Attenuated Effects of Bile Acids on Glucose Metabolism and Insulin Sensitivity in a Male Mouse Model of Prenatal Undernutrition

Huijuan Ma,[1,2]* Vicencia M. Sales,[1]* Ashley R. Wolf,[3] Sathish Subramanian,[3] Tucker J. Matthews,[1] Michael Chen,[1] Aparna Sharma,[1] Walt Gall,[4] Wim Kulik,[5] David E. Cohen,[6] Yusuke Adachi,[1] Nicholas W. Griffin,[3] Jeffrey I. Gordon,[3] Mary-Elizabeth Patti,[1] and Elvira Isganaitis[1]

[1]Research Division, Joslin Diabetes Center and Harvard Medical School, Boston, Massachusetts 02115; [2]Department of Endocrinology and Metabolism, Hebei General Hospital, Shijiazhuang, Hebei, 050051, China; [3]Center for Genome Sciences and Systems Biology, and Center for Gut Microbiome and Nutrition Research, Washington University School of Medicine, St. Louis, Missouri 63110; [4]Metabolon, Durham, North Carolina 27713; [5]Laboratory of Genetic Metabolic Diseases, Departments of Pediatrics and Clinical Chemistry, University of Amsterdam, 1105 AZ Amsterdam-Zuidoost, The Netherlands; and [6]Division of Gastroenterology and Hepatology, Joan & Sanford I. Weill Department of Medicine, Weill Cornell Medical College, New York, New York 10021

Prenatal undernutrition and low birth weight are associated with risk of type 2 diabetes and obesity. Prenatal caloric restriction results in low birth weight, glucose intolerance, obesity, and reduced plasma bile acids (BAs) in offspring mice. Because BAs can regulate systemic metabolism and glucose homeostasis, we hypothesized that BA supplementation could prevent diet-induced obesity and glucose intolerance in this model of developmental programming. Pregnant dams were food restricted by 50% from gestational days 12.5 to 18.5. Offspring of both undernourished (UN) and control (C) dams given unrestricted diets were weaned to high-fat diets with or without supplementation with 0.25% w/w ursodeoxycholic acid (UDCA), yielding four experimental groups: C, UN, C + UDCA, and UN + UDCA. Glucose homeostasis, BA composition, liver and intestinal gene expression, and microbiota composition were analyzed in the four groups. Although UDCA supplementation ameliorated diet-induced obesity in C mice, there was no effect in UN mice. UDCA similarly lowered fasting insulin, and improved glucose tolerance, pyruvate tolerance, and liver steatosis in C, but not UN, animals. BA composition differed significantly, and liver and ileal expression of genes involved in BA metabolism (*Cyp7b1*, *Shp*) were differentially induced by UDCA in C vs UN animals. Bacterial taxa in fecal microbiota correlated with treatment groups and metabolic parameters. In conclusion, prenatal undernutrition alters responsiveness to the metabolic benefits of BA supplementation, with resistance to the weight-lowering and insulin-sensitizing effects of UDCA supplementation. Our findings suggest that BA metabolism may be a previously unrecognized contributor to developmentally programmed diabetes risk. (***Endocrinology*** **158: 2441–2452, 2017)**

Low birth weight, a marker for undernutrition (and other forms of stress) *in utero*, is associated with later risk of insulin resistance, type 2 diabetes, and cardiovascular disease (1–3). Chronic disease risk can be further exacerbated by postnatal obesogenic diet (4). The dual burden of undernutrition and obesity represents a major health challenge in low- and middle-income countries, where large populations experience suboptimal prenatal nutrition, but are increasingly exposed to calorie-dense Western diets (5).

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in USA

Received 26 March 2017. Accepted 9 June 2017.
First Published Online 15 June 2017

*These authors contributed equally to this study.

Abbreviations: ANOVA, analysis of variance; C, control; GTT, glucose tolerance test; HFD, high-fat diet; ID, identity; ITT, insulin tolerance testing; LC, liquid chromatography; MS, mass spectrometry; OTU, operational taxonomic unit; PTT, pyruvate tolerance test; rRNA, ribosomal RNA; TNF-α, tumor necrosis factor α; UDCA, ursodeoxycholic acid; UN, undernourished.

We have previously described a mouse model of prenatal nutritional programming of postnatal metabolism. Caloric restriction of ICR mouse dams during pregnancy results in low birth weight, adiposity, and glucose intolerance in offspring (6), in parallel with dysfunction in multiple organs, including adipocyte hypertrophy, impaired muscle stem cell function, and dysregulated insulin secretion (6–9). Another feature of our model is a thrifty metabolism, in which prenatal stress results in impaired mitochondrial metabolism, with protection from weight loss in the face of caloric restriction during adult life (10).

A rapidly evolving body of literature has established that bile acids function as pivotal regulators of systemic metabolism. For example, bile acid-binding resins improve insulin resistance and obesity (11, 12), and bile acid composition is altered in individuals with type 2 diabetes (13). Serum bile acids are higher after gastric bypass surgery (14, 15), and bile acid signaling through FXR (16), TGR5 (17), and endoplasmic reticulum stress pathways (18) may contribute to the metabolic improvements following such procedures. Moreover, bile acid supplementation increases muscle and brown adipose tissue thermogenesis in both mice and humans (19, 20). Less is known about the impact of bile acids on the growth and metabolism of infants. However, infants born either preterm or small for gestational age (both risk factors for later development of insulin resistance) manifest altered bile acid composition and increased plasma bile acids in the perinatal period, raising the possibility that low birth weight infants may have altered enterohepatic circulation or altered responsiveness to bile acids (21, 22). In the current study, we use a mouse model of prenatal undernutrition to examine how bile acids contribute to developmentally programmed disease susceptibility.

Materials and Methods

Animals and husbandry

We used a model of fetal programming developed in our laboratory (6); the model uses the ICR outbred mouse strain, chosen because of its large litter size and infrequent infanticide after being subjected to gestational undernutrition, as compared with other mouse strains. This late-gestation undernutrition model has been used by several groups in mice (23–25) and rats (26) and has been well described to contribute to impaired glucose tolerance and obesity in adulthood (6, 8, 10, 26).

Adult (6- to 8-week-old) ICR females were caged with ICR males, and pregnancies were dated by the presence of a vaginal plug (day 0.5). Pregnant dams had *ad libitum* access to a standard mouse chow (Purina 9F; Purina Mills, St. Louis, MO) with 23% of kcal from protein, 21% from fat, and 56% from carbohydrate. On a per-gram basis, this diet contains 21.5% protein and 9% fat.

On day 12.5, pregnant females were randomly assigned to either control (C) or undernourished (UN) groups. UN dams were food restricted by 50% from days 12.5 to 18.5 (calculated from intake in gestational day–matched controls). The food ration was given between 9 and 11 AM, and mice typically began consumption immediately. By contrast, mice fed *ad lib* generally ate during the dark cycle (7 PM to 7 AM). Dams were returned to *ad lib* chow diet upon delivery and were allowed unrestricted access to food throughout the lactation period until weaning at 3 weeks.

At birth, litters were equalized to eight pups per dam by removing the heaviest and lightest mice. At 3 weeks, pups were weaned to a 45% high-fat diet [(HFD) No. D12451; Research Diets, New Brunswick, NJ] with or without ursodeoxycholic acid [(UDCA) No. U5127; 0.25% w/w; Sigma-Aldrich, St. Louis, MO) and given these diets for 16 weeks. This experimental diet contained 20% of calories from protein (casein), 35% from carbohydrates (corn starch, maltodextrin, and sucrose), and 45% of calories from fat (lard and soybean oil). On a per-gram basis, this diet consists of 24% protein, 41% carbohydrate, and 24% fat. Cholesterol content was 195.5 mg/kg.

We chose to supplement the diets with UDCA after pilot studies in male C57BL/6J mice demonstrated that UDCA is well tolerated, tends to increase plasma bile acid levels, and reduces plasma glucose and insulin levels without hepatotoxicity, whereas supplementation with cholate (0.1% or 0.25%) was associated with increased alanine aminotransferase levels (Supplemental Fig. 1). Thus, the design yielded four treatment groups, as follows: C (control offspring, HFD without UDCA), C + UDCA (control offspring, HFD with UDCA), UN (undernourished offspring, HFD without UDCA), and UN + UDCA (undernourished offspring, HFD with UDCA) [see Experimental Scheme in Fig. 1(a)]. Only males were studied because metabolic phenotypes following our prenatal undernutrition protocol are more pronounced in this sex. Mice were housed in a National Institutes of Health Office of Laboratory Animal Welfare–approved vivarium, with controlled temperature, humidity, and light–dark cycle (7 AM to 7 PM). All protocols were approved by the Joslin Institutional Animal Use and Care Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Metabolic assessments

Resting energy expenditure, VO_2, VCO_2, food intake, and activity level were measured in metabolic cages (CLAMS, Columbus, OH) in the Joslin Animal Physiology Core; VO_2 and VCO_2 were normalized to lean body mass assessed by dual-energy x-ray absorptiometry (GE Lunar; GE Healthcare, Chicago, IL). Glucose and insulin tolerance were assessed by oral glucose tolerance test (GTT; 2 g/kg glucose via gavage), or by intraperitoneal insulin tolerance test [1 U/kg Humulin R (Eli Lilly, Indianapolis, IN)]. Pyruvate tolerance was assessed by intraperitoneal injection of pyruvate [pyruvate tolerance test (PTT); 1.5 mg/g]. GTT and PTT were performed after a 16-hour fast; insulin tolerance testing (ITT) was done 2 hours after food removal. Commercial kits were used to measure plasma insulin (Crystal Chem, Downers Grove, IL; No. 90010), nonesterified fatty acids (Wako, Richmond, VA; No. 994-75409), triglycerides (Stanbio Laboratory, Boerne, TX; No. 2200-430), cholesterol (Stanbio Laboratory; No.1010-430), β-hydroxybutyrate (Stanbio Laboratory; No. 2440), adipokines (Millipore, Billerica, MA; EZMADP-60K), leptin (Crystal Chem; No. 90030), and interleukin-6 (R&D Systems, Minneapolis, MN; No. MTA00B).

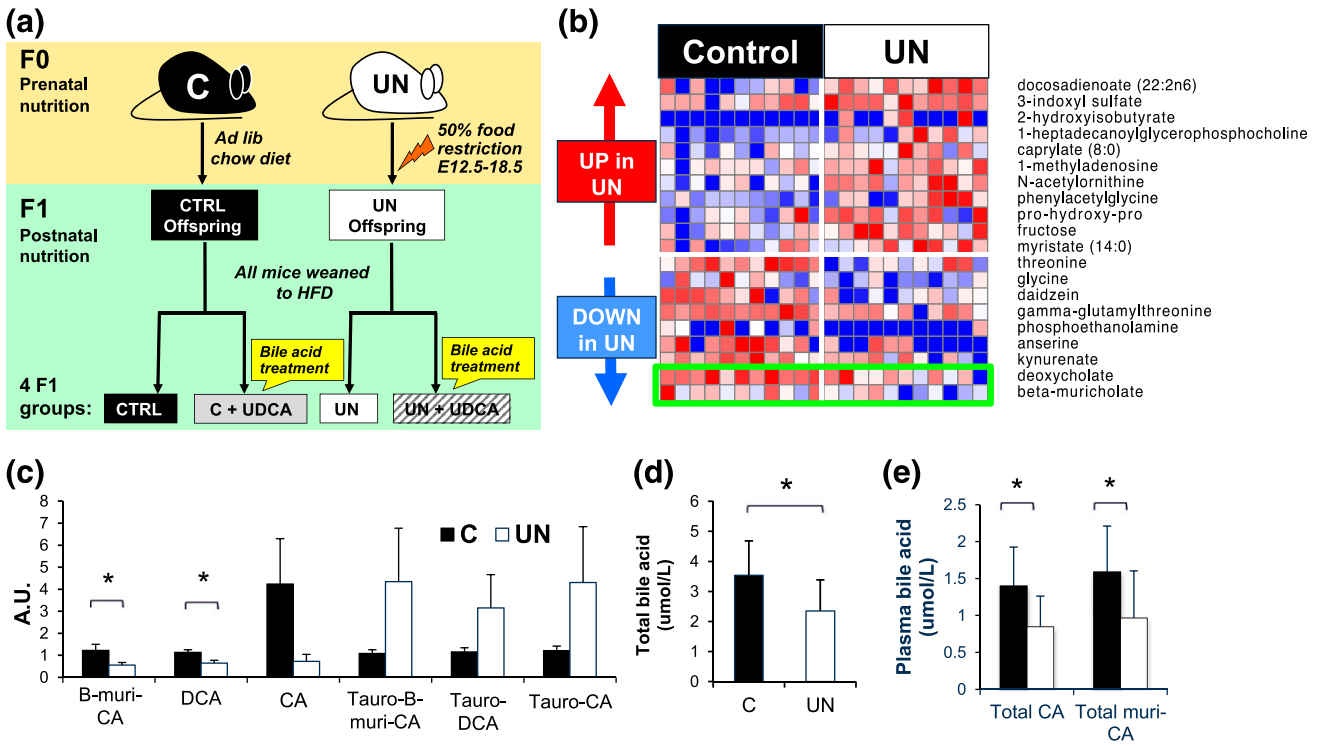

Figure 1. Prenatal undernutrition is associated with reduced plasma bile acids in weanling mice. (a) Experimental design. (b) Untargeted, semiquantitative plasma metabolomic profiling, males, age 3 weeks, n = 11 per group, two litters per group. Random fed. Heat map includes only metabolites altered between UN and C mice ($P < 0.05$). (c) Bile acids detected in semiquantitative metabolomic profiling, males, age 3 weeks, n = 11 per group. Concentrations normalized to the median for each metabolite. (d) Targeted liquid chromatography–mass spectroscopy quantitative analysis of total plasma bile acids in an independent cohort, males, age 3 weeks, n = 10 to 15 per group representing four control and seven UN litters. (e) Targeted analysis of plasma bile acid subsets, males, age 3 weeks, n = 10 to 15 per group representing four control litters and seven UN litters. Total CA = tauro-cholate + glyco-cholate + cholate; total muri-CA = tauro-muri-CA + glyco-muri-CA + muri-CA. Controls are depicted in black, and UN in white. *Denotes $P < 0.05$ in panels (c), (d), and (e). DCA, deoxycholate.

Plasma metabolites were assayed using liquid chromatography (LC)–mass spectroscopy (MS) (Metabolon, Inc., Durham, NC), as described (27). Plasma levels of bile acid species were measured in 3-week-old mice by high-performance LC tandem MS and quantified using deuterium-labeled standards (28). Total plasma bile acid levels were measured in 11-week-old mice using a colorimetric kit (Diazyme Laboratories, Inc., Poway, CA; No. DZ042A-K01). Bile acid pool composition in 16-week-old mice was measured by high-performance LC (29). Triglycerides were measured in liver using chloroform/methanol lipid extraction, followed by a colorimetric assay (Cayman Chemical, Ann Arbor, MI; No. 10010303).

Quantitative real-time polymerase chain reaction

RNA was isolated from liver and ileum (TRIzol; Life Technologies) for complementary DNA synthesis (Applied Biosystems High Capacity cDNA Reverse Transcription Kit; Thermo Fisher Scientific, Waltham, MA) and quantitative real-time polymerase chain reaction (Applied Biosystems SYBR Green Mastermix and 7900HT Thermocycler; Thermo Fisher Scientific). Values were normalized to the housekeeping gene TBP for liver gene expression and 36B4 for ileum gene expression. Primer sequences are available upon request.

16S ribosomal RNA analyses of fecal samples

Fecal samples were collected and stored at −80°C. DNA was isolated by bead beating in phenol/chloroform, purified (QIAquick column; Qiagen, Germantown, MD), and quantified (Qubit; Thermo Fisher Scientific) for sequencing, as previously described (30). Briefly, barcoded primers 515F and 806R were used to generate polymerase chain reaction amplicons covering the V4 region of bacterial 16S ribosomal RNA (rRNA) genes present in the fecal samples. Multiplex sequencing of pooled amplicons with sample-specific barcodes was performed using an Illumina MiSeq instrument (250-bp paired-end reads). Reads were trimmed *in silico* to 200 bases to retain the highest-quality base calls, assembled using FLASH v1.2.11, and demultiplexed in QIIME v1.9.0 (31). The 16S rRNA sequence datasets were first analyzed using open-reference operational taxonomic unit (OTU) picking [97% identity (ID) OTUs, uclust-ref against the Greengenes reference database]. For all subsequent analyses, OTU tables were rarefied to 10,000 reads per sample and filtered to retain only those OTUs with relative abundances in fecal microbiota >0.1% in at least 7 of 130 (5.4%) samples sequenced. Taxonomies were assigned using RDP classifier 2.4 trained on the manually curated Greengenes database Isolated named strains 16S. Principal coordinates analysis was performed using unweighted UniFrac and the QIIME software package (version 1.9). To identify bacterial OTUs associated with the different treatment groups after 2, 4, and 16 weeks of UDCA treatment, we performed an indicator species analysis using the "indicspecies" package in R (32). In this analysis, we tested for OTUs positively associated (more prevalent or more frequently detected) with particular treatment groups or pairs of treatment groups. Spearman

rank correlations between the relative abundances of 97% ID OTUs and various metabolic parameters were computed within each UDCA treatment group. Bacterial 16S rRNA datasets in raw format, prior to postprocessing and data analysis, have been deposited in the European Nucleotide Archive (ENA accession number PRJEB21354).

Statistics

Data are expressed as mean ± standard error of the mean. For two-group analyses, statistical analysis was performed using a two-tailed, unequal variance Student *t* test. For four-group analyses, we performed two-way analysis of variance (ANOVA) examining effects of prenatal nutritional status, postnatal UDCA, and the interaction of the two factors. For those models in which the interaction had a significant effect ($P < 0.05$), we next performed Tukey–Kramer tests (JMP Pro 12; SAS). $P < 0.05$ was considered statistically significant. Glucose area under the curve values during GTT, ITT, and PTT were calculated using the trapezoidal integration method. For plasma metabolomics data, undetectable values were replaced by half the minimum value detected for each metabolite; raw metabolite abundances were log transformed to minimize the effect of outliers; and differences were analyzed using fold changes, *t* tests, and false discovery rates (www.MetaboAnalyst.ca) (33). For analysis of differentially abundant OTUs and Spearman correlations of OTUs and metabolic parameters, $P < 0.05$ after correction for false discovery rate was considered significant.

Results

Prenatal undernutrition reduces plasma bile acids in weanling mice

Pregnant ICR mice were food restricted by 50% during pregnancy days 12.5 to 18.5, reducing birth weight by 10% in UN offspring compared with C [Supplemental Fig. 2(a)]. Untargeted LC-MS analysis of plasma obtained from 3-week-old weanling mice yielded 266 identified metabolites, 20 of which had levels that were significantly different between C and UN animals (Supplemental Table 1; $P < 0.05$, *t* test; n = 11 to 12 mice per group). Among these 20 metabolites, two bile acid species, β-muricholate and deoxycholate, were reduced >twofold in UN [$P < 0.05$; Fig. 1(b) and 1(c)]. By contrast, tauro-conjugated bile acids were not significantly different between C and UN mice [Fig. 1(c)]. Bile acids were also positively correlated with birth weight (β-muricholate: Pearson $R = 0.42$, $P = 0.049$; deoxycholate: $R = 0.49$, $P = 0.02$, cholate: $R = 0.43$, $P = 0.047$; data not shown).

We next performed quantitative targeted LC-MS analysis of bile acid composition in an independent cohort of 3-week-old weanling mice, which similarly revealed that total bile acids were significantly reduced in UN compared with C animals ($P = 0.03$), as were levels of cholate- and muricholate-related bile acids [Fig. 1(d) and 1(e); Supplemental Table 2].

UDCA supplementation protects control, but not prenatally undernourished, mice from diet-induced obesity

UDCA has been used clinically in humans with primary biliary cirrhosis and other hepatobiliary diseases (34, 35), and has been shown to improve glucose tolerance and hepatic steatosis in mice and humans (36–38). Moreover, our pilot studies demonstrated that UDCA reduced glucose and insulin without hepatotoxicity [in contrast to cholic acid, which resulted in higher alanine aminotransferase levels (Supplemental Fig. 1)]. We therefore chose UDCA for supplementation experiments in our preclinical model. After weaning at 3 weeks, UN or C mice were fed a HFD (45% calories from fat) with or without 0.25% w/w UDCA for 16 weeks [Fig. 1(a)]. Body weight was similar at the onset of supplementation, and food intake was similar across the four groups [Supplemental Fig. 2(b) and 2(c)]. By the eighth week of supplementation (11 weeks of age), significant body weight differences between C and C + UDCA emerged, with 7.2% reduction in body weight ($P = 0.01$). C + UDCA mice maintained a significantly lower average body weight than that of untreated controls from 8 to 14 weeks ($P < 0.05$), whereas no differences in weight between UN + UDCA and untreated UN mice were observed throughout the experiment [Fig. 2(a) and 2(b)]. We found no effects of UDCA treatment on oxygen consumption (VO_2) or CO_2 production, but did note reduced respiratory quotient in both UDCA-treated and untreated UN offspring during fasting, suggesting preferential utilization of fat [Supplemental Fig. 3(a–c)].

Insulin sensitivity is improved by UDCA supplementation in C, but not UN, offspring

To test whether UDCA altered glucose homeostasis, we performed oral GTTs 14 weeks after initiation of the experimental diets. Glucose area under the curve during oral GTT did not differ among the four groups, which is similar to our previous findings (using standard chow rather than HFD) that glucose intolerance in the UN model typically emerges after 6 months of age (6) [Fig. 2(c)].

UDCA supplementation reduced both fasting and glucose-stimulated insulin levels in C, but not UN, animals. Fasting insulin levels were threefold lower in C + UDCA mice vs C mice, with a significant interaction of UDCA supplementation and prenatal nutritional status [Fig. 2(d)]. Similarly, glucose-stimulated insulin levels (30-minute postgavage) were lower in C + UDCA, as compared with all other groups [Fig. 2(d)]. Given that glucose and insulin levels were not modified by UDCA in UN mice, we concluded that UN mice were resistant to the benefits of UDCA treatment observed in the C animals, as

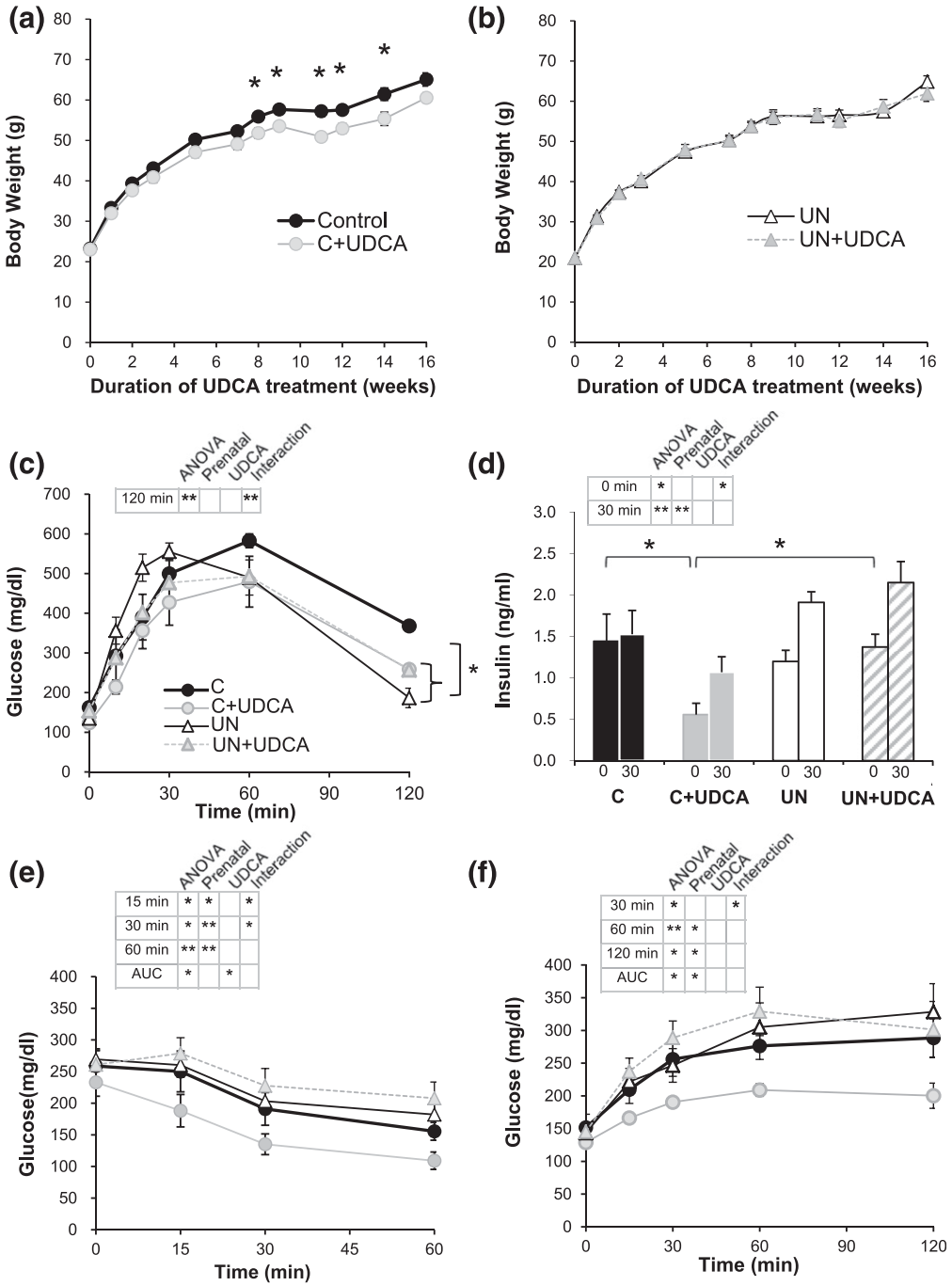

Figure 2. Effects of bile acid supplementation on diet-induced obesity, glucose homeostasis, and insulin sensitivity. Weight gain on experimental diets in (a) control and (b) UN offspring. Males, 11 to 12 per group (three to five litters per group); HFD with or without UDCA (as indicated) begun at age 3 weeks, $*P < 0.05$ by *t* test. (c) Oral GTT, 2 mg/kg glucose gavage. $*P = 0.005$ for 120-minute time point, two-way ANOVA; significant effect of prenatal nutrition ($P = 0.01$) and interaction of prenatal condition and postnatal UDCA [*P*(interaction) = 0.01]. Untreated controls are statistically different from all other groups at 120 minutes (Tukey–Kramer $P < 0.05$). (d) Fasted insulin levels and insulin levels 30 minutes after oral glucose gavage (2 mg/kg). Fasted: two-way ANOVA, $P = 0.02$; interaction of prenatal condition and postnatal UDCA, $P = 0.02$. Thirty minutes: two-way ANOVA, $P = 0.01$; significant effect of prenatal condition, $P = 0.004$. (c and d) Males, age 17 weeks (14 weeks of experimental diet), n = 6 per group, two litters per group. (e) Insulin tolerance test (1 U/kg, intraperitoneal). Males, age 11 weeks (8 weeks of experimental diet); n = 11 to 12 per group, three litters per group. Fifteen minutes: two-way ANOVA, $P = 0.02$; *P*(prenatal) = 0.02; *P*(interaction) = 0.04; 30 minutes: two-way ANOVA, $P = 0.02$; *P*(prenatal) = 0.01; *P*(interaction) = 0.04; 60 minutes: two-way ANOVA, $P = 0.007$; *P*(prenatal) = 0.003; *P*(interaction) = 0.053; area under the curve: two-way ANOVA, $P = 0.049$; *P*(UDCA) = 0.04; *P*(prenatal) = 0.07. (f) Pyruvate tolerance test (1.5 mg/kg, intraperitoneal). Males, age 15 weeks (12 weeks of experimental diet); n = 6 per group, two litters per group. Thirty minutes: two-way ANOVA, $P = 0.05$; *P*(interaction) = 0.03; 60 minutes: two-way ANOVA, $P = 0.01$; *P*(prenatal) = 0.006; 120 minutes: two-way ANOVA, $P = 0.047$; *P*(prenatal) = 0.035; *P*(UDCA) = 0.08; area under the curve: two-way ANOVA, $P = 0.02$; *P*(prenatal) = 0.015; *P*(interaction) = 0.1. *Denotes two-way ANOVA, $P < 0.05$. Tables embedded in the figures summarize results of two-way ANOVA, which tested for effects of prenatal nutrition group, UDCA treatment, and the interaction between the two. $*P < 0.05$ and $**P < 0.01$ for the two-way ANOVA, or for each of the factors, as indicated. AUC, area under the curve.

measured by improved glucose metabolism and decreased insulin levels in the context of a HFD.

We next examined the effects of prenatal undernutrition and postnatal UDCA on systemic and hepatic insulin sensitivity. ITT revealed that C + UDCA animals had the lowest glucose levels after intraperitoneal insulin injection; prenatal nutrition status significantly interacted with UDCA treatment at 15 and 30 minutes. We observed no change in the UN animals with UDCA treatment [Fig. 2(e)]. Because hepatic insulin resistance can be associated with increased gluconeogenesis, we assessed gluconeogenic responses to pyruvate. C + UDCA mice also had the lowest glucose levels after pyruvate injection, consistent with reduced gluconeogenesis and improved hepatic insulin sensitivity. Glucose levels 30, 60, and 120 minutes after pyruvate injection, and the area under the glucose curve, differed across the four groups, with significant interactions between prenatal condition and postnatal UDCA at 30 minutes [Fig. 2(f)]. However, hepatic expression of genes involved in gluconeogenesis was largely unaltered across the four groups, except for *Pepck*, which was highest in the C + UDCA group (Supplemental Fig. 4). We concluded that UDCA improves insulin sensitivity in HFD-fed control mice, but this effect is blocked by prenatal undernutrition.

Given that UDCA treatment reduced body weight and improved insulin sensitivity in control mice, we next examined levels of adipokines. Although plasma adiponectin and interleukin-6 did not differ across the four treatment groups, leptin and tumor necrosis factor α (TNF-α) were highest in the UN group, with a significant interaction of prenatal nutrition and postnatal UDCA on TNF-α (leptin, two-way ANOVA, $P = 0.0037$; TNF-α, two-way ANOVA, $P = 0.048$; Supplemental Table 3). Proinflammatory cytokines were not significantly lower in the C + UDCA group, which exhibited the greatest improvement in insulin sensitivity. We concluded that effects of UDCA on insulin sensitivity were independent of adipokines.

Effects of UDCA treatment on bile acid pools and metabolism

Bile acid modulation of gluconeogenic responses and insulin sensitivity suggests a robust effect on hepatic metabolism. We therefore evaluated the impact of UDCA treatment on lipids and hepatic gene expression. Plasma cholesterol was significantly reduced with UDCA treatment, independent of prenatal nutritional status [P(ANOVA) = 0.003; Supplemental Table 3]. No differences in plasma triacylglycerides, free fatty acids, or β-hydroxybutyrate were observed. The effect of UDCA on liver lipid accumulation was more pronounced in C animals; liver triacylglycerides were reduced by 60% in C + UDCA vs C, and there was a significant interaction between prenatal nutrition and postnatal UDCA [$P = 0.046$; Fig. 3(a) and 3(b)]. Quantitative reverse-transcription polymerase chain reaction assays of liver RNA disclosed that UDCA supplementation changes expression of several genes involved in the regulation of lipid metabolism independent of prenatal nutrition status (*e.g.*, *Hmgcr*, the rate-limiting enzyme in cholesterol synthesis, is significantly increased in both C and UN [P(ANOVA) = 0.0003]), whereas expression of *Srebf*, a key regulator of lipogenesis, *Scd1*, an enzyme essential for *de novo* lipogenesis, and *Mcad*, which catalyzes the first step in fatty acid β-oxidation, is reduced in both the C and UN UDCA-supplemented groups [*Srebf*: P(ANOVA) = 0.008; *Scd1*: P(ANOVA) = 0.04; *Mcad*: P(ANOVA) < 0.0001; Fig. 3(c)].

UDCA supplementation produced a trend toward increased fasting plasma total BA levels at 11 weeks of age, with a similar effect size in both C and UN animals [Fig. 3(d)]. Although the total bile acid pool did not differ in absolute size [Fig. 3(e)], nor when normalized to the animal's body weight [Supplemental Fig. 5(a)], bile acid composition differed markedly across the four groups. Untreated UN mice had the highest levels of taurocholate, and the lowest levels of tauro-ursodeoxycholate and taurochenodeoxycholate, with significant interactions of prenatal nutrition and postnatal UDCA on abundance of taurocholate, tauro-ursodeoxycholate, and taurochenodeoxycholate [Fig. 3(e); Supplemental Fig. 5(b)]. Expression of bile acid transporters in liver and ileum was largely unaltered by either prenatal undernutrition or UDCA [Supplemental Fig. 6(a) and 6(b)]. Ileal expression of the bile acid receptor *Tgr5* or of Preproglucagon (*Gcg*) was also not significantly altered by prenatal undernutrition or UDCA treatment [Supplemental Fig. 6(c) and 6(d)].

Cyp7a1, the rate-limiting enzyme for bile acid synthesis, was reduced by UDCA in both the C and UN groups [P(ANOVA) = 0.0004] [Fig. 3(f)], whereas expression of *Cyp7b1*, an enzyme involved in alternative pathway bile acid synthesis, was significantly increased in C + UDCA, but not in UN + UDCA [P(ANOVA) = 0.004, P(interaction) = 0.02]. These findings raise the possibility that differences in UDCA-triggered upregulation of *Cyp7b1* might contribute to differences in bile acid composition across the four groups, although we did not directly measure the products of *Cyp7b1* (*i.e.*, 6- or 7-hydroxysteroids and oxysterols). We found no significant differences in liver expression of the bile acid receptor *Fxr*, or in *Shp*, an inhibitor of nuclear receptor signaling [Supplemental Fig. 6(e)]. By contrast, expression of *Shp* in the ileum was markedly upregulated by UDCA

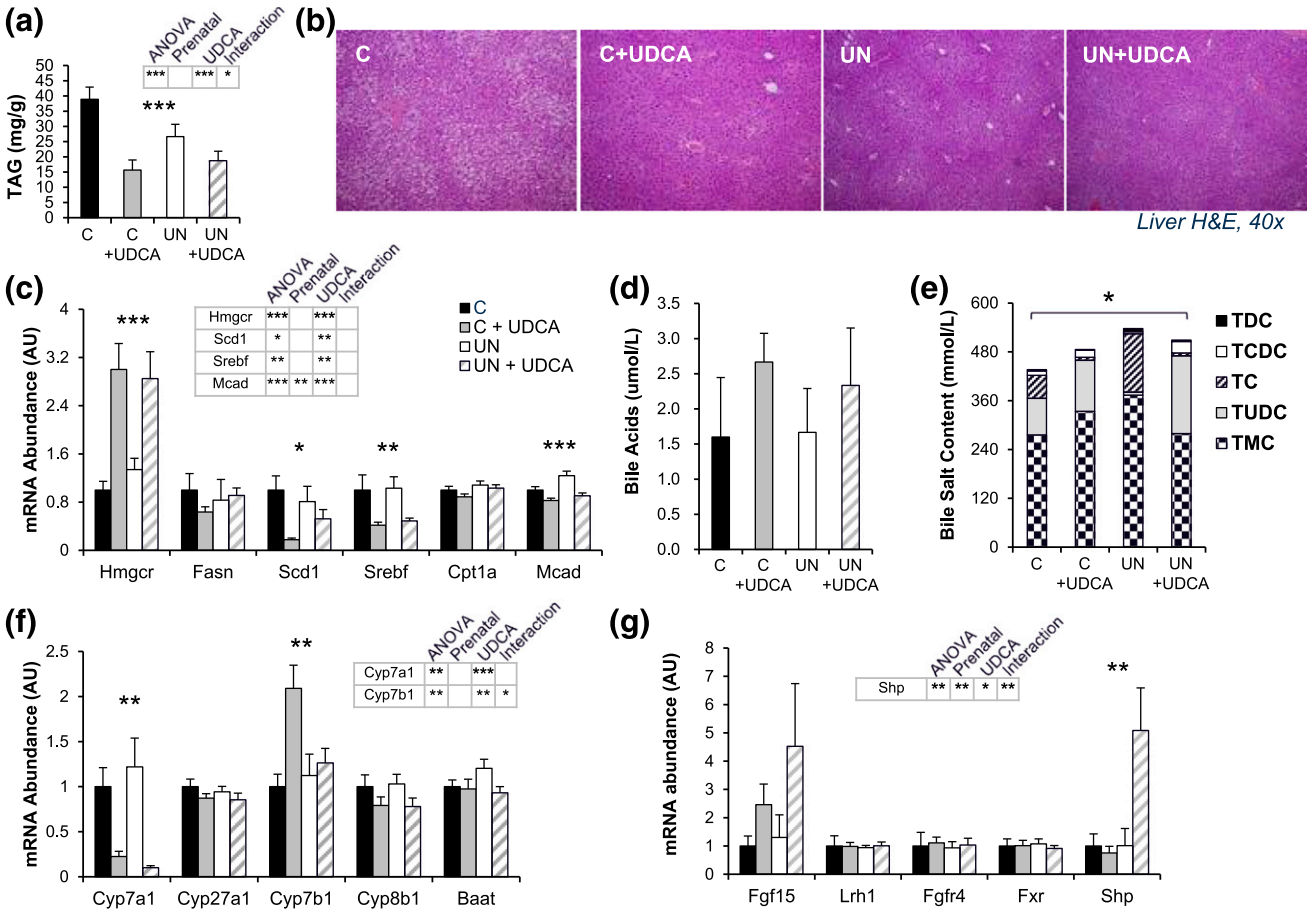

Figure 3. Effects of bile acid supplementation on liver lipid metabolism. (a) Liver triglyceride content, mg per gram tissue. Two-way ANOVA, $P = 0.0008$; significant effect of UDCA = 0.0002; significant interaction of prenatal condition and UDCA, P(interaction) = 0.046. (b) Representative liver histology images, hematoxylin and eosin stain, ×40 magnification. (c) Liver mRNA expression of lipid metabolism genes. Hmgcr: two-way ANOVA, $P = 0.0003$; significant effect of UDCA, $P < 0.0001$. Scd1: two-way ANOVA, $P = 0.04$; significant effect of UDCA, $P = 0.007$. Srebf: two-way ANOVA, $P = 0.007$, significant effect of UDCA, $P = 0.0006$. Mcad: two-way ANOVA, $P < 0.0001$, significant effect of UDCA, $P = 0.0001$; significant effect of prenatal condition, $P = 0.01$. Males, age 19 weeks (16 weeks of experimental diet); n = 8 to 11 per group, three to five litters per group. (d) Plasma total bile acids in the fasted state. Males, age 11 weeks, n = 4 to 5 per group, two litters per group. (e) Composition of the bile acid pool, expressed as absolute abundance (mmol/L) in liver, gallbladder, and small intestine. n = 4 to 7 per group, two to three litters per group, males, age 19 weeks. Tauro-ursodeoxycholate (TUDC): two-way ANOVA, $P < 0.0001$; effect of UDCA, $P < 0.0001$; interaction of prenatal condition and UDCA, $P = 0.002$. Taurochenodeoxycholate (TCDC): two-way ANOVA, $P = 0.0004$; effect of UDCA, $P = 0.0004$; interaction of prenatal condition and UDCA, $P = 0.03$. Taurocholate (TC): two-way ANOVA, $P < 0.0001$; effect of UDCA, $P < 0.0001$; effect of prenatal condition, $P = 0.0002$; interaction of prenatal condition and UDCA, $P = 0.0002$. (f) Liver mRNA expression of genes involved in bile acid synthesis. Males, age 19 weeks (16 weeks of experimental diet); n = 8 to 12 per group, three to five litters per group. Cyp7a1: two-way ANOVA, $P = 0.0004$; effect of UDCA, $P < 0.0001$. Cyp7b1: two-way ANOVA, $P = 0.004$; effect of UDCA, $P = 0.009$; interaction of prenatal condition and UDCA, $P = 0.02$. (g) Distal ileum mRNA expression of genes involved in bile acid and FGF15 signaling. Males, age 19 weeks (16 weeks of experimental diet); n = 6 to 11 per group, three to five litters per group. Shp: two-way ANOVA, $P = 0.002$; effect of UDCA, $P = 0.02$; effect of prenatal, $P = 0.01$; interaction of prenatal condition and UDCA, $P = 0.01$. For all graphs, controls are depicted in black, UDCA-treated controls in gray, UN in white, and UDCA-treated UN in hatched gray. An asterisk above the bar graphs denotes $P < 0.05$ (two-way ANOVA). Tables embedded in the figures summarize results of two-way ANOVA, which tested for effects of prenatal nutrition group, UDCA treatment, and the interaction between the two. Key: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ for the two-way ANOVA, or for each of the factors, as indicated. TDC, taurodeoxycholate; TMC, tauromuricholate.

in prenatally undernourished mice, but not in controls [P(ANOVA) = 0.002, P(interaction) = 0.02; Fig. 3(g)]. Ileal expression of *Fgf15*, a major repressor of *Cyp7a1* activity and bile acid synthesis (39), also tended to be upregulated by UDCA in both C and UN mice [Fig. 3(g)]. Together, these data demonstrate that expression of messenger RNAs encoding key regulators of bile acid metabolism and responsiveness is differentially induced by UDCA, depending on prenatal nutritional status.

Effects of prenatal undernutrition and UDCA supplementation on microbiota configuration

Given that the gut microbiota can influence bile acid metabolism, weight gain, and insulin resistance, we examined effects of UDCA treatment on microbiota configuration in both C and UN offspring. We sequenced bacterial 16S rRNA genes in 130 fecal samples collected from C and UN mice 2, 4, and 16 weeks after initiating the experimental diets (*i.e.*, at 5, 7, and 19 weeks of age),

and identified 205 OTUs. Across all time points, UDCA supplementation had a pronounced effect on α-diversity, as judged by OTU richness (overall number of OTUs observed), phylogenetic diversity (branch length on the bacterial tree), and Simpson diversity index (how many OTUs are present and how evenly they are represented) (Supplemental Table 4). At 16 weeks, UDCA treatment explained a greater degree of the variance in phylogenetic community structure (as defined by principal coordinates analysis of unweighted UniFrac distances) than did prenatal nutritional status [PERMANOVA; 25.8% vs 6%, respectively; Supplemental Table 5; Fig. 4(a)].

We performed indicator species analysis to identify OTUs differing in their representation among the four treatment groups (40). Seventy OTUs were positively associated with at least one group or pair of treatment groups at >1 time point [Fig. 4(b); Supplemental Table 6]. At 2 weeks, there were far more OTUs indicative of UDCA treatment, regardless of prenatal nutritional status, than there were OTUs that differentiated nutritional status across UDCA treatments. Only five OTUs were indicative of C nutritional status at this time point (*e.g.*, OTU 1106101, Porphyromonadaceae), and two OTUs were indicative of UN (*e.g.*, OTU 262095, *Clostridium cocleatum*), whereas the vast majority were indicative of UDCA (*e.g.*, OTU 363731, *Akkermansia muciniphila*) or no UDCA (*e.g.*, OTU 837473, Clostridiales) [Fig. 4(b)]. The pattern of a greater number of significant indicators being related to UDCA treatment than to prenatal undernutrition persisted over the course of the entire 16-week treatment period [Fig. 4(b); Supplemental Table 6].

To examine associations between gut microbiota and metabolic parameters, we performed Spearman rank correlation between OTU abundance and metabolic measurements at the 16-week time point for each treatment group.

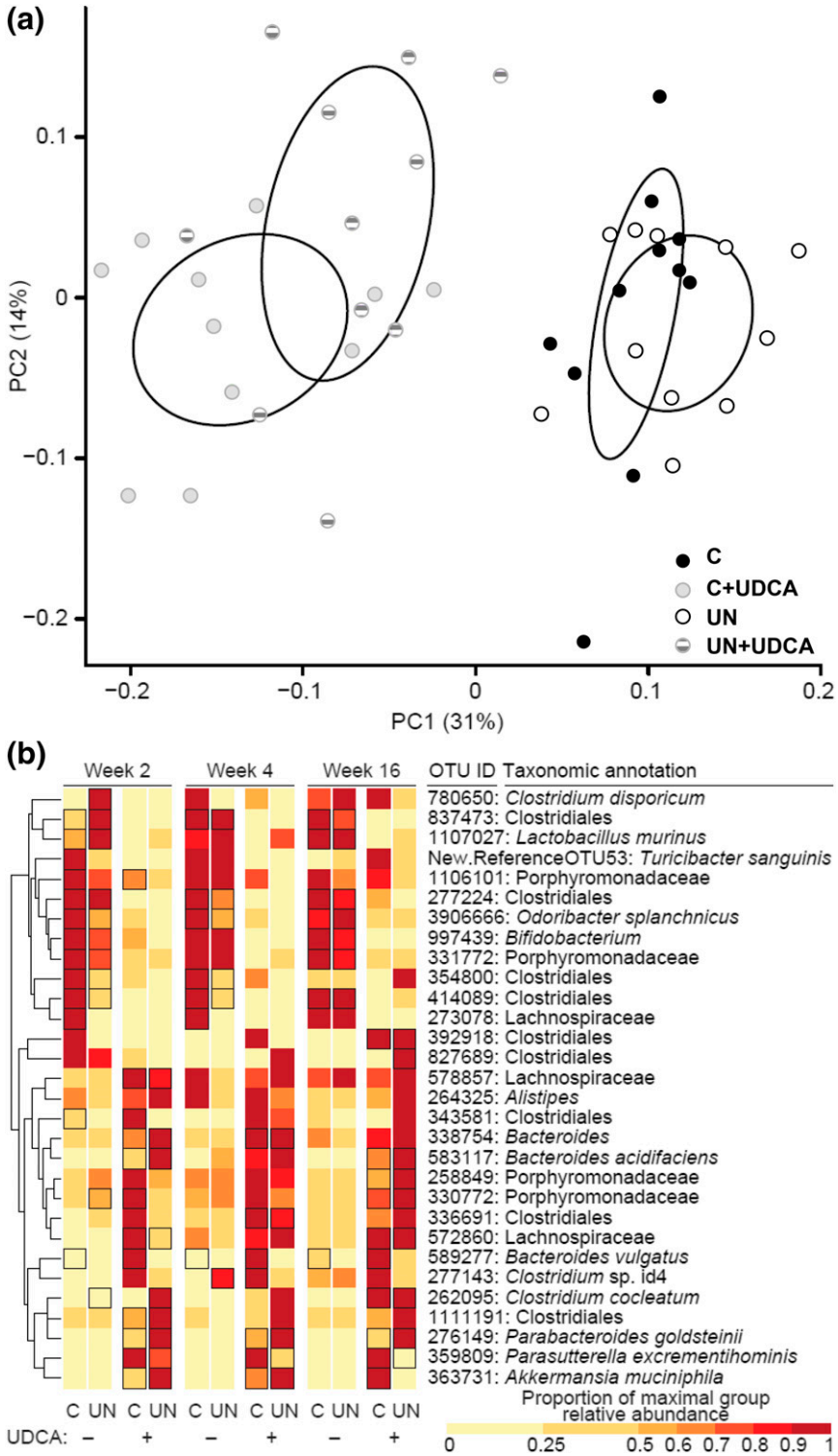

Figure 4. Effects of UDCA supplementation and prenatal nutrition (C vs UN) on fecal microbiota composition. (a) Principal coordinates analysis of unweighted UniFrac distances for all fecal samples from mice at 16 weeks of treatment. Values for C ± UDCA and UN ± UDCA animals are plotted for principal coordinates 1 and 2 (PC1 and PC2). Dispersion ellipses represent the standard deviations around the centroid within each category of samples. (b) Mean relative abundances of selected OTUs at each time point, normalized to the maximum treatment group mean at that time point, are plotted for each group of mice (n = 9 to 12 per group). The 97% ID OTUs displayed are significant indicators of one or two groups at a given time point, as defined by indicator species analysis of fecal samples collected from mice [boxes represent significant indicators at a given time point with a false discovery rate (FDR)–corrected *P* value <0.05; OTUs displayed have a mean abundance of at least 1% in at least one treatment group in at least one time point; see Supplemental Table 6 for complete list].

Within the UN + UDCA group, one OTU in the family Porphyromonadaceae (330772) was significantly positively correlated with body weight ($P = 0.035$, Spearman rank correlation; Supplemental Table 7; Supplemental Fig. 7). Additionally, we identified three OTUs, including two members of the order Clostridiales, that were significantly associated with serum adiponectin levels within the C + UDCA group (Supplemental Table 7). Because UDCA treatment had a larger effect on the relative abundances of OTUs than did nutritional status (C vs UN), and because we wanted to have more power to identify correlations, we also combined the C and UN groups within each treatment arm and performed Spearman rank analysis. This approach yielded two OTUs that correlated with fasting serum glucose; one was positively correlated (338796, *Oscillibacter* sp. g2, a member of the Clostridiales), whereas the other was negatively correlated (a *de novo* OTU assigned to *Olsenella*; see Supplemental Table 7 for these and all other significant correlations). We found no significant associations between metabolic markers and OTUs in untreated mice.

Discussion

Recent studies in humans and rodents have suggested possible links between bile acid metabolism and developmental programming. For example, untargeted metabolomic analysis following protein–calorie restriction in mice revealed a marked reduction in the plasma bile acid deoxycholate (41), and conjugated bile acids were reduced in a mouse model of moderate malnutrition and enteropathy (42). Moreover, data from humans suggest that early exposure to bile acids can have long-term effects on metabolism. Indeed, infants of women with gestational cholestasis—a rare pregnancy complication characterized by elevated bile salts in the maternal and fetal circulations—have increased risk for insulin resistance and obesity in adolescence (43). Similarly, cholate supplementation during pregnancy increases diet-induced obesity risk in offspring mice (43). By contrast, Watanabe *et al.* (20) demonstrated that postnatal cholate supplementation reduces diet-induced obesity through *Tgr5*- and *Dio2*-mediated thermogenesis. Our results demonstrate that the plasma bile acids deoxycholate and β-muricholate are reduced in weanling mice exposed to prenatal undernutrition, and that postnatal UDCA supplementation improved obesity, glycemia, and insulin sensitivity in control, but not in prenatally undernourished, offspring. Collectively, these studies implicate bile acid signaling and metabolism in developmental programming. Whether elevated bile acids are protective or deleterious remains unclear, but these data suggest that nutritional history and timing of exposure may critically alter the metabolic effects of bile acids.

Given that bile acids can have multisystem effects on metabolism, we explored several potential mechanisms for the observed differences in responsiveness of control vs undernourished mice to UDCA supplementation. We noted striking differences in the composition of the bile acid pool across the four experimental groups, which is interesting given that differences in bile acid composition can alter secretion of intestinal hormones, including Glp1 (44) [most likely mediated through differences in individual bile acid affinity for Fxr (45) and Tgr5 (44)]. By contrast, we did not observe any differences in expression of *Fxr* or *Tgr5*, or in *Fgf15*, a circulating regulator of bile acid metabolism. We did, however, note a significant increase in expression of *Shp*, a nuclear receptor corepressor known to repress bile acid and cholesterol metabolism (46), in UDCA-treated UN but not control mice, raising the possibility that differences in *Shp*-mediated repression of bile acid signaling might underpin the diminished responsiveness to UDCA in UN mice. This hypothesis remains to be tested in our model.

We also assessed whether UDCA supplementation affected expression of genes involved in bile acid metabolism and signaling. Regardless of prenatal nutritional group, UDCA supplementation resulted in downregulation of *Cyp7a1*, the rate-limiting enzyme in bile acid synthesis, consistent with previous reports (47). By contrast, we found that hepatic expression of other enzymes involved in bile acid synthesis (*e.g.*, *Cyp27a1*, *Cyp8b1*, and *Baat*) was largely unaltered across the four experimental groups, as was ileal and hepatic expression of bile acid transporters. However, we observed differential induction of the bile acid synthesis enzyme *Cyp7b1* in the C vs UN groups. The effect on *Cyp7b1* is notable given recent data showing that overexpression of *Cyp7b1* lowers fasting glucose levels and improves hepatic steatosis (48), raising the possibility that increased *Cyp7b1* expression may be related to the metabolic improvements in the C + UDCA group. Levels of 6- or 7-hydroxysteroid or oxysterol intermediates will need to be measured to confirm that increased expression of *Cyp7b1* is accompanied by demonstrable increases in enzymatic activity.

Given the key role of the gut microbiota in bile acid metabolism (49) and thus bile acid signaling (50, 51), we examined temporal changes in gut microbiota composition in response to prenatal undernutrition and postnatal UDCA treatment. We identified several OTUs indicative of the various treatment groups, many of which belonged to Clostridiales. Interestingly, members of this bacterial order have been linked to fasting insulin and other metabolic markers of diabetes in humans (52, 53).

We also noted increased abundance of *Akkermansia muciniphila* in the C + UDCA group at 16 weeks, which is intriguing as several studies link reductions in this bacterium to obesity and diabetes risk (54, 55), and a recent report demonstrated its alteration during postnatal catch-up growth in mice (56). Moreover, the OTU Porphyromonadaceae, previously linked to lipid metabolism and obesity (57), was significantly associated with body weight in UDCA-treated mice. However, it is unclear whether the microbiota is a primary determinant of the differential responses of C vs UN mice to UDCA supplementation, or whether the microbiota reflects differences in obesity or glucose metabolism. Future studies, including transplantation of microbiota to germfree recipient mice, will be needed to establish a causal role for the gut community in determining the different metabolic phenotypes produced by UDCA treatment of C vs UN animals.

Obesity is associated with a decrease in the diversity of the gut microbiota in several preclinical models (58, 59). Increased diversity has been linked to increased fiber intake and protection from long-term weight gain in humans (60). Some data suggest that HFD, and not obesity, is the primary driver of reduced microbial diversity (61). Interestingly, in our experiments, UDCA-treated control mice, which are less obese than untreated controls, exhibited reduced diversity, raising the question of how UDCA, in the context of a HFD, exerts this effect on community composition.

We acknowledge several limitations to our study. First, UDCA has minimal affinity for the bile acid receptors FXR and TGR5 (62), so we cannot assess whether the resistance to metabolic improvements that we noted in prenatally undernourished mice is related to bile acid signaling through these pathways. We chose the bile acid UDCA because it has been used clinically in humans (35) and has been shown to improve glucose tolerance and hepatic steatosis (37, 38). Examining how prenatal nutrition alters responsiveness to other bile acids and assessing the role of FXR, TGR5, and other signaling pathways will be important questions for future studies. We were also unable to fully examine the role of FGF15, a key regulator of bile acid metabolism (39), in the differential responses to UDCA treatment. Although we did note a trend for increased *Fgf15* mRNA expression in the UDCA-treated groups, we could not corroborate as there is currently no reliable assay for mouse FGF15 in plasma. Second, we used the outbred ICR mouse strain; in the absence of detailed genomic analyses, we cannot define the contributions of genetic variation or gene-by-environment interactions to the observed differential responsiveness to bile acids. Third, we observed only a transient effect of UDCA treatment on body weight and are still working to identify a mechanistic basis for this time course. Finally, as noted previously, our analysis did not include tests for a causal role for the gut microbiota in the host responses to bile acid supplementation.

In summary, our findings in UDCA-supplemented control mice are consistent with prior observations that bile acids can have protective effects on diet-induced obesity and insulin resistance. By contrast, prenatally undernourished offspring had an altered response to UDCA supplementation, including lack of effect on weight gain, glucose tolerance, and insulin resistance. These discordant metabolic phenotypes were linked to pronounced differences in bile acid composition and associated with changes in gut microbiota configuration. Our results suggest that altered bile acid metabolism may be a previously unrecognized mediator of metabolic disease risk following prenatal undernutrition.

Acknowledgments

We are grateful to Martin Meier and Jessica Hoisington-Lopez for technical assistance with 16S rRNA library preparation and sequencing.

Address all correspondence and requests for reprints to: Elvira Isganaitis, MD, MPH, Research Division and Pediatric, Adolescent, and Young Adult Unit, Joslin Diabetes Center, One Joslin Place, Boston, Massachusetts 02215. E-mail: Elvira.Isganaitis@joslin.harvard.edu.

This work was supported in part by National Institutes of Health Grants K99/R00 HD064793 (to E.I.); DK106193 (to M.-E.P.); DK036836 [Joslin Diabetes and Endocrinology Research Center (DRC) Grant]; DK048872, DK056626, and DK103046 (to D.E.C.); and DK30292 (to J.I.G.). A.R.W. is the recipient of a postdoctoral fellowship from the American Diabetes Association (Grant No. 1-16-PDF-125).

Author contributions: H.M. collected/analyzed the majority of the data and wrote portions of the manuscript. V.M.S. and A.R.W. collected/analyzed data and edited the manuscript. S.S., T.J.M., M.C., A.S., W.G., W.K., D.E.C., and Y.A. collected data. N.W.G. provided input about data analysis and statistics. J.I.G. provided input about study design and data analysis and edited the manuscript. M.-E.P. was involved in study design, oversaw data analysis, and edited the manuscript. E.I. was involved in study design, oversaw data collection/analysis, and wrote/edited the manuscript.

Disclosure Summary: J.I.G. is a cofounder of Matatu, a company characterizing the role of diet-by-microbiota interactions in animal health. W.G. was an employee of Metabolon at the time the data were collected. None of the authors have conflicts of interest related to the current work.

References

1. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BE, Carlsson S, de Rooij SR, Dyck RF, Eriksson JG, Falkner B, Fall C, Forsén T, Grill V, Gudnason V, Hulman S, Hyppönen E, Jeffreys M, Lawlor DA, Leon DA, Minami J, Mishra G, Osmond C, Power C, Rich-Edwards JW, Roseboom TJ, Sachdev HS, Syddall H, Thorsdottir I, Vanhala M,

Wadsworth M, Yarbrough DE. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA*. 2008;**300**(24):2886–2897.
2. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. *Lancet*. 1998;**351**(9097):173–177.
3. de Rooij SR, Painter RC, Roseboom TJ, Phillips DI, Osmond C, Barker DJ, Tanck MW, Michels RP, Bossuyt PM, Bleker OP. Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. *Diabetologia*. 2006;**49**(4):637–643.
4. Gluckman PD, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, Anokhin KV, Bougnères P, Chandak GR, Dasgupta P, Smith GD, Ellison PT, Forrester TE, Gilbert SF, Jablonka E, Kaplan H, Prentice AM, Simpson SJ, Uauy R, West-Eberhard MJ. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet*. 2009;**373**(9675):1654–1657.
5. Hanson M, Gluckman P. Developmental origins of noncommunicable disease: population and public health implications. *Am J Clin Nutr*. 2011;**94**(Suppl 6)1754S–1758S.
6. Jimenez-Chillaron JC, Hernandez-Valencia M, Reamer C, Fisher S, Joszi A, Hirshman M, Oge A, Walrond S, Przybyla R, Boozer C, Goodyear LJ, Patti ME. Beta-cell secretory dysfunction in the pathogenesis of low birth weight-associated diabetes: a murine model. *Diabetes*. 2005;**54**(3):702–711.
7. Radford EJ, Ito M, Shi H, Corish JA, Yamazawa K, Isganaitis E, Seisenberger S, Hore TA, Reik W, Erkek S, Peters AH, Patti ME, Ferguson-Smith AC. In utero effects: in utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science*. 2014;**345**(6198):1255903.
8. Isganaitis E, Jimenez-Chillaron J, Woo M, Chow A, DeCoste J, Vokes M, Liu M, Kasif S, Zavacki AM, Leshan RL, Myers MG, Patti ME. Accelerated postnatal growth increases lipogenic gene expression and adipocyte size in low-birth weight mice. *Diabetes*. 2009;**58**(5):1192–1200.
9. Woo M, Isganaitis E, Cerletti M, Fitzpatrick C, Wagers AJ, Jimenez-Chillaron J, Patti ME. Early life nutrition modulates muscle stem cell number: implications for muscle mass and repair. *Stem Cells Dev*. 2011;**20**(10):1763–1769.
10. Beauchamp B, Ghosh S, Dysart MW, Kanaan GN, Chu A, Blais A, Rajamanickam K, Tsai EC, Patti ME, Harper ME. Low birth weight is associated with adiposity, impaired skeletal muscle energetics and weight loss resistance in mice. *Int J Obes*. 2015;**39**(4):702–711.
11. Kobayashi M, Ikegami H, Fujisawa T, Nojima K, Kawabata Y, Noso S, Babaya N, Itoi-Babaya M, Yamaji K, Hiromine Y, Shibata M, Ogihara T. Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid-binding resin. *Diabetes*. 2007;**56**(1):239–247.
12. Watanabe M, Morimoto K, Houten SM, Kaneko-Iwasaki N, Sugizaki T, Horai Y, Mataki C, Sato H, Murahashi K, Arita E, Schoonjans K, Suzuki T, Itoh H, Auwerx J. Bile acid binding resin improves metabolic control through the induction of energy expenditure. *PLoS One*. 2012;**7**(8):e38286.
13. Wewalka M, Patti ME, Barbato C, Houten SM, Goldfine AB. Fasting serum taurine-conjugated bile acids are elevated in type 2 diabetes and do not change with intensification of insulin. *J Clin Endocrinol Metab*. 2014;**99**(4):1442–1451.
14. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, Badman MK, Maratos-Flier E, Mun EC, Pihlajamaki J, Auwerx J, Goldfine AB. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obesity (Silver Spring)*. 2009;**17**(9):1671–1677.
15. Simonen M, Dali-Youcef N, Kaminska D, Venesmaa S, Käkelä P, Pääkkönen M, Hallikainen M, Kolehmainen M, Uusitupa M, Moilanen L, Laakso M, Gylling H, Patti ME, Auwerx J, Pihlajamäki J. Conjugated bile acids associate with altered rates of glucose and lipid oxidation after Roux-en-Y gastric bypass. *Obes Surg*. 2012;**22**(9):1473–1480.
16. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Pérez HE, Sandoval DA, Kohli R, Bäckhed F, Seeley RJ. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature*. 2014;**509**(7499):183–188.
17. Penney NC, Kinross J, Newton RC, Purkayastha S. The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: a systematic review. *Int J Obes*. 2015;**39**(11):1565–1574.
18. Kohli R, Setchell KD, Kirby M, Myronovych A, Ryan KK, Ibrahim SH, Berger J, Smith K, Toure M, Woods SC, Seeley RJ. A surgical model in male obese rats uncovers protective effects of bile acids post-bariatric surgery. *Endocrinology*. 2013;**154**(7):2341–2351.
19. Broeders EP, Nascimento EB, Havekes B, Brans B, Roumans KH, Tailleux A, Schaart G, Kouach M, Charton J, Deprez B, Bouvy ND, Mottaghy F, Staels B, van Marken Lichtenbelt WD, Schrauwen P. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. *Cell Metab*. 2015;**22**(3):418–426.
20. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature*. 2006;**439**(7075):484–489.
21. Kumagai M, Kimura A, Takei H, Kurosawa T, Aoki K, Inokuchi T, Matsuishi T. Perinatal bile acid metabolism: bile acid analysis of meconium of preterm and full-term infants. *J Gastroenterol*. 2007;**42**(11):904–910.
22. Senger H, Boehm G, Beyreiss K, Braun W. Bile acids in the serum and duodenal content of newborn infants of different classification. *Biomed Biochim Acta*. 1986;**45**(7):931–939.
23. Martínez D, Pentinat T, Ribó S, Daviaud C, Bloks VW, Cebrià J, Villalmanzo N, Kalko SG, Ramón-Krauel M, Díaz R, Plösch T, Tost J, Jiménez-Chillarón JC. In utero undernutrition in male mice programs liver lipid metabolism in the second-generation offspring involving altered Lxra DNA methylation. *Cell Metab*. 2014;**19**(6):941–951.
24. Chu A, Thamotharan S, Ganguly A, Wadehra M, Pellegrini M, Devaskar SU. Gestational food restriction decreases placental interleukin-10 expression and markers of autophagy and endoplasmic reticulum stress in murine intrauterine growth restriction. *Nutr Res*. 2016;**36**(10):1055–1067.
25. Ganguly A, Touma M, Thamotharan S, De Vivo DC, Devaskar SU. Maternal calorie restriction causing uteroplacental insufficiency differentially affects mammalian placental glucose and leucine transport molecular mechanisms. *Endocrinology*. 2016;**157**(10):4041–4054.
26. Desai M, Babu J, Ross MG. Programmed metabolic syndrome: prenatal undernutrition and postweaning overnutrition. *Am J Physiol Regul Integr Comp Physiol*. 2007;**293**(6):R2306–R2314.
27. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP, Walter K, Menni C, Chen L, Vasquez L, Valdes AM, Hyde CL, Wang V, Ziemek D, Roberts P, Xi L, Grundberg E, Waldenberger M, Richards JB, Mohney RP, Milburn MV, John SL, Trimmer J, Theis FJ, Overington JP, Suhre K, Brosnan MJ, Gieger C, Kastenmüller G, Spector TD, Soranzo N; Multiple Tissue Human Expression Resource (MuTHER) Consortium. An atlas of genetic influences on human blood metabolites. *Nat Genet*. 2014;**46**(6):543–550.
28. Argmann CA, Houten SM, Champy MF, Auwerx J. Lipid and bile acid analysis. *Curr Protoc Mol Biol*. 2006;**Chapter 29**:Unit 29B.2. 10.1002/0471142727.mb29b02s75.
29. VanPatten S, Ranginani N, Shefer S, Nguyen LB, Rossetti L, Cohen DE. Impaired biliary lipid secretion in obese Zucker rats: leptin promotes hepatic cholesterol clearance. *Am J Physiol Gastrointest Liver Physiol*. 2001;**281**(2):G393–G404.
30. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA, Jr, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature*. 2014;**510**(7505):417–421.
31. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;**7**(5):335–336.

32. De Cáceres M, Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology*. 2009; **90**(12):3566–3574.
33. Xia J, Wishart DS. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nat Protoc*. 2011;**6**(6):743–760.
34. Makino I, Shinozaki K, Yoshino K, Nakagawa S. [Dissolution of cholesterol gallstones by long-term administration of ursodeoxycholic acid]. *Nippon Shokakibyo Gakkai Zasshi*. 1975;**72**(6): 690–702.
35. Gurung V, Middleton P, Milan SJ, Hague W, Thornton JG. Interventions for treating cholestasis in pregnancy. *Cochrane Database Syst Rev*. 2013;(6):CD000493.
36. Quintero P, Pizarro M, Solís N, Arab JP, Padilla O, Riquelme A, Arrese M. Bile acid supplementation improves established liver steatosis in obese mice independently of glucagon-like peptide-1 secretion. *J Physiol Biochem*. 2014;**70**(3):667–674.
37. Murakami M, Une N, Nishizawa M, Suzuki S, Ito H, Horiuchi T. Incretin secretion stimulated by ursodeoxycholic acid in healthy subjects. *Springerplus*. 2013;**2**(1):20.
38. Tsuchida T, Shiraishi M, Ohta T, Sakai K, Ishii S. Ursodeoxycholic acid improves insulin sensitivity and hepatic steatosis by inducing the excretion of hepatic lipids in high-fat diet-fed KK-Ay mice. *Metabolism*. 2012;**61**(7):944–953.
39. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab*. 2005;**2**(4):217–225.
40. Marc Dufrene PL. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr*. 1997; **67**(3):345–366.
41. Preidis GA, Keaton MA, Campeau PM, Bessard BC, Conner ME, Hotez PJ. The undernourished neonatal mouse metabolome reveals evidence of liver and biliary dysfunction, inflammation, and oxidative stress. *J Nutr*. 2014;**144**(3):273–281.
42. Brown EM, Wlodarska M, Willing BP, Vonaesch P, Han J, Reynolds LA, Arrieta MC, Uhrig M, Scholz R, Partida O, Borchers CH, Sansonetti PJ, Finlay BB. Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model. *Nat Commun*. 2015;**6**:7806.
43. Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, Briz O, Owen BM, Nikolova V, Ovadia C, Huang X, Vaarasmaki M, Baumann M, Jansen E, Albrecht C, Jarvelin MR, Marin JJ, Knisely AS, Williamson C. Maternal cholestasis during pregnancy programs metabolic disease in offspring. *J Clin Invest*. 2013;**123**(7):3172–3181.
44. Morimoto K, Watanabe M, Sugizaki T, Irie J, Itoh H. Intestinal bile acid composition modulates prohormone convertase 1/3 (PC1/3) expression and consequent GLP-1 production in male mice. *Endocrinology*. 2016;**157**(3):1071–1081.
45. Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota-farnesoid X receptor axis modulates metabolic disease. *Gastroenterology*. 2016;**151**(5):845–859.
46. Zhi X, Zhou XE, He Y, Zechner C, Suino-Powell KM, Kliewer SA, Melcher K, Mangelsdorf DJ, Xu HE. Structural insights into gene repression by the orphan nuclear receptor SHP. *Proc Natl Acad Sci USA*. 2014;**111**(2):839–844.
47. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, Kliewer SA. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell*. 2000;**6**(3):517–526.
48. Fu S, Fan J, Blanco J, Gimenez-Cassina A, Danial NN, Watkins SM, Hotamisligil GS. Polysome profiling in liver identifies dynamic regulation of endoplasmic reticulum translatome by obesity and fasting. *PLoS Genet*. 2012;**8**(8):e1002902.
49. Reijnders D, Goossens GH, Hermes GD, Neis EP, van der Beek CM, Most J, Holst JJ, Lenaerts K, Kootte RS, Nieuwdorp M, Groen AK, Olde Damink SW, Boekschoten MV, Smidt H, Zoetendal EG, Dejong CH, Blaak EE. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial [published correction appears in Cell Metab. 2016;24(2):341]. *Cell Metab*. 2016;**24**(1):63–74.
50. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab*. 2013;**17**(2):225–235.
51. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab*. 2016;**24**(1):41–50.
52. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature*. 2013;**498**(7452):99–103.
53. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, Prifti E, Vieira-Silva S, Gudmundsdottir V, Krogh Pedersen H, Arumugam M, Kristiansen K, Voigt AY, Vestergaard H, Hercog R, Igor Costea P, Kultima JR, Li J, Jørgensen T, Levenez F, Dore J, Nielsen HB, Brunak S, Raes J, Hansen T, Wang J, Ehrlich SD, Bork P, Pedersen O; MetaHIT Consortium. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*. 2015;**528**(7581):262–266.
54. Yassour M, Lim MY, Yun HS, Tickle TL, Sung J, Song YM, Lee K, Franzosa EA, Morgan XC, Gevers D, Lander ES, Xavier RJ, Birren BW, Ko G, Huttenhower C. Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. *Genome Med*. 2016;**8**(1):17.
55. Schneeberger M, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, Gomis R, Claret M, Cani PD. Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Sci Rep*. 2015;**5**:16643.
56. Preidis GA, Ajami NJ, Wong MC, Bessard BC, Conner ME, Petrosino JF. Microbial-derived metabolites reflect an altered intestinal microbiota during catch-up growth in undernourished neonatal mice. *J Nutr*. 2016;**146**(5):940–948.
57. Marques TM, Wall R, O'Sullivan O, Fitzgerald GF, Shanahan F, Quigley EM, Cotter PD, Cryan JF, Dinan TG, Ross RP, Stanton C. Dietary trans-10, cis-12-conjugated linoleic acid alters fatty acid metabolism and microbiota composition in mice. *Br J Nutr*. 2015;**113**(5):728–738.
58. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature*. 2009;**457**(7228):480–484.
59. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA*. 2005;**102**(31):11070–11075.
60. Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain [published online ahead of print April 4, 2017]. *Int J Obes*.
61. Xiao L, Sonne SB, Feng Q, Chen N, Xia Z, Li X, Fang Z, Zhang D, Fjære E, Midtbø LK, Derrien M, Hugenholtz F, Tang L, Li J, Zhang J, Liu C, Hao Q, Vogel UB, Mortensen A, Kleerebezem M, Licht TR, Yang H, Wang J, Li Y, Arumugam M, Wang J, Madsen L, Kristiansen K. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. *Microbiome*. 2017;**5**(1):43.
62. Sepe V, Renga B, Festa C, D'Amore C, Masullo D, Cipriani S, Di Leva FS, Monti MC, Novellino E, Limongelli V, Zampella A, Fiorucci S. Modification on ursodeoxycholic acid (UDCA) scaffold: discovery of bile acid derivatives as selective agonists of cell-surface G-protein coupled bile acid receptor 1 (GP-BAR1). *J Med Chem*. 2014;**57**(18):7687–7701.