

Western Diet Changes Gut Microbiota and Ameliorates Liver Injury in a Mouse Model with Human-Like Bile Acid Composition

Junichi Iwamoto ¹, Akira Honda ^{1,2}, Teruo Miyazaki ², Tadakuni Monma,¹ Hajime Ueda,¹ Yukio Morishita,³ Sho-ichiro Yara,¹ Takeshi Hirayama,¹ and Tadashi Ikegami ¹

Western-style high-fat/high-sucrose diet (HFHSD) changes gut microbiota and bile acid (BA) profiles. Because gut microbiota and BAs could influence each other, the mechanism of changes in both by HFHSD is complicated and remains unclear. We first aimed to clarify the roles of BAs in the HFHSD-induced change of gut microbiota. Then, we studied the effects of the changed gut microbiota on BA composition and liver function. Male wild-type (WT) and human-like *Cyp2a12/Cyp2c70* double knockout (DKO) mice derived from C57BL/6J were fed with normal chow or HFHSD for 4 weeks. Gut microbiomes were analyzed by fecal 16S ribosomal RNA gene sequencing, and BA composition was determined by liquid chromatography–tandem mass spectrometry. The DKO mice exhibited significantly reduced fecal BA concentration, lacked muricholic acids, and increased proportions of chenodeoxycholic and lithocholic acids. Despite the marked difference in the fecal BA composition, the profiles of gut microbiota in the two mouse models were quite similar. An HFHSD resulted in a significant increase in the BA pool and fecal BA excretion in WT mice but not in DKO mice. However, microbial composition in the two mouse models was drastically but similarly changed by the HFHSD. In addition, the HFHSD-induced change of gut microbiota inhibited BA deconjugation and 7 α -dehydroxylation in both types of mice, which improved chronic liver injury observed in DKO mice. **Conclusion:** The HFHSD itself causes the change of gut microbiota due to HFHSD, and the altered composition or concentration of BAs by HFHSD is not the primary factor. On the contrary, the gut microbiota formed by HFHSD affects BA composition and ameliorates liver injury in the mouse model with human-like hydrophobic BA composition. (*Hepatology Communications* 2021;5:2052–2067).

The Western-style high-fat and usually high-sucrose (sugar) diet (HFHSD) changes the gut microbiota in mammals.^(1–4) This diet-induced imbalance of microbial composition (dysbiosis) is suspected as the cause of several gastrointestinal diseases,⁽⁵⁾ including nonalcoholic steatohepatitis (NASH),⁽⁶⁾ inflammatory bowel diseases,⁽⁷⁾ and colorectal cancer.^(8,9) However, the mechanism of

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; BA, bile acid; Bsep, bile salt export pump; CA, cholic acid; CDCA, chenodeoxycholic acid; CYP, cytochrome P450; DCA, deoxycholic acid; DKO, double knockout; FFA, free fatty acids; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; HFD, high-fat diet; HFHSD, high-fat high-sucrose diet; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; Hmgcr, HMG-CoA reductase; HSD, high-sucrose diet; LCA, lithocholic acid; LC/MS-MS, liquid chromatography–tandem mass spectrometry; LXR α , liver X receptor α ; MCA, muricholic acid; mRNA, messenger RNA; OTU, operational taxonomic unit; PPAR α , peroxisome proliferator-activated receptor α ; PXR, pregnane X receptor; SCEFA, short-chain fatty acid; Shp, small heterodimer partner; SULT2A1, sulfotransferase 2A1; TCA, taurocholic acid; TCDCa, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, tauroolithocholic acid; TMCA, tauromuricholic acid; UDCA, ursodeoxycholic acid; WT, wild type.

Received January 25, 2021; accepted June 20, 2021.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1778/supinfo.

Supported by the Japan Society for the Promotion of Science (17H04167, 18K07920, and 18K08017) and Japan Agency for Medical Research and Development (JP21ek0109416 and JP22fk0210073).

© 2021 The Authors. *Hepatology Communications* published by Wiley Periodicals LLC on behalf of American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution–NonCommercial–NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

dysbiosis due to HFHSD is not fully understood. Bile acids (BAs) are suggested as compounds that connect HFHSD with dysbiosis,⁽¹⁰⁾ but the relationship between BAs and microbiota is complicated because microbiota also regulate BA composition.⁽¹¹⁾

Bile acids are the end products of cholesterol metabolism and are synthesized in the liver. The BAs are called biological detergents because they are amphipathic molecules and facilitate digestion and absorption of lipids in the small intestine. Because of their detergent properties, BAs, especially deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA), have antimicrobial activity due to damage to the bacterial membrane.^(12,13) A Western diet enhances biliary secretion and fecal excretion of BAs including DCA.⁽¹⁴⁾ Therefore, it is tempting to speculate that BAs are a major factor that regulates the profile of gut microbiota on a HFHSD.

Indeed, when 3-week-old rats were fed cholic acid (CA) for 10 days, Islam et al. observed vastly expanded cecal Firmicutes and reduced Bacteroidetes proportions at the phylum level, and markedly increased Clostridia at the class level.⁽¹⁵⁾ However, the fecal amount of DCA, a bacterial metabolite of CA, was raised at least 15-fold in the CA-fed rats, which is far beyond physiological elevation due to a Western diet (less than 2-fold).⁽¹⁴⁾ A mouse model with a mildly elevated cecal BA concentration was also created by feeding control chow supplemented with a low amount of CAs.⁽¹⁰⁾ However, the alteration of the cecal microbial profile in this mouse model was limited as compared with that in mice with a high-fat diet (HFD).

In addition, increased fecal BAs on gut microbiota were studied in patients with chronic constipation treated with elobixibat, an inhibitor of apical sodium-dependent BA transporter.⁽¹⁶⁾ This treatment significantly augmented fecal concentrations of total BAs by 38% and DCA by 27%, but did not change gut microbiota at the phylum and genus levels. These results together suggest that the higher amounts of fecal BAs at a physiological level have little effect on microbiota, and the Western diet itself may control the composition of gut microbiota.

The present study aimed to clarify the roles of BAs on the HFHSD-induced changes in gut microbiota and the effects of the altered gut microbiota on BA metabolism. A mouse is the most generally used animal to extrapolate human metabolism. However, BA metabolism between mice and humans is very different.⁽¹⁷⁾ First, CDCA, an end product in the human liver, is metabolized to highly hydrophilic muricholic acids (MCAs) by *Cyp2c70* in mice liver. Second, gut microbiota convert the primary BAs, CA and CDCA, into more hydrophobic secondary BAs, DCA and lithocholic acid (LCA). However, mice revert these secondary BAs to primary BAs by hepatic *Cyp2a12*. Therefore, a conventional mouse is not an ideal animal to study the effects of BAs on human health and diseases.

To overcome the problem, we recently generated *Cyp2a12/Cyp2c70* double knockout (DKO) mice with human-like BA composition.⁽¹⁷⁾ The mice exhibit a completely different BA composition from wild-type (WT) C57BL/6J mice (i.e., they lacked MCAs and markedly increased proportions of CDCAs, DCAs,

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1778

Potential conflict of interest: Nothing to report.

ARTICLE INFORMATION:

From the ¹Division of Gastroenterology and Hepatology, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan; ²Joint Research Center, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan; ³Diagnostic Pathology Division, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Akira Honda, M.D., Ph.D.
Division of Gastroenterology and Hepatology
Tokyo Medical University Ibaraki Medical Center
3-20-1 Ami-machi Chuo

Inashiki-gun, Ibaraki 300-0395, Japan
E-mail: akihonda@tokyo-med.ac.jp
Tel.: +81-29-887-1161

and LCAs). The comparison of gut microbiota among WT and DKO mice fed with normal chow or HFHSD showed that BA was not a major determinant of gut microbial composition.

Animals and Methods

ANIMALS

Cyp2a12^{-/-}*Cyp2c70*^{-/-} DKO mice were generated as described previously,⁽¹⁷⁾ and C57BL/6J WT mice were obtained from Charles River Laboratories (Kanagawa, Japan). All mice were kept under pathogen-free conditions and a regular 12-hour light-dark cycle (light period: 6:00-18:00), with free access to normal chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. This experimental protocol was approved by the Animal Experiment Committees of Charles River Laboratories Japan and Tokyo Medical University (Permission #R2-0013).

Twelve-week-old male WT (n = 10) and DKO (n = 10) mice were divided into two groups, respectively. One group was fed a normal chow diet (CRF-1), and the other group was fed an HFHSD (F2HFHSD; Oriental Yeast Co.) for 4 weeks. The nutritional contents of both diets are given in Supporting Table S1.

After fasting for 4 hours with free access to water, the animals were sacrificed under combination anesthesia with medetomidine, midazolam, and butorphanol. Their serum, gallbladder, liver, small intestine, cecal contents, and feces were collected and frozen at -80°C until analysis.

DETERMINATION OF LIVER FUNCTION TESTS

The activities of alanine transaminase (ALT) and alkaline phosphatase (ALP) in sera were measured by colorimetric assays using Transaminase CII-Test Wako and LabAssay ALP (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan).

DETERMINATION OF BA CONCENTRATIONS

BA concentrations in the liver, bile, small intestine, feces, and serum were determined by the liquid chromatography-tandem mass spectrometry (LC/MS-MS) method as described previously.⁽¹⁷⁾

DETERMINATION OF LIPID CONCENTRATIONS

Serum and hepatic concentrations of triglycerides, total cholesterol, and free fatty acids (FFA) were measured by colorimetric assays using Triglyceride E-Test Wako, Cholesterol E-Test Wako, and NEFA C-Test Wako (FUJIFILM Wako Pure Chemical Corp.), respectively. Serum and hepatic concentrations of sterols and oxysterols were measured as described previously.⁽¹⁷⁾ Fecal sterol concentrations were quantified using our previously described LC/MS-MS method⁽¹⁸⁾ with minor modifications. Briefly, 5 mg of wet feces were incubated in 250 µL of 1N ethanolic KOH at 60°C for 1 hour. After the addition of 1 µg of [²H₇]cholesterol (internal standard) to a 10 µL aliquot of the hydrolysate, sterols were extracted with n-hexane, derivatized to picolinyl esters, and analyzed by LC/MS-MS. The fecal fatty acid concentration was determined as follows: Fatty acids were extracted from a 5-µL aliquot of this hydrolysate by the Bligh-Dyer method⁽¹⁹⁾ and quantified using NEFA C-Test Wako.

DETERMINATION OF ORGANIC ACID CONCENTRATIONS

The concentrations of acetate, propionate, and n-butyrate in cecal contents were determined as described by Nagatomo et al.,⁽²⁰⁾ except that [¹³C₄]3-hydroxybutyrate was used as an internal standard (IS). The selected reaction monitoring (SRM) for a 2-picolylamine derivative of [¹³C₄]3-hydroxybutyrate was conducted using *m/z* 199.0 → 108.9 (collision energy: 20 V). To measure lactate concentration, an aliquot of methanol extract from cecal contents was mixed with [²H₃]lactate as an IS. After evaporation of the solvent at 37°C under nitrogen, the residue was redissolved in H₂O and injected into the LC/MS-MS system. The same column and mobile phases as this method for 2-picolylamine derivatives were used, and the SRM was conducted using *m/z* 89.0 → 43.0 for lactate and 92.0 → 45.0 for the [²H₃] variant (collision energy: 12 V).

MICROBIAL OPERATIONAL TAXONOMIC UNIT ANALYSIS BY SEQUENCING THE 16S rRNA GENE

The fecal bacterial population was analyzed by the TechnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan)

according to a method described previously.⁽²¹⁾ Briefly, whole bacterial DNA was extracted from feces, and the hypervariable V3-4 region of the 16S rRNA was amplified by polymerase chain reaction (PCR). The amplicons were sequenced using a paired-end strategy on a MiSeq sequencing system (Illumina, San Diego, CA). Sequence data analysis was performed using the quantitative insights into microbial ecology (QIIME) ver. 1.8.0, and the reads were clustered into operational taxonomic units (OTUs) at 97% pairwise identity. To analyze the bacterial composition, Greengenes Database ver. 13.8⁽²²⁾ was used. α -Diversity and β -diversity were calculated using QIIME.

HISTOPATHOLOGICAL EXAMINATION

An aliquot of the liver was fixed in 10% neutral buffered formalin and embedded in a paraffin block. Each paraffin block was sectioned at 3 μ m, and the paraffin sections were stained using hematoxylin and eosin or Masson's trichrome.

MESSENGER RNA MEASUREMENTS

Another aliquot of the liver was stored in RNAlater (Thermo Fisher Scientific, Waltham, MA) at -80°C until RNA isolation. Then, the extraction of total RNA, reverse transcription, and real-time quantitative PCR were performed as described previously.⁽¹⁷⁾ The sequences of the primer pairs used are provided in Supporting Table S6.

ENZYME ASSAYS

Hepatic microsomes and mitochondria were prepared, and activities of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, CYP7A1, CYP8B1, CYP27A1, and CYP7B1 were measured as described previously.⁽¹⁷⁾

DETERMINATION OF SERUM FIBROBLAST GROWTH FACTOR 15 CONCENTRATIONS

Serum concentrations of fibroblast growth factor 15 (FGF15) were quantified by mouse FGF15 ELISA kit (Catalog # MBS2700661; MyBiosource, Inc., San Diego, CA), according to the manufacturer's instruction.

STATISTICS

Data are expressed as the mean \pm SEM. The statistical significance of differences between the different groups was evaluated using the Tukey-Kramer test. For all analyses, significance was accepted at the level of $P < 0.05$. Correlations were tested by calculating nonparametric Spearman's rank correlation coefficient, r_s . All statistical analyses were conducted using Prism (ver. 9.1.0) software (GraphPad Software, San Diego, CA).

Results

EFFECTS OF HFHSD ON BA POOL SIZE AND COMPOSITION IN WT AND DKO MICE

The total BA pool in each group was calculated by combining the amounts of all BAs in the liver, gallbladder, and small intestine. As we reported previously,⁽¹⁷⁾ BA compositions of the total BA pool were markedly different between WT and DKO mice (Fig. 1A). Glycine-conjugated BAs were also detected in both WT and DKO mice, but the proportion was less than 1% of taurine-conjugated BAs. The DKO mice lacked MCAs and had increased proportions of CDCAs, DCAs, and LCAs. The HFHSD significantly reduced deconjugated BAs in both WT and DKO mice and significantly decreased secondary BAs in DKO mice only (Supporting Table S2). The total BA pool was significantly reduced in DKO mice, and the HFHSD increased the pool to nearly twice that in WT mice (Fig. 1B). In contrast, the collective did not change significantly in DKO mice fed with HFHSD. BA content by the organ is also shown (Fig. 1C-E), and hepatic BA content was generally elevated in DKO mice with a normal diet, which may reflect mild liver injury in these mice.⁽¹⁷⁾

EFFECTS OF HFHSD ON FECAL BA COMPOSITION AND CONCENTRATION IN WT AND DKO MICE

As shown in Fig. 2A, fecal BA compositions were also completely different between WT and

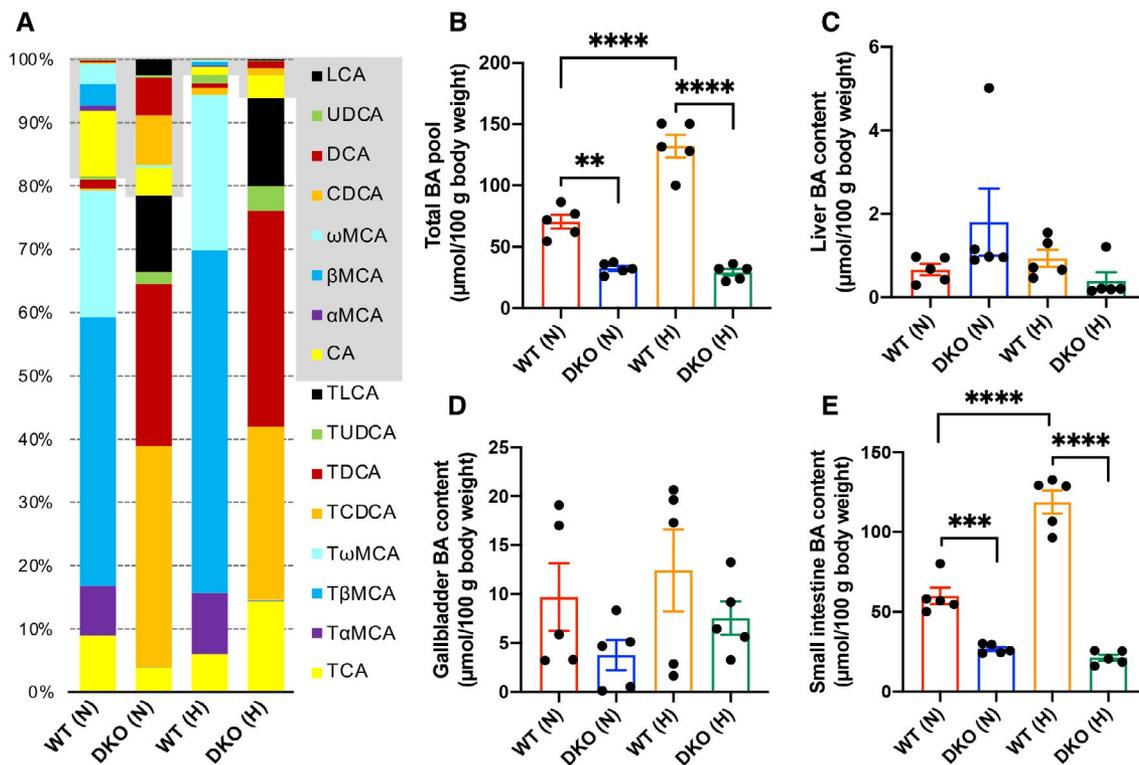


FIG. 1. BA composition and concentration in WT and DKO mice treated with different diets. A total of 20 male mice ($n = 5$ in each group) were analyzed. The total BA pool in the enterohepatic circulation was calculated by adding total BAs in the liver, gallbladder, and small intestine. (A) Composition of major BAs in the total BA pool. Deconjugated BAs are shaded gray. BA amounts in the total pool (B), liver (C), gallbladder (D), and small intestine (E). Each column and error bar represents the mean and SEM. $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ were considered significantly different by the Tukey-Kramer test. Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet.

DKO mice regardless of diets. DKO mice lacked MCAs and had an increased proportion of LCA (Supporting Table S3). The effects of HFHSD on fecal concentrations of total and each BAs are presented in Fig. 2B-E. The HFHSD significantly increased fecal concentrations of total BAs in WT mice but not in DKO mice. DCA and CDCA are the most bactericidal BAs,⁽¹³⁾ but HFHSD did not significantly affect the concentrations of DCA or CDCA in both types of mice. α MCA, β MCA, and ursodeoxycholic acid (UDCA) have intermediate activities, whereas CA and ω MCA have mild bactericidal activities,⁽¹³⁾ and α MCA, CA, and ω MCA concentrations significantly increased after HFHSD only in WT mice. Although the bactericidal activity of LCA is still controversial,^(23,24) the concentration of this BA was not significantly altered by HFHSD.

EFFECTS OF HFHSD ON GUT MICROBIOTA IN WT AND DKO MICE

Fecal microbial compositions at the phylum level are compared in Fig. 3A and Supporting Table S4. The compositions were not significantly different between WT and DKO mice fed with normal diet, nor those animals fed with HFHSD. However, significantly decreased Bacteroidetes and increased Firmicutes were observed in WT mice fed with HFHSD as compared to those with a normal diet. In contrast, HFHSD did not modify the composition much at the phylum level in DKO mice. The Bacteroidetes/Firmicutes ratio is an index of dysbiosis.⁽²⁵⁾ When we calculated this ratio, HFHSD significantly decreased the ratio in WT mice but not in DKO mice (Supporting Fig. S1D).

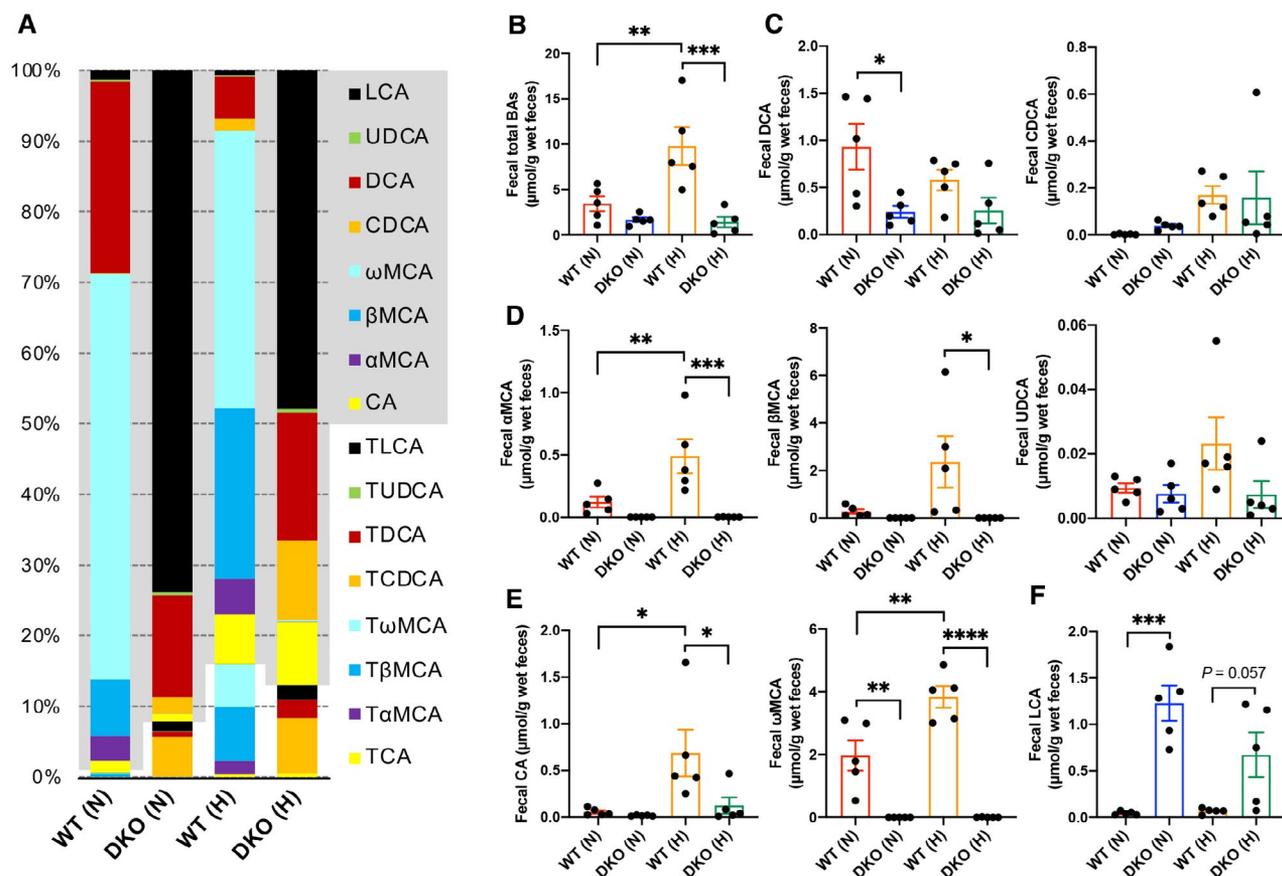


FIG. 2. Fecal BA composition and concentration in WT and DKO mice treated with different diets. Feces from 20 male mice ($n = 5$ in each group) were analyzed. (A) Composition of major BAs in feces. Deconjugated BAs are gray shaded. (B) Total BA concentrations in feces. Fecal concentrations of the most bactericidal BAs, DCA, and CDCA (C), intermediate bactericidal BAs, α MCA, β MCA, and ursodeoxycholic acid (D), (E) less bactericidal BAs, CA and ω MCA, and LCA whose bactericidal activity is in question (F). Each column and error bar represents the mean and SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ were considered significantly different by the Tukey-Kramer test. Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet.

The microbial compositions at class, order, family, and genus levels are provided in Supporting Fig. S1A-C, Fig. 3B, and Supporting Table S5. Bacterial compositions at the genus level were virtually the same between WT and DKO mice fed with normal diet despite the marked difference of fecal BA profiles. However, both in WT and DKO mice, HFHSD drastically but similarly induced a change in the microbial compositions, showing a significantly elevated proportion of the genera, *Dorea*, *rc4-4*, *f_Clostridiaceae*; *g_Other* and *g_Unclassified*, and *f_Peptostreptococcaceae*; *g_Unclassified*, and significantly reduced the proportion of the genera, *Lactobacillus* and *f_S24-7*; *g_Unclassified*. Exceptionally, *Bacteroides* increased only in DKO mice fed with HFHSD and *f_Erysipelotrichaceae*; *g_Unclassified* increased only in WT mice fed with HFHSD.

We compared α -diversity among the four groups by calculating Chao 1 index (OTU richness estimation; Fig. 4A) and Shannon index (OTU evenness estimation; Fig. 4B). These indices tended to be low in both HFHSD-treated types of mice, but were not statistically significant.

β -Diversity was compared among the four groups by calculating UniFrac distances. Principal coordinate analysis exhibited significant microbial structural differences along the PC1 axis between the mice fed with normal diet and those fed an HFHSD in unweighted ($P < 0.0001$) and weighted ($P < 0.0001$) UniFrac distances (Fig. 4C,D). However, microbial composition between WT and DKO mice fed with the same diet type were not separated along the PC1 axis.

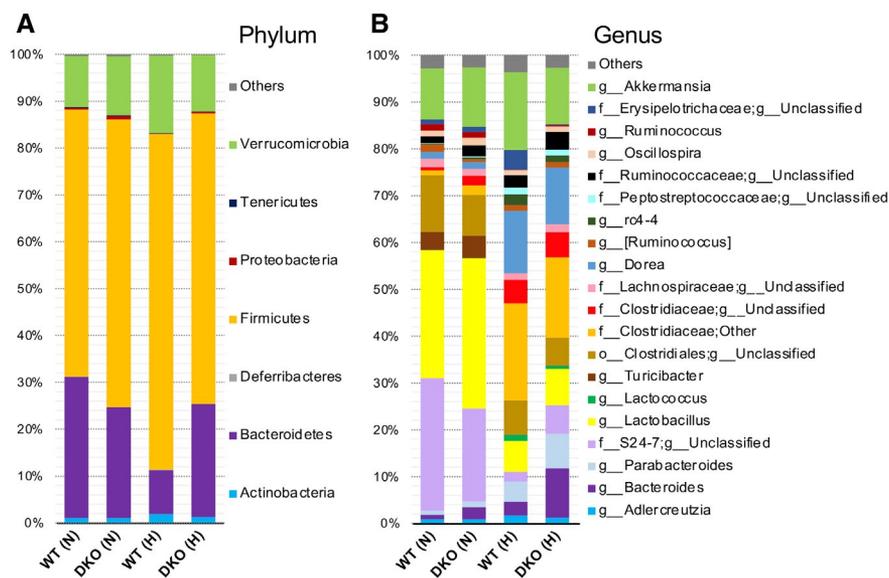


FIG. 3. Gut microbial composition in WT and DKO mice treated with different diets. Feces from 20 male mice ($n = 5$ in each group) were analyzed. The compositions are shown at the phylum level (A) and the genus level (B). Each column represents the mean of five mice. Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet.

EFFECTS OF ALTERATION OF GUT MICROBIOTA ON BA, STEROL, AND ORGANIC ACID METABOLISM

The change in gut microbiota by HFHSD appeared to diminish the proportion of deconjugated BA pool in both WT and DKO mice (Supporting Table S2). Looking at each organ, the reduction of deconjugated BAs was the most apparent in the small intestine (Fig. 5A). The proportion of secondary BAs, which was calculated by $(DCA+LCA)/(CA+CDCA+DCA+LCA)$, was generally higher in DKO mice than in WT mice (Fig. 5B), because of the disruption of *Cyp2a12* (BA 7α -rehydroxylase). The HFHSD significantly reduced biliary and small intestinal secondary BAs in DKO mice and fecal secondary BAs in WT mice.

The concentrations of fecal animal sterols (cholesterol+coprostanol) tended to increase in both types of mice fed with HFHSD but were not statistically significant (Fig. 5C). However, the proportion of coprostanol was markedly reduced in the mice fed with HFHSD. Our HFHSD contains less plant sterols than a normal diet, and fecal plant sterol concentrations were significantly decreased in WT mice fed with HFHSD but

were not modified by HFHSD in DKO mice. However, in normal chow fed mice, sitosterol and campesterol may convert to 24β -ethyl-coprostanol and 24α -methyl-coprostanol, although we did not quantify these sterols. If the conversion rates are equal to that of cholesterol to coprostanol, all plant sterol concentrations in WT and DKO mice with regular diet are estimated to be 1.80 and 1.37 $\mu\text{mol/g}$ wet feces, respectively. Therefore, the actual excretion of fecal plant sterols may be decreased in both mice fed with HFHSD. On the other hand, fecal fatty acid concentrations tended to increase in both types of mice with HFHSD but the difference was not statistically significant.

Cecal organic acid concentrations were determined (Fig. 5D). Although lactate and n-butyrate concentrations did not change among the groups, acetate and propionate were significantly increased in DKO mice as compared with those in WT mice, regardless of diet types.

CORRELATIONS BETWEEN GUT MICROBIOTA AND BA, STEROL, OR ORGANIC ACID METABOLISM

To explore the genera that relate to fecal BA, sterol, and organic acid metabolism, correlations

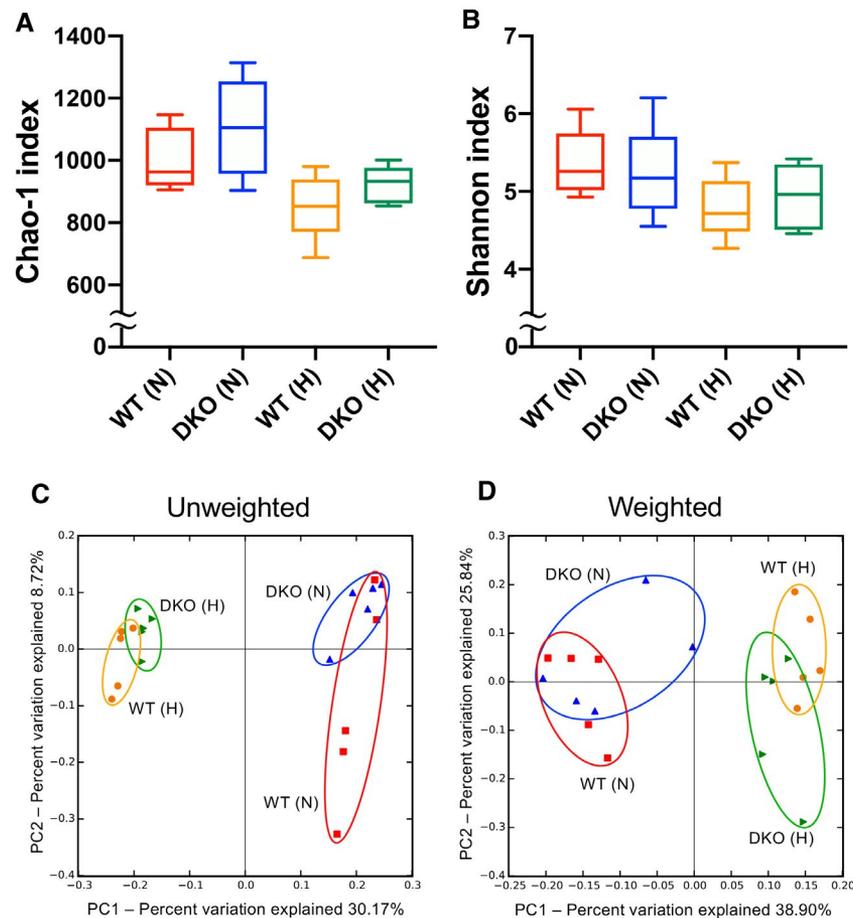


FIG. 4. α -Diversity and β -diversity of gut microbiota in WT and DKO mice treated with different diets. Feces from 20 male mice ($n = 5$ in each group) were analyzed. (A) The Chao-1 index estimates OUT richness (α -diversity). (B) The Shannon index estimates OUT evenness (α -diversity). Principal coordinate analysis plots of unweighted (C) and weighted (D) UniFrac distances (β -diversity). Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet.

between gut microbiota (major 20 genera) and metabolic markers were studied. As shown in Fig. 6, the percentage of deconjugated BAs, percentage of secondary BAs, and percentage of coprostanol are all positively linked to *f_S24-7*; *g_Unclassified*, *Ruminococcus*, *Lactobacillus*, and *Turicibacter*. The positive relationship of percentage of secondary BAs and these genera were supported by the negative correlations of fecal CA or CDCA (primary BA) concentrations and these genera. Regarding cecal organic acid concentrations, acetate and propionate were positively associated with *Bacteroides*, and n-butyrate was positively correlated with *Ruminococcus* and *Oscillospira*.

EFFECTS OF HFHSD ON LIVER INJURY AND LIPID METABOLISM

As we showed previously,⁽¹⁷⁾ DKO mice had a liver injury that may be due to the increased hepatic concentrations of deconjugated CDCA (and DCA). Histologic sections of liver from DKO mice fed with normal diet revealed bile ductular proliferation with moderate infiltration of lymphocytes, neutrophils, and eosinophils, suggesting chronic inflammation (Fig. 7A). In addition, pericellular or perisinusoidal fibrosis and bridging fibrosis were also shown by staining with Masson's trichrome. However, in DKO mice fed with HFHSD, these inflammatory reactions

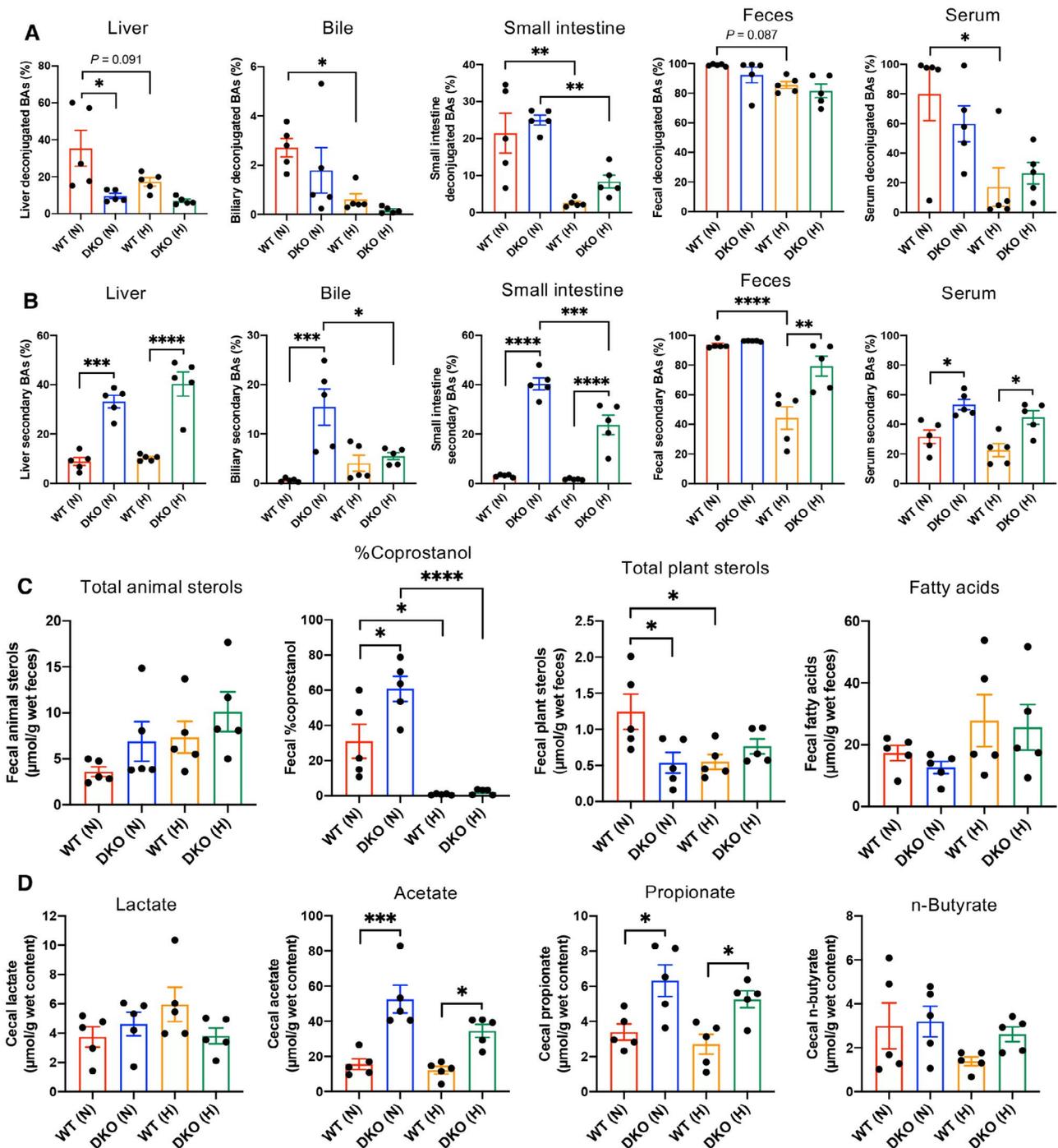


FIG. 5. Effects of the change in gut microbiota on the composition of BAs, and the concentrations of neutral sterols and organic acids in WT and DKO mice treated with different diets. A total of 20 male mice ($n = 5$ in each group) were analyzed. Proportions of deconjugated BAs (A), secondary (7α -dehydroxylated) BAs (B), and fecal concentrations of sterols and fatty acids (C) are shown. The percentage of secondary BAs were calculated by $(DCA+LCA)/(CA+CDCA+DCA+LCA)$, and percentage of coprostanol was calculated by $\text{coprostanol}/\text{total animal sterols (cholesterol+coprostanol)}$. (D) The concentrations of major organic acids in cecal contents were determined. Each column and error bar represents the mean and SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ were considered significantly different by the Tukey–Kramer test. Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet.

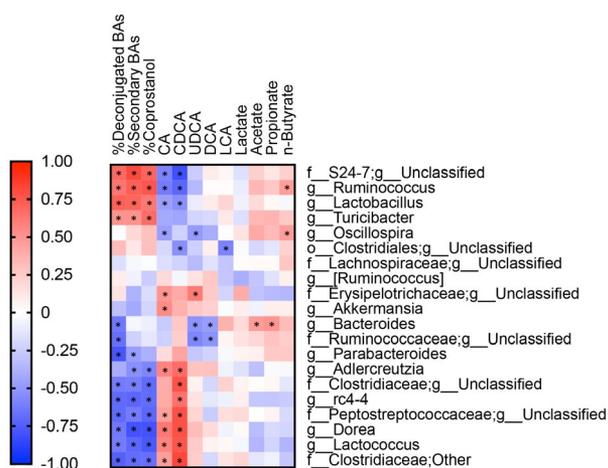


FIG. 6. A heat map demonstrating the Spearman's rank correlation coefficients among the abundance of gut microbiota at the genus level and fecal BA and sterol compositions, fecal BA concentrations, and cecal organic acid concentrations (n = 20). Red and blue denote positive and negative correlations, respectively. * $P < 0.05$.

and fibrotic changes disappeared, and only medium-sized fat droplets were observed in less than 10% of hepatocytes. These histologic findings were supported by significantly reduced serum ALT activities (Fig. 7C) and significantly increased liver triglyceride concentrations (Fig. 7E) in DKO mice with HFHSD.

In WT mice, the high hepatic levels of triglycerides, FFA (Fig. 7E), and messenger RNA (mRNA) expressions of inflammatory cytokines, tumor necrosis factor α (*Tnfa*), chemokine (C-C motif) ligand 2 (*Ccl2*), and transforming growth factor β 1 (*Tgfb1*) (Fig. 7F) were associated with increased serum ALT activity by HFHSD. On the other hand, hepatic lipid concentrations or inflammatory cytokine levels did not explain marked liver injury with normal chow and improvement with HFHSD in DKO mice.

To assess how the HFHSD influences lipid metabolism, hepatic mRNA expression levels of related genes were determined (Supporting Fig. S2). Sterol regulatory element-binding protein 1 (*Srebp1*), acetyl-CoA carboxylase 1 (*Acc1*), fatty acid synthase (*Fas*), ATP binding cassette A1 (*Abca1*), *Abcg5*, and *Abcg8* are all target genes for liver X receptor α (LXR α). *Acc1* and *Fas*, which are critical enzymes in the fatty acid biosynthetic pathway, did not change significantly among the groups. Although HFHSD up-regulated the other LXR α target genes, the increase

of sterols that are ligands of LXR α was not apparent (Supporting Fig. S3A). *ABCG5* and *ABCG8* form a sterol transporter expressed in the liver and intestine to excrete cholesterol and plant sterols. The up-regulation of this transporter by HFHSD was supported by decreased serum concentrations of plant sterols (sitosterol and campesterol), surrogate markers for intestinal cholesterol absorption (Supporting Fig. S3B). Gene expression of hepatic HMG-CoA reductase (*Hmgcr*), the rate-limiting enzyme in the cholesterol biosynthetic pathway, was up-regulated by HFHSD in both mice (Fig. 8C). However, this treatment does not appear to increase cholesterol biosynthesis *in vivo*, because HMGCR activity and serum lathosterol and desmosterol concentrations, surrogate markers for cholesterol biosynthesis, did not rise by HFHSD (Supporting Fig. S3B).

EFFECTS OF HFHSD ON THE REGULATION OF BA METABOLISM

We compared the percentage of deconjugated each BA in enterohepatic circulation (total pool) among the groups. As shown in Fig. 8A, the percentage of deconjugated hydrophobic BAs (CDCA, DCA, and LCA) in DKO mice with HFHSD were all markedly lower than those with a normal diet. To explore the reason for the reduction of deconjugated hydrophobic BAs in DKO mice with HFHSD, we determined the ratios of CA-derived BAs (CA and DCA) to CDCA-derived BAs (CDCA, LCA, and MCAs) and critical enzyme activities in BA biosynthetic pathways. In DKO mice, HFHSD significantly increased the CA/CDCA ratio in the BA pool (Fig. 8B). HFHSD significantly increased CYP7A1 activities in both mice groups, but it increased CYP8B1 activity only in WT mice (Fig. 8C). In contrast, CYP27A1 and CYP7B1 activities did not change significantly among the groups. mRNA expression levels of these enzymes were not wholly consistent with the enzyme activities, but they were enough to indicate the change in enzyme activities. Hepatic expression levels of other target genes for farnesoid X receptor (*Fxr*) were studied (Fig. 8D). HFHSD tended to increase the expression of small heterodimer partner (*Shp*) and bile salt export pump (*Bsep*) in both WT and DKO mice, although only *Bsep* in DKO mice was significantly different. Serum FGF15 level reflects the activation of intestinal *Fxr* and tended to increase by HFHSD,

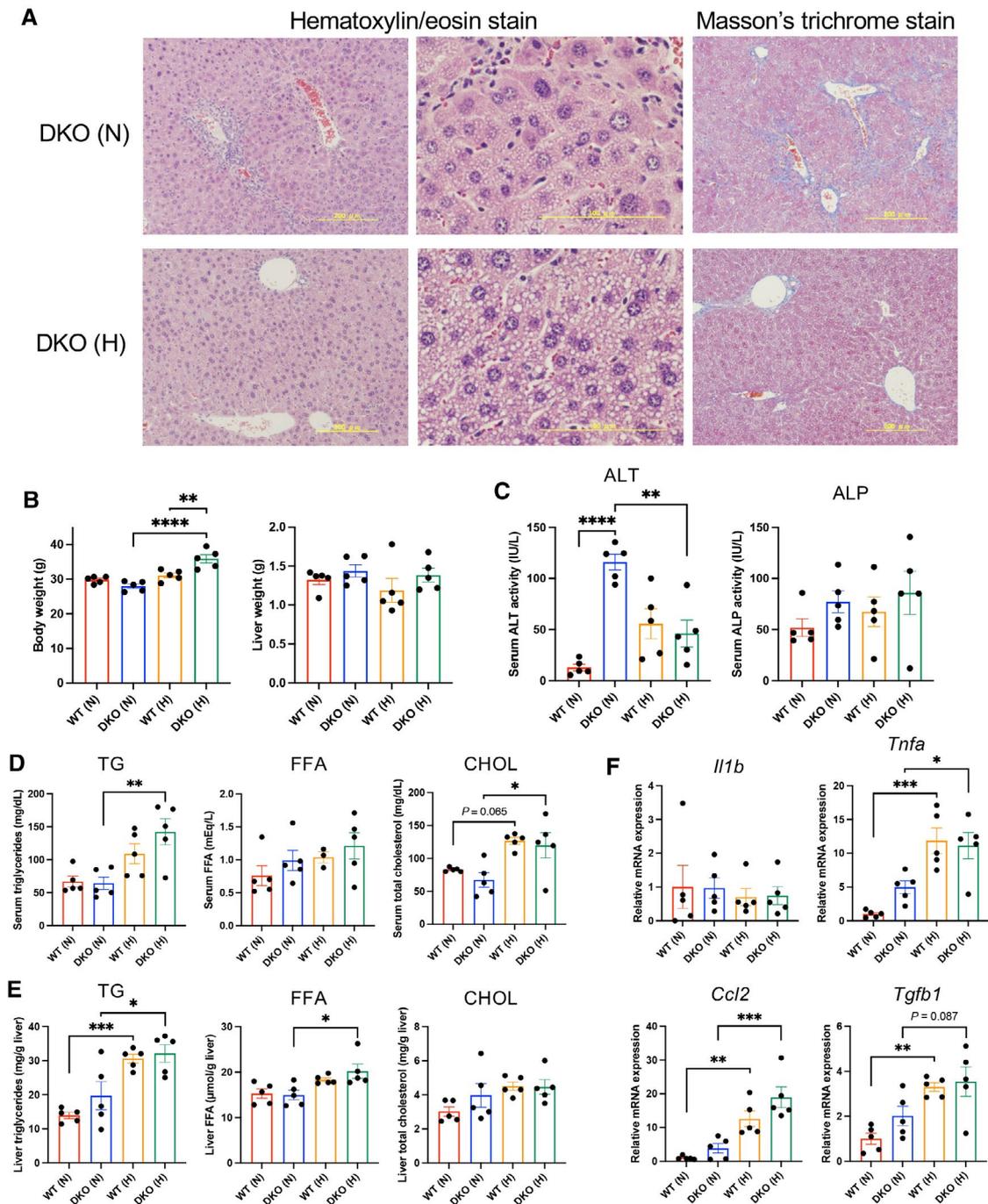


FIG. 7. Effects of HFHSD on liver injury and lipid metabolism in WT and DKO mice treated with different diets. A total of 20 male mice (n = 5 in each group) were analyzed. (A) Representative histopathologic features of the livers from male DKO mice treated with normal chow diet and HFHSD. Hematoxylin and eosin or Masson's trichrome stain. Comparison of body and liver weights (B), serum activities of ALT and ALP (C), serum (D) and liver lipid concentrations (E), and hepatic mRNA expression levels of inflammatory cytokines (F) among groups. Each column and error bar represents the mean and SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001 were considered significantly different by the Tukey–Kramer test. Abbreviations: *Ccl2*, chemokine (C–C motif) ligand 2; CHOL, cholesterol; H, high-fat/high-sucrose diet; N, normal chow diet; TG, triglycerides; *Tgfb1*, transforming growth factor β1.

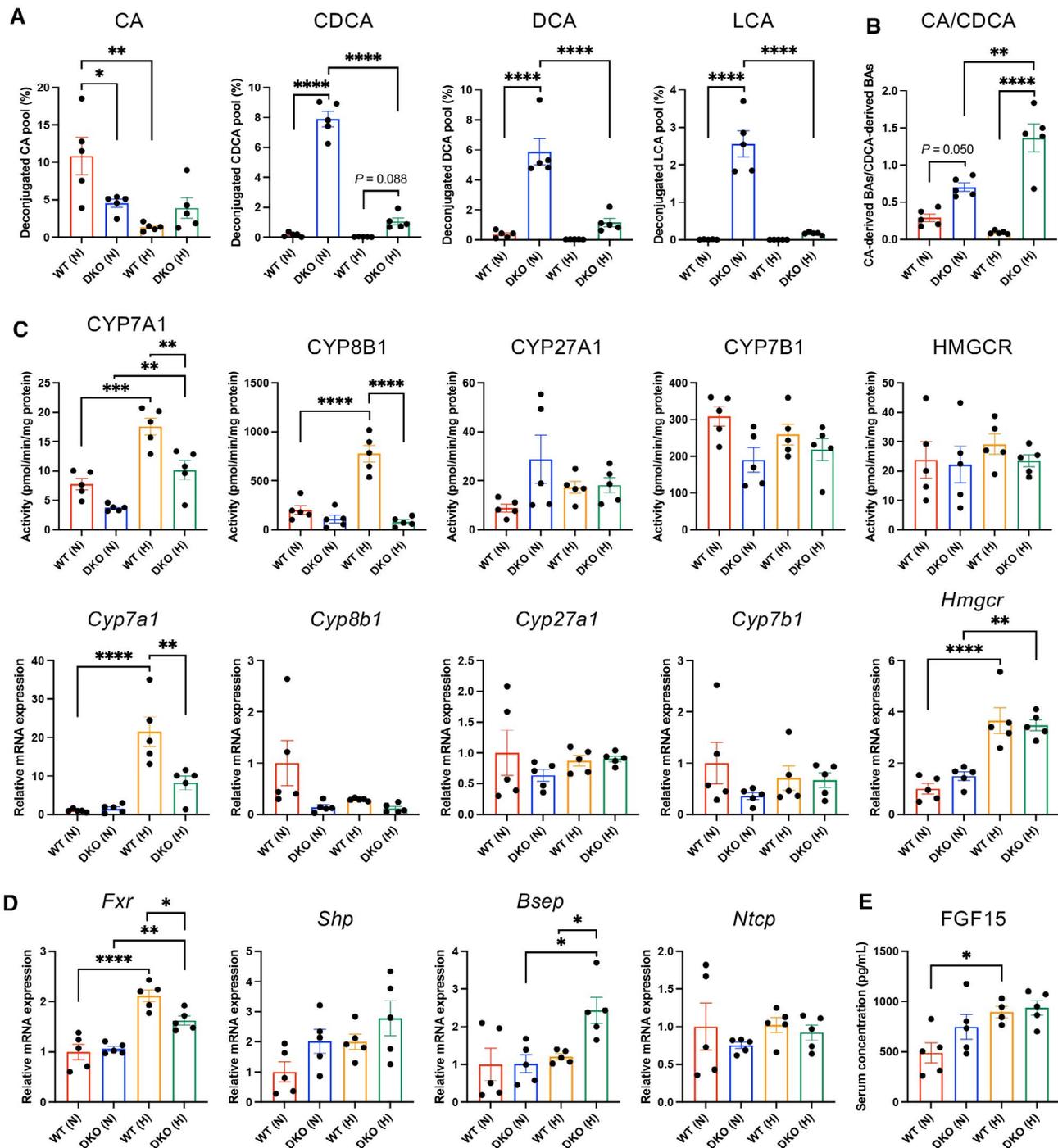


FIG. 8. Effects of HFHSD on BA metabolism in WT and DKO mice treated with different diets. A total of 20 male mice ($n = 5$ in each group) were analyzed. (A) Proportions of deconjugated CA, CDCA, DCA, and LCA in the total BA pool. (B) The ratios of CA-derived BAs (CA and DCA) to CDCA-derived BAs (CDCA, LCA, and MCAs) in total pool (enterohepatic circulation). (C) Hepatic activities and mRNA expression levels of key enzymes in the BA and cholesterol biosynthetic pathways. (D) Hepatic mRNA expression levels of *Fxr* and related genes. (E) Serum concentrations of FGF15. Each column and error bar represents the mean and SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ were considered significantly different by the Tukey–Kramer test. Abbreviations: H, high-fat/high-sucrose diet; N, normal chow diet; and *Ntcp*, sodium/taurocholate cotransporting polypeptide.

but a significant difference was detected only in WT mice (Fig. 8E).

Discussion

BA s are potential bactericidal compounds,^(12,13) and a large amount of BA feeding in rats alters microbial composition in the colon.⁽¹⁵⁾ On the other hand, reduced bile flow due to bile duct ligation causes bacterial overgrowth in the small intestine of rats.⁽²⁶⁾ In addition, bacterial overgrowth in the ileum and cecum is inhibited by the administration of non-steroid FXR agonist in mice with bile duct ligation.⁽²⁷⁾ Therefore, BA s can control gut microbiota through the bactericidal activity and the activation of FXR. However, our data strongly suggest that the effects of BA s on microbial composition in the large intestine are limited under physiological conditions. Under a normal chow diet, BA compositions were completely different between WT and DKO mice, but gut microbial compositions in both types of mice were quite similar. No major changes in the microbial compositions were also reported between WT and *Cyp2c70*^{-/-} mice,⁽²⁸⁾ although some genera were differently abundant.⁽²⁹⁾ The similarity between WT and DKO with a regular diet may be due to the nearly equivalent concentrations of fecal hydrophobic BA s (DCA+CDCA+LCA) between these mice. However, HFHSD did not alter fecal hydrophobic BA concentrations significantly in both mice. Nonetheless, gut microbial compositions changed drastically and similarly in both types of mice.

On the other hand, one might speculate that the increased proportion of fecal CA affected gut microbial composition under HFHSD (Fig. 2A). However, it is more likely that the reduced transformation from CA to DCA due to altered gut microbiota increased CA proportion because the CA/DCA ratios in feces are 0.06 and 0.08 in WT and DKO mice with regular chow, but 1.19 and 0.49 in WT and DKO mice with HFHSD.

The adaptation of gut microbiota due to HFHSD appears to be caused by certain nutrients in the HFHSD rather than BA s. HFD and high-sucrose diet (HSD) play crucial roles in modifying the gut microbial composition.⁽³⁰⁾ Both the HFD and HSD decrease select beneficial bacteria and augment conditional pathogenic bacteria with the reduction of microbial diversity. Regarding the HFD, long-chain

fatty acid-rich HFD decreases the Bacteroidetes/Firmicutes ratio, whereas HFD rich in medium-chain fatty acids, monounsaturated fatty acids, or n-3 polyunsaturated fatty acids increase the Bacteroidetes/Firmicutes ratio.⁽³¹⁾ As for cholesterol, our HFHSD is not laden with cholesterol (Supporting Table S1), and there is a report that a high-cholesterol diet alone does not alter the composition of gut microbiota.⁽³²⁾

Conversely, the alteration of gut microbiota by HFHSD markedly affects the intestinal metabolism of BA s. The taurine or glycine moiety of primary BA s is deconjugated by bile salt hydrolases in various genera of intestinal microbiota including *Clostridium*, *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*.⁽³³⁾ An HFHSD appears to decrease the proportion of deconjugated BA s in both WT and DKO mice (Fig. 5A). In the liver, almost all BA s are conjugated with amino acids, such that bile has only a few percent of deconjugated BA s. In the small intestine, deconjugated BA s are about 20% under a normal chow diet but less than 10% under HFHSD in both types of mice. In feces, most BA s are deconjugated, but the deconjugation rates were low under HFHSD. The hydroxyl group at the C-7 α position of the deconjugated primary BA s is dehydroxylated to form secondary BA s by multistep reactions of specific *Clostridium spp.* such as *C. hiranonis*, *C. hylemonae*, *C. sordelli*, and *C. scindens*.^(11,34) Our results suggest that the synthesis of secondary BA s in the intestine is inhibited under HFHSD (Fig. 5B). An increased proportion of serum primary conjugated BA s is a characteristic feature of patients with nonalcoholic fatty liver disease or NASH.^(35,36) Alterations of gut microbiota have been reported in these diseases,^(37,38) but the mechanism of the dysbiosis has yet to be clarified. Our data suggest that the changes in gut microbiota and BA composition in patients with these diseases are caused in part by Westernized dietary habits.

Dysbiosis due to HFHSD affects not only BA metabolism but also neutral sterol metabolism. Cholesterol is converted to coprostanol by intestinal various genera, such as *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, *Peptostreptococcus*, and *Bacteroides sp.* strain D8.⁽³⁹⁾ In contrast to cholesterol, coprostanol is a nonabsorbable sterol because its uptake and esterification are limited to the intestinal mucosa.⁽⁴⁰⁾ One might expect that efficient conversion of cholesterol to coprostanol reduces cholesterol absorption from the intestine. In fact, an inverse relationship between blood cholesterol concentrations and the fecal

coprostanol/cholesterol ratio was noted in human subjects.⁽⁴¹⁾ However, coprostanol production occurs predominantly in the large intestine,⁽⁴²⁾ whereas cholesterol is mostly absorbed in the small intestine.⁽⁴³⁾ Our data show that HFHSD markedly reduced the fecal proportion of coprostanol regardless of BA composition (Fig. 5C). Therefore, this inverse relationship between blood cholesterol and fecal coprostanol levels may simply reflect gut dysbiosis and hypercholesterolemia due to HFD.

In contrast to BAs and sterols, organic acid concentrations in the cecal contents were not significantly affected by HFHSD (Fig. 5D). However, acetate and propionate concentrations in DKO mice were significantly higher than those in WT mice regardless of dietary intervention. Acetate, propionate, and n-butyrate are also classified as short-chain fatty acids (SCFAs). Because 95% of SCFAs are absorbed from the colon, fecal concentrations of SCFAs appear to reflect the absorption from the colon rather than production.⁽⁴⁴⁾ We used cecal contents instead of feces to measure organic acids because the cecal concentrations appear to reflect their production rates more than disappearance rates. Among the three major SCFAs, acetate is produced by many groups of bacteria, whereas propionate and n-butyrate are synthesized by specific bacteria.⁽⁴⁵⁾ One of the genera that synthesize both acetate and propionate is *Bacteroides*.⁽⁴⁵⁾ Actually, only the proportion of *Bacteroides* was positively correlated with the concentrations of acetate and propionate (Fig. 6). Thus, although microbial compositions were quite similar between WT and DKO mice, some specific genera were affected by the difference in BA composition (Supporting Table S5).

DKO mice exhibit liver injury probably due to lack of hydrophilic and hepatoprotective MCAs and markedly increased hydrophobic BAs in the pool, as in humans. Surprisingly, liver injury of DKO mice was improved after feeding HFHSD for 4 weeks, although hepatic concentrations of triglycerides and FFA were increased. We found that even mild steatosis caused significant expressions of inflammatory cytokines (Fig. 7F). However, liver injury assessed by serum ALT activity and histologic findings was calm in mice fed with HFHSD. Thus, neither hepatic lipids nor liver inflammatory cytokines can explain the amelioration of liver injury in HFHSD fed DKO mice. In addition, we previously measured serum concentrations of lipopolysaccharides (LPS) and TNF α

in normal chow-fed WT and DKO mice.⁽¹⁷⁾ They were not increased in DKO mice, suggesting that liver injury in DKO mice was not the consequence of LPS or pro-inflammatory cytokines derived from the gut.

The toxicity of LCA can be reduced by sulfotransferase 2A1 (SULT2A1) and CYP3A11 in mice. SULT2A1 converts LCA into LCA-3-sulfate, while CYP3A11 metabolizes LCA into hyodeoxycholic acid or murideoxycholic acid. However, male mice do not express *Sult2a1* much,⁽¹⁷⁾ and hepatic *Cyp3a11* expression and its serum surrogate marker, 4 β -hydroxycholesterol, were not up-regulated by HFHSD (Supporting Fig. S2C and S3B). Therefore, SULT2A1 and CYP3A11 do not contribute to the improvement of liver injury in DKO mice fed with HFHSD.

Deconjugated hydrophobic BAs are more hepatotoxic than conjugated ones.⁽¹⁷⁾ The analysis of BA composition revealed that deconjugated CDCA, DCA, and LCA in enterohepatic circulation markedly reduced after feeding HFHSD (Fig. 8A). It suggests that the change of gut microbiota by HFHSD inhibited deconjugation followed by 7 α -dehydroxylation of these BAs, especially in the small intestine, and ultimately improved liver injury. Increased CA/CDCA ratio may also contribute to reducing deconjugated CDCA and LCA in the pool. One might think that CYP8B1 is a major determinant of the CA/CDCA ratio. However, we observed a conflicting relationship between CA/CDCA ratios and CYP8B1 activities in our mice models, suggesting that CA/CDCA ratio regulates CYP8B1 rather than CYP8B1 determines CA/CDCA ratio.

It is known that liver injury inhibits FXR activity by posttranslational modifications.^(46–48) In DKO mice with HFHSD, hepatic expressions of *Fxr*, *Shp*, and *Bsep* and the serum concentrations of FGF15 were marginally elevated compared to those with a normal diet (Fig. 8D,E). Although *Cyp7a1* was moderately up-regulated in the mice, it may be due to the activation of LXR α by HFHSD (Supporting Fig. S2B). Thus, improvement of liver injury by HFHSD may restore feedback regulation of BA metabolism through FXR and inhibit the enlargement of the BA pool in DKO mice.

Because HFHSD contributes to the progression of various lifestyle diseases, we speculate that the gut microbiota formed under HFHSD is less desirable

for host health.⁽⁴⁹⁾ However, our results demonstrated that the alteration of gut microbiota by HFHSD could have some beneficial effects on specific liver injury by reducing deconjugation followed by 7 α -dehydroxylation of BAs. Our study suggests that deconjugation of BAs is associated with the increased proportion of *f_S24-7*; *g_Unclassified*, *Ruminococcus*, *Lactobacillus*, and *Turicibacter* (Fig. 6). Therefore, the methods for reducing these genera except for HFHSD may be worthy of consideration.

In summary, there are multiple interactions among diet, gut microbiota, and BAs. Our data revealed that the difference in BA profiles after normal chow diet and HFHSD was much less between WT and DKO mice. Nonetheless, the difference of gut microbiota between normal chow diet and HFHSD was much greater between WT and DKO mice. Therefore, our data suggest that the change of gut microbiota due to HFHSD is not mediated by the alteration of the BA profile but is caused by HFHSD itself. In addition, the HFHSD-induced change of gut microbiota ameliorated chronic liver injury in DKO mice by inhibiting intestinal BA deconjugation and 7 α -dehydroxylation. In conclusion, BA is not a major determinant of gut microbial composition under physiological conditions, such as a Western-style HFHSD. Furthermore, the gut microbiota formed by HFHSD improved liver injury in DKO mice by altering BA composition.

Acknowledgment: The authors thank Hideto Takahashi, Raku Kato, Sachiko Murayama, and Takahiro Machiura (Charles River Laboratories Japan, Inc.) for the breeding and care of the mice.

REFERENCES

- Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008;3:213-223
- Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen Y, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009;137:1716-1724.
- Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009;1:6-14.
- David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559-563.
- Zhang M, Yang XJ. Effects of a high fat diet on intestinal microbiota and gastrointestinal diseases. *World J Gastroenterol* 2016;22:8905-8909.
- Yamada S, Kamada N, Amiya T, Nakamoto N, Nakaoka T, Kimura M, et al. Gut microbiota-mediated generation of saturated fatty acids elicits inflammation in the liver in murine high-fat diet-induced steatohepatitis. *BMC Gastroenterol* 2017;17:136.
- Knight-Sepulveda K, Kais S, Santaolalla R, Abreu MT. Diet and inflammatory bowel disease. *Gastroenterol Hepatol* 2015;11:511-520.
- Zeng H, Umar S, Rust B, Lazarova D, Bordonaro M. Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer. *Int J Mol Sci* 2019;20:1214.
- Gaines S, van Praagh JB, Williamson AJ, Jacobson RA, Hyoju S, Zaborin A, et al. Western diet promotes intestinal colonization by collagenolytic microbes and promotes tumor formation after colorectal surgery. *Gastroenterology* 2020;158:958-970.
- Zheng X, Huang F, Zhao A, Lei S, Zhang Y, Xie G, et al. Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice. *BMC Biol* 2017;15:120.
- Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 2006;47:241-259.
- Floch MH, Binder HJ, Filburn B, Gershengoren W. The effect of bile acids on intestinal microflora. *Am J Clin Nutr* 1972;25:1418-1426.
- Watanabe M, Fukiya S, Yokota A. Comprehensive evaluation of the bactericidal activities of free bile acids in the large intestine of humans and rodents. *J Lipid Res* 2017;58:1143-1152.
- Reddy BS. Diet and excretion of bile acids. *Cancer Res* 1981;41:3766-3768.
- Islam KBMS, Fukiya S, Hagi M, Fujii N, Ishizuka S, Ooka T, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* 2011;141:1773-1781.
- Misawa N, Higurashi T, Takatsu T, Iwaki M, Kobayashi T, Yoshihara T, et al. The benefit of elobixibat in chronic constipation is associated with faecal deoxycholic acid but not effects of altered microbiota. *Aliment Pharmacol Ther* 2020;52:821-828.
- Honda A, Miyazaki T, Iwamoto J, Hirayama T, Morishita Y, Monma T, et al. Regulation of bile acid metabolism in mouse models with hydrophobic bile acid composition. *J Lipid Res* 2020;61:54-69.
- Honda A, Yamashita K, Miyazaki H, Shirai M, Ikegami T, Xu G, et al. Highly sensitive analysis of sterol profiles in human serum by LC-ESI-MS/MS. *J Lipid Res* 2008;49:2063-2073.
- Blish EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911-917.
- Nagatomo R, Okada Y, Ichimura M, Tsuneyama K, Inoue K. Application of 2-picolyamine derivatized ultra-high performance liquid chromatography tandem mass spectrometry for the determination of short-chain fatty acids in feces samples. *Anal Sci* 2018;34:1031-1036.
- Yanagi H, Tsuda A, Matsushima M, Takahashi S, Ozawa G, Koga Y, et al. Changes in the gut microbiota composition and the plasma ghrelin level in patients with *Helicobacter pylori*-infected patients with eradication therapy. *BMJ Open Gastroenterol* 2017;4:e000182.
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006;72:5069-5072.
- do Nascimento PGG, Lemos TLG, Almeida MCS, de Souza JMO, Bizerra AMC, Santiago GMP, et al. Lithocholic acid and derivatives: antibacterial activity. *Steroids* 2015;104:8-15.
- Tian Y, Gui W, Koo I, Smith PB, Allman EL, Nichols RG, et al. The microbiome modulating activity of bile acids. *Gut Microbes* 2020;11:979-996.

- 25) Davis SC, Yadav JS, Barrow SD, Robertson BK. Gut microbiome diversity influenced more by the Westernized dietary regime than the body mass index as assessed using effect size statistic. *Microbiologyopen* 2017;6:e476.
- 26) Ding JW, Andersson R, Soltész V, Willén R, Bengmark S. The role of bile and bile acids in bacterial translocation in obstructive jaundice in rats. *Eur Surg Res* 1993;25:11-19.
- 27) Inagaki T, Moschetta A, Lee Y-K, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci USA* 2006;103:3920-3925.
- 28) de Boer JF, Verkade E, Mulder NL, de Vries HD, Huijkman N, Koehorst M, et al. A human-like bile acid pool induced by deletion of hepatic *Cyp2c70* modulates effects of FXR activation in mice. *J Lipid Res* 2020;61:291-305.
- 29) de Boer JF, de Vries HD, Palmiotti A, Li R, Doestzada M, Hoogerland JA, et al. Cholangiopathy and biliary fibrosis in *Cyp2c70*-deficient mice are fully reversed by ursodeoxycholic acid. *Cell Mol Gastroenterol Hepatol* 2021;11:1045-1069.
- 30) Kong C, Gao R, Yan X, Huang L, Qin H. Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition* 2019;60:175-184.
- 31) Machate DJ, Figueiredo PS, Marcelino G, Guimarães RdCA, Hiane PA, Bogo D, et al. Fatty acid diets: regulation of gut microbiota composition and obesity and its related metabolic dysbiosis. *Int J Mol Sci* 2020;21:4093.
- 32) Dimova LG, Zlatkov N, Verkade HJ, Uhlin BE, Tietge UJF. High-cholesterol diet does not alter gut microbiota composition in mice. *Nutr Metab* 2017;14:15.
- 33) Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. *FEMS Microbiol Rev* 2005;29:625-651.
- 34) Winston JA, Theriot CM. Diversification of host bile acids by members of the gut microbiota. *Gut Microbes* 2020;11:158-171.
- 35) Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. *Hepatology* 2018;67:534-548.
- 36) Yara S-I, Ikegami T, Miyazaki T, Murakami M, Iwamoto J, Hirayama T, et al. Circulating bile acid profiles in Japanese patients with NASH. *GastroHep* 2019;1:302-310.
- 37) Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013;11:868-875.
- 38) Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764-775.
- 39) Kriaa A, Bourgin M, Potiron A, Mkaouer H, Jablaoui A, Gérard P, et al. Microbial impact on cholesterol and bile acid metabolism: current status and future prospects. *J Lipid Res* 2019;60:323-332.
- 40) Bhattacharyya AK. Differences in uptake and esterification of saturated analogues of cholesterol by rat small intestine. *Am J Physiol* 1986;251:G495-G500.
- 41) Sekimoto H, Shimada O, Makanishi M, Nakano T, Katayama O. Interrelationship between serum and fecal sterols. *Jpn J Med* 1983;22:14-20.
- 42) Kellogg TF. On the site of the microbiological reduction of cholesterol to coprostanol in the rat. *Lipids* 1973;8:658-659.
- 43) Swell L, Trout EC Jr, Hopper JR, Field H Jr, Treadwell CR. Mechanism of cholesterol absorption. II. Changes in free and esterified cholesterol pools of mucosa after feeding cholesterol-4-C14. *J Biol Chem* 1958;233:49-53.
- 44) Sakata T. Pitfalls in short-chain fatty acid research: a methodological review. *Anim Sci J* 2019;90:3-13.
- 45) Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* 2015;74:13-22.
- 46) Kemper JK. Regulation of FXR transcriptional activity in health and disease: emerging roles of FXR cofactors and post-translational modifications. *Biochim Biophys Acta* 2011;1812:842-850.
- 47) Balasubramaniyan N, Luo Y, Sun AQ, Suchy FJ. SUMOylation of the farnesoid X receptor (FXR) regulates the expression of FXR target genes. *J Biol Chem* 2013;288:13850-13862.
- 48) Byun S, Jung H, Chen J, Kim Y-C, Kim D-H, Kong BO, et al. Phosphorylation of hepatic farnesoid X receptor by FGF19 signaling-activated Src maintains cholesterol levels and protects from atherosclerosis. *J Biol Chem* 2019;294:8732-8744.
- 49) Murphy EA, Velazquez KT, Herbert KM. Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care* 2015;18:515-520.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1778/suppinfo.