.

**Real-time PCR analysis**

Total RNA was isolated from mouse tissue using TRI-Reagent (Sigma-Aldrich). Quantitative real-time PCR was performed using TaqMan gene expression assays according to the manufacturer’s instructions (ThermoFisher Scientific Inc., Waltham, MA). Relative mRNA levels were calculated using the comparative Ct method and experimental values were normalized to Gapdh as an internal control.

**Lipid analysis**

Lipids were extracted from homogenized liver tissue and dried feces with 2:1 chloroform:isopropanol followed by evaporation at 60°C. The resulting lipids were dissolved in water with 2% Triton X-100. Liver and serum cholesterol, triglycerides (ThermoFisher), and free fatty acids (Wako Diagnostics, Richmond, VA) were analyzed using commercially available kits.

**Bile acid analysis**

Bile acids were isolated from 100 mg liver, whole intestine, and 200 mg feces by a series of ethanol and methanol extractions overnight at 65°C. Bile acid content was quantified by kit (Genzyme Diagnostic, Cambridge, MA) and bile acid pool size was determined by totaling bile acids in liver, gallbladder, and intestine. Content measured in liver was back-calculated to whole liver weight to determine total liver bile acid content. Bile acid composition was determined by ultra (U) HPLC-quadrupole (Q)TOFMS (25). Gallbladder bile (25 μl) was incubated in 500 μl isopropanol overnight in a 55°C water bath. After vortexing for 30 s, the resulting mixture was centrifuged at 15,000 rcf for 15 min. Twenty microliters of the supernatant was diluted with 980 μl of water:methanol (1:1). Following centrifugation at 14,000 rcf for 20 min, the supernatant was transferred to a sample vial for analysis. Five microliters of the supernatant was injected to a UHPLC-QTOFMS setup for analysis. Bile acid separation was achieved using a 1260 Infinity Binary LC System (Agilent Technologies, Santa Clara, CA) equipped with a 100 × 2.7 mm (Agilent XDB C18) column. The column temperature was maintained at 45°C. The flow rate was 0.3 ml/min with a gradient ranging from 2% to 98% aqueous acetoni-trile containing ammonia acetate (pH 8.6) in a 25 min run. QTOFMS was operated in negative mode with electrospray ionization.

Ultra-highly pure nitrogen was applied as the drying gas (12 l/min) and the collision gas. The drying gas temperature was set at 325°C and nebulizer pressure was kept at 35 psi. During the analysis of samples, real-time mass correction and accurate mass were achieved by continuously measuring standard reference ions at m/z 119.0363 (m/z 966.0072 for the negative mode). Bile acid hydrophobicity indices of mouse gallbladder bile composition was calculated using the following values (24): tauro-α-MCA (T-α-MCA) = −0.84, tauro-β-MCA (T-β-MCA) = −0.78, tauro-holistic acid (TCA) = 0, tauro-CDCA (TCDCA) = +0.46, tauro-DCA (TDC) = +0.59, tauro-litholic acid = +1.

**RESULTS**

**Bile acid pool size is reduced and composition is altered in Cyp7a1−/− mice**

Bile acid pool size, defined as total liver, gallbladder, and intestinal bile acids, was determined. Figure 1A (upper left panel) demonstrates that bile acid content in liver, gallbladder, and intestine was reduced and total bile acid pool size in Cyp7a1−/− mice was significantly reduced to approximately 50–60% of WT mice, which is ~2-fold larger than the previously reported pool size in Cyp7a1−/− mice (B6/129Sv). After supplementation with 0.03% CA, intestinal bile acid content and overall bile acid pool size in Cyp7a1−/− mice remained significantly reduced compared with chow-fed mice (Fig. 1B, upper left panel). Gallbladder bile acid composition analysis (Fig. 1A, lower panels) revealed Cyp7a1−/− mice have markedly reduced TCA (32%) and TDC (6%) compared with WT mice (45% and 11%, respectively) and roughly doubled content of T-α-MCA (25%) and TCDCA (7%) compared with WT (11% and 3%, respectively), while T-β-MCA was not changed. As a result, the bile acid hydrophobicity index was reduced to −0.5764 in Cyp7a1−/− mice compared with −0.2477 in WT mice. Real-time PCR analysis of liver gene expression demonstrated that mRNA expression levels of Cyp8b1 in the classic pathway and Cyp7b1 in the alternative bile acid synthesis pathway were significantly increased, while Fxr and Shp mRNA levels were not changed (Fig. 1A, upper right panel). Increasing Cyp8b1, Cyp7b1, and Cyp27a1 expression is due to reduced bile acid pool and, thus, reduced feedback inhibition in Cyp7a1−/− mice. Surprisingly, feeding Cyp7a1−/− mice with 0.03% CA resulted in further induction of Cyp8b1 and Cyp7b1, while Cyp27a1 and Shp gene expression were moderately increased compared with WT mice (Fig. 1B, upper right panel). This may be because CA reduced Cyp8b1 and Cyp7b1 mRNA levels in WT mice. Supplementation with CA increased TCA (from 32% to 59%) and decreased T-α-MCA (from 25% to 8%) in Cyp7a1−/− mice, while composition in WT mice indicates a slight increase in TCA (from 45% to 52%), reduced TCDCA (from 7% to ~1%), and no change in T-β-MCA. Thus, CA supplementation largely restored bile acid composition in Cyp7a1−/− mice to that of WT mice. Breeding Cyp7a1−/− mice into a C57/BL6J background may increase the bile acid pool by stimulating the alternative pathway, resulting in reduced TCA and TDC and increased TCDCA and T-α-MCA in Cyp7a1−/− mice.
Cyp7a1<sup>−/−</sup> mice in a C57BL/6J genetic background exhibit normal physiological characteristics with improved glucose tolerance that is reversed with CA supplementation

The original Cyp7a1-deficient mice (B6/129Sv) had high mortality unless pups were nutritionally supplemented with vitamins or bile acids (17). The Cyp7a1<sup>−/−</sup> mice we generated by backcrossing to a nearly pure C57BL/6J (99.6%) genetic background exhibit normal pup survival rate, as well as normal body weight/growth, liver weight, white adipose weight, and food intake comparable to WT controls maintained on normal rodent chow diet (supplementary Fig. 1). Following an ip injection of glucose after an overnight fast, Cyp7a1<sup>−/−</sup> mice responded with reduced blood glucose levels within 60 min, and blood glucose remained significantly lower than WT counterparts through 150 min after injection, indicating increased glucose tolerance (Fig. 2A, upper panel). When ip injected with insulin after a 5 h fast, blood glucose levels in Cyp7a1<sup>−/−</sup> mice were significantly reduced compared with WT mice (Fig. 2A, lower panel), though fasting glucose levels prior to the test were reduced in Cyp7a1<sup>−/−</sup> mice.

Cyp7a1<sup>−/−</sup> mice fed Western diet have reduced and altered bile acid composition

Under Western diet feeding, Cyp7a1<sup>−/−</sup> mice had significantly reduced intestinal bile acid content, which resulted in approximately 30% reduction in pool size, largely due to reduced bile acid content in the intestine (Fig. 3A, upper left panel). Real-time PCR analysis indicated that...
Cyp7b1 mRNA levels were markedly increased by ~5-fold and Cyp27a1 mRNA levels were also significantly elevated in Cyp7a1−/− mice compared with WT mice; additionally, liver Fxr mRNA expression remained unchanged, but Shp mRNA expression was significantly elevated in Cyp7a1−/− mice fed a Western diet (Fig. 3A, upper right panel). In Western diet-fed WT mice, TCA content was similar to chow-fed controls, while TCDCA content tripled with Western diet feeding (9%) compared with chow diet (3%; compare Fig. 1A and Fig. 3A). TDCA decreased from 11% in chow-fed WT mice to 2% in Western diet-fed WT mice, possibly indicating a high-fat diet-induced dysbiosis in the microbial communities responsible for the conversion of CA to DCA in the intestine, or reduced reabsorption of DCA into the bile acid pool (Fig. 3A). Western diet feeding in Cyp7a1−/− mice resulted in an increase of T-β-MCA from 23% to 8% and increased TCA and T-β-MCA in Cyp7a1−/− mice, while bile acid composition in WT mice fed Western diet plus CA remained unchanged (Fig. 3B, lower panel). Thus, CA supplementation only partially restored bile acid composition in Cyp7a1−/− mice, and it is surprising that CA supplementation did not increase CA content in both Western diet-fed WT and Cyp7a1−/− mice. These data suggest that the high cholesterol content in Western diet may further stimulate the alternative bile acid synthesis pathway to produce more T-β-MCA and increase bile acid hydrophobicity in Cyp7a1−/− mice.

Cyp7a1−/− mice fed Western diet gain weight but maintain improved glucose tolerance

When fed a high-fat/high-cholesterol Western diet for 18 weeks, body weight increased in Cyp7a1−/− mice, which was statistically insignificant from WT controls. Liver and adipose tissue weight did not significantly differ between genotypes; though interestingly, Cyp7a1−/− mice ate significantly more food compared with WT controls (supplementary Fig. 2). Glucose tolerance was significantly improved in Western diet-induced obese Cyp7a1−/− mice beginning 60 min after glucose injection and remained lower than WT controls up to 150 min after injection (Fig. 4A, upper panel). Insulin tolerance test results did not differ between Cyp7a1−/− and WT mice, though 5 h fasting blood glucose levels in Cyp7a1−/− mice prior to insulin injection were significantly lower than WT mice (Fig. 4A, lower panel). After 10 weeks of 0.03% CA supplementation, glucose and insulin tolerance (Fig. 4B) were not different statistically between WT and Cyp7a1−/− mice.

Lipid profiles in Cyp7a1−/− mice are altered and results in increased fecal lipid excretion

Liver and serum lipids were analyzed in chow- and Western diet-fed WT and Cyp7a1−/− mice. On chow diet, liver cholesterol and liver triglycerides were unaltered, while liver free fatty acid content was increased in Cyp7a1−/− mice (Fig. 5A), and serum cholesterol, triglycerides, and free fatty acids were unaltered, consistent with previous studies done in Cyp7a1−/− (6J/129Sv) mice (18) (Fig. 5B). When fed a Western diet, liver cholesterol, triglycerides, and free fatty acids increased in both WT and Cyp7a1−/− mice (Fig. 5A), but Cyp7a1−/− mice had significantly reduced serum cholesterol. An oral fat tolerance test was conducted in chow-fed mice, which resulted in significantly reduced serum triglyceride levels in Cyp7a1−/− mice for 2 h indicating...
When mice were fed a normal chow diet, resting RER, energy expenditure (heat), and locomotor activity did not differ between Cyp7a1−/− mice and WT controls (supplementary Fig. 3), indicating that lack of the Cyp7a1 gene does not produce a lethal metabolic phenotype in mice and their overt physiology is similar to WT mice.

After Western diet feeding, 24 h RER was significantly elevated in Cyp7a1−/− mice (φ0.9) compared with WT controls (φ0.84); it was determined that RER was increased during both the day (inactive period) and night (active period) (Fig. 7A), indicating that the elevation in RER was not an artifact due to the normal diurnal increase in nighttime locomotor activity and was not associated with energy expenditure (supplementary Fig. 4). Following dietary supplementation with 0.03% CA, RER of Cyp7a1−/− mice was reduced to φ0.81 and that of WT mice was reduced to φ0.82 (Fig. 7B).

EchoMRI was used to measure body composition (percentage of fat and lean mass normalized to body weight) in live mice. Under chow-fed or Western diet-fed conditions, there were no differences in fat or lean mass between WT and Cyp7a1−/− mice (Fig. 5C). Fecal bile acid and cholesterol contents were similar in Cyp7a1−/− mice and WT mice (Fig. 6A, B). Western diet feeding significantly increased fecal cholesterol secretion in both WT and Cyp7a1−/− mice. However, Western diet markedly increased fecal fatty acid secretion in Cyp7a1−/− mice (Fig. 6C). CA supplementation markedly increased fecal bile acid excretion, though there was no significant difference between genotypes (Fig. 6D). Thus, Cyp7a1−/− mice in a C57BL/6J background may reabsorb more bile acids to maintain a larger bile acid pool size compared with Cyp7a1−/− mice in a mixed genetic background and increased bile acid excretion after CA feeding may explain the slightly reduced bile acid pool size and similar CA levels in bile after Western diet feeding (Fig. 3A, B).

**Resting metabolic rate is increased in Cyp7a1−/− mice maintained on Western diet**

Next, we used CLAMS equipment to determine metabolic phenotype in chow-fed and Western diet-fed mice. When mice were fed a normal chow diet, resting RER, energy expenditure (heat), and locomotor activity did not differ between Cyp7a1−/− mice and WT controls (supplementary Fig. 3), indicating that lack of the Cyp7a1 gene does not produce a lethal metabolic phenotype in mice and their overt physiology is similar to WT mice.

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increased in genes were all significantly increased or trending toward Cpt1α, carnitine palmityltransferase 1 (Cpt1), and Scd1 expression levels of scavenger receptor class B member 4 (Stard4) (StAR, Srb1 or Srb11) were significantly upregulated in chow-fed mice and Western diet feeding compared with WT mice (Fig. 8B), suggesting glycolysis may be stimulated to reduce serum glucose level in Cyp7a1−/− mice. In the ileum, mRNA expression levels of apical sodium-dependent bile acid transporter (Asbt) and Fxr were significantly elevated in Cyp7a1−/− mice independent of diet, while Fgf15 was reduced under chow-fed conditions in Cyp7a1−/− mice (Fig. 8C). Increased Asbt expression is correlated to increased bile acid reabsorption in the ileum (25). Decreasing Fgf15 may reduce activation of hepatic FGFR4 signaling to induce Cyp8b1 expression (Fig. 1A). Expression of Shp in the ileum was highly upregulated by Western diet in WT mice and was suppressed in Cyp7a1−/− mice, while expression of sinusoidal bile acid efflux transporters organic solute transporter α (Osta) and β (Ostβ) and Tgr5 was not differentially regulated in Cyp7a1−/− mice or by Western diet (Fig. 8C).

We examined canonical genes involved in bile acid, lipid, and energy metabolism in liver and brown adipose tissue (supplementary Fig. 6). Bile acid transport genes in the liver of Cyp7a1−/− and WT mice remained relatively unchanged, though sinusoidal bile acid uptake transporter Na+-taurocholate cotransport polypeptide (Ntcp) mRNA was reduced in Western diet-fed WT mice, while Ostβ mRNA was significantly increased by Western diet in Cyp7a1−/− mice (supplementary Fig. 6A). HMG-CoA reductase (Hmgcr) mRNA expression was upregulated in Western diet-fed WT mice and was further increased in Western diet-fed Cyp7a1−/− mice, while proprotein convertase subtilisin/kexin type 9 (Pcsk9) was suppressed by Western diet (supplementary Fig. 6B). Pcsk9 is an inhibitor of Ldlr (26); decreased Pcsk9 may increase Ldlr-mediated uptake of serum cholesterol in Cyp7a1−/− mice. Acetyl-CoA carboxylase (Acc) mRNA was not differentially regulated in WT and Cyp7a1−/− mice, while glucokinase regulator protein (Gckr) mRNA expression was significantly elevated under both chow and Western diet feeding compared with WT mice (Fig. 8B), suggesting glycolysis may be stimulated to reduce serum glucose level in Cyp7a1−/− mice. In the ileum, mRNA expression levels of apical sodium-dependent bile acid transporter (Asbt) and Fxr were significantly elevated in Cyp7a1−/− mice independent of diet, while Fgf15 was reduced under chow-fed conditions in Cyp7a1−/− mice (Fig. 8C). Increased Asbt expression is correlated to increased bile acid reabsorption in the ileum (25). Decreasing Fgf15 may reduce activation of hepatic FGFR4 signaling to induce Cyp8b1 expression (Fig. 1A). Expression of Shp in the ileum was highly upregulated by Western diet in WT mice and was suppressed in Cyp7a1−/− mice, while expression of sinusoidal bile acid efflux transporters organic solute transporter α (Osta) and β (Ostβ) and Tgr5 was not differentially regulated in Cyp7a1−/− mice or by Western diet (Fig. 8C).

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Fig. 4. Cyp7a1−/− mice fed Western diet maintain improved glucose tolerance, which is reversed with CA supplementation. A: Cyp7a1−/− mice fed Western diet have significantly improved response to glucose (upper panel) but not insulin (lower panel), though the baseline 5 h fasting blood glucose levels were significantly reduced in Cyp7a1−/− mice (n = 6–7). GTT, glucose tolerance test; ITT, insulin tolerance test. B: Following feeding a Western diet supplemented with 0.03% CA for 10 weeks, glucose (upper panel) tolerance was worsened in Cyp7a1−/− mice. WT (white triangle or bar), Cyp7a1−/− (black triangle or bar), WT + CA (white diamond), Cyp7a1−/− + CA (black diamond). Data were analyzed by Student’s t-test; *p < 0.05.

Expression of genes involved in lipid and glucose metabolism is altered in Cyp7a1−/− mice

Next, we measured mRNA expression levels of several genes involved in lipid metabolism. In the liver, mRNA expression levels of scavenger receptor class B member 1 (Scarb1 or Srb1), sterol regulatory element-binding protein (SREBP), SREBP, SREBP-related lipid transfer domain containing 2 (Srd2), and carnitine palmitoyltransferase 1a (Cpt1a) genes were all significantly increased or trending toward increased in Cyp7a1−/− mice while expression of sterol-CoA desaturase (Sd1) was reduced in Cyp7a1−/− mouse liver (Fig. 8A). Expression of glucose-6-phosphatase (G6pc) was significantly upregulated in chow-fed Cyp7a1−/− mice and Western diet feeding significantly suppressed its expression in both WT and Cyp7a1−/− mice, while glucokinase (Gck) and glucokinase regulatory protein (Gkrr) mRNA expression was significantly elevated under both chow and Western diet feeding compared with WT mice (Fig. 8B), suggesting glycolysis may be stimulated to reduce serum glucose level in Cyp7a1−/− mice. In the ileum, mRNA expression levels of apical sodium-dependent bile acid transporter (Asbt) and Fxr were significantly elevated in Cyp7a1−/− mice independent of diet, while Fgf15 was reduced under chow-fed conditions in Cyp7a1−/− mice (Fig. 8C). Increased Asbt expression is correlated to increased bile acid reabsorption in the ileum (25). Decreasing Fgf15 may reduce activation of hepatic FGFR4 signaling to induce Cyp8b1 expression (Fig. 1A). Expression of Shp in the ileum was highly upregulated by Western diet in WT mice and was suppressed in Cyp7a1−/− mice, while expression of sinusoidal bile acid efflux transporters organic solute transporter α (Osta) and β (Ostβ) and Tgr5 was not differentially regulated in Cyp7a1−/− mice or by Western diet (Fig. 8C).

and Cyp7a1−/− mice; when normalized to total body weight, Western diet significantly increased fat mass and reduced total lean mass (supplementary Fig. 5).

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DISCUSSION

There is sparse information regarding the physiology of Cyp7a1<sup>−/−</sup> mice, which were first characterized in a mixed genetic background. These mice had low survival rates and required vitamin supplementation to reach adulthood, and had reduced bile acid pool size, reduced fecal excretion of bile acids, and reduced fecal sterol and intestinal cholesterol absorption. It was also more recently reported that Cyp7a1<sup>−/−</sup> mice exhibited induction of Cyp8b1 gene expression and repression of genes involved in the alternative synthesis pathway, and that these mice were physiologically responsive to low-dose CA and CDCAsupplementation (19, 20). The mice used in the present study were obtained by backcrossing the mixed background B6/129Sv strain with C57BL/6J mice for seven generations to obtain a nearly pure C57 background and represent a model suitable for use in dietary studies. Here, we report an alternative phenotype in which both Cyp8b1 and alternative bile acid synthesis genes (Cyp7b1, Cyp27a1) are upregulated under both chow- and Western diet-fed conditions, which resulted in a 2-fold increase in bile acid pool size compared with the original colony of Cyp7a1<sup>−/−</sup> mice in the mixed genetic background, altered gallbladder bile acid composition, and reduced bile acid hydrophobicity. The mice also have improved glucose and/or insulin sensitivity when maintained on either normal chow or Western diet and increased RER when maintained on Western diet.

Several studies demonstrate a causative link between increased 12α-hydroxylated bile acid (CA+DCA) levels and insulin insensitivity in rodents (9) and humans (29). Cyp7a1<sup>−/−</sup> mice have a reduced CA+DCA:CDCA+MCA ratio under both chow (0.61 compared with 1.27 in WT mice) and Western diet (0.54 compared with 0.92 in WT mice) feeding conditions. Therefore, the increased glucose sensitivity inherent to Cyp7a1<sup>−/−</sup> mice may be due to the dramatically altered bile acid composition, despite having a reduced bile acid pool. Increased TCDCA and Tα-MCA in Cyp7a1<sup>−/−</sup> mice indicates an induction of the alternative pathway of bile acid synthesis, which produces mainly CDCA that is then converted to α- and β-MCA. This is consistent with the significantly increased Cyp7b1 and Cyp27a1 mRNA expression we observed in the liver. We also observed an increase in Cyp8b1 gene expression in Cyp7a1<sup>−/−</sup> mice, possibly due to a reduction in feedback inhibition via FGF15/FGFR4 signaling, which inhibits Cyp7a1 and Cyp8b1 (30). Reduced ileal Fgf15 and Shp gene expression may result in derepression of Cyp8b1. In addition, MCAs were recently identified as potent FXR antagonists (2); therefore, increasing MCAs may antagonize feedback inhibition of Cyp8b1 expression in both chow- and Western diet-fed Cyp7a1<sup>−/−</sup> mice. Despite stimulation of Cyp8b1 gene expression, the composition of CA in the bile acid pool was reduced 10% in Cyp7a1<sup>−/−</sup> mice, indicating that upregulation of the alternative bile acid synthesis pathway may dominate the classic pathway in these mice. Supplementation of CA (0.03%) reversed the glucose tolerant phenotype in Cyp7a1<sup>−/−</sup> mice, and altered bile acid composition to resemble that of WT mice. CA has low critical micellar concentration that enhances mixed micelle formation with phospholipids and cholesterol (31) and may increase fat absorption and facilitate the reduction in glucose tolerance after CA feeding. These data support our hypothesis that reducing CA, a 12α-hydroxylated bile acid, in both Cyp7a1<sup>−/−</sup> mice in this study and in Cyp7a1-transgenic mice in our previous study may improve...
We also observed differential regulation of several key genes involved in cholesterol and lipid metabolism. Expression of *Stard1* and *Stard4* were increased in *Cyp7a1<sup>−/−</sup>* mice, independent of diet. Overexpression of *Cyp27a1* was shown to increase StAR protein, while overexpression of *Stard1* in HepG2 cells increased bile acid synthesis and the production of 27-hydroxycholesterol (35). Likewise, overexpression of *Stard4* in primary mouse hepatocytes increased bile acid synthesis (36). CYP27A1 is located in the mitochondrial inner membrane, which does not contain cholesterol. STARD1 facilitates the transport of cholesterol to mitochondria to provide substrate for CYP27A1. Increased expression of *Stard1* and *Stard4* genes was observed in *Cyp7a1<sup>−/−</sup>* mice, indicating increasing fat catabolism for energy production.

CA supplementation, indicating increasing fat catabolism for energy production. Western diet-fed *Cyp7a1<sup>−/−</sup>* mice have increased RER (i.e., the ratio of CO<sub>2</sub> produced/O<sub>2</sub> consumed) that is not due to typical diurnal variation or night-time induction of locomotor activity. RER is used as a tool to estimate respiratory quotient, the proportion of carbohydrate-to-fat utilization of metabolic oxygen. Accordingly, an RER of ~0.84 in WT mice maintained on Western diet indicates a near-equal utilization of carbohydrates and fats, such that approximately 49% of O<sub>2</sub> is consumed by carbohydrate catabolism while 51% of O<sub>2</sub> is consumed by fat (33), while an RER of ~0.9 in *Cyp7a1<sup>−/−</sup>* mice shifts O<sub>2</sub> utilization to approximately 66% for carbohydrate catabolism and 34% for fat catabolism. This shift was only observed in Western diet-fed *Cyp7a1<sup>−/−</sup>* mice, which ate significantly more food despite an equal gain in body weight compared with control mice. This may indicate a reduced ability to absorb dietary fat due to a smaller bile acid pool with lower CA. CA is the most efficacious bile acid in mixed micelle formation for emulsification of fats and cholesterol absorption (34). Indeed, RER was reduced in Western diet-fed *Cyp7a1<sup>−/−</sup>* mice from ~0.90 to 0.81 after CA supplementation, indicating increasing fat catabolism for energy production.

We also observed differential regulation of several key genes involved in cholesterol and lipid metabolism. Expression of *Stard1* and *Stard4* were increased in *Cyp7a1<sup>−/−</sup>* mice, independent of diet. Overexpression of *Cyp27a1* was shown to increase StAR protein, while overexpression of *Stard1* in HepG2 cells increased bile acid synthesis and the production of 27-hydroxycholesterol (35). Likewise, overexpression of *Stard4* in primary mouse hepatocytes increased bile acid synthesis (36). CYP27A1 is located in the mitochondrial inner membrane, which does not contain cholesterol. STARD1 facilitates the transport of cholesterol to mitochondria to provide substrate for CYP27A1. Increased expression of *Stard1* and *Stard4* genes was observed in *Cyp7a1<sup>−/−</sup>* mice, indicating increasing fat catabolism for energy production.
may contribute to stimulation of the alternative bile acid synthesis pathway in Cyp7a1−/− mice.

In the ileum of Cyp7a1−/− mice, Asbt gene expression was upregulated. Reduced FXR/FGF15 in the ileum may contribute to an increase in intestinal Asbt in Cyp7a1−/− mice (37). ASBT is a key ileal bile acid reabsorption transporter known to be inhibited by FXR and increased intestinal ASBT has been linked to increased bile acid pool and reduced fecal excretion of bile acids (25, 38). Therefore, increased ileum Asbt expression could account for the relative increase in bile acid pool size in our Cyp7a1−/− mice compared with the 70% reduction in bile acid pool size in Cyp7a1/B6/129Sv mice (17).

Interestingly, we previously reported that Cyp7a1-transgenic mice (with 2-fold increase in CYP7A1 enzyme activity and 2.5-fold increase in bile acid pool size) have a hydrophobic bile acid pool consisting of markedly increased CDCA and ursoodeoxycholic acid and reduced CA (22). These mice are protected against diet-induced obesity and have improved glucose sensitivity. Here, we demonstrate that Cyp7a1−/− mice have a reduced bile acid pool with reduced hydrophobicity coupled with improved glucose tolerance. Both bile acid sequestrants (reducing bile acid pool size) and bile acid supplementation (increasing bile acid pool size) have beneficial effects in improving insulin sensitivity and glucose tolerance in human studies (39, 40). Our current study points to a critical role of bile acid composition, rather than bile acid pool size, in the regulation of bile acid signaling in control of glucose, lipid, and energy metabolism.

We conclude that the Cyp7a1−/− mice characterized here represent a useful mouse model suitable for dietary studies and the study of bile acid and lipid metabolism. They present with a unique phenotype, such that survivability, body weight, and body composition are comparable to that of normal WT mice, but when challenged with a physiological stimulus (glucose or insulin bolus, Western diet) have an improved response that we speculate is due to a uniquely altered bile acid composition, and may be used to study the interactions of bile acids and lipid homeostasis.

REFERENCES

1. Chiang, J. Y. L. 2009. Bile acids: regulation of synthesis. J. Lipid Res. 50:1955–1966.
2. Sayin, S. I., A. Wahlström, J. Felin, S. Jäntti, H. U. Marschall, K. Bamberg, B. Angelin, T. Hyyöläinen, M. Orešič, and F. Bäckhed. 2013. Gut microbiota regulates bile acid metabolism by reducing the levels of tauo-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. 17:225–235.
3. 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.
4. Duane, W. C., and N. B. Javitt. 1999. 27-Hydroxycholesterol: production rates in normal human subjects. J. Lipid Res. 40:1194–1199.
5. Li, T., and J. Y. L. Chiang. 2014. Bile acid signaling in metabolic disease and drug therapy. Pharmacol. Rev. 66:948–983.
6. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Anwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol. Cell. 6:507–515.
7. Song, K. H., T. Li, E. Owsley, S. Strom, and J. Y. L. Chiang. 2009. Bile acids activate fibroblast growth factor 19 signaling in human hepatocytes to inhibit cholesterol 7a-hydroxylase gene expression. Hepatology. 49:297–305.
8. Li, T., J. M. Franchi, S. Boehme, A. Ochoa, Y. Zhang, C. D. Klaassen, S. K. Erickson, and J. Y. L. Chiang. 2012. Glucose and insulin induction of bile acid synthesis: mechanisms and implications in diabetes and obesity. J. Biol. Chem. 287:1861–1873.
9. Qi, Y., C. Jiang, J. Cheng, K. W. Krausz, T. Li, J. M. Ferrell, F. J. Gonzalez, and J. Y. L. Chiang. 2015. Bile acid signaling in lipid metabolism: metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. Biochim. Biophys. Acta. 1851:19–29.
10. Broeders, E. P., E. B. Nascimento, B. Havekes, B. Brans, K. H. Roumans, A. Tailleux, G. Schaart, M. Kousch, J. Charton, B. Deprez, et al. 2015. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. Cell Metab. 22:418–426.
11. Prawitt, J., S. Caron, and B. Staels. 2014. Glucose-lowering effects of intestinal bile acid sequestration through enhancement of splanchnic glucose utilization. Trends Endocrinol. Metab. 25:235–244.
12. Kobayashi, M., H. Iyegami, T. Fujisawa, K. Noyama, Y. Kawahata, S. Nosu, N. Babaya, M. Itoi-Babaya, K. Yamaji, Y. Hiromine, et al. 2007. Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid–binding resin. Diabetes. 56:239–247.
13. Ma, K., P. K. Saha, L. Chan, and D. D. Moore. 2006. Farnesoid X receptor is essential for normal glucose homeostasis. J. Clin. Invest. 116:1102–1109.
25. Xu, G., B. L. Shneider, S. Shefer, L. B. Nguyen, A. K. Batta, G. S. Tint, M. Arrese, S. Thevananther, L. Ma, S. Stengelin, et al. 2000. Ileal bile acid transport regulates bile acid pool, synthesis, and plasma cholesterol levels differently in cholesterol-fed rats and rabbits. *J. Lipid Res.* 41: 298–304.

26. Horton, J. D., J. C. Cohen, and H. H. Hobbs. 2009. PCSK9: a converter that coordinates LDL catabolism. *J. Lipid Res.* 50: S172–S177.

27. Kersten, S. 2014. Integrated physiology and systems biology of PPARY. *Mol. Metab.* 3: 554–571.

28. Hou, X., S. Xu, K. A. Maitland-Toodan, K. Sato, B. Jiang, Y. Idó, F. Lan, K. Walsh, M. Wierzbički, T. J. Verbeuren, et al. 2008. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. *J. Biol. Chem.* 283: 20015–20026.

29. Haesler, R. A., B. Astiarraga, S. Camstra, D. Accili, and E. Ferrannini. 2013. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. *Diabetes.* 62: 4184–4191.

30. Kong, B., L. Wang, J. Y. Chiang, Y. Zhang, C. D. Klaassen, and G. L. Guo. 2012. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology.* 56: 1034–1043.

31. Wang, D. Q.-H., S. Tazuma, D. E. Cohen, and M. C. Carey. 2003. Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse. *Am. J. Physiol. Gastrointest. Liver Physiol.* 285: G149–G502.

32. Zhang, Y., F. Y. Lee, G. Barrera, H. Lee, C. Vales, F. J. Gonzalez, T. M. Wilsson, and P. A. Edwards. 2006. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc. Natl. Acad. Sci. USA.* 103: 1006–1011.

33. McLean, J. A., and G. Tobin. 1987. Animal and Human Calorimetry. Cambridge University Press, Cambridge, UK.

34. Wang, D. Q.-H., F. Lammert, D. E. Cohen, B. Paigen, and M. C. Carey. 1999. Cholic acid aids absorption, biliary secretion, and phase transitions of cholesterol in murine cholelithogenesis. *Am. J. Physiol. Cell Physiol.* 276: C751–C760.

35. Hall, E. A., S. Ren, P. B. Hylemon, D. Rodriguez-Aguado, K. Redford, D. Marques, D. Kang, G. Gil, and W. M. Pandak. 2005. Detection of the steroidaligenic acute regulatory protein, ScAR, in human liver cells. *Biochem. Biophys. Acta.* 1733: 111–119.

36. Rodriguez-Aguado, D., S. Ren, E. Wong, D. Marques, K. Redford, G. Gil, P. Hylemon, and W. M. Pandak. 2008. Intracellular cholesterol transporter StarD4 binds free cholesterol and increases cholesteryl ester formation. *J. Lipid Res.* 49: 1409–1419.

37. Sinha, J., F. Chen, T. Miloh, R. C. Burns, Z. Yu, and B. L. Shneider. 2008. beta-Klotho and FGF-15/19 inhibit the apical sodium-dependent bile acid transporter in enterocytes and cholangiocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295: G996–G1003.

38. Li, H., F. Chen, Q. Shang, L. Pan, B. L. Shneider, J. Y. L. Chiang, B. M. Forman, M. Ananthanarayan, G. S. Tint, G. Salen, et al. 2005. FXR-activating ligands inhibit rabbit ASBT expression via FXR-SHP-PTC cascade. *Am. J. Physiol. Gastrointest. Liver Physiol.* 288: G660–G666.

39. Ratziu, V., V. de Ledinghen, F. Oberpi, P. Mathurin, C. Wartelle-Bladou, C. Renou, P. Sogni, M. Maynard, D. Larrey, L. Serfaty, et al. 2011. A randomized controlled trial of high-dose ursodeoxycholic acid for nonalcoholic steatohepatitis. *J. Hepatol.* 54: 1011–1019.

40. Staels, B. 2009. A review of bile acid sequestrants: potential mechanism(s) for glucose-lowering effects in type 2 diabetes mellitus. *Postgrad. Med.* 121: 25–30.