DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Effect of Gender and Various Diets on Bile Acid Profile and Related Genes in Mice

Chong Ma[1,2], Ying Guo[1,2,3*], and Curtis D. Klaassen[3*]

[1] *Department of Clinical Pharmacology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha 410008, P. R. China; Institute of Clinical Pharmacology, Central South University, Hunan Key Laboratory of Pharmacogenetics, 110 Xiangya Road, Changsha 410078, P. R. China; Engineering Research Center of Applied Technology of Pharmacogenomics, Ministry of Education, 110 Xiangya Road, Changsha 410078, P. R. China*

[2] *National Clinical Research Center for Geriatric Disorders , Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China, 410008*

[3] *Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160, USA*

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Effect of Gender and Various Diets on Bile Acid Homeostasis

Address correspondence to: Ying Guo: Department of Clinical Pharmacology, Xiangya Hospital, Institute of Clinical Pharmacology, Central South University, Hunan Key Laboratory of Pharmacogenetics, 110 Xiangya Road, Changsha 410078, P. R. China; Engineering Research Center of Applied Technology of Pharmacogenomics, Ministry of Education, 110 Xiangya Road, Changsha 410078, P. R. China. Tel: +86-731-84805380, E-mail: guoying881212@csu.edu.cn; Curtis D. Klaassen: University of Kansas Medical Center, Kansas City, KS 66160, USA; 2617 W 112th Street, Leawood KS 66211, E-mail: curtisklaassenphd@gmail.com

The number of text pages: 20;

The number of tables: 1;

The number of figures: 5;

The number of references: 63;

The number of words in the Abstract: 246;

The number of words in the Introduction: 754;

The number of words in the Discussion: 1557.

ABBREVIATIONS: 12α-OH, 12α-hydroxylated; ABCA1, ATP binding cassette subfamily A member 1; ABCG5/8, ATP-binding cassette subfamily G members 5/8; ASBT, Apical sodium-dependent bile salt transporter; ATP8B1, P-type ATPase, class 1, type 8B, member 1; BA, Bile acid; BAAT, Bile acid-CoA: amino acid *N*-acyltransferase; BACS, Bile acyl-CoA synthetase; BCRP, Breast cancer resistance

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

protein; bDNA, Multiplexed Branched-DNA; BSEP, Bile salt export pump; CDCA, Chenodeoxycholic acid; CA, Cholic acid; CAR, Constitutive androstane receptor; CYP, Cytochrome p450; DCA, Deoxycholic acid; EFA, Essential fatty acid; FA, Fatty acid; FGF15, Fibroblast growth factor 15; FGFR4, Fibroblast growth factor receptor 4; FXR, Farnesoid x nuclear receptor; H-Cpr, Hepatocyte-specific deletion of NADPH cytochrome P450 reductase; HDCA, Hyodeoxycholic acid, LCA, Lithocholic acid; LRH, Liver receptor homolog; LXR, Liver x receptor; MDR, Multidrug resistance protein; MRP, Multidrug resistance-associated protein; MCA, Muricholic acid; MDCA, Murideoxycholic acid, NTCP, Sodium/taurocholate co-transporting polypeptide, OATP, Organic anion transporter polypeptide, OST, Organic solute transporter; PFNA, Perfluorononanoic acid; PPAR, Peroxisome proliferator-activated receptor; SHP, Small heterodimer partner; STAR, Steroidogenic acute regulatory protein; T, taurine; UDCA, Ursodeoxycholic acid; UHPLC-MS/MS, Ultra high performance liquid chromatography/ tandem mass spectrometry.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Diet is an important factor for many diseases. Previous studies have demonstrated that several diets had remarkable effects on bile acid (BA) homeostasis but no comprehensive information for both genders has been reported. Therefore, the current study characterized the 9 most used laboratory animal diets fed to both genders of mice for a comparable evaluation of the topic. The results revealed that marked gender difference of BA homeostasis is ubiquitous in mice fed the various diets, and of the 9 diets fed to mice, the atherogenic and calorie-restricted diets had the most marked effects on BA homeostasis, followed by the lab chow and EFA-deficient diets. More specifically, females had higher concentrations of total BAs in serum when fed 6 of the 9 diets compared to male mice, and 26 of the 35 BA-related genes had marked gender difference in mice fed at least one diet. Although mice fed the calorie-restricted and atherogenic diets had increased BAs, which was more pronounced in serum than liver, the intestinal *Fxr-Fgf15* axis changed in the opposite direction, and resulted in different hepatic expression patterns of *Cyp7a1*. Compared with AIN-93M purified diet, higher hepatic expression of *Mrp3* was the only alteration in mice fed the lab chow diet. The other diets had little or no effect on BA concentrations in the liver and plasma, nor the expression of BA related genes. This study indicates that gender, the atherogenic diet, and the calorie-restricted diet have the most marked effects on BA homeostasis.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Significance Statement.

Previous evidence suggested that various diets have effect on BA homeostasis; however, it is not possible to directly compare these findings as they are all from different studies. The current study was the first to systematically investigate the influence of the 9 most used experimental mouse diets on BA homeostasis and potential mechanism in both genders of mice, and indicates that gender, the atherogenic, and calorie-restricted diets have the most marked effects on BA homeostasis, which will aid future investigations.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Diets have important effects on organisms. Diets high in saturated fat and glucose, or with high ratios of n-6:n-3 polyunsaturated fatty acids, can cause disorders in body lipid, glucose, and energy metabolism, which may contribute to the development of metabolic syndrome (Berr et al., 1993; Vamecq et al., 1993; Bae et al., 2013; Luo and Yang, 2016; Luo et al., 2016; Bertaggia et al., 2017; Jiang et al., 2019; Liao et al., 2019). In contrast, a calorie-restricted diet fed to mice improves the metabolic syndrome by altering the metabolism of bile acids (BAs) (De Guzman et al., 2013; Kok et al., 2018). BAs are important signaling molecules and metabolic regulators that can alter glucose and lipid homeostasis as well as insulin resistance in humans and mice (Makishima et al., 1999; Trauner et al., 2010; Tsuchida et al., 2012; Guo et al., 2016; Liu et al., 2018; Wang et al., 2019). Therefore, it is important to explore the effects of various diets on endogenous active substances such as BAs.

The primary BA chenodeoxycholic acid (CDCA) is synthesized from cholesterol in hepatocytes via two pathways: the classical pathway that utilizes cytochrome P450 7a1 (CYP7A1) and the alternative pathway that involves CYP7B1 (Chen et al., 2019) (see Figure 1). CDCA is then converted to cholic acid (CA) via sterol 12α-hydroxylase (CYP8B1) or 6β-hydroxylated and epimerized to α-muricholic acid (αMCA) and β-muricholic acid (βMCA) in rodents by CYP2C70 (Takahashi et al., 2016; Donepudi et al., 2017). These primary BAs are conjugated predominantly with taurine in rodents and with glycine and taurine in humans by bile acyl-CoA synthetase (BACS) and bile acid-CoA: amino acid *N*-acyltransferase (BAAT) and transferred to

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

the intestine primarily via the bile salt export pump (BSEP /ABCB11) (Xie et al., 2016; Droge et al., 2017).

Primary BAs are converted into secondary BAs by intestinal microbial enzymes with the oxidation and epimerization of the hydroxyl groups at C3, C7 and C12. Almost 95% of the BAs are reabsorbed from the intestine with the help of the apical sodium-dependent bile acid transporter (ASBT) and organic solute transporter α/β (OSTα/β). The portal vein transfers BAs to the liver where they are taken up by the sodium/taurocholate co-transporting polypeptide (NTCP) and the organic anion-transporting polypeptide 1b2 (OATP1B2). While most of the BAs are excreted into bile by BSEP, a small amount of BAs is secreted by the liver back into the systemic circulation via OSTα/β and multidrug resistance-associated proteins 3 and 4 (MRP3 and MRP4).

BAs regulate their own homeostasis and perform other functions by binding to receptors in the intestine and liver. Farnesoid X nuclear receptor (FXR) is a ligand-activated transcription factor belonging to the nuclear receptor superfamily and is essential for regulating BA and lipid homeostasis. Activation of intestinal FXR suppresses the expression of CYP7A1 and CYP8B1, which inhibit BA synthesis in the liver (Goodwin et al., 2000; Holt et al., 2003). The primary BA, CDCA, activates FXR expression that can alter obesity and reverse insulin resistance caused by a high-fat diet (Thomas et al., 2008; Shihabudeen et al., 2015). The BA metabolizing pathway is shown in Figure 1.

Scattered reports have demonstrated that diet had an effect on BA homeostasis in

mice fed high-fat, western, atherogenic, lab chow, AIN-93M purified and calorie-restricted diets (Wanon et al., 1998; Phan et al., 2002; Fu and Klaassen, 2013; Dermadi et al., 2017; Ichimura et al., 2017), but some of them focused on the serum and/or liver BA profile or were only reported to influence total BAs, and it is not possible to directly compare these findings as they are all from different studies. Additionally, although gender differences in BA profiles have been reported in mice fed the normal laboratory, high-fat, and western diets, the results are inconsistent. For example, one study found that female mice had a higher hepatic concentration of BAs than males when fed a laboratory diet (Fu et al., 2012); however, another study found the opposite effect in mice fed the high-fat and western diets (Jena et al., 2017; Xie et al., 2017). Therefore, gender diversity of BAs may be altered by diets, however, very limited comparable information has been reported on this topic. Thus, in the current study, 9 frequently used experimental diets were given to male and female mice simultaneously for 3 weeks in order to assess early diet-induced changes on the concentration of 20 individual BAs in livers and serum, and the relative mRNA expression of major BA-related genes in livers and ileum, which will provide systematic evidence for the further investigation of dietary effects.

Materials and Methods

Ethics Statement. The animal housing facility at the University of Kansas Medical Center is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the University of Kansas Medical Center's Institutional Animal Care and Use Committee.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Animals and treatments. Seven-week-old male and female C57BL/6 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA), housed according to the American Animal Association Laboratory Animal Care guidance under a standard 12-hour dark-light cycle and humidity-controlled environment with a room temperature at approximately 25°C, and acclimated for at least 1 week before treatment. Mice (n = 5 per diet, gender = male & female) were divided into 9 groups and fed one of the following diets, which were all purchased from Harlan Laboratories (Madison, WI). The diets included AIN-93M purified diet (TD.94048), high fructose diet (TD.89247), high-fat diet (TD.97070), western diet (TD.88137), EFA-deficient diet (TD.8422), low n-3 FA diet (TD.00235 + 7% sunflower oil), lab chow (TD.8604, natural ingredient diet), diet restriction (75% of the diet TD.8604 consumed by ad lib feeding) and atherogenic diet (a modification of the western diet plus 1.25% cholesterol and 0.5% cholic acid) for 3 weeks, which was described in detail previously (Renaud et al., 2014) . Mice were euthanized in the morning (8:00-10:00 A.M.), and their blood and tissues were sampled as described in our previous study (Zhang and Klaassen, 2010).

BA extraction and quantification. Sample extraction and quantification of individual BAs by UHPLC-MS/MS were performed according to methods described previously (Alnouti et al., 2008; Zhang and Klaassen, 2010). In this study, a total number of 20 BAs in serum and liver were detected by UHPLC-MS/MS simultaneously. The standards included tauromurideoxycholic acid (TMDCA) (were kind gifts from Dr. Alan F. Hofmann (University of California, San Diego).), CA,

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

CDCA, deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), TCDCA, TDCA, TUDCA, TLCA, (were purchased from Sigma-Aldrich (St. Louis, MO)), hyodeoxycholic acid (HDCA), murideoxycholic acid (MDCA), α, β, ωMCA, TCA, THDCA, Tα, βMCA (were purchased from Steraloids, Inc. (Newport, RI)), and TωMCA (were synthesized according to previous methods (Zhang et al., 2012c)) were diluted with 50% methanol and spiked with internal standards ^{2}H4-CDCA to construct standard curves between 5 and 20,000 ng/ml. All standard curves were constructed using a 1/concentration^{2} weighted quadratic regression, and the correlation coefficient (r^{2}) for all BAs was above 0.99 (Table S1). The limit of detection (signal/noise ratio=3) for the various BAs was in the range of 5-10 ng/ml, which equals 0.01-0.02 nmol/ml.

RNA extraction. RNA from liver and ileum was extracted using RNA-Bee reagent (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's protocol. RNA concentrations were quantified using a NanoDrop1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at a wavelength of 260 nm. RNA integrity was confirmed by agarose gel electrophoresis and ethidium bromide staining of 5 μg of total RNA to visualize intact 18S and 28S bands.

Multiplexed Branched-DNA (bDNA) Assay. The multiplexed bDNA technique is a robust, high-throughput method used for characterizing mRNA expression was used. Relative expression of genes in the livers of mice fed the various diets was quantified using the bDNA assay (QuantiGene high volume branched DNA signal amplification kit; Panomics/Affymetrix), according to a previously published method (Cheng et al.,

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

2005). The gene sequences were accessed from GenBank.

Quantification of *Fxr, Ostα, Ostβ, Asbt, Fgf15* mRNA expression by RT-qPCR assay. The mRNA expression of *Fxr, Ostα, Ostβ, Asbt*, and *Fgf15* in the ileum of mice were quantified by real-time PCR assay as described previously (Renaud et al., 2014). Reverse transcription of RNA to cDNA was performed with the Applied Biosystems High Capacity Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). All primers were synthesized by Integrated DNA Technologies (Coralville, IA).

Statistical analysis. Data are expressed as mean ± S.D. (n= 4-5). Statistical analyses were performed with an IBM-SPSS 21.0 computer program (IBM, Armonk, NY) and SIMCA (v14, Umetrics, UmeÅ, Sweden). Two-way analysis of variance (ANOVA) followed by Duncan's post hoc test was used to analyze the differences between male and female mice fed the various diets. Principal component analysis (PCA) of the BAs and relative mRNA level of various diet treatments was determined by SIMCA. Statistical significance was set at $P<0.05$ for all analyses. AIN-93M purified diet is referred to as the control.

Results

PCA profile of BAs and major BA-related genes in livers of mice fed the 9 diets. In order to visualize patterns in the data and highlight similarities and differences between diets, PCA of the liver BAs and relative mRNA level of mice fed the various diets in both genders are shown in Figure 2. Liver BAs and related mRNA profiles were substantially altered by the atherogenic and restricted diet compared to the AIN-93M purified diet (control) and the other diets. The profile of serum BAs had no

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

significant change within mice fed the different diets in both genders (data not show).

Concentrations of BAs in serum of mice fed the 9 diets.

Gender. Ten T-conjugated and 10 unconjugated BAs in serum were quantified in the current study, while only 17 BAs were detected (Supplemental Table S2). As shown in Figure 3A, gender differences in total BAs, total T-conjugated BAs, and total unconjugated BAs in serum were observed in mice fed the various diets. The concentration of total BAs in the serum were higher in female than male mice fed 6 of the 9 diets: the AIN-93M purified diet, the lab chow diet, the high fructose diet, the high-fat diet, the western diet, and the low n-3 FA diet. Total T-conjugated BAs were also higher in the serum of female than male mice fed 6 of the 9 diets: the AIN-93M purified diet, the lab chow diet, the high fructose diet, the high-fat diet, the low n-3 FA diet, and the EFA-deficient diet. Total unconjugated BAs were higher in the serum of female than male mice fed 4 of the 9 diets: the AIN-93M purified diet, the high fructose diet, the western diet, and the low n-3 FA diet.

As depicted in Figure 3B, of the 17 individual BAs in the serum of mice fed the various diets in this study, 14 BAs show a significant gender difference in at least one diet. The concentrations of 14 BAs were higher in female than male mice fed the various diets, except that TCDCA was higher in male than female mice fed the atherogenic diet.

Female mice fed the AIN-93M purified diet had higher serum concentrations of primary BAs αMCA, βMCA, UDCA, TCA, TCDCA, TαMCA, TβMCA, and TUDCA as well as the secondary BAs DCA, HDCA, TDCA, TωMCA, THDCA, and TMDCA

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

than male mice. Female mice fed the high-fat diet had higher serum concentrations of BAs including αMCA, βMCA, UDCA, TCA, TCDCA, TαMCA, TβMCA, TUDCA, DCA, HDCA, TDCA, TωMCA, THDCA, and TMDCA. Female mice fed the restricted diet had higher serum concentrations of TUDCA than male mice, while female mice fed the atherogenic diet also had higher concentrations of αMCA, βMCA, ωMCA, and HDCA.

Diets. Some of the diets also affected BAs in serum. In male mice, the total BA concentrations in serum were increased in female mice by the atherogenic diet and the restricted diet, whereas the EFA-deficient diet decreased it (Figure 3A). Meanwhile, total T-conjugated BAs were similarly increased in male mice fed the restricted diet and the atherogenic diet, and was decreased in female mice by the EFA-deficient diet. However, total unconjugated BA concentrations in the serum of female mice did not change significantly with any diet, whereas in male mice, they were increased by the atherogenic diet.

Figure 3B indicates that 16 of the 17 individual BAs in serum of mice were affected by at least one diet. Moreover, female mice had higher serum BAs than male mice fed the various diets compared with the AIN-93M purified diet. Specifically, the restricted diet significantly increased the serum concentration of primary BAs in male mice: CA, TCA, UDCA, αMCA, TαMCA, and TβMCA; as well as the secondary BAs DCA, TDCA, TMDCA, HDCA, THDCA, ωMCA, and TωMCA. While in female mice, the atherogenic diet significantly decreased the concentrations of TCDCA, TαMCA, TβMCA, TUDCA, TωMCA, TMDCA, and THDCA, and, in male

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

mice, increased the serum concentrations of CA, TCA, TαMCA, DCA, and TDCA and decreased the concentration of TUDCA.

The diets, other than the calorie-restricted diet and the atherogenic diet, also decreased BAs in the serum of female mice (Figure 3B). Specifically, the EFA-deficient diet decreased serum concentrations of TαMCA, TUDCA, TCA, TCDCA, and TDCA; the lab chow diet decreased βMCA, TUDCA, DCA, and TDCA; the high fructose diet decreased TωMCA, DCA, and TDCA; the western diet decreased TCA and THDCA; the high-fat diet and the low n-3 FA diet decreased the concentrations of DCA and TCA. In contrast to female mice, there were only two diets that had a significant influence on the concentrations of BAs in the serum of male mice. The lab chow diet increased the serum concentration of TωMCA, and the EFA-deficient diet decreased TDCA.

Concentrations of BAs in livers of mice fed the 9 diets

Gender. Ten T-conjugated BAs and 10 unconjugated BAs were detected in livers (Supplemental Table S3). As shown in Figure 4A, only a few gender differences in total BAs, total T-conjugated BAs, and total unconjugated BAs in livers were observed in mice fed the various diets. Specifically, a gender difference was detected in mice fed the high-fat diet in which the total BAs in the livers of females was higher than in males, and the T-conjugated BAs were higher in the livers of females than males. In contrast, unconjugated BAs were higher in livers of male than in female mice fed the low n-3 FA diet and the EFA-deficient diet.

As is shown in Figure 4B, of the 20 individual BAs in livers of male and female

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

mice that were investigated in this study, 17 BAs show a significant gender difference after one or more of the diets. Specifically, female mice fed the AIN-93M purified diet had higher liver concentrations of the primary BAs CA, CDCA, UDCA, and TCA, as well as the secondary BAs DCA, MDCA, and TDCA than male mice, but the concentration of secondary BA TωMCA was higher in the livers of male mice. Male mice which were fed with the high fructose diet had a higher liver concentration of TωMCA compared to female mice, but the concentration of CA was higher in the livers of female mice. However, mice fed the atherogenic diet, which produced a unique pattern of BAs in the livers compared to the other diets in both genders, did not show a significant gender difference of any BA.

Diets. Mice fed the various diets were compared to those fed the AIN-93M purified diet, to determine the effect of the various diets on BA homeostasis. As is shown in Figure 4A, total BAs and unconjugated BA concentrations in livers remained relatively constant in male mice fed the various diets, whereas in female mice, total BAs significantly decreased when fed the lab chow diet, and the unconjugated BAs were decreased by the calorie-restricted diet, the low n-3 FA diet, and the EFA-deficient diet. T-conjugated BAs did not change significantly in the livers of either gender of mice fed the 9 diets.

Also shown in Figure 4B, 19 of the 20 individual BAs in livers had gender differences in their response to at least one diet. Specifically, male mice fed the atherogenic diet had increased liver primary BAs CA, and TCA, but decreased βMCA, TβMCA, and TUDCA. In the liver of male mice, the atherogenic diet increased

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

secondary BAs DCA, and TDCA, but decreased ωMCA, TωMCA, TMDCA, and THDCA. In comparison to the AIN-93M purified diet, female mice fed the atherogenic diet had decreased hepatic primary BAs CDCA, TCDCA, TαMCA, βMCA, TβMCA, UDCA, and TUDCA, and the secondary BAs ωMCA, TωMCA, MDCA, TMDCA, and THDCA.

The concentration of BAs in the livers of male and female mice were also affected by the diets (Figure 4B). Compared to the AIN-93M purified diet, the calorie-restricted diet increased liver concentrations of BAs in male mice including CA, TCA, DCA, and TDCA, the western diet increased CDCA, and the high-fat diet decreased TLCA, TωMCA, and THDCA. Meanwhile, in female mice, the calorie-restricted diet increased liver concentrations of TωMCA, but decreased CDCA, αMCA, βMCA, ωMCA, and MDCA. The high-fat diet decreased the liver concentrations of BAs in female mice including DCA, ωMCA, MDCA, THDCA, and TDCA; the low n-3 FA diet decreased CA, CDCA, αMCA, UDCA, DCA, and MDCA; the lab chow diet decreased the liver concentrations of TβMCA, TUDCA, TDCA, and TMDCA; the EFA-deficient diet decreased the liver concentrations of CDCA, UDCA, TDCA, and MDCA; the western diet decreased the liver concentrations of CA, TCA, and THDCA; and the high fructose diet decreased the liver concentrations of DCA and ωMCA.

The ratio of 12α-OH BAs to the non-12α-OH BAs. Compared to the AIN-93 purified diet, the atherogenic diet increased the ratio of 12α-OH BAs to non-12α-OH BAs in serum and livers of both genders (Table 1). The calorie-restricted diet also

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

increased this ratio in livers of both genders, and in the serum of male mice. The other 6 diets did not affect this ratio.

The mRNA expression of BA-synthetic enzymes in livers of mice fed the 9 diets. Figure 5 and Supplemental Table S4 depict the effect of the 9 diets on the mRNAs of the 6 BA-synthetic enzymes. The most pronounced gender difference was the expression of *Cyp7b1*, where female mice had little or no mRNA for this enzyme. This enzyme is a 7α-hydroxylase, that is responsible for the synthesis of 7-OH BAs in the alternative BA pathway (Chiang, 1998). This suggests that female mice produce little or no BAs by the alternative pathway. In contrast, the gene *Cyp39a1* had higher expression in female mice when fed the AIN-93M purified diet, the calorie-restricted diet, the high-fat diet, and the low n-3 FA diet.

As shown in Figure 5, the mRNA expression of *Cyp7a1* was increased by the calorie-restricted diet in female mice, but was decreased by the atherogenic diet in both genders. *Cyp8b1* was increased by the lab chow diet in male mice, but was decreased by the calorie-restricted diet and the atherogenic diet in both male and female mice. *Cyp27a1* was decreased by the high fructose diet and the EFA-deficient diet in female mice. *Cyp7b1* in male mice was increased by the lab chow diet, but was decreased by the restricted and the atherogenic diet. *Cyp39a1* was only increased by the calorie-restricted diet in both genders. *Baat* was decreased by the calorie-restricted diet and the high fructose diet in male mice.

The mRNA expression of BA-transporters in livers of mice fed the 9 diets. Figure 5 and Supplemental Table S4 depict the effect of the 9 diets on the mRNA

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

expression of 12 transporters in livers. The most pronounced gender difference, was the expression of *Bcrp*, in which female mice had much less mRNA compared to males (Cheng and Klaassen, 2009; Klaassen and Aleksunes, 2010). The other gene that has a gender difference in expression is *Oatp1a1*, in which the expression is about 2.5-fold higher in male than in female mice fed the various diets (Gong et al., 2011; Zhang et al., 2011; Zhang et al., 2012a; Zhang et al., 2012b; Zhang et al., 2013).

The mRNA expression of *Ntcp* was decreased by the western diet in male mice, and by the atherogenic diet in both genders. *Oatp1a1* was decreased by the high fructose diet and the atherogenic diet in male mice, and by the calorie-restricted diet in both genders. *Bsep* was decreased by the calorie-restricted diet in female mice and increased by the atherogenic diet in male mice. *Bcrp* was decreased by the calorie-restricted diet in male mice. *Atp8b1* was decreased by the high fructose diet in female mice, and the calorie-restricted diet in both genders. *Ostβ* was increased by the calorie-restricted diet in female mice, and the atherogenic diet in both genders. *Mrp3* was increased by the high-fat diet in female mice, and the lab chow and the calorie-restricted diet in both genders. *Mrp4* was increased by the calorie-restricted and the atherogenic diet.

The mRNA expression of cholesterol and phosphatidylcholine transporters in livers of mice fed the 9 diets. Figure 5 and Supplemental Table S4 depict the effect of the 9 diets on the mRNA expression of the 6 cholesterol and phosphatidylcholine transporters. The most pronounced gender difference, was the expression of *Mdr1b*, in which male mice had almost 1-fold higher levels compared to females (Lu and

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Klaassen, 2008; Cui et al., 2009). Mitochondrial cholesterol transporter *Star* was expressed higher in female mice fed the AIN-93 purified diet, the calorie-restricted diet, and the low n-3 diet. The mRNA expression of *Abcg5* and *Abcg8* were increased by the atherogenic diet in both genders. *Abca1* was decreased by the high fructose diet and the EFA-deficient diet in female mice, and by the calorie-restricted diet in both genders. Liver *Mdr2* was not affected by any of the diets.

The mRNA expression of BA receptors in livers of mice fed the 9 diets. Figure 5 and Supplemental Table S4 depict the effect of the 9 diets on the mRNAs of 5 important receptors in BA homeostasis in the liver. The most pronounced gender difference was the expression of *Fgfr4*, where female mice fed 5 of the 9 diets had more mRNA for the receptor than males. This is the receptor for *Fgf15* in the liver, which represses transcription of *Cyp7a1*. Female mice fed the calorie-restricted and low n-3 diets had increased *Fxr* expression compared to males. Compared to the AIN-93 purified diet, male mice fed the atherogenic diet had decreased *Fxr* expression, and *Lxr* was decreased by the restricted diet. Additionally, *Shp* was increased by the atherogenic diet in both genders, *Fgfr4* was decreased in female mice by the calorie-restricted diet, *Lrh-1* was decreased in female mice on the high fructose diet, and both *Fgfr4* and *Lrh-1* were decreased in females by the EFA-deficient diet.

The mRNA expression of genes involved in BA homeostasis in duodenum of mice fed the 9 diets. As shown in Figure 5 and Supplemental Table S4, the mRNA expression of 5 genes involved in BA homeostasis in the ileum of mice were quantified by RT-qPCR assay, and 3 of the genes were affected by at least one diet.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Asbt was increased by the calorie-restricted diet, but was decreased by the atherogenic diet and the western diet in the ileum of male mice. *Ostβ* was decreased by the western diet in male mice. Fibroblast growth factor 15 (*Fgf15*), which was higher in the ileum of female than male mice fed the AIN-93 purified diet, was increased by the atherogenic diet, but was decreased by the lab chow diet and the restricted diet in both genders.

Discussion

Previous studies reported the influence of gender and diet on serum and/or liver BA profile, and fewer studies discussed the classic BA regulatory pathways *Fxr-Fgf15* and *Fxr-Shp* after diet intervention. Therefore, the influence of the 9 most used experimental mouse diets on BA homeostasis were investigated simultaneously in the present study in both genders of mice. This data provides information on the importance of gender and diets on BA homeostasis in mice.

In serum, females had higher concentrations of total BAs than males when fed 6 of the 9 diets (Fig. 3A), which is consistent with previous reports for normal laboratory and high-fat diets (Fu et al., 2012; Sheng et al., 2017; Wankhade et al., 2018). Our study is the first to show that females have higher concentrations of total BAs in mice fed the AIN-93M purified, high fructose, western, and low n-3 FA diets. This higher concentration of BAs in serum of female mice may due to increased BA synthesis (Fig. 5, *Cyp7a1* and *Cyp39a1*), BA reabsorption (Fig. 5, *Ntcp* and *Oatp2b1*), or decreased BA efflux (Fig. 5 and Supplemental Table S5, *Bsep*). In addition, FXR is a critical regulatory factor of BA homeostasis via feedback mechanisms (Sheng et al.,

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

2017). When BAs activate FXR in the intestine, there is an increase in FGF15 secreted into the portal circulation to interact with FGFR4 in the liver, and to downregulate BA synthesis and transport (Mencarelli and Fiorucci, 2010). In the present study, *Fxr* may also contribute to the gender differences of BA metabolism in the above 5 diets, except the lab chow diet, since the altered expression of at least one gene involved in the *Fxr-Fgf15-Fgfr4* feed-back axis was detected (Fig. 5). Our study revealed this effect in mice fed the AIN-93M purified, restricted, high-fat, atherogenic, and western diets for the first time. This feedback mechanism may contribute to inhibit the global increase of serum BA concentration, and it is possible that the activation of this feedback axis may require longer time in mice fed the lab chow diet (Fu et al., 2012).

In liver, no obvious gender difference is first demonstrated for mice fed the AIN-93M purified, restricted, atherogenic, high fructose, low n-3 FA and EFA-deficient diets in this study, and is consistent with earlier report for the western diet (Sheng et al., 2017). Previous studies have reported higher hepatic total BAs in female mice fed the lab chow and in male mice fed the high-fat diet; however, these effects were not observed in the current study, which may be due to the differences in the amount of time the mice were fed the diets and/or the type of experimental animal model used (Fu et al., 2012; Xie et al., 2017). In addition, although no gender difference of *Fxr* was found in livers of mice fed the high-fat diet, its target gene *Shp*, which has been reported to represses BA biosynthesis (Goodwin et al., 2000), was higher in female mice fed the high-fat diet. Thus, the hepatic *Fxr-Shp* feedback

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

mechanism may be involved in the gender difference for mice fed the high-fat diet.

BAs are often quantified in serum and it is often assumed that BA concentrations in the serum reflects BA concentrations in the liver (Jiao et al., 2018). In the present study, BAs in the serum did not predict BA concentrations in the livers of mice. For example, the serum concentrations of αMCA, UDCA, HDCA, ωMCA, TαMCA, and TMDCA were increased in male mice fed the calorie-restricted diet, while the hepatic concentrations of all these BAs remained stable (Fig. 3 and 4). Meanwhile, a review of the published data from a number of previous studies in mice also found a lack of correlation of changes in serum and liver BA concentrations in same and different mouse models as well as varied pathological situations. For example, in the same C57BL/6J mice, the serum concentrations of CA, CDCA, αMCA, and βMCA were increased in mice fed 30 mg/kg berberine compared to the control mice, while the liver concentrations of all of these BAs were not altered by berberine (Guo et al., 2016). While the same phenomenon was found in different mice models (germ-free and hepatocyte-specific deletion of NADPH cytochrome P450 reductase null mice), or in varied pathological situations (mice after bile duct ligation) (Zhang et al., 2012b; Cheng et al., 2014; Selwyn et al., 2015). Thus, it is apparent from these numerous studies that changes in concentrations of BAs in the serum do not predict what happens to BA concentrations in the liver.

Of the 9 diets fed to mice in the present study, the atherogenic and calorie-restricted diets had the largest effects on BA homeostasis. The atherogenic diet increased numerous BAs in the serum and liver, and the increase of total BAs in serum was

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

more pronounced than that in the liver, which are novel findings. Meanwhile, the intestinal *Fxr-Fgf15* negative feed-back mechanism was apparent in mice fed the atherogenic diet as there was a marked increase of *Fgf15* in the intestine (Fig. 5), and resulted in a decrease in BA synthetase (*Cyp7a1* and *Cyp8b1*) (Fig 5), which was consistent with a previous report (Gutierrez et al., 2006). In addition, *Abcg5*/*Abcg8* are *Fxr* target genes and transporters that efflux cholesterol into the lumen of the intestine, and they were markedly increased in mice fed the atherogenic diet (Fig. 5), which was consistent with previous reports of CA fed mice (Cui et al., 2009; Song et al., 2015). The addition of CA increased Abcg5/Abcg8 expression, which might inhibit the accumulation of cholesterol caused by the atherogenic diet, thereby delaying the occurrence of high cholesterol related chronic diseases.

The atherogenic diet also increased the concentration of the 12α-hydroxylated BAs CA and DCA, and altered the ratio of 12α-OH to non-12α-OH BAs in livers and serum of both genders of mice (Table 1). However, mice displayed a decreased expression of *Cyp8b1*, which is a sterol 12α-hydroxylase and *Fxr-Fgf15-Fgfr4* target gene. A previous study reported that the addition of 0.5% CA to the diet suppresses the expression of Cyp8b1 in the livers of mice (Murphy et al., 2005). In addition, the increase of hepatic *Shp* might also be a potential regulator for this feedback inhibition of *Cyp8b1* in mice fed the atherogenic diet (Yang et al., 2014).

The calorie-restricted diet did not have a consistent effect on the BA concentrations in serum or liver in the two genders. However, the calorie-restricted diet decreased *Fgf15* in the intestine, and decreased *Cyp7b1*, *Cyp8b1*, *Atp8b1*, and *Abca1* as well as

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

increased *Cyp39a1* and *Mrp3* in the livers of both genders of mice. In addition, as discussed above, the serum BA concentration of female were higher than male mice, the present study found that the calorie-restricted diet closes the gap of the gender difference in serum by increasing BA concentration in male mice (Total, T-conjugated BA, CA, TCA, DCA, TDCA). In addition, the calorie-restricted diet increased the concentration of 12α-OH BAs CA and DCA, and the ratio of 12α-OH to the non-12α-OH BAs in livers and serum of male mice (Table 1), and induced the feedback mechanism of *Fxr-Fgf15* in the intestine, which resulted in a decrease of *Cyp8b1*.

As for the influence of diet, this is the first study to report the effect of high fructose, low n-3 FA, and EFA-deficient diets on the serum and hepatic BA profile in mice. Compared with the AIN-93M purified diet, there is little or no effects of these diets on BA homeostasis and related genes. This is also the first study to report that the only difference between mice fed the lab chow vs AIN-93M purified diet was that hepatic *Mrp3* was higher in mice fed the lab chow diet. Previous studies in mice fed a high-fat or EFA-deficient diet reported that these diets inhibit the intestinal Fxr-Fgf15 pathway (Lukovac et al., 2009; Li et al., 2020); however, these results were not repeated in the current study. Reasons for this discrepancy could be due to a relatively short duration of time on the diet. Therefore, the effects of the length of time on various diets should be considered in future studies to provide comprehensive insight into BA homeostasis.

In summary, present study systematically investigated the influence of the 9 most used experimental mouse diets on BA homeostasis in male and female mice. Several

new findings were made: 1) marked gender differences in BA homeostasis is ubiquitous in mice fed various diets; 2) female mice had higher total BAs in serum than male mice fed the AIN-93M purified, high fructose, and low n-3 FA diets; 3) of the 9 diets used, the atherogenic and calorie-restricted diets had the largest effects on BA homeostasis, followed by the lab chow and EFA-deficient diets, while the other 4 diets had less or no influence on BA homeostasis; 4) in serum, females had higher concentrations of total BAs than male mice fed the AIN-93M purified, high fructose, western, and low n-3 FA diet; 5) changes in the concentrations of BAs in the serum do not always predict BA concentrations in the liver; 6) the calorie-restricted diet closes the gap of the gender difference in serum and hepatic BA homeostasis found in controlled AIN-93M purified diet fed mice. In conclusion, this study reveals the important role of gender and diet on BA homeostasis and underlying mechanisms, which will aid future investigations.

Conflict of interest

The authors have no potential conflict of interest to declare.

Acknowledgments

The authors would like to thank all the graduate students, postdoctoral fellows, and Xiaohong Lei who were in Dr. Klaassen's lab for technical support of the experiments.

Author Contributions

Participated in research design: Guo, and Klaassen.

Conducted experiments: Guo.

Performed data analysis: Ma.

Wrote or contributed to the writing of the manuscript: Ma, Guo, and Klaassen.

Reference

Alnouti Y, Csanaky IL, and Klaassen CD (2008) Quantitative-profiling of bile acids and their conjugates in mouse liver, bile, plasma, and urine using LC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* **873:**209-217.

Bae JS, Oh AR, and Cha JY (2013) Regulation of Cholesterol Metabolism in Liver: Link to NAFLD and Impact of n-3 PUFAs. *J Lifestyle Med* **3:**19-25.

Berr F, Goetz A, Schreiber E, and Paumgartner G (1993) Effect of dietary n-3 versus n-6 polyunsaturated fatty acids on hepatic excretion of cholesterol in the hamster. *J Lipid Res* **34:**1275-1284.

Bertaggia E, Jensen KK, Castro-Perez J, Xu Y, Di Paolo G, Chan RB, Wang L, and Haeusler RA (2017) Cyp8b1 ablation prevents Western diet-induced weight gain and hepatic steatosis because of impaired fat absorption. *Am J Physiol Endocrinol Metab* **313:**E121-e133.

Chen G, Zhang Q, Ai C, Huang S, Zhang H, Guo X, Wang W, Hua W, Bi H, and Wang H (2019) Serum metabolic profile characteristics of offspring rats before and after birth caused by prenatal caffeine exposure. *Toxicology* **427:**152302.

Cheng X and Klaassen CD (2009) Tissue distribution, ontogeny, and hormonal regulation of xenobiotic transporters in mouse kidneys. *Drug Metab Dispos* **37:**2178-2185.

Cheng X, Maher J, Chen C, and Klaassen CD (2005) Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). *Drug Metab Dispos* **33:**1062-1073.

Cheng X, Zhang Y, and Klaassen CD (2014) Decreased bile-acid synthesis in livers of hepatocyte-conditional NADPH-cytochrome P450 reductase-null mice results in increased bile acids in serum. *J Pharmacol Exp Ther* **351:**105-113.

Chiang JY (1998) Regulation of bile acid synthesis. *Front Biosci* **3:**d176-193.

Cui YJ, Cheng X, Weaver YM, and Klaassen CD (2009) Tissue distribution, gender-divergent expression, ontogeny, and chemical induction of multidrug resistance transporter genes (Mdr1a, Mdr1b, Mdr2) in mice. *Drug Metab Dispos* **37:**203-210.

De Guzman JM, Ku G, Fahey R, Youm YH, Kass I, Ingram DK, Dixit VD, and Kheterpal I (2013) Chronic caloric restriction partially protects against age-related alteration in serum metabolome. *Age (Dordr)* **35:**1091-1104.

Dermadi D, Valo S, Ollila S, Soliymani R, Sipari N, Pussila M, Sarantaus L, Linden J, Baumann M, and Nystrom M (2017) Western Diet Deregulates Bile Acid Homeostasis, Cell Proliferation, and Tumorigenesis in Colon. *Cancer Res* **77:**3352-3363.

Donepudi AC, Boehme S, Li F, and Chiang JY (2017) G-protein-coupled bile acid receptor plays a key role in bile acid metabolism and fasting-induced hepatic steatosis in mice. *Hepatology* **65:**813-827.

Droge C, Bonus M, Baumann U, Klindt C, Lainka E, Kathemann S, Brinkert F,

Grabhorn E, Pfister ED, Wenning D, Fichtner A, Gotthardt DN, Weiss KH, McKiernan P, Puri RD, Verma IC, Kluge S, Gohlke H, Schmitt L, Kubitz R, Haussinger D, and Keitel V (2017) Sequencing of FIC1, BSEP and MDR3 in a large cohort of patients with cholestasis revealed a high number of different genetic variants. *J Hepatol* **67:**1253-1264.

Fu ZD, Csanaky IL, and Klaassen CD (2012) Gender-divergent profile of bile acid homeostasis during aging of mice. *PLoS One* **7:**e32551.

Fu ZD and Klaassen CD (2013) Increased bile acids in enterohepatic circulation by short-term calorie restriction in male mice. *Toxicol Appl Pharmacol* **273:**680-690.

Gong L, Aranibar N, Han YH, Zhang Y, Lecureux L, Bhaskaran V, Khandelwal P, Klaassen CD, and Lehman-McKeeman LD (2011) Characterization of organic anion-transporting polypeptide (Oatp) 1a1 and 1a4 null mice reveals altered transport function and urinary metabolomic profiles. *Toxicol Sci* **122:**587-597.

Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, and Kliewer SA (2000) A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* **6:**517-526.

Guo Y, Zhang Y, Huang W, Selwyn FP, and Klaassen CD (2016) Dose-response effect of berberine on bile acid profile and gut microbiota in mice. *BMC Complement Altern Med* **16:**394.

Gutierrez A, Ratliff EP, Andres AM, Huang X, McKeehan WL, and Davis RA (2006) Bile acids decrease hepatic paraoxonase 1 expression and plasma high-density lipoprotein levels via FXR-mediated signaling of FGFR4. *Arterioscler Thromb Vasc Biol* **26:**301-306.

Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, Donahee M, Wang DY, Mansfield TA, Kliewer SA, Goodwin B, and Jones SA (2003) Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev* **17:**1581-1591.

Ichimura M, Masuzumi M, Kawase M, Sakaki M, Tamaru S, Nagata Y, Tanaka K, Suruga K, Tsuneyama K, Matsuda S, and Omagari K (2017) A diet-induced Sprague-Dawley rat model of nonalcoholic steatohepatitis-related cirrhosis. *J Nutr Biochem* **40:**62-69.

Jena PK, Sheng L, Liu HX, Kalanetra KM, Mirsoian A, Murphy WJ, French SW, Krishnan VV, Mills DA, and Wan YY (2017) Western Diet-Induced Dysbiosis in Farnesoid X Receptor Knockout Mice Causes Persistent Hepatic Inflammation after Antibiotic Treatment. *Am J Pathol* **187:**1800-1813.

Jiang M, Li C, Liu Q, Wang A, and Lei M (2019) Inhibiting Ceramide Synthesis Attenuates Hepatic Steatosis and Fibrosis in Rats With Non-alcoholic Fatty Liver Disease. *Front Endocrinol (Lausanne)* **10:**665.

Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, Mastrandrea L, Buck MJ, Baker RD, Genco RJ, Zhu R, and Zhu L (2018) Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* **67:**1881-1891.

Klaassen CD and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol

transporters: function and regulation. *Pharmacol Rev* **62:**1-96.

Kok DEG, Rusli F, van der Lugt B, Lute C, Laghi L, Salvioli S, Picone G, Franceschi C, Smidt H, Vervoort J, Kampman E, Muller M, and Steegenga WT (2018) Lifelong calorie restriction affects indicators of colonic health in aging C57Bl/6J mice. *J Nutr Biochem* **56:**152-164.

Li H, Xi Y, Xin X, Tian H, and Hu Y (2020) Salidroside improves high-fat diet-induced non-alcoholic steatohepatitis by regulating the gut microbiota-bile acid-farnesoid X receptor axis. *Biomedicine & Pharmacotherapy* **124**.

Liao J, Liu B, Zhong W, Wang GD, Xu YL, and Chen X (2019) Protective effect of Lycium barbarum polysaccharides against high-fat diet-induced renal injury and lipid deposition in rat kidneys. *J Biol Regul Homeost Agents* **33:**7-17.

Liu T, Yang H, Fan W, Tu J, Li TWH, Wang J, Shen H, Yang J, Xiong T, Steggerda J, Liu Z, Noureddin M, Maldonado SS, Annamalai A, Seki E, Mato JM, and Lu SC (2018) Mechanisms of MAFG Dysregulation in Cholestatic Liver Injury and Development of Liver Cancer. *Gastroenterology* **155:**557-571.e514.

Lu H and Klaassen C (2008) Gender differences in mRNA expression of ATP-binding cassette efflux and bile acid transporters in kidney, liver, and intestine of 5/6 nephrectomized rats. *Drug Metab Dispos* **36:**16-23.

Lukovac S, Los EL, Stellaard F, Rings EHHM, and Verkade HJ (2009) Effects of essential fatty acid deficiency on enterohepatic circulation of bile salts in mice. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **297:**G520-G531.

Luo L and Yang T (2016) Triptolide inhibits the progression of atherosclerosis in apolipoprotein E(-)/(-) mice. *Exp Ther Med* **12:**2307-2313.

Luo T, Miranda-Garcia O, Adamson A, Hamilton-Reeves J, Sullivan DK, Kinchen JM, and Shay NF (2016) Consumption of Walnuts in Combination with Other Whole Foods Produces Physiologic, Metabolic, and Gene Expression Changes in Obese C57BL/6J High-Fat-Fed Male Mice. *J Nutr* **146:**1641-1650.

Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, and Shan B (1999) Identification of a nuclear receptor for bile acids. *Science* **284:**1362-1365.

Mencarelli A and Fiorucci S (2010) FXR an emerging therapeutic target for the treatment of atherosclerosis. *J Cell Mol Med* **14:**79-92.

Murphy C, Parini P, Wang J, Bjorkhem I, Eggertsen G, and Gafvels M (2005) Cholic acid as key regulator of cholesterol synthesis, intestinal absorption and hepatic storage in mice. *Biochim Biophys Acta* **1735:**167-175.

Phan J, Pesaran T, Davis RC, and Reue K (2002) The Diet1 locus confers protection against hypercholesterolemia through enhanced bile acid metabolism. *J Biol Chem* **277:**469-477.

Renaud HJ, Cui JY, Lu H, and Klaassen CD (2014) Effect of diet on expression of genes involved in lipid metabolism, oxidative stress, and inflammation in mouse liver-insights into mechanisms of hepatic steatosis. *PLoS One* **9:**e88584.

Selwyn FP, Csanaky IL, Zhang Y, and Klaassen CD (2015) Importance of Large Intestine in Regulating Bile Acids and Glucagon-Like Peptide-1 in Germ-Free

Mice. *Drug Metab Dispos* **43:**1544-1556.

Sheng L, Jena PK, Liu HX, Kalanetra KM, Gonzalez FJ, French SW, Krishnan VV, Mills DA, and Wan YY (2017) Gender Differences in Bile Acids and Microbiota in Relationship with Gender Dissimilarity in Steatosis Induced by Diet and FXR Inactivation. *Sci Rep* **7:**1748.

Shihabudeen MS, Roy D, James J, and Thirumurugan K (2015) Chenodeoxycholic acid, an endogenous FXR ligand alters adipokines and reverses insulin resistance. *Mol Cell Endocrinol* **414:**19-28.

Song P, Rockwell CE, Cui JY, and Klaassen CD (2015) Individual bile acids have differential effects on bile acid signaling in mice. *Toxicol Appl Pharmacol* **283:**57-64.

Takahashi S, Fukami T, Masuo Y, Brocker CN, Xie C, Krausz KW, Wolf CR, Henderson CJ, and Gonzalez FJ (2016) Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. *J Lipid Res* **57:**2130-2137.

Thomas C, Pellicciari R, Pruzanski M, Auwerx J, and Schoonjans K (2008) Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* **7:**678-693.

Trauner M, Claudel T, Fickert P, Moustafa T, and Wagner M (2010) Bile acids as regulators of hepatic lipid and glucose metabolism. *Dig Dis* **28:**220-224.

Tsuchida T, Shiraishi M, Ohta T, Sakai K, and Ishii S (2012) Ursodeoxycholic acid improves insulin sensitivity and hepatic steatosis by inducing the excretion of hepatic lipids in high-fat diet-fed KK-Ay mice. *Metabolism* **61:**944-953.

Vamecq J, Vallee L, de la Porte PL, Fontaine M, de Craemer D, van den Branden C, Lafont H, Grataroli R, and Nalbone G (1993) Effect of various n-3/n-6 fatty acid ratio contents of high fat diets on rat liver and heart peroxisomal and mitochondrial beta-oxidation. *Biochim Biophys Acta* **1170:**151-156.

Wang C, Yang M, Zhao J, Li X, Xiao X, Zhang Y, Jin X, and Liao M (2019) Bile salt (glycochenodeoxycholate acid) induces cell survival and chemoresistance in hepatocellular carcinoma. *J Cell Physiol* **234:**10899-10906.

Wankhade UD, Zhong Y, Kang P, Alfaro M, Chintapalli SV, Piccolo BD, Mercer KE, Andres A, Thakali KM, and Shankar K (2018) Maternal High-Fat Diet Programs Offspring Liver Steatosis in a Sexually Dimorphic Manner in Association with Changes in Gut Microbial Ecology in Mice. *Sci Rep* **8:**16502.

Wanon J, Guertin F, Brunet S, Delvin E, Gavino V, Bouthillier D, Lairon D, Yotov W, and Levy E (1998) The effects of cholesterol uptake from high-density lipoprotein subfractions on biliary sterol secretion in rats with essential fatty-acid deficiency. *Hepatology* **27:**779-786.

Xie G, Wang X, Huang F, Zhao A, Chen W, Yan J, Zhang Y, Lei S, Ge K, Zheng X, Liu J, Su M, Liu P, and Jia W (2016) Dysregulated hepatic bile acids collaboratively promote liver carcinogenesis. *Int J Cancer* **139:**1764-1775.

Xie G, Wang X, Zhao A, Yan J, Chen W, Jiang R, Ji J, Huang F, Zhang Y, Lei S, Ge K, Zheng X, Rajani C, Alegado RA, Liu J, Liu P, Nicholson J, and Jia W (2017) Sex-dependent effects on gut microbiota regulate hepatic carcinogenic outcomes. *Sci Rep* **7:**45232.

Yang F, He Y, Liu HX, Tsuei J, Jiang X, Yang L, Wang ZT, and Wan YJ (2014) All-trans retinoic acid regulates hepatic bile acid homeostasis. *Biochem Pharmacol* **91:**483-489.

Zhang Y, Csanaky IL, Cheng X, Lehman-McKeeman LD, and Klaassen CD (2012a) Organic anion transporting polypeptide 1a1 null mice are sensitive to cholestatic liver injury. *Toxicol Sci* **127:**451-462.

Zhang Y, Csanaky IL, Lehman-McKeeman LD, and Klaassen CD (2011) Loss of organic anion transporting polypeptide 1a1 increases deoxycholic acid absorption in mice by increasing intestinal permeability. *Toxicol Sci* **124:**251-260.

Zhang Y, Csanaky IL, Selwyn FP, Lehman-McKeeman LD, and Klaassen CD (2013) Organic anion-transporting polypeptide 1a4 (Oatp1a4) is important for secondary bile acid metabolism. *Biochem Pharmacol* **86:**437-445.

Zhang Y, Hong JY, Rockwell CE, Copple BL, Jaeschke H, and Klaassen CD (2012b) Effect of bile duct ligation on bile acid composition in mouse serum and liver. *Liver Int* **32:**58-69.

Zhang Y and Klaassen CD (2010) Effects of feeding bile acids and a bile acid sequestrant on hepatic bile acid composition in mice. *J Lipid Res* **51:**3230-3242.

Zhang Y, Limaye PB, Lehman-McKeeman LD, and Klaassen CD (2012c) Dysfunction of organic anion transporting polypeptide 1a1 alters intestinal bacteria and bile acid metabolism in mice. *PLoS One* **7:**e34522.

Footnotes

This work was supported by the National Natural Scientific Foundation of China (grant number No.81503563), Hunan Provincial Natural Science Foundation of China (2019JJ60074) and Hunan Provincial Innovation Foundation for Postgraduate (CX20190250).

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

FIGURE CAPTIONS

Fig. 1 Bile acid biosynthesis, transport and metabolism. The left arrow (solid line) shows the trend of male mice, and the right arrow (dashed line) shows the trend of female mice.

Fig. 2 Three-dimensional scatter plots of the principal component analysis (PCA) for the influence of different diets on liver bile acid (BA) profile and BA-related gene mRNA expression in both genders of mice. A. PCA based on the BA-related gene mRNA expression in livers of male mice (R2=0.797, Q2=0.532); B. PCA based on the BA-related gene mRNA expression in livers of female mice (R2=0.828, Q2=0.584); C. PCA based on liver BA concentrations of male mice (R2=0.988, Q2=0.664); D. PCA based on liver BA concentrations of female mice (R2=0.956, Q2=0.618). Each color represents a single diet. PCA was performed using the software SIMCA.

Fig. 3 Effect of 9 diets on the concentrations of BAs in the serum of mice. The amount of T-conjugated, unconjugated, and total BAs in the serum of mice were quantified and are shown in **A**; Heatmap of BAs in the serum of mice fed 9 various diets are shown in **B**. Average values of 5 replicates per diet are given by colored squares. High BA concentration is represented in red, whereas low BA concentration is in blue, and "*" means $P<0.05$ when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#" indicates $P<0.05$ when data is compared between genders fed the same diet.

Fig. 4 Effect of 9 diets on the concentrations of BAs in the livers of mice. The amount of T-conjugated, unconjugated, and total BAs in the livers of mice were quantified

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

and are shown in **A**; Heatmap of BAs in the livers of mice fed 9 various diets are shown in **B**. Average values of 5 replicates per diet are given by colored squares. High BA concentration is represented in red, whereas low BA concentration is in blue, and "*" means $P<0.05$ when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#" indicates $P<0.05$ when data is compared between genders fed the same diet.

Fig. 5 Effect of 9 diets on mRNA expression of BA-related genes in the livers and ileum of mice. Heatmap of BA-related genes in the livers and ileum of mice fed 9 various diets. Average values of 5 replicates per diet are given by colored squares. High mRNA abundance is represented in red, whereas low mRNA abundance is in blue. Relative mRNA levels were calculated with male controls (male, AIN-93M purified diet) set as 100%. Data were analyzed by two-way ANOVA, and "*" means $P<0.05$ when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#" indicates $P<0.05$ when data from the two genders fed the same diet are compared.

DMD Fast Forward. Published on October 22, 2020 as DOI: 10.1124/dmd.120.000166
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. The ratio of 12α-OH BAs to non-12α-OH BAs in livers and serum of two genders.

	12α-OH BAs to non-12α-OH BAs			
	Male		**Female**	
	Liver	**Serum**	**Liver**	**Serum**
AIN-93M Purified	0.24±0.05	0.53±0.15	0.57±0.05	1.55±0.2
Lab chow	0.61±0.1	0.69±0.15	0.8±0.1	1.28±0.25
Diet-restriction	2.12±0.55*	2.14±0.6*	1.65±0.15*	2.02±0.35
High fructose	0.47±0.45	0.33±0.15	0.8±0.05	1.5±0.15
High-fat	0.29±0.05	0.5±0.20	0.8±0.25	1.51±0.35
Atherogenic	30.09±3.25*	48.47±9.8*	21.73±8.75*	41.24±16.35*
Western	0.26±0.1	0.61±0.2	0.3±0.05	0.82±0.2
Low n-3 FA	0.23±0.05	0.56±0.25	0.51±0.2	1.53±0.35
EFA Deficient	0.28±0.1	0.22±0.1	0.56±0.15	1.05±0.3

Note: 12α-OH BAs: CA, DCA, TCA, TDCA; non-12α-OH BAs: CDCA, LCA, UDCA, HDCA, MDCA, TCDCA, TLCA, TUDCA, TMDCA, THDCA, MCAs and TMCAs. "*": indicates $p<0.05$ when compare with the AIN-93M purified diet. Mean values ± S.D. are shown.

Figure 1

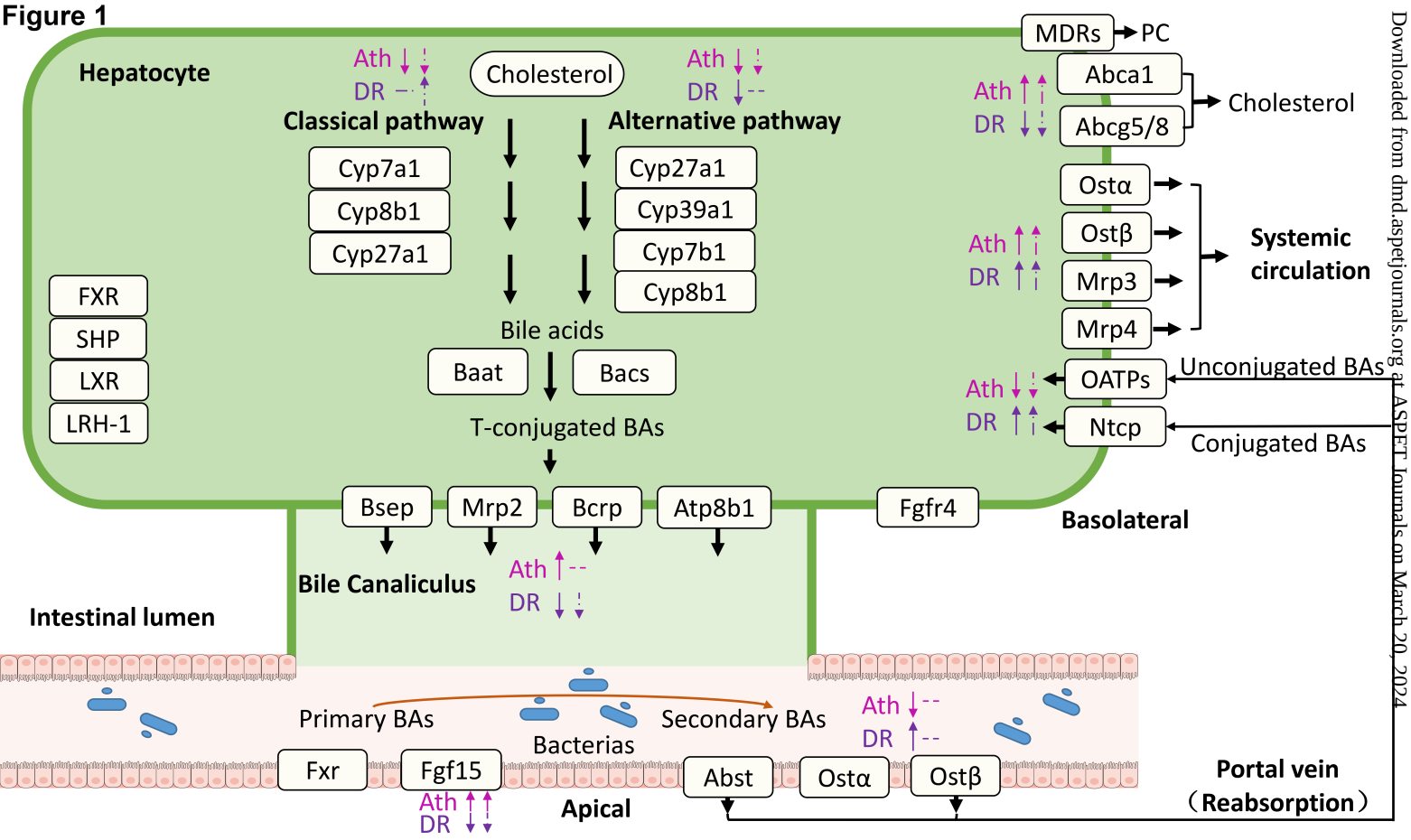


Figure 2

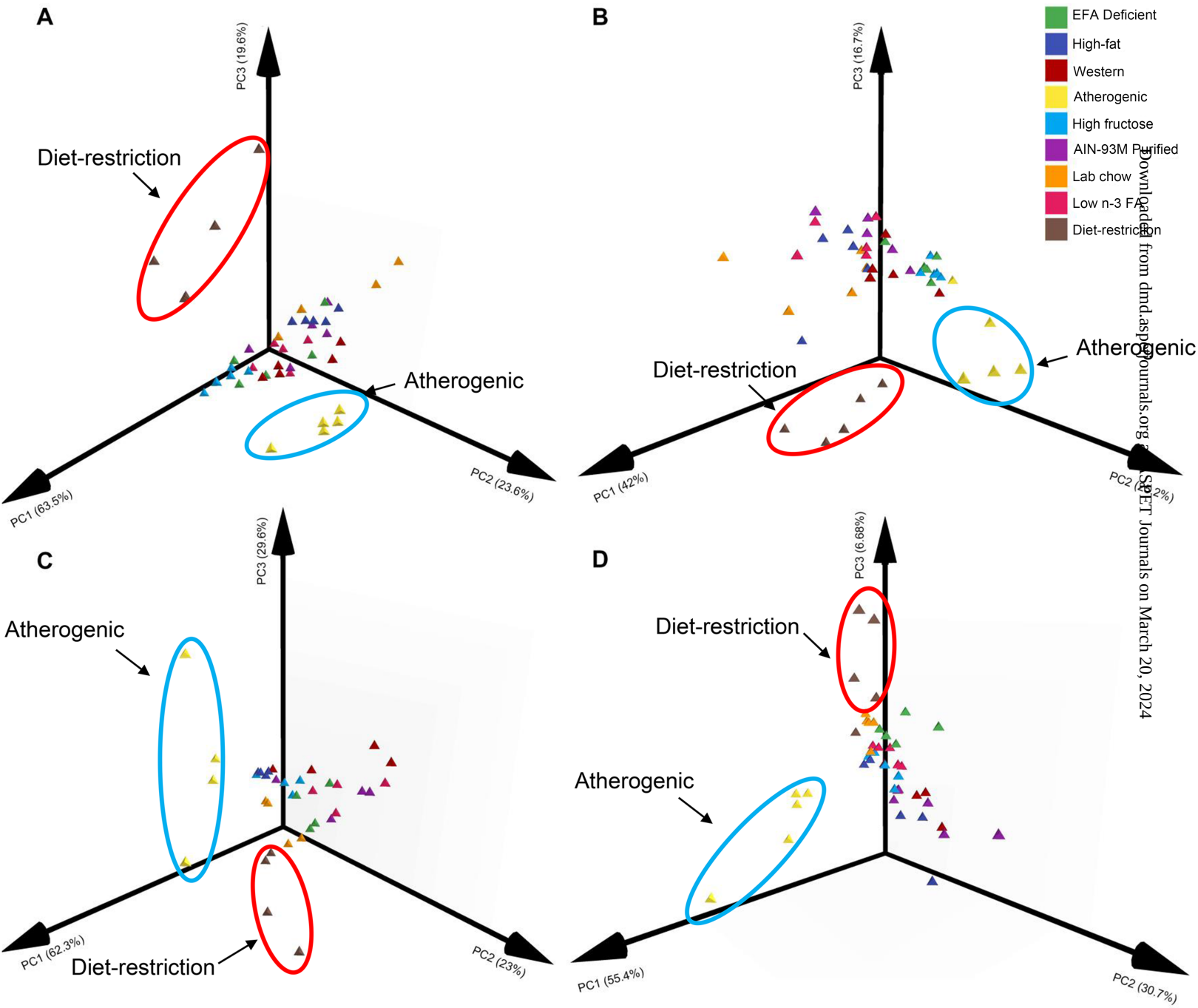


Figure 3

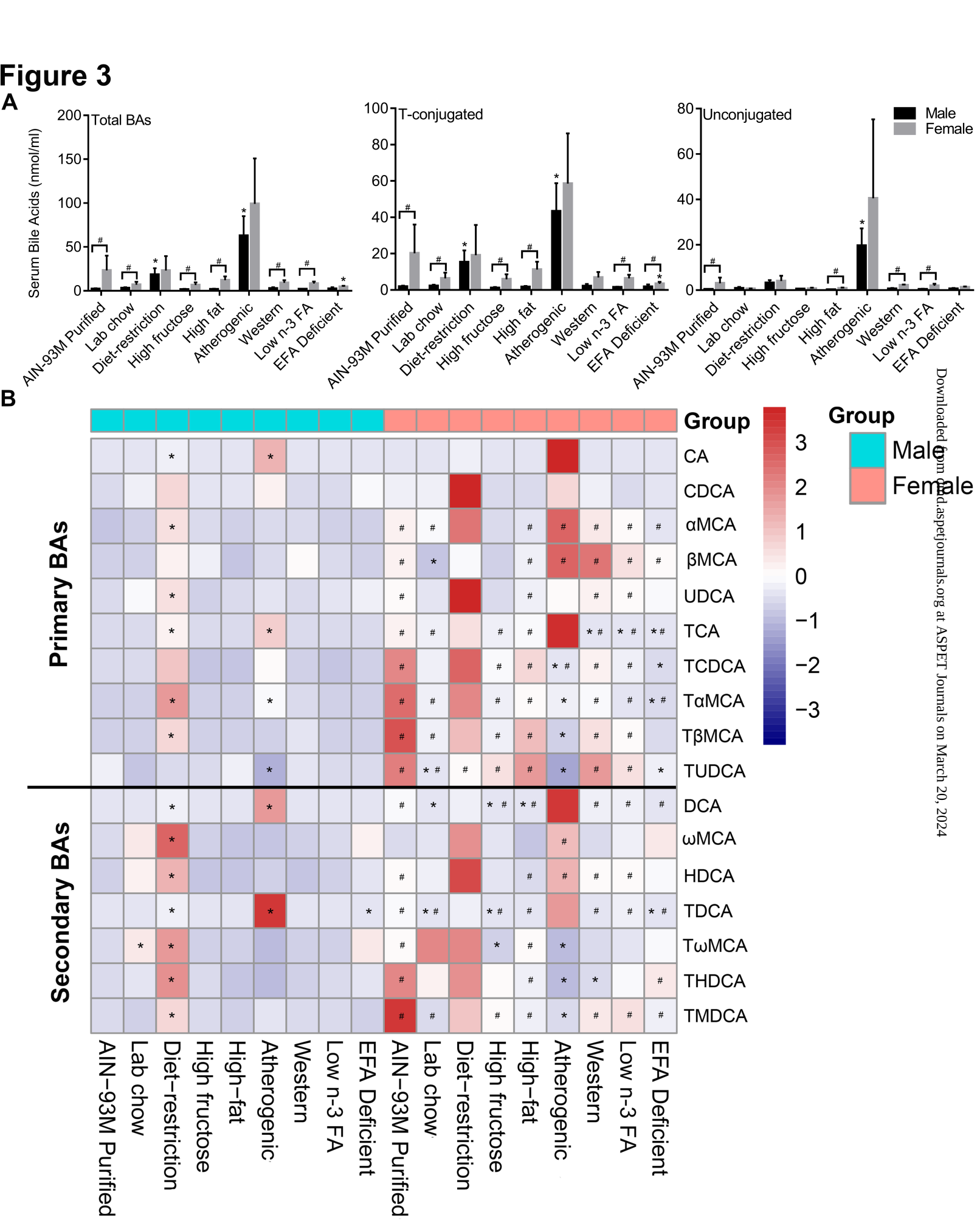


Figure 4

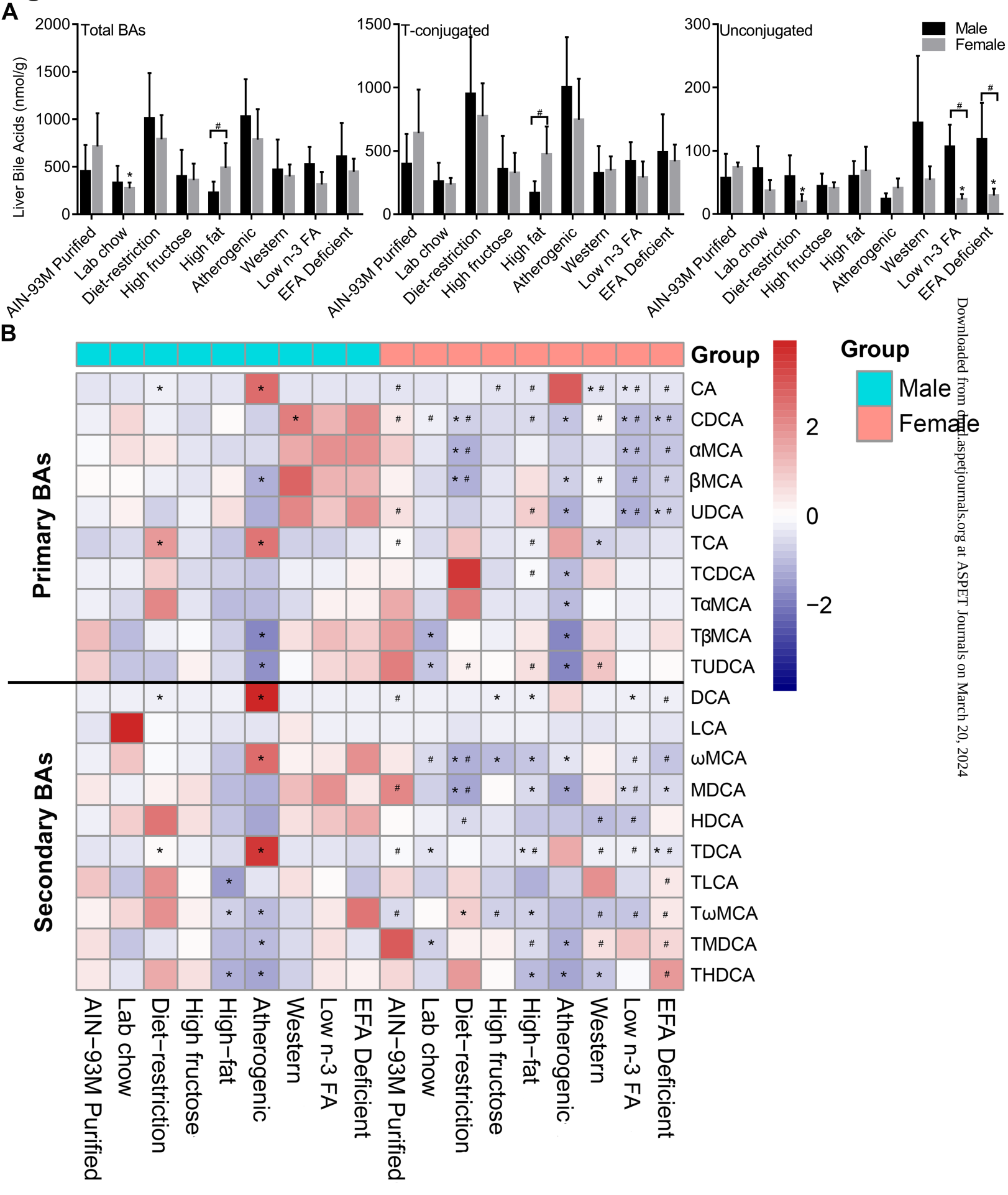
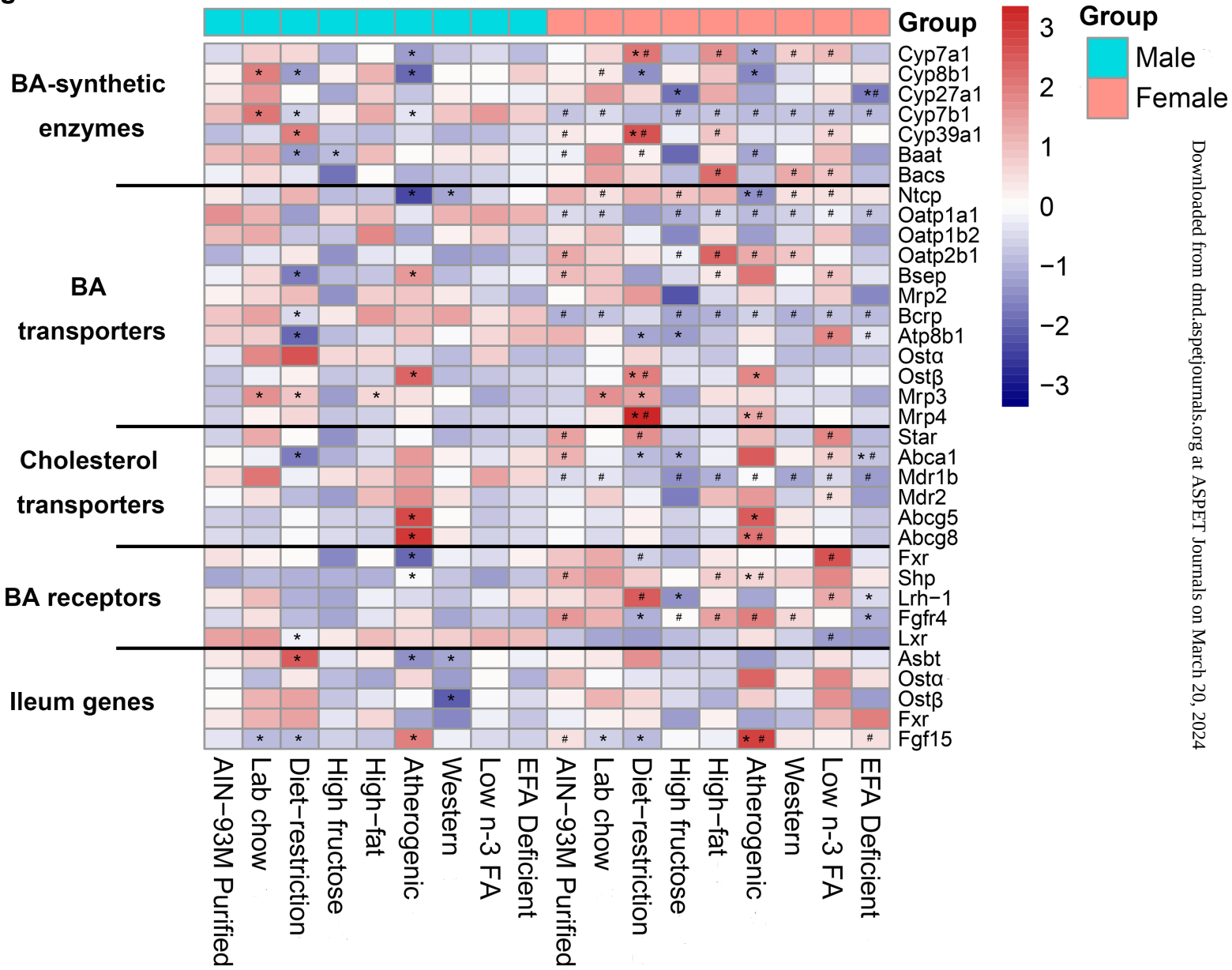


Figure 5



Supplemental Material

Effect of Gender and Various Diets on Bile Acid Profile and Related Genes in Mice

Chong Ma[1,2], Ying Guo[1,2,3*], and Curtis D. Klaassen[3*]

[1] *Department of Clinical Pharmacology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha 410008, P. R. China; Institute of Clinical Pharmacology, Central South University, Hunan Key Laboratory of Pharmacogenetics, 110 Xiangya Road, Changsha 410078, P. R. China; Engineering Research Center of Applied Technology of Pharmacogenomics, Ministry of Education, 110 Xiangya Road, Changsha 410078, P. R. China*

[2] *National Clinical Research Center for Geriatric Disorders , Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China, 410008*

[3] *Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160, USA*

Table S1. Standard curve data of the different bile acids.

Bile Acids	Groups	Retention Time (min)	Calibration Curve	Internal Standards	R^2
CA	Primary BAs	14.02	y = 0.0037x - 0.0107	CDCA-d4	0.9999
CDCA	Primary BAs	18.15	y = 0.0017x + 0.0003	CDCA-d4	0.9999
αMCA	Primary BAs	6.5	y = 0.0022x - 0.0001	CDCA-d4	0.9999
βMCA	Primary BAs	7.03	y = 0.003x - 0.0027	CDCA-d4	0.9999
UDCA	Primary BAs	13.08	y = 0.0017x + 0.0005	CDCA-d4	0.9999
TCA	Primary BAs	14.22	y = 0.0032x + 0.0049	CDCA-d4	0.9991
TCDCA	Primary BAs	16.84	y = 0.002x + 0.0027	CDCA-d4	0.9992
TαMCA	Primary BAs	6.44	y = 0.0036x + 0.0058	CDCA-d4	0.9996
TβMCA	Primary BAs	6.7	y = 0.0038x + 0.0024	CDCA-d4	1
TUDCA	Primary BAs	12.5	y = 0.0018x + 0.0028	CDCA-d4	0.9991
DCA	Secondary BAs	18.56	y = 0.0022x + 0.0029	CDCA-d4	0.9999
LCA	Secondary BAs	20.24	y = 0.0023x + 0.0009	CDCA-d4	0.9985
ωMCA	Secondary BAs	5.86	y = 0.0022x - 0.0046	CDCA-d4	0.9999
MDCA	Secondary BAs	6.62	y = 0.0109x - 0.0108	CDCA-d4	0.9999
HDCA	Secondary BAs	14.39	y = 0.0016x + 0.0009	CDCA-d4	0.9999
TDCA	Secondary BAs	17.56	y = 0.002x + 0.0031	CDCA-d4	0.9987
TLCA	Secondary BAs	18.82	y = 0.0042x - 0.0043	CDCA-d4	0.9995
TωMCA	Secondary BAs	6.51	y = 0.0036x + 0.0027	CDCA-d4	0.9998
TMDCA	Secondary BAs	8.42	y = 0.001x + 0.0014	CDCA-d4	0.9992
THDCA	Secondary BAs	13.43	y = 0.002x + 0.0041	CDCA-d4	0.999

Table S2. The effects of nine diets on serum individual, total, T-conjugated, unconjugated BAs.

	AIN-93M Purified		Lab chow		Diet-restriction		High fructose		High-fat		Atherogenic		Western		low n-3 FA		EFA Deficient	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
CA	0.02±0.001	0.07±0.07	0.08±0.08	0.05±0.05	0.56±0.49*	0.66±0.59	0.03±0.01	0.02±0.01	0.02±0	0.03±0.02	5.86±3.28*	14.51±19.24	0.02±0	0.03±0.01	0.03±0.04	0.07±0.07	0.04±0.03	0.06±0.07
CDCA	0.08±0.02	0.1±0.09	0.1±0.04	0.09±0.06	0.19±0.07	0.52±0.51	0.08±0.03	0.07±0.02	0.08±0.02	0.06±0.03	0.16±0.12	0.2±0.17	0.07±0.02	0.1±0.02	0.08±0.03	0.07±0.03	0.11±0.12	0.09±0.03
αMCA	0.001±0.001	0.02±0.01#	0.002±0.002	0.01±0.01#	0.02±0.01*	0.05±0.05	0.01±0.002	0.004±0.004	0.002±0.002	0.01±0.01#	0.01±0.004	0.06±0.06#	0.004±0.002	0.02±0.1#	0.004±0.004	0.02±0.01#	0.004±0.004	0.01±0.002#
βMCA	0.09±0.07	0.36±0.24#	0.11±0.12	0.04±0.03*	0.35±0.34	0.25±0.07	0.21±0.12	0.12±0.08	0.04±0.03	0.22±0.23#	0.15±0.14	1.04±1.01#	0.33±0.32	0.96±0.7#	0.12±0.12	0.46±0.26#	0.09±0.06	0.3±0.18#
UDCA	0.01±0.01	0.05±0.03#	0.04±0.04	0.02±0.01	0.08±0.07*	0.29±0.29	0.01±0.004	0.01±0.01	0.01±0.004	0.03±0.02#	0.02±0.01	0.05±0.04	0.01±0	0.06±0.3#	0.02±0.02	0.05±0.05#	0.03±0.03	0.04±0.04
TCA	0.43±0.22	8.84±6.57#	0.81±0.31	3.2±1.85#	8.31±3.32*	11.37±10.72	0.11±0.05	2.91±1.43#	0.47±0.26	5.7±2.1#1	14.24±4.24*	41.47±2.2	0.53±0.28	2.24±1.1*#	0.35±0.16	2.53±0.83*#	0.27±0.18	1.5±0.47*#
TCDCA	0.05±0.04	0.34±0.23#	0.05±0.02	0.09±0.04	0.22±0.16	0.4±0.26	0.02±0.02	0.1±0.09#	0.03±0.03	0.18±0.16#	0.12±0.02	0.06±0.06*#	0.04±0.02	0.14±0.05#	0.01±0.01	0.08±0.02#	0.04±0.02	0.05±0.04*
TαMCA	0.06±0.04	1.37±1.15#	0.09±0.05	0.24±0.17#	1.06±0.59*	1.22±1.14	0.05±0.03	0.25±0.18#	0.05±0.03	0.4±0.27#	0.33±0.21*	0.22±0.24*	0.1±0.07	0.34±0.2#	0.09±0.03	0.21±0.05#	0.04±0.03	0.14±0.07*#
TβMCA	0.28±0.12	4.26±3.63#	0.27±0.11	0.71±0.56#	1.85±1.32*	2.32±2.65	0.26±0.16	0.83±0.46#	0.36±0.13	2.34±1.53#	0.27±0.09	0.34±0.22*	0.58±0.41	1.64±0.86#	0.28±0.12	1.15±0.45#	0.37±0.33	0.41±0.1
TUDCA	0.46±0.15	1.39±0.9#	0.21±0.1	0.41±0.08*#	0.34±0.29	0.58±0.27#	0.32±0.24	0.77±0.2#	0.44±0.16	1.24±0.5#	0.1±0.1*	0.05±0.02*	0.42±0.26	1.25±0.4 2#	0.32±0.09	0.73±0.22#	0.32±0.28	0.43±0.19*
DCA	0.1±0.07	2.33±2.32#	0.19±0.15	0.3±0.15*	1.21±0.48*	1.59±1.1	0.08±0.01	0.38±0.27*#	0.03±0.02	0.42±0.15*#	13.41±6.04*	24.16±17.32	0.18±0.07	0.93±0.24#	0.06±0.02	1.11±0.51#	0.02±0.01	0.46±0.22
ωMCA	0.07±0.09	0.09±0.26	0.26±0.09	0.09±0.72	0.72±0.58	0.58±0.05	0.05±0.09	0.09±0.02	0.02±0.03	0.03±0.03	0.03±0.43	0.43±0.05	0.05±0.08	0.08±0.06	0.06±0.14	0.14±0.23	0.23±0.25	0.25±0

	03	06	25	05	31*	32	04	06	01	02	01	4#	04	5	04	15	23	12
HDCA	0.004±	0.02±0.	0.02±0.	0.01±0.	0.04±0.	0.07±0.	0.004±	0.01±0.	0.003±	0.01±0.	0.005±0	0.04±0.	0.004±	0.02±0.0	0.01±0.	0.02±0.	0.01±0.	0.02±0.
	0.001	01#	02	01	04*	02	0.001	01	0.001	01#	.004	04#	0.001	1#	004	02#	01	01
TDCA	0.2±0.1	2.81±2.	0.17±0.	0.43±0.	2.03±0.	1.67±0.	0.14±0.	0.67±0.	0.1±0.0	0.86±0.	28.18±1	16.35±6	0.27±0.	0.91±0.5	0.2±0.0	1.18±0.	0.03±0.	0.31±0.
	3	79#	1	18*#	68*	63	06	35*#	3	33#	4.33*	.87	1	5#	9	52#	03*	13*#
TωMCA	0.2±0.0	0.43±0.	0.54±0.	1.12±0.	0.99±0.	1.08±1	0.13±0.	0.16±0.	0.17±0.	0.39±0.	0.03±0.	0.05±0.	0.13±0.	0.18±0.1	0.17±0.	0.26±0.	0.52±0.	0.37±0.
	7	14#	2*	46	49*		09	12*	07	31#	01	04*	1	7	04	15	57	1
THDCA	0.05±0.	0.29±0.	0.06±0.	0.12±0.	0.28±0.	0.28±0.	0.05±0.	0.1±0.0	0.01±0.	0.07±0.	0.01±0.	0.01±0.	0.03±0.	0.04±0.0	0.04±0.	0.09±0.	0.06±0.	0.13±0.
	04	27#	02	09	23*	18	02	9	01	06#	004	003*	03	4*	02	07	03	05#
TMDCA	0.03±0.	0.51±0.	0.01±0.	0.03±0.	0.17±0.	0.22±0.	0.03±0.	0.1±0.0	0.01±0.	0.06±0.	0.04±0.	0.02±0.	0.04±0.	0.14±0.0	0.02±0.	0.16±0.	0.02±0.	0.06±0.
	03	55#	01	01#	1*	23	02	8#	01	03#	03	02*	03	3#	01	1#	004	03#
Unconju	0.37±0.	3.03±2.	0.8±0.6	0.62±0.	3.18±1.	4.01±2.	0.47±0.	0.71±0.	0.21±0.	0.8±0.3	19.64±7	40.49±3	0.67±0.	2.19±0.3	0.39±0.	1.93±0.	0.54±0.	1.23±0.
gated	2	55#	5	17	23*	37	14	38	05	9	.63*	4.81#	33	2	24	91#	42	41#
BAs																		
T-conjug	1.76±0.	20.23±1	2.21±0.	6.35±3.	15.25±	19.13±	1.11±0.	5.88±2.	1.63±0.	11.24±	43.31±1	58.57±2	2.12±1.	6.88±2.9	1.48±0.	6.39±2.	1.67±1.	3.4±1.0
ated	64	5.77#	69	2#	6.52	16.68	52	75#	55	4.34#	5.46*	7.64	2	8	32	03#	38	4*#
Total	2.12±0.	23.26±1	3.01±1.	6.97±3.	18.43±	23.15±	1.57±0.	6.59±2.	1.83±0.	12.04±	62.95±2	99.06±5	2.79±1.	9.07±3.0	1.87±0.	8.32±2.	2.21±1.	4.63±1.
BAs	8	6.81#	01	27#	7.35*	16.47	65	99#	57	4.34#	2.18*	1.79	23	6#	29	31#	79	16*

All values are expressed as mean ± S.D., and “*” indicates P<0.05 when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#"means P<0.05 statistical significance between genders fed the same diet.

Table S3. The effects of nine diets on liver individual, total, T-conjugated, unconjugated BAs.

	AIN-93M Purified		Lab chow		Diet-restriction		High fructose		High-fat		Atherogenic		Western		low n-3 FA		EFA Deficient	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
CA	1.54±1.38	3.74±1.13#	4.24±2.43	1.97±0.7	5.55±2.82*	2.64±1.44	1.22±0.72	2.95±1.47#	1.27±0.36	2.69±1.65#	16.31±2.09*	30.07±9.82	2.8±1.98	0.75±0.38*#	2.56±0.92	0.82±0.29*#	2.89±0.62	1.65±0.81#
CDCA	0.53±0.39	0.79±0.18#	0.89±0.29	0.52±0.11#	0.57±0.27	0.34±0.2*#	0.41±0.15	0.43±0.06	0.66±0.09	0.41±0.14#	0.34±0.23	0.27±0.11*	1.52±1.05*	0.64±0.21#	1.17±0.53	0.22±0.04*#	1.46±1.21	0.3±0.06*#
αMCA	3.71±1.73	5.32±1.63	4.64±2.62	2.71±0.89	4.21±2.02	1.08±0.66*#	2.48±1.15	2.16±0.45	2.26±1.06	2.84±2.64	0.54±0.23	0.96±0.87	4.19±2.52	2.54±1.13	7.35±3	1.2±0.58*#	7.5±4.85	1.61±0.71#
βMCA	41.12±30.19	48.41±4.27	41.34±21.58	23.43±13.12	42.96±24.03	7.77±4.46*#	29.47±13.32	29.14±6.85	48.94±21.68	55.84±3.5	4.11±2.61*	10.41±5.93*	142.07±84.19	37.26±14.5#	79.56±31.15	12.63±4.95#	80.46±37.3	19.37±8.26#
UDCA	0.71±0.42	1.04±0.15#	0.9±0.4	0.65±0.25	0.53±0.27	0.52±0.27	0.69±0.33	0.63±0.16	1.04±0.24	1.19±0.55#	0.42±0.26	0.36±0.09*	1.7±1.28	0.71±0.18	1.22±0.51	0.34±0.09*#	1.61±0.73	0.51±0.12*#
TCA	58.72±37.3	238.71±132.14#	112.88±63.98	114.98±33.63	652.22±311.71*	462.12±164.13	54.33±27.4	150.4±67.89	42.96±11.28	194.7±102.68#	809.7±372.85*	636.09±283.18	54.03±33.71	75.5±21.42*	89.1±42.79	95.75±62.11	78.46±15.85	146.21±38.6
TCDCA	3.65±1.9	5.19±2.27	3.44±1.44	3.5±0.45	6.99±2.69	10.43±8.11	2.65±1.75	2.47±0.85	1.97±0.32	3.78±1.92#	1.2±0.73	0.71±0.38*	3.58±1.99	6.45±2.98	3.45±1.91	3.37±1.44	3.29±1.35	3.6±1.57
TαMCA	18.34±10.66	26.39±10.96	15.13±7.55	13.03±3.42	50.85±23.1	53.03±21.39	18.04±10.84	15.87±6.28	4.45±1.16	8.98±4.54	5.21±2.75	4.37±3.08*	16.29±10.49	19.96±6.97	24±9.05	16.9±9.24	23.59±17.74	17.78±6.82
TβMCA	287.8±114.5	288.5±14.26	90.14±45.05	65.12±14.22*	135.82±66.78	156.3±62.74	168.7±18.83	136.1±62.58	67.67±15.43	182.9±114.3	16.06±7.55*	13.86±4.56*	150.5±111.3	207.3±78.65	245.1±98.19	139.3±49.42	221.3±75.8	190.5±79.32
TUDCA	9.06±4.5	11.45±5.06	2.85±1.32	2.86±0.37*	3.15±1.24	6.05±1.55#	5.81±2.06	5.5±1.49	3.64±1.42	7±3.59#	1.12±0.6*	0.66±0.51*	4.93±2.75	8.1±2.47#	7.03±2.42	4.94±2.18	8.69±3.76	5.69±0.76
DCA	0.17±0.13	0.56±0.21#	0.26±0.14	0.22±0.08	0.97±0.49*	1.1±1.13	0.35±0.44	0.14±0.08*	0.14±0.08	0.17±0.11*	2.54±0.36*	10.3±7.44	0.33±0.14	0.31±0.1	0.17±0.07	0.14±0.1*	0.07±0.04	0.25±0.13#

LCA	0.13±0.11	0.19±0.12	0.17±0.14	0.14±0.15	0.3±0.13	0.12±0.18	0.13±0.04	0.28±0.18	0.15±0.14	0.21±0.11	0.11±0.04	0.07±0.03	0.74±0.25	0.18±0.13	0.21±0.18	0.37±0.26	0.18±0.07	0.15±0.13
ωMCA	7.84±3.81	11.07±3.43	15.2±6.21	6.3±1.96#	9.1±4.81	2.48±1.12*#	7.52±3.89	3.8±1.95*	4.61±1.5	4.1±1.16*	0.41±0.25*	1.63±1.32*	10.1±5.82	10.68±6.28	11.57±0.87	6.78±3.26#	13.8±2.53	4.45±1.07#
MDCA	1.19±0.78	1.86±0.85#	0.7±0.29	0.48±0.24	0.9±0.53	0.17±0.05#	1.08±0.77	0.83±0.38	0.31±0.05	0.57±0.23*	0.24±0.08	0.13±0.08*	1.6±0.74	1.03±0.34	1.78±0.72	0.57±0.3*#	0.98±0.26	0.55±0.19*
HDCA	0.44±0.38	0.58±0.19	0.83±0.29	0.46±0.3	1.41±0.9	0.33±0.2#	0.72±0.89	0.38±0.13	0.22±0.12	0.25±0.13	0.07±0.01	0.21±0.3	0.76±0.43	0.16±0.1#	0.92±0.56	0.2±0.13#	1.07±0.47	0.59±0.29
TDCA	3.05±1.35	20.04±9.36#	4.77±2.73	4.92±1.54*	22.89±11.62*	20.55±8.63	4.02±0.75	8.24±4.99	1.83±0.33	6.76±3.71*#	165.17±73.5*	84.81±4.95	6.44±2.29	13.68±5#	4.9±0.84	11.02±4.85#	1.37±0.68	6.87±2.36*#
TLCA	0.83±0.31	0.76±0.44	0.32±0.18	0.37±0.13	1.04±0.24	0.75±0.52	0.59±0.24	0.38±0.25	0.19±0.12*	0.25±0.12	0.55±0.14	0.36±0.23	0.76±0.27	1.05±0.39	0.58±0.3	0.42±0.13	0.31±0.09	0.64±0.27#
TωMCA	36.89±17.96	14.14±6.32#	48.52±18.62	27.37±3.9	43.01±7.29*	47.07±23	16.87±12.5	8.73±4.87*#	9.67±7.35	7.79±3.17	0.77±0.47*	0.93±0.63*	14.65±9.44	5.34±2.33#	33.31±8.28	6.82±2.35#	53.71±16.72	33.24±11.96#
TMDCA	9.02±4.55	17.51±13.2	2.31±1	2.2±1.02*	3.65±1.5	6.14±1.57	4.13±1.93	6.08±4.09	0.61±0.08	3.03±1.86#	1.5±0.69*	0.78±0.25*	2.82±1.52	7.28±1.4	7.37±2.71	9.65±4.4#	3.72±1.49	8.31±2.36#
THDCA	3.74±1.67	3.69±1.68	1.74±0.76	1.46±0.51	3.58±0.91	5.36±3.5	2.21±1.36	2.47±1.74	0.43±0.2*	0.74±0.34*	0.14±0.1*	0.08±0.04*	1.23±0.83	0.95±0.24*	3.01±1.08	2.2±1.18	2.75±1.41	5.38±1.92#
Unconjugated BAs	396.3±237.8	640.5±343.55	256.9±149.4	235.8±51.05	950.2±448.65	773.2±261.6*	355.2±264.1	326.3±158.9	166.3±93.79	421.5±223.0	1002.9±395.5	745±32.5.3	323.4±215.5	345.6±110.9	417.8±152.1	290.3±126.2*#	488.1±301	418.2±132.21*#
T-conjugated	56.55±38.88	73.53±8.15	71.8±3.5.57	36.86±16.95	59.16±33.73	19.26±12.32	43.89±20.32	40.6±9.85	59.51±24.25	68.22±3.8.24#	23.97±8.8	40.88±15.49	143.8±106.3	54.21±21.07	106.5±34.94	23.06±8.57	117.8±58.07	29.36±11.11
Total BAs	452.8±275.6	714.0±349.8	328.7±182.2	272.7±62.23*	1009.4±477.2	788.6±256.3	399.1±278.5	358.8±176.2	225.8±117.7	489.7±260.1#	1026.9±394.0	785.9±319.8	467.2±319.8	399.8±123.8	524.3±185.4	313.4±131.6	605.9±356.0	447.5±137.8

All values are expressed as mean ± S.D., and “*” indicates P<0.05 when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#"means P<0.05 statistical significance between

genders fed the same diet.

Table S4. The effects of nine diets on BA-related genes in liver and ileum.

	AIN-93M Purified		Lab chow		Diet-restriction		High fructose		High-fat		Atherogenic		Western		low n-3 FA		EFA Deficient	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Cyp7	1±0.6	1.36±0.	2.1±1	2.07±1.	1.94±0.	3.44±1.7	0.46±0.	0.55±0.	1.49±0.	3.09±1.	0.13±0.11	0.28±0.2	0.73±0.	2.07±1.	1±0.67	2.43±1.	0.59±0	0.64±0.2
a1	7	66		29	96	8*#	31	24	43	92#	*	5*	43	46#		49#	.32	6
Cyp8	1±0.2	1.02±0.	1.81±0.	1.07±0.	0.31±0.	0.28±0.2	0.98±0.	1.02±0.	1.41±0.	1.3±0.8	0.01±0.0	0.2±0.43	0.96±0.	0.7±0.1	0.96±0	0.88±0.	1.26±0	1.08±0.1
b1	6	33	42*	29#	23*	1*	23	29	11		1*	*	15	9	.25	14	.43	8
Cyp2	1±0.1	1.03±0.	1.22±0.	1.21±0.	0.94±0.	1.04±0.1	0.72±0.	0.59±0.	1.07±0.	1.18±0.	0.79±0.1	0.73±0.2	0.96±0.	0.9±0.1	0.91±0	1.01±0.	0.9±0.	0.64±0.0
7a1	4	21	2	16	2	8	08	02*	07	09	3	2	12	7	.09	15	21	6*#
Cyp7	1±0.2	0.2±0.0	1.47±0.	0.3±0.1	0.23±0.	0.13±0.0	0.62±0.	0.1±0.0	1.12±0.	0.18±0.	0.37±0.1	0.12±0.0	0.93±0.	0.17±0.	1.21±0	0.2±0.0	0.83±0	0.1±0.02
b1	1	6#	3*	#	04*	4	23	1#	24	05#	3	7#	17*	05#	.44	4#	.21	#
Cyp3	1±0.2	3.36±0.	1.71±0.	3.22±0.	6.54±1.	8.06±2.9	1.28±0.	2.18±0.	0.81±0.	4.34±3.	1.88±0.5	2.12±0.3	0.66±0.	1.95±0.	0.85±0	4.03±1.	1.62±0	2.88±0.4
9a1	7	87#	53	41	42*	7*#	8	17	15	02#	7		19	53	.19	49#	.25	2
Baat	1±0.1	0.82±0.	1.04±0.	1.09±0.	0.65±0.	0.87±0.1	0.71±0.	0.55±0.	0.99±0.	0.89±0.	0.86±0.1	0.69±0.1	0.9±0.1	0.83±0.	0.91±0	0.99±0.	0.81±0	0.65±0.1
	5	1#	08	26	11*	3#	09*	03	12	07		5#	5	12	.1	1	.2	1
Bacs	1±0.1	1.06±0.	1.1±0.1	1.21±0.	0.99±0.	1.1±0.13	0.81±0.	0.92±0.	1.04±0.	1.3±0.1	0.89±0.2	0.93±0.1	0.94±0.	1.19±0.	0.96±0	1.14±0.	1.01±0	0.92±0.1
	1	11	1	05	24		13	04	06	6#	1	3	1	11#	.07	08	.07	#
Ntcp	1±0.2	1.18±0.	0.83±0.	1.05±0.	1.2±0.2	1.2±0.2	0.76±0.	1.12±0.	0.78±0.	1.21±0.	0.42±0.1	0.63±0.1	0.69±0.	1.03±0.	0.84±0	1.04±0.	0.92±0	1.01±0.1
		21	15	15#	2		12	08#	04	04#	*	8#	08*	12#	.07	1#	.21	3
Oatp1	1±0.2	0.28±0.	0.82±0.	0.24±0.	0.01±0*	0.01±0.0	0.68±0.	0.12±0.	0.78±0.	0.23±0.	0.34±0.2	0.13±0.0	0.84±0.	0.25±0.	0.94±0	0.36±0.	0.83±0	0.2±0.1#
a1	3	13#	24	09#		1*	2*	03#	05	15#	2*	9#	15	08#	.07	14#	.22	
Oatp1	1±0.1	0.91±0.	1.05±0.	1.01±0.	0.75±0.	0.83±0.0	0.74±0.	0.63±0.	1.11±0.	0.93±0.	0.69±0.1	0.67±0.1	0.88±0.	0.79±0.	0.97±0	0.97±0.	0.77±0	0.66±0.0
b2	6	16	18	31	22	9	14	09	09	09	7		11	14	.08	1	.27	9
Oatp2	1±0.1	1.64±0.	1.2±0.2	1.5±0.5	1.4±0.3	1.37±0.0	0.86±0.	1.23±0.	1.21±0.	1.96±0.	1.22±0.2	1.65±0.5	0.94±0.	1.54±0.	0.97±0	1.26±0.	1.13±0	1.08±0.1
b1	9	45#	4		5	8	22	16#	06	15#	6	#	16	34#	.23	28	.18	7

Bsep	1±0.15	1.37±0.27#	1.26±0.42	1.35±0.19	0.57±0.12	0.68±0.22*	0.8±0.09	0.94±0.04	0.84±0.02	1.17±0.22#	1.54±0.22	1.72±0.59	0.82±0.11*	1.05±0.16	0.97±0.13	1.36±0.34	1.02±0.08	0.98±0.1#
Mrp2	1±0.1	0.99±0.19	1.05±0.17	1.1±0.34	1.11±0.13	1.19±0.19	0.78±0.13	0.68±0.08	1.07±0.07	0.91±0.09	1.09±0.07	1.05±0.32	1.01±0.13	0.93±0.15	0.89±0.07	1.07±0.07	0.84±0.13	0.77±0.07
Bcrp	1±0.25	0.36±0.13#	1.17±0.32	0.43±0.13#	0.52±0.04*	0.53±0.19	0.82±0.24	0.29±0.07#	1.23±0.14	0.34±0.04#	1.03±0.08	0.49±0.1#	1.16±0.23	0.37±0.05#	0.86±0.08	0.44±0.13#	1±0.39	0.39±0.08#
Atp8b1	1±0.17	1.06±0.41	1±0.31	0.88±0.17	0.41±0.05*	0.59±0.18*	0.66±0.14*	0.56±0.06	0.73±0.03	0.68±0.06	1.02±0.13	0.91±0.21	0.83±0.16	0.69±0.14	0.95±0.09	1.21±0.24#	1.03±0.29	0.75±0.16#
Ostα	1±0.24	0±0	6.17±4.48	1.7±0.67	7.78±6.44	2.04±2.21	3.25±2.14	0.15±0.33	3.17±1.85	0.07±0.15	N.D.	0.68±0.94	N.D.	N.D.	N.D.	N.D.	N.D.	0.05±0.11
Ostβ	1±0.7	2.57±0.91	3.33±0.94	4.38±3.18	5.45±2.94	14.2±10.18*#	1.3±0.73	2.67±0.73	1.58±0.36	2.74±0.48	16.29±15.19*	13.58±8.97*	0.6±0.25	1.84±0.37	0.75±0.44	4.42±1.81	1.64±0.55	4.5±2.19
Mrp3	1±0.38	1.12±0.45	2.86±0.59*	2.89±0.41*	2.25±0.71*	2.63±0.47*	0.64±0.04	0.71±0.19	2.06±0.29*	1.99±0.53	1.91±0.25	1.96±0.85	1.59±0.53	1.3±0.32	0.67±0.23	1.21±0.41	0.97±0.23	0.69±0.23
Mrp4	1±0.28	1.44±0.31	2.42±0.47	2.68±1.26	3.21±0.67	8.36±2.36*#	0.73±0.2	1.19±0.3	0.91±0.17	1.13±0.38	2.43±2.16	4.53±2.86*#	0.7±0.14	1.25±0.3	0.99±0.39	2.29±1.67	1.18±0.12	1.15±0.17
Star	1±0.29	1.55±0.42#	1.52±0.16	1.17±0.16	1.18±0.21	1.64±0.38#	0.74±0.19	0.94±0.21	1.01±0.29	1.07±0.24	1.14±0.36	1.43±0.46	0.88±0.19	1±0.46	0.94±0.24	1.68±0.71#	1±0.22	0.9±0.33
Abca1	1±0.13	1.29±0.45#	0.93±0.19	0.92±0.09	0.53±0.08*	0.74±0.24*	0.71±0.09	0.71±0.1*	0.86±0.05	0.92±0.06	1.41±0.13	1.65±0.52	1.04±0.1	1.02±0.16	0.89±0.13	1.18±0.27#	1.1±0.12	0.76±0.17*#
Mdr1b	1±0.52	0.58±0.26#	1.61±0.13	0.62±0.25#	0.73±0.32	0.49±0.15	0.98±0.19	0.23±0.02#	1.09±0.16	0.38±0.17#	1.36±0.38	0.73±0.27#	0.75±0.21	0.3±0.2#	1.32±0.57	0.61±0.2#	1±0.34	0.28±0.11#
Mdr2	1±0.18	0.97±0.2	1.12±0.24	1.18±0.34	0.79±0.18	0.97±0.2	0.69±0.05	0.6±0.06	1.27±0.11	1.26±0.18	1.43±0.08	1.39±0.53	1.11±0.19	0.88±0.15	0.84±0.09	1.14±0.29#	0.9±0.27	0.7±0.17
Abcg5	1±0.09	1.67±0.81	0.89±0.16	1.4±0.29	1.78±0.36	2.08±0.39	1.02±0.74	0.97±0.12	0.97±0.15	1.52±0.24	5.56±1.07*	5.29±2.84*	1.99±0.59	2.27±0.67	0.83±0.14	1.61±0.47	1.14±0.24	0.84±0.21

Abcg8	1±0.1	1.3±0.4	0.9±0.1	1.06±0.	1.33±0.	1.6±0.5	1.03±0.	0.81±0.	0.95±0.	1.07±0.	3.84±0.5	3.1±1.35	1.7±0.4	1.58±0.	0.89±0	1.31±0.	1.03±0	0.79±0.1
	1	6	4	29	23		73	13	13	16	2*	*#	3	32	.05	38	.21	6
Fxr	1±0.2	0.99±0.	0.95±0.	1.07±0.	0.92±0.	1.38±0.2	0.59±0.	0.63±0.	0.92±0.	0.94±0.	0.53±0.1	0.68±0.0	0.88±0.	0.9±0.1	0.81±0	1.14±0.	0.95±0	0.81±0.1
	7	32	23	28	29	5#	1	13	1	12	2*	5	17	6	.14	29#	.23	5
Shp	1±0.1	2.12±0.	1.38±0.	1.47±0.	1.24±0.	1.22±0.3	1.14±0.	1.75±0.	1.27±0.	2.25±0.	2.89±0.2	3.79±1.2	1.64±0.	2.22±0.	0.79±0	1.27±0.	1.76±0	1.14±0.2
	7	55#	23	76	87	3	38	28	43	54#	6*	4*#	39	79	.33	33	.49	8
Lrh-1	1±0.1	1.18±0.	1.14±0.	1.26±0.	0.73±0.	0.97±0.1	0.7±0.0	0.71±0.	0.89±0.	0.96±0.	0.83±0.0	0.92±0.2	0.78±0.	0.95±0.	0.9±0.	1.39±0.	1.02±0	0.8±0.08
	5	26	26	26	13	5#	9	03*	07	11	7	6	1	13	16	35#	.2	*
Fgfr4	1±0.1	1.63±0.	1.3±0.2	1.52±0.	0.84±0.	0.83±0.1	0.76±0.	1.17±0.	1.03±0.	1.59±0.	1.3±0.28	1.77±0.6	0.77±0.	1.33±0.	0.92±0	1.16±0.	0.84±0	0.81±0.2
	9	41#	7	17	3	6*	25	28	11	16#		#	12	4#	.33	26#	.22	1*
Lxr	1±0.1	1±0.21	1.05±0.	1.08±0.	0.54±0.	0.74±0.1	0.69±0.	0.69±0.	0.9±0.0	0.9±0.0	0.79±0.0	0.84±0.1	0.82±0.	0.82±0.	0.94±0	1.32±0.	0.9±0.	0.78±0.1
	7		25	28	07*	4	05	09	6	7	7	4	08	09	.16	5#	14	8
Asbt	1±0.1	0.96±0.	1.18±0.	1.04±0.	1.85±0.	1.54±0.4	0.77±0.	0.59±0.	1.04±0.	0.65±0.	0.36±0.0	0.41±0.0	0.46±0.	0.69±0.	0.92±0	1.06±0.	0.83±0	0.77±0.2
	3	25	07	1	05*		08	14	11	21	2*	4	14*	06	.23	09	.15	1
Ostα	1±0.1	1.32±0.	0.72±0.	0.97±0.	1.08±0.	0.9±0.21	0.73±0.	0.84±0.	0.67±0.	0.81±0.	1.17±0.0	1.69±0.2	0.61±0.	1.1±0.1	1.01±0	1.54±0.	0.69±0	1.16±0.2
	6	32	09	11	05		06	14	05	17	7	8	12	6	.23	12	.08	9
Ostβ	1±0.0	1.02±0.	1.2±0.0	1.21±0.	1.26±0.	1.11±0.2	0.85±0.	0.9±0.0	0.94±0.	0.8±0.1	0.97±0.0	1.14±0.0	0.59±0.	0.92±0.	0.98±0	1.31±0.	0.91±0	0.75±0.2
	8	25	5	04	04		08	7	04	1	5	8	09	08	.18	1	.12	2
Fxr	1±0.1	0.9±0.2	1.17±0.	0.98±0.	1.22±0.	1.02±0.2	0.89±0.	0.71±0.	1.06±0.	0.99±0.	0.73±0.0	0.74±0.0	0.65±0.	0.78±0.	0.92±0	1.14±0.	0.82±0	1.33±0.3
		7	05	1	1		11	09	07	17	9	3	11*	1	.18	08	.06	8
Fgf15	1±0.1	2.08±0.	0.24±0.	0.69±0.	0.23±0.	0.17±0.0	0.61±0.	1.33±0.	0.42±0.	1.17±0.	3.9±0.24	5.22±0.4	1.1±0.2	1.77±0.	0.85±0	1.64±0.	0.66±0	2.08±0.6
	3	46#	03*	03*	08*	5*	13*	16*	07	31		6#	1	18	.22	15	.12	4#

Relative mRNA levels were calculated with male controls (male, AIN-93M purified diet) set as 100%. All values are expressed as mean ± S.D. Data were analyzed by two-way ANOVA, and "*" means $P<0.05$ when compared with control (AIN-93M Purified diet) in male and female mice respectively, while "#" indicates $P<0.05$ when data from the two genders fed the same diet are compared.

Table S5. The correlation analysis of BAs (total, conjugated and unconjugated) and BA-related genes involved in the synthesis, metabolism and transport of BAs in the livers of mice fed the 9 diets.

	Male						Female					
	Serum			Liver			Serum			Liver		
	Unconjugated BAs	T-conjugated BAs	Total BAs	Unconjugated BAs	T-conjugated BAs	Total BAs	Unconjugated BAs	T-conjugated BAs	Total BAs	Unconjugated BAs	T-conjugated BAs	Total BAs
Cyp7a1	-0.11	0.1	0.04	0.29	0.01	0.04	-0.22	-0.37	-0.35	-0.34	0.37	0.34
Cyp8b1	-0.52*	-0.56**	-0.6**	0.24	-0.66**	-0.6**	-0.74**	-0.62*	-0.65*	0.06	-0.59*	-0.59*
Cyp27a1	-0.17	-0.08	-0.1	0.44*	-0.17	-0.13	-0.35	-0.45	-0.43	0.11	-0.28	-0.27
Cyp7b1	-0.36	-0.46*	-0.44*	0.26	-0.58**	-0.54*	-0.43	-0.38	-0.39	0.17	-0.61*	-0.6*
Cyp39a1	0.24	.47*	0.42	-0.01	0.65**	0.62**	0.03	-0.23	-0.18	-0.12	0.4	0.39
Baat	-0.15	-0.22	-0.21	0.07	-0.63**	-0.6**	-0.31	-0.37	-0.36	0.03	-0.34	-0.34
Bacs	0.11	0.23	0.21	0.32	-0.01	0.02	-0.41	-0.29	-0.32	0.08	-0.51	-0.51
Ntcp	-0.12	0.13	0.07	-0.02	0.25	0.24	-0.57*	-0.74**	-0.7**	-0.13	-0.1	-0.11
Oatp1a1	-0.32	-0.48*	-0.45*	0.08	-0.72**	-0.7**	-0.3	-0.16	-0.19	0.18	-0.72**	-0.71**
Oatp1b2	-0.21	-0.2	-0.21	0.16	-0.53*	-0.5*	-0.33	-0.37	-0.37	-0.18	-0.36	-0.37
Oatp2b1	0.03	0.21	0.17	0.31	0.29	0.31	0.27	0.28	0.28	0.66*	0.09	0.14
Bsep	0.18	0.02	0.06	0.16	-0.4	-0.37	0.54	0.57*	0.57*	0.41	-0.15	-0.12
Mrp2	0.38	0.49*	0.47*	0.06	0.11	0.11	0.35	0.21	0.24	-0.05	0.18	0.18
Bcrp	-0.14	-0.28	-0.25	-0.11	-0.71**	-0.7**	0.29	0.02	0.08	-0.07	0.2	0.19
Atp8b1	0.08	-0.08	-0.04	0.13	-0.44*	-0.41	0.14	0.07	0.09	0.2	-0.44	-0.43
Ostα	0.04	0.16	0.13	-0.04	0.13	0.12	0.11	0.04	0.06	-0.15	0.72**	0.71**
Ostβ	0.47*	0.46*	0.47*	-0.32	0.36	0.32	0.45	0.19	0.24	0.04	0.39	0.39
Mrp3	0.15	0.28	0.25	0.18	0.05	0.07	-0.05	-0.08	-0.07	0.11	-0.18	-0.17
Mrp4	0.19	0.31	0.29	0.14	0.55*	0.54*	0.36	0.12	0.17	-0.15	0.45	0.44
Star	0.37	0.33	0.35	0.17	-0.05	-0.03	0.29	0.07	0.11	0.09	0.48	0.48

Abca1	0.24	0.06	0.1	0.12	-0.43*	-0.41	0.72**	0.69**	0.7**	0.37	0.09	0.11
Mdr1b	0.02	-0.07	-0.05	0.08	-0.53*	-0.5*	0.47	0.41	0.43	0.14	0.03	0.04
Mdr2	0.26	0.2	0.22	0.02	-0.4	-0.38	0.39	0.3	0.32	0.24	-0.06	-0.05
Abcg5	0.93**	0.85**	0.88**	-0.17	0.37	0.34	0.77**	0.7**	0.72**	0.32	0.37	0.39
Abcg8	0.86**	0.75**	0.78**	-0.14	0.33	0.31	0.87**	0.8**	0.82**	0.18	0.33	0.34
Fxr	-0.04	0.08	0.05	0.02	-0.21	-0.2	-0.16	-0.35	-0.31	-0.27	0.22	0.2
Shp	0.58**	0.55**	0.57**	0.21	0.26	0.26	0.61*	0.62*	0.62*	0.52	0.08	0.12
Lrh-1	-0.02	-0.06	-0.06	0.3	-0.33	-0.29	-0.08	-0.13	-0.12	0.23	-0.32	-0.31
Fgfr4	0.01	0.03	0.02	0.32	-0.26	-0.22	0.48	0.52	0.52	0.54	-0.18	-0.14
Lxr	-0.22	-0.32	-0.3	0.28	-0.51*	-0.47*	-0.19	-0.22	-0.21	0.12	-0.43	-0.42

“*” means significant coefficient <0.05; “**” means significant coefficient <0.05.