Cholestyramine Administration Induces Gut Dysbiosis and Severe Liver Injury in ApoE^{-/-} Mice

Jin Chen

First People's Hospital of Yancheng <https://orcid.org/0000-0003-2675-2780>

Yifan Sun

Fudan University

Yan Luo

Hangzhou Normal University Affiliated Hospital

Xiangsu Li

The First People's Hospital of Yancheng

Zhaoxu Zuo

The First People's Hospital of Yancheng

Dongxue Bian

Yancheng TCM Hospital

Xudong Wu

The First People's Hospital of Yancheng

Shufei Zang

The Fifth People's Hospital of Shanghai

Junping Shi (✉ chenjin0837@126.com)

Hangzhou Normal University Affiliated Hospital <https://orcid.org/0000-0002-8188-4594>

Research

Keywords: cholestyramine, liver injury, obesity, bile acids, intestinal flora

Posted Date: December 17th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-129379/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background & Aim

Bile acids, as hydrophilicity/ hydrophobic molecules and natural ligands of bile acids receptors, are involved in the pathogenesis of liver injury and metabolic disorders. Cholestyramine acts as a bile acid chelating agent and is used to reduce cholesterol in clinic, but its hepatotoxicity and effects on metabolism are still unclear.

Methods

ApoE^{-/-} mice were continuously administered with low fat diet with or without 2% cholestyramine 12 weeks to determine the hepatotoxicity and metabolic influences of cholestyramine on these mice. Serum transaminases, liver histological features and hepatic inflammatory markers expression were used to assess cholestyramine hepatotoxicity. Body weight, liver weight, serum lipids profile and liver lipid metabolism related genes expression were used to evaluate effects of cholestyramine on metabolism. Differences of endogenous bile acids composition, bile acids metabolism related genes and intestinal flora structure between groups were also analyzed by an ultraperformance liquid chromatography coupled to tandem mass spectrometry system, quantitative real time PCR and 16S rDNA sequencing.

Results

After 12 weeks of dietary intervention, although cholestyramine decreased ApoE^{-/-} mice serum cholesterol level, it significantly increased the body and liver weight of these mice, and caused severe liver injury: increased serum transaminases levels, promoted liver vesicular steatosis and inflammatory cells infiltration, and enhanced the expression of lipid synthesis gene: *FASN* and inflammatory markers genes: *TNF-α*, *MCP-1*, *F4/80* and *CD68*. Moreover, cholestyramine administration caused hydrophobic transformation of bile acids pool and severe gut dysbiosis, which were characterized by decreased hydrophilic bile acids: muricholic acid (MCA) and ursodeoxycholic acid (UDCA), and increased abundance of potential pathogenic bacteria: *s_Bacteroides_vulgatus*.

Conclusion

This study revealed that cholestyramine had the ability to induce severe liver injury and metabolic disorders. These effects might be attributed to inducing hydrophobic transformation of bile acids pool and gut dysbiosis by cholestyramine. Furthermore, our study provided a warning of hepatotoxicity and metabolic disorders for clinical application of cholestyramine, and suggested that cholestyramine was not a good scavenging tool to study the role of bile acids alternations in diseases progression.

Introduction

Bile acids are amphoteric metabolic molecules derived from cholesterol. In the liver, at least 17 enzymes are involved in the de novo synthesis of primary bile acids by hepatocytes using cholesterol as the

substrate [1]. Among them, cholesterol 7 α -hydroxylase (CYP7A1) is considered to be the rate limiting enzyme for bile acids synthesis, which determines the size of the bile acids pool [2]. Primary bile acids were synthesized in hepatocytes, which include cholic acid (CA) (human and mice), chenodeoxycholic acid (CDCA) (human and mice) and α/β muricholic acid (MCA) (mice). These bile acids continue to be conjugated with taurine (T) and glycine (G) to form conjugated primary bile acids: T (G) CA, T (G) CDCA, T α/β MCA in the liver [3]. Then, conjugated primary bile acids enter into the intestinal tract through the bile duct system and further form the secondary bile acids: deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) through bacterial biotransformation. About 95% of intestinal bile acids metabolites are reabsorbed by the portal vein circulation system at the jejunum end and ileum, and most of them are reuptake by hepatocytes to repeat the “enterohepatic circulation”.

The number, position and direction of hydroxyl groups in the amphoteric structure of bile acids determine their hydrophilicity/ hydrophobicity. It is reported that DCA, LCA, CDCA and CA are hydrophobic bile acids, while MCA and UDCA are hydrophilic. Hydrophobic bile acids have been proved to be hepatotoxic, resulting in oxidative stress and inflammatory death of hepatocytes [4]. Previous studies have shown that hydrophobic transformation of bile acids pool usually occurred in liver diseases such as nonalcoholic steatohepatitis (NASH), cirrhosis and cholestatic hepatitis [5–7]. In addition, bile acids are also natural ligands of some receptors in vivo, such as the nuclear receptor: farnesoid X receptor (FXR), and G protein coupled receptors: takeda G-protein-coupled receptor 5 (TGR5) and sphingosine-1-phosphate receptor 2 (S1PR2) [8]. The abnormality of these signals has recently been considered to be involved in the pathogenesis of metabolic diseases due to their abilities to regulating glucose and lipid metabolism and inflammatory response. In endogenous bile acids pool, CDCA is the most potent ligand for FXR activation, followed by CA, DCA and LCA, while TaMCA and T β MCA have been identified as natural FXR antagonists; and TCA is a weak agonist of S1PR2; and TGR5 is activated mainly by the secondary bile acids: LCA and DCA [8]. Although bile acids signaling biofunction has been confirmed by many studies, the causal relationship between endogenous bile acids alternations and diseases progression has not been established.

Cholestyramine is a bile acids sequestrant, which is used to treat hypercholesterolemia in clinic [9]. Besides, in basic experiments, it is also used as a method to remove endogenous bile acids in laboratory animals to investigate the role of bile acids in regulating diseases progression [10–11]. However, as a strong CYP7A1 inducer, bile acids may not be removed by cholestyramine since overexpression of CYP7A1 can lead to an enlarged and hydrophobic bile acids pool [12]. Based on the above, we speculate that cholestyramine may cause hepatotoxicity and metabolic disorders by inducing hydrophobic transformation of endogenous bile acids pool and altering bile acids signaling. Therefore, we conducted this study to evaluate the relationship between the use of cholestyramine and liver injury and/ or metabolic disorders in mice.

Methods

Animals

8-week-old male ApoE knockout (ApoE^{-/-}) mice on a C57BL/6 background purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd were divided into two groups according to their diets: the low fat diet (LFD) control group and the cholestyramine supplement (LFD + Chy) group. Mice in groups of LFD and LFD + Chy were fed with a low fat diet (LF10B, Dyets, USA) for 12 weeks and a low fat diet (LF10B, Dyets, USA) supplemented with 2% cholestyramine (C4650, Sigma, USA) for 12 weeks. After 12 weeks feeding, mice were sacrifice after fasted overnight and then serum, liver and intestinal tissues were collected.

Biochemical and histopathologic analyses

Fasting serum samples were obtained from orbital blood and. Mice serum biochemical parameters including total cholesterol (TC), total triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by Hitachi 7180 automatic biochemical analyzer. Formalin-fixed liver paraffin samples were cut into successive 4um sections and stained with hematoxylineosin (HE). The characteristics of liver tissues were observed by the light microscope.

Quantitative real-time PCR (Q-PCR)

RNA was extracted from frozen liver and intestine tissues using TRIzol reagent (190906, Ambion, USA). Complementary DNA was synthesized from 1 mg of total RNA using Transcriptor cDNA Synth. kit (32460620, Roche, GER) following the instruction. Q-PCR was performed using SYBR green PCR master mix (04913914001, Roche, GER) in an ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Each mRNA level was normalized to those of *GAPDH* and expressed as fold change relative to those of control group. Primer sequences used for Q-PCR were listed in Table 1.

Table 1
Primers used in this study.

ID	Forward (5'-3')	Reverse (5'-3')
SREBP1c	CGACTACATCCGCTTCTTGCAG	GCTCTCCAGATGGCGTTGTTCA
SCD1	GCAAGCTCTACACCTGCCTCTT	CGTGCCTTGTAAGTTCTGTGGC
FASN	CACAGTGCTCAAAGGACATGCC	CACCAGGTGTAGTGCCTTCCTC
PPAR- α	TCTGGAAGCTTTGGTTTTGC	TTCGACACTCGATGTTCAAG
TNF- α	CCAGGCGGTGCCTATGTCTC	CAGCCACTCCAGCTGCTCCT
TLR4	AATGAGGACTGGGTGAGAAATGAG	AGACACTACCACAATAACCTTCCG
MCP-1	TCCCAATGAGTAGGCTGGAG	TCTGGACCCATTCTTCTTG
F4/80	TAGTGGAGGCAGTGATGCTC	TATGACCACCAGGACTGGAA
CD68	TCCAAGATCCTCCACTGTTG	ATTTGAATTTGGGCTTGGAG
FXR	CAAAATGACTCAGGAGGAGTACG	GCCTCTCTGTCCTTGATGTATTG
SHP	TCTCTTCTTCCGCCCTATCA	AAGGGCTTGCTGGACAGTTA
CYP7A1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
CYP7B1	TAGCCCTCTTTCCTCCACTCATA	GAACCGATCGAACCTAAATTCCT
CYP27A1	GCCTCACCTATGGGATCTTCA	TCAAAGCCTGACGCAGATG
CYP8B1	GGCTGGCTTCCTGAGCTTATT	ACTTCCTGAACAGCTCATCGG
BACS	ACCCTGGATCAGCTCCTGGAT	GTTCTCAGCTAGCAGCTTGG
BAAT	GGAAACCTGTTAGTTCTCAGGC	GTGGACCCCCATATAGTCTCC
NTCP	ATGACCACCTGCTCCAGCTT	GCCTTTGTAGGGCACCTTGT
OATP	CAGTCTTACGAGTGTGCTCCAGAT	ATGAGGAATACTGCCTCTGAAGTG
BSEP	CTGCCAAGGATGCTAATGCA	CGATGGCTACCCTTTGCTTCT
ASBT	ACCACTTGCTCCACACTCTT	CCCGAGTCAACCCACATCTT
OST α	TGTTCCAGGTGCTTGTCATCC	CCACTGTTAGCCAAGATGGAGAA
OST β	GATGCGGCTCCTTGGAATTA	GGAGGAACATGCTTGTCATGAC
GAPDH	CTCATGACCACAGTCCATGC	CACATTGGGGGTAGGAACAC

Bile acids detection and appendiceal feces 16 s rDNA sequencing

Bile acids were isolated from serum, liver and intestine tissues using acetonitrile/methanol (8:2) mix, and an ultraperformance liquid chromatography coupled to tandem mass spectrometry system (ACQUITY UPLC-Xevo TQ-S; Waters Corp., Milford, Massachusetts) was used to measure bile acids absolute concentration in serum (nM), liver (fmol/mg) and intestine (fmol/mg) as described previously [13]. Each bile acid concentration was then normalized to the ratio of its absolute concentration to total bile acids. Fecal samples were collected from mouse appendix and frozen at -80°C. Bacterial genomic DNA of appendiceal feces was extracted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, USA), and then bacterial 16 s rDNA was amplified by employing BIO-RAD thermal cycler (BIO-RAD Inc., USA). 16 s recombinant DNA sequencing data was generated using Illumina MiSeq (Illumina, San Diego, CA) sequencing. The sequencing data was processed by the ucluster method of Quantitative Insights Into Microbial Ecology (QIIME) (version 1.9.0; <http://qiime.org/index.html>). High similar ($\geq 97\%$) sequences were assigned into the same operational taxonomic units (OUT) and the most abundant sequence was elected as the OTU representative sequence.

Statistical analysis

Student t test was performed to examine significance of measurement data between groups using GraphPad Prism, version 7.00 (GraphPad Software, CA, USA). Similarity of bacterial community structure was conducted by principal co-ordinates analysis (PCoA). LEfSe analysis was performed to identify the potential biomarker intestinal flora. Results were considered to be statistical significant with a P-value < 0.05.

Results

1. Cholestyramine administration induces obesity and severe liver injury in ApoE^{-/-} mice fed with LFD

After 12 weeks of continuous feeding, the effect of cholestyramine on reducing levels of serum TC ($p < 0.001$) and LDL ($p < 0.01$) was confirmed in this study. While, serum TG and HDL levels were not affected by cholestyramine administration (Fig.1 C-F). Interestingly, we found that cholestyramine administration significantly increased body ($p < 0.01$) and liver ($p < 0.001$) weight of these mice (Fig.1 A-B).

Further serological tests showed that levels of liver enzymes: ALT ($p < 0.001$) and AST ($p < 0.01$) levels were much higher in LFD+Chy group than those in LFD group. (Fig.2 A). HE staining of liver tissues showed mice in LFD+Chy group had more severe vesicular steatosis and inflammation compared with these in LFD group (Fig.2 B). Furthermore, we investigated whether cholestyramine administration altered hepatic mRNA expression of lipid metabolism related genes and inflammatory factors genes in these mice. Fig.2 C-D showed that cholestyramine administration significantly promoted hepatic expression of *FASN* ($p < 0.01$), but did not altered the expression of *SREBP1* and *SCD1* and *PPAR- α* ($p > 0.05$) in liver tissues of these mice. Moreover, cholestyramine administration increased the expression of hepatic *TNF- α* ($p < 0.05$) and *MCP-1* ($p < 0.01$) and *F4/80* ($p < 0.05$) and *CD68* ($p < 0.05$) in these mice.

2. Cholestyramine does not remove endogenous bile acids, but induces a hydrophobic bile acid pool in ApoE^{-/-} mice fed with LFD

As shown in Fig.3 A-C, cholestyramine administration did not completely eliminate serum, hepatic and intestinal bile acids. Only hepatic total bile acids level was reduced by cholestyramine administration and there was no differences in serum and intestinal total bile acids between LFD and LFD+Chy groups.

Alterations of bile acids composition in serum, liver and intestine caused by cholestyramine were further analyzed. Bile acids which could be detected were listed as follows: α MCA, β MCA, CA, CDCA, DCA, GCA, HDCA, LCA, TaMCA, T β MCA, TCA, TCDCA, TDCA, THDCA, TUDCA, TwMCA, UDCA, wMCA (serum); α MCA, β MCA, CA, CDCA, DCA, GCA, HCA, HDCA, LCA, TaMCA, T β MCA, TCA, TCDCA, TDCA, THCA, THDCA, TLCA, TUDCA, TwMCA, UDCA, wMCA (liver); CDCA, DCA, GCA, GHCA, GHCA, GUDCA, LCA, TaMCA, T β MCA, TCA, THCA, THDCA, TLCA, TUDCA, TwMCA (intestine). CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA used for comparison in this study were the sum of their free and conjugated types.

Results showed that the percentage of hydrophilic bile acids in serum, liver and intestinal bile acids pools decreased significantly, while the percentage of hydrophobic bile acids increased in the cholestyramine administration group (Fig.4 A-C). Specifically, in the serum bile acids pool, hydrophobic bile acid CA ($p < 0.001$) percentage increased, while the percentages of MCA ($p < 0.001$), UDCA ($p < 0.001$) and HDCA ($p < 0.05$) decreased significantly in the cholestyramine administration group (Fig.4 A). CA ($p < 0.001$) and DCA ($p < 0.05$) were the main elevated hydrophobic bile acids in the liver by cholestyramine administration, and hepatic hydrophilic bile acids: MCA ($p < 0.001$), UDCA ($p < 0.001$), HDCA ($p < 0.01$) and HCA ($p < 0.05$) were also decreased significantly in the cholestyramine administration group (Fig.4 B). In intestinal tissues, CDCA ($p < 0.05$), LCA ($p < 0.01$) and DCA ($p < 0.01$) were the main elevated hydrophobic bile acids, while MCA ($p < 0.001$) and UDCA ($p < 0.001$) were the main decreased hydrophilic bile acids in the cholestyramine administration group (Fig.4 C).

In addition, we also analyzed the expression of molecules involved in bile acids enterohepatic circulation. The results indicated that cholestyramine administration significantly decreased the expression of the bile acid signaling molecule: SHP ($p < 0.05$) in the liver (Fig.5 A), increased the expression of the rate-limiting enzyme of bile acids synthesis: CYP7A1 ($p < 0.05$) (Fig.5 B) and decreased the expression of molecules responsible for hepatic bile acids reuptake: NTCP ($p < 0.001$) and OATP ($p < 0.05$) (Fig.5 C). However, in the intestinal tissue, we did not find that cholestyramine administration had any effects on the expression of molecules responsible for intestinal bile acids transport: ASBT and OST α/β .

3. Cholestyramine administration causes the increase of *Bacteroides* abundance that closely associated with the decreased hydrophilic bile acids in ApoE^{-/-} mice fed with LFD

Considering that bile acids profile and signaling alterations were closely related to gut microbiota, we further evaluated the effect of cholestyramine on intestinal flora in mice. The unweighted and weighted PCoA revealed significant differences in the structure of intestinal flora between cholestyramine intervention and non-intervention groups (Fig.6 A). The cladogram showed that cholestyramine

intervention caused significant changes of *Firmicutes* and *Bacteroides* abundances in phylum, class, order, family, genus and species, especially increased *p_Bacteroidetes*, *c_Bacteroidia*, *o_Bacteroidales*, *f_Bacteroidaceae*, *g_Bacteroides* and *s_Bacteroides_vulgatus* abundances. (Fig.6 B-C). Further analysis showed the correlation between changes of hydrophilic bile acids and *Bacteroides*. As shown in Figure 6, the decrease of hydrophilic bile acids: MCA and UDCA in vivo showed a negative correlation with the rise of *Bacteroides* abundances, especially in the family and genus (Fig.6 D).

Discussion

Previous studies have shown that elimination of endogenous bile acids by cholestyramine could improve liver injury and metabolic abnormalities [11, 14]. Conversely, our study for the first time has demonstrated that cholestyramine was able to induced obesity and promoted severe liver injury in ApoE^{-/-} mice. More importantly, cholestyramine could not remove endogenous bile acids, but induce hydrophobic transformation of endogenous bile acids pool and imbalance of bacterial flora, which were characterized by decreased hydrophilic bile acids: MCA and UDCA percentages in serum, liver and intestine, and increased abundance of pathogenic bacteria: *s_Bacteroides_vulgatus*. These results suggested that hydrophobic toxic bile acid pool formation and the increase of pathogenic bacteria might be the main cause of hepatotoxicity and metabolic disorders caused by cholestyramine in ApoE^{-/-} mice.

Cholestyramine reduced cholesterol mainly by chelating intestinal bile acids. However, this effect would feedback activate liver CYP7A1 to promote bile acids synthesis [15]. In our study, cholestyramine not only upregulated the expression of *CYP7A1*, but also reduced the expression of *NTCP* and *OATP* to inhibit bile acids reuptake by hepatocytes. And, we only found total bile acids level was decreased in the liver rather than the serum and intestine. This might be attributed to insufficient uptake of bile acids by hepatocytes and unchanged intestinal bile acids output. Li et al.'s study [16] indicated that over-expression of CYP7A1 resulted in bile acids pool enlargement and increased hydrophobic bile acids proportions, especially CDCA. In our study, cholestyramine caused the increase of CYP7A1 expression and bile acids pool hydrophobicity. And, this hydrophobic bile acids pool was mainly characterized by decreased proportions of MCA and UDCA. Unlike CYP7A1 overexpression, there might be other mechanisms for explaining the increase of bile acids pools hydrophobicity caused by cholestyramine.

Intestinal flora was responsible for secondary bile acids production. But, the specific bacteria for bile acids metabolism was not clear. We observed a significant decrease in *Bacteroides* abundances in this study: *p_Bacteroidetes*, *c_Bacteroidia*, *o_Bacteroidales*, *f_Bacteroidaceae*, *g_Bacteroides* and *s_Bacteroides_vulgatus* and these decreased *Bacteroides* abundances showed a negative correlation with hydrophilic bile acids: MCA and UDCA. *Bacteroides* have been shown a strong metabolic capacity for primary bile acids in previous studies due to containing bile salt hydrolases [17]. Qi et al. [18] found that elevated *s_Bacteroides_vulgatus* in polycystic ovary syndrome (PCOS) patients was negatively correlated with reduced TUDCA level, and moreover *s_Bacteroides_vulgatus* administration could reduce TUDCA level in mice. Based on the above, besides liver enzymes expression alternations, decreased *Bacteroides*

abundances might be another important reason for the hydrophobicity transformation of bile acids pool caused by cholestyramine administration.

MCA and UDCA were considered to be the most hydrophilic bile acids, which both decreased significantly in our study. Functionally, Denk et al. [19] have shown that MCA could restricted hepatocellular apoptosis induced by GCDCA and palmitate through preserving the mitochondrial membrane potential. Protective effects of UDCA for hepatocytes were realized through a variety of mechanisms: reducing oxidative stress, inhibiting apoptosis, detoxification of cholephilic compounds and hydrophobic bile acids, and immunomodulation [4]. We also observed some hydrophobic bile acids: CA, CDCA, DCA and LCA were increased in circulation, liver or intestine. These hydrophobic bile acids play an important role in inducing oxidative stress, necrosis, apoptosis and plasma membrane damage of hepatocytes [4]. Therefore, we speculated that the formation of hydrophobic bile acids pool might be responsible for the severe liver injury induced by cholestyramine. Small heterodimer partner (SHP) is a unique orphan nuclear receptor and acts as an important factor for regulating metabolism and inflammation [20-21]. In our study, SHP expression was decreased by cholestyramine administration. This might be a reason for cholestyramine inducing obesity and liver inflammation. Gut dysbiosis was closely related with liver injury and metabolic disorders. We found a significant increase in the abundance of *s_Bacteroides_vulgatus* in mice fed with cholestyramine.

Based on the adverse effects of *s_Bacteroides_vulgatus* on inflammation and metabolic abnormalities reported in previous studies [18, 22], we speculated that elevated abundance of *s_Bacteroides_vulgatus* might also participate in liver injury and obesity caused by cholestyramine.

Our research had some limitations: we have not investigated the role of intestinal flora in cholestyramine induced liver injury and obesity phenotype through eliminating intestinal flora; and the function of single bacteria and/ or bile acid in cholestyramine induced phenotype has not been studied in this study.

Conclusion

In summary, our study for the first time confirmed that cholestyramine induced severe liver injury and gut dysbiosis in ApoE^{-/-} mice fed with low fat diet, which not only provided a warning of hepatotoxicity and metabolic disorders for clinical application of cholestyramine, but also suggested that cholestyramine was not a good bile acids scavenger in basic research, and better tools were needed to study the role of bile acids in diseases progression.

Abbreviations

CYP7A1: cholesterol 7 α -hydroxylase

CA: cholic acid

CDCA: chenodeoxycholic acid

MCA: muricholic acid

T: taurine

G: glycine

DCA: deoxycholic acid

LCA: lithocholic acid

UDCA: ursodeoxycholic acid

NASH: nonalcoholic steatohepatitis

FXR: farnesoid X receptor

TGR5: takeda G-protein-coupled receptor 5

S1PR2: sphingosine-1-phosphate receptor 2

LFD: low fat diet

Chy: cholestyramine

TC: total cholesterol

TG: total triglyceride

LDL: low density lipoprotein

HDL: high density lipoprotein

ALT: alanine aminotransferase

AST: aspartate aminotransferase

HE: hematoxylin-eosin

PCoA: principal co-ordinates analysis

SREBP: sterol-regulatory element binding protein

SCD: stearoyl-CoA desaturase

PPAR: peroxisome proliferators-activated receptor

TNF: tumor necrosis factor

MCP: monocyte chemoattractant protein

NTCP: sodium taurocholate co-transporting polypeptide

OATP: organic-anion-transporting polypeptide

ABST: apical sodium dependent bile salt transporter

OST: organic solute transporter

Declarations

Junping Shi, Shufei Zang and Xudong Wu conceived and designed this study. Jin Chen, Yifan Sun, Yan Luo and Dongxue Bian performed the experiments. Jin Chen, Xiangsu Li and Zhaoxu Zuo analyzed the data. Jin Chen wrote the manuscript. Junping Shi and Shufei Zang revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the Natural Science Research Funds of Minhang District, Shanghai (2018MHZ027), Health Profession Clinical Research Funds of Shanghai Municipal Health Commission, (201940295), Scientific Research Project funded by Shanghai Fifth People's Hospital, Fudan University, (2019WYZD02), Natural Science Foundation of China (81700510), Natural Science Foundation of Jiangsu Province (BK20200265) and Medical Research Project of Jiangsu Health Commission (ZDB2020033).

Availability of data and materials

All data and materials used in this study are available when requested.

Ethics approval and consent to participate

The animal study was conducted in accordance with the guidelines of the Laboratory Animal Ethical and Welfare Committee at Hangzhou Normal University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

Not applicable.

References

1. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab.* 2013; 17 (5): 657-69.
2. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov.* 2008; 7 (8): 678-93.
3. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab.* 2016; 24 (1): 41-50.
4. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol.* 2009; 15 (14): 1677-89.
5. Chen J, Zheng M, Liu J, Luo Y, Yang W, Yang J, Liu J, Zhou J, Xu C, Zhao F, Su M, Zang S, Shi J. Ratio of Conjugated Chenodeoxycholic to Muricholic Acids is Associated with Severity of Nonalcoholic Steatohepatitis. *Obesity (Silver Spring).* 2019; 27 (12): 2055-2066.
6. Wang X, Xie G, Zhao A, Zheng X, Huang F, Wang Y, Yao C, Jia W, Liu P. Serum Bile Acids Are Associated with Pathological Progression of Hepatitis B-Induced Cirrhosis. *J Proteome Res.* 2016; 15 (4): 1126-34.
7. Cai SY, Ouyang X, Chen Y, Soroka CJ, Wang J, Mennone A, Wang Y, Mehal WZ, Jain D, Boyer JL. Bile acids initiate cholestatic liver injury by triggering a hepatocyte-specific inflammatory response. *JCI Insight.* 2017; 2 (5): e90780.
8. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease. *Gastroenterology.* 2017; 152 (7): 1679-1694. e3.
9. Kane JP, Malloy MJ. Treatment of hypercholesterolemia. *Med Clin North Am.* 1982; 66 (2): 537-50.
10. Wang Y, Li J, Matye D, Zhang Y, Dennis K, Ding WX, Li T. Bile acids regulate cysteine catabolism and glutathione regeneration to modulate hepatic sensitivity to oxidative injury. *JCI Insight.* 2018; 3 (8): e99676.
11. 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, Jia W. Dysregulated hepatic bile acids collaboratively promote liver carcinogenesis. *Int J Cancer.* 2016; 139 (8): 1764-75.
12. Pandak WM, Schwarz C, Hylemon PB, Mallonee D, Valerie K, Heuman DM, Fisher RA, Redford K, Vlahcevic ZR. Effects of CYP7A1 overexpression on cholesterol and bile acid homeostasis. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281 (4): G878-89.
13. Zheng X, Huang F, Zhao A, Lei S, Zhang Y, Xie G, Chen T, Qu C, Rajani C, Dong B, Li D, Jia W. Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice. *BMC Biol.* 2017; 15 (1): 120.
14. Kobayashi M, Ikegami H, Fujisawa T, Nojima K, Kawabata Y, Noso S, Babaya N, Itoi-Babaya M, Yamaji K, Hiromine Y, Shibata M, Ogiwara T. Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid-binding resin. *Diabetes.* 2007; 56 (1): 239-47.

15. Qiu DN, Shang Q, Sun DY, Ding WQ, Luo ZG, Chen J, Jiang WR, Huang JP, Jiang XY. Cholestyramine alters bile acid amounts and the expression of cholesterol-related genes in rabbit intestinal and hepatic tissues. *J Dig Dis*. 2017; 18 (2): 107-114.
16. Li T, Matozel M, Boehme S, Kong B, Nilsson LM, Guo G, Ellis E, Chiang JY. Overexpression of cholesterol 7 α -hydroxylase promotes hepatic bile acid synthesis and secretion and maintains cholesterol homeostasis. *Hepatology*. 2011; 53 (3): 996-1006.
17. Yao L, Seaton SC, Ndousse-Fetter S, Adhikari AA, DiBenedetto N, Mina AI, Banks AS, Bry L, Devlin AS. A selective gut bacterial bile salt hydrolase alters host metabolism. *Elife*. 2018; 7: e37182.
18. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, Wang L, Zhang Y, Liang X, Wang L, Gonzalez FJ, Patterson AD, Liu H, Mu L, Zhou Z, Zhao Y, Li R, Liu P, Zhong C, Pang Y, Jiang C, Qiao J. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med*. 2019; 25 (8): 1225-1233.
19. Denk GU, Kleiss CP, Wimmer R, Vennegeerts T, Reiter FP, Schulz S, Zischka H, Rust C. Tauro- β -muricholic acid restricts bile acid-induced hepatocellular apoptosis by preserving the mitochondrial membrane potential. *Biochem Biophys Res Commun*. 2012; 424 (4): 758-64.
20. Yuk JM, Jin HS, Jo EK. Small Heterodimer Partner and Innate Immune Regulation. *Endocrinol Metab (Seoul)*. 2016; 31 (1): 17-24.
21. Barranco-Fragoso B, Almeda-Valdes P, Aguilar-Olivos N, Méndez-Sánchez N. The role of small heterodimer partner in hepatic lipid homeostasis. *Ann Hepatol*. 2015; 14 (2): 286-7.
22. Ó Cuív P, de Wouters T, Giri R, Mondot S, Smith WJ, Blottière HM, Begun J, Morrison M. The gut bacterium and pathobiont *Bacteroides vulgatus* activates NF- κ B in a human gut epithelial cell line in a strain and growth phase dependent manner. *Anaerobe*. 2017; 47: 209-217.

Figures

Figure 1

Cholestyramine administration reduces serum total cholesterol (TC) and low density lipoprotein (LDL) levels but induces obesity in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of body weight (A), liver weight (B) and serum lipid (C-F) indicators between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 1

Cholestyramine administration reduces serum total cholesterol (TC) and low density lipoprotein (LDL) levels but induces obesity in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of body weight (A), liver weight (B) and serum lipid (C-F) indicators between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 1

Cholestyramine administration reduces serum total cholesterol (TC) and low density lipoprotein (LDL) levels but induces obesity in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean ± SD showed the differences of body weight (A), liver weight (B) and serum lipid (C-F) indicators between groups. *p < 0.05, **p < 0.01, ***p < 0.001. LFD: low fat diet; Chy: cholestyramine.

Figure 1

Cholestyramine administration reduces serum total cholesterol (TC) and low density lipoprotein (LDL) levels but induces obesity in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of body weight (A), liver weight (B) and serum lipid (C-F) indicators between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 1

Cholestyramine administration reduces serum total cholesterol (TC) and low density lipoprotein (LDL) levels but induces obesity in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean ± SD showed the differences of body weight (A), liver weight (B) and serum lipid (C-F) indicators between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 2

Cholestyramine administration induces severe liver injury in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of serum transaminases (A), hepatic lipid metabolism genes (C) and inflammatory genes (D) between groups. HE staining showed the histological difference of liver tissues (B) between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 2

Cholestyramine administration induces severe liver injury in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of serum transaminases (A), hepatic lipid metabolism genes (C) and inflammatory genes (D) between groups. HE staining showed the histological difference of liver tissues (B) between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 2

Cholestyramine administration induces severe liver injury in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of serum transaminases (A), hepatic lipid metabolism genes (C) and inflammatory genes (D) between groups. HE staining showed the histological difference of liver tissues (B) between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 2

Cholestyramine administration induces severe liver injury in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of serum transaminases (A), hepatic lipid metabolism genes (C) and inflammatory genes (D) between groups. HE staining showed the histological difference of liver tissues (B) between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 2

Cholestyramine administration induces severe liver injury in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of serum transaminases (A), hepatic lipid metabolism genes (C) and inflammatory genes (D) between groups. HE staining showed the histological difference of liver tissues (B) between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 3

The effect of cholestyramine administration on total bile acids levels. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of total bile acids levels in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 3

The effect of cholestyramine administration on total bile acids levels. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of total bile acids levels in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 3

The effect of cholestyramine administration on total bile acids levels. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of total bile acids levels in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 3

The effect of cholestyramine administration on total bile acids levels. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of total bile acids levels in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 3

The effect of cholestyramine administration on total bile acids levels. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of total bile acids levels in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 4

Cholestyramine administration altered the composition of bile acids pool in ApoE^{-/-} mice fed with LFD. Pie charts drawn with mean showed the profile changes of bile acids pool composition between two

groups in serum (A), liver (B) and intestine (C). Scatter and bar plots drawn with mean $\pm$ SD showed the differences of CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA between two groups in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine. CA: cholic acids; CDCA: chenodeoxycholic acids; LCA: lithocholic acids, DCA: deoxycholic acids, MCA: muricholic acids, UDCA: ursodeoxycholic acids, HCA: hyocholic acids; HDCA: hyodeoxycholic acids.

Figure 4

Cholestyramine administration altered the composition of bile acids pool in ApoE^{-/-} mice fed with LFD. Pie charts drawn with mean showed the profile changes of bile acids pool composition between two groups in serum (A), liver (B) and intestine (C). Scatter and bar plots drawn with mean $\pm$ SD showed the differences of CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA between two groups in serum (A), liver (B) and intestine (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine. CA: cholic acids; CDCA: chenodeoxycholic acids; LCA: lithocholic acids, DCA: deoxycholic acids, MCA: muricholic acids, UDCA: ursodeoxycholic acids, HCA: hyocholic acids; HDCA: hyodeoxycholic acids.

Figure 4

Cholestyramine administration altered the composition of bile acids pool in ApoE^{-/-} mice fed with LFD. Pie charts drawn with mean showed the profile changes of bile acids pool composition between two groups in serum (A), liver (B) and intestine (C). Scatter and bar plots drawn with mean $\pm$ SD showed the differences of CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA between two groups in serum (A), liver (B) and intestine (C). *p < 0.05, **p < 0.01, ***p < 0.001. LFD: low fat diet; Chy: cholestyramine. CA: cholic acids; CDCA: chenodeoxycholic acids; LCA: lithocholic acids, DCA: deoxycholic acids, MCA: muricholic acids, UDCA: ursodeoxycholic acids, HCA: hyocholic acids; HDCA: hyodeoxycholic acids.

Figure 4

Cholestyramine administration altered the composition of bile acids pool in ApoE^{-/-} mice fed with LFD. Pie charts drawn with mean showed the profile changes of bile acids pool composition between two groups in serum (A), liver (B) and intestine (C). Scatter and bar plots drawn with mean $\pm$ SD showed the differences of CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA between two groups in serum (A), liver (B) and intestine (C). *p < 0.05, **p < 0.01, ***p < 0.001. LFD: low fat diet; Chy: cholestyramine. CA: cholic acids; CDCA: chenodeoxycholic acids; LCA: lithocholic acids, DCA: deoxycholic acids, MCA: muricholic acids, UDCA: ursodeoxycholic acids, HCA: hyocholic acids; HDCA: hyodeoxycholic acids.

Figure 4

Cholestyramine administration altered the composition of bile acids pool in ApoE^{-/-} mice fed with LFD. Pie charts drawn with mean showed the profile changes of bile acids pool composition between two groups in serum (A), liver (B) and intestine (C). Scatter and bar plots drawn with mean $\pm$ SD showed the differences of CA, CDCA, LCA, DCA, MCA, UDCA, HCA and HDCA between two groups in serum (A), liver (B) and intestine (C). *p < 0.05, **p < 0.01, ***p < 0.001. LFD: low fat diet; Chy: cholestyramine. CA: cholic acids; CDCA: chenodeoxycholic acids; LCA: lithocholic acids, DCA: deoxycholic acids, MCA: muricholic acids, UDCA: ursodeoxycholic acids, HCA: hyocholic acids; HDCA: hyodeoxycholic acids.

Figure 5

Cholestyramine administration altered the expression of bile acids metabolism related genes in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of FXR signaling molecules mRNA expression (A), hepatic bile acids transport associated proteins mRNA expression (B) and intestinal bile acids transport associated proteins mRNA expression (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 5

Cholestyramine administration altered the expression of bile acids metabolism related genes in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of FXR signaling molecules mRNA expression (A), hepatic bile acids transport associated proteins mRNA expression (B) and intestinal bile acids transport associated proteins mRNA expression (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 5

Cholestyramine administration altered the expression of bile acids metabolism related genes in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of FXR signaling molecules mRNA expression (A), hepatic bile acids transport associated proteins mRNA expression (B) and intestinal bile acids transport associated proteins mRNA expression (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 5

Cholestyramine administration altered the expression of bile acids metabolism related genes in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of FXR signaling molecules mRNA expression (A), hepatic bile acids transport associated proteins mRNA expression (B) and intestinal bile acids transport associated proteins mRNA expression (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 5

Cholestyramine administration altered the expression of bile acids metabolism related genes in ApoE^{-/-} mice fed with LFD. Scatter and bar plots drawn with mean $\pm$ SD showed the differences of FXR signaling molecules mRNA expression (A), hepatic bile acids transport associated proteins mRNA expression (B) and intestinal bile acids transport associated proteins mRNA expression (C). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. LFD: low fat diet; Chy: cholestyramine.

Figure 6

Cholestyramine administration induces gut dysbiosis in ApoE^{-/-} mice fed with LFD. PCoA scatter plots reflected differences of intestinal flora profile between groups (A); Cladogram and LDA bar plots showed intestinal flora biomarkers between groups (B-C); The correlation's matrix drawn with correlation coefficient showed the correlation between differential Bacteroides and hydrophilic bile acids (D). LFD: low fat diet; Chy: cholestyramine; PCoA: principal co-ordinates analysis; LDA: linear discriminant analysis.

Figure 6

Cholestyramine administration induces gut dysbiosis in ApoE^{-/-} mice fed with LFD. PCoA scatter plots reflected differences of intestinal flora profile between groups (A); Cladogram and LDA bar plots showed intestinal flora biomarkers between groups (B-C); The correlation's matrix drawn with correlation coefficient showed the correlation between differential Bacteroides and hydrophilic bile acids (D). LFD: low fat diet; Chy: cholestyramine; PCoA: principal co-ordinates analysis; LDA: linear discriminant analysis.

Figure 6

Cholestyramine administration induces gut dysbiosis in ApoE^{-/-} mice fed with LFD. PCoA scatter plots reflected differences of intestinal flora profile between groups (A); Cladogram and LDA bar plots showed intestinal flora biomarkers between groups (B-C); The correlation's matrix drawn with correlation coefficient showed the correlation between differential Bacteroides and hydrophilic bile acids (D). LFD: low fat diet; Chy: cholestyramine; PCoA: principal co-ordinates analysis; LDA: linear discriminant analysis.

Figure 6

Cholestyramine administration induces gut dysbiosis in ApoE^{-/-} mice fed with LFD. PCoA scatter plots reflected differences of intestinal flora profile between groups (A); Cladogram and LDA bar plots showed intestinal flora biomarkers between groups (B-C); The correlation's matrix drawn with correlation coefficient showed the correlation between differential Bacteroides and hydrophilic bile acids (D). LFD: low fat diet; Chy: cholestyramine; PCoA: principal co-ordinates analysis; LDA: linear discriminant analysis.

Figure 6

Cholestyramine administration induces gut dysbiosis in ApoE^{-/-} mice fed with LFD. PCoA scatter plots reflected differences of intestinal flora profile between groups (A); Cladogram and LDA bar plots showed intestinal flora biomarkers between groups (B-C); The correlation's matrix drawn with correlation coefficient showed the correlation between differential Bacteroides and hydrophilic bile acids (D). LFD: low fat diet; Chy: cholestyramine; PCoA: principal co-ordinates analysis; LDA: linear discriminant analysis.