Gut Microbiota-Regulated Bile Acids Metabolism Features In The Aging Process In Mice

Duosheng Luo

Guangdong Pharmaceutical University

Kechun Chen

Guangdong Pharmaceutical University

Yating Zhao

Guangdong Pharmaceutical University

Lexun Wang

Guangdong Pharmaceutical University

Yifan Yin

Guangdong Pharmaceutical University

Ling Yang

Guangdong Pharmaceutical University

Xianglu Rong

Guangdong Pharmaceutical University

Jiao Guo (✉ gyguoyz@163.com)

Guangdong Pharmaceutical University

Research Article

Keywords: Aging, gut microbiota, bile acid composition

Posted Date: February 23rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1371784/v1>

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

[Read Full License](#)

Abstract

Aging is usually characterized with imbalanced gut microbiota and bile acids (BAs) dyshomeostasis. However, the gut microbiota-regulated BA metabolism features in the aging process remains unclear. To establish a direct link between gut microbiota and BA profile changes in the liver, plasma and intestinal contents. UPLC-MS/MS, 16S rDNA sequencing, Western blotting, and HE staining methods were used. The plasma content of TC, TG and LDL-C gradually increased during aging, the *Lactobacillus*, *Bacteroides*, *Bacillus*, *Ruminococcus*, *Blautia* and *Streptococcus* which can produce BSH were increased. The concentration of total BAs was increased, especially unconjugated BAs, and the ratio of unconjugated BAs to conjugated BAs was increased. The expression of bile acid transporter receptors and intestinal tight junction proteins were significantly decreased. The presented data revealed that the changes of BA profile mediated by gut microbiota were closely related to aging, and highlights that prevention and treatment of aging-related disorders, especially disorders of lipid metabolism by targeting gut microbiota-regulated BAs metabolism should be paid more attention.

In the current study, we established a direct link between gut microbiota and BA profile changes in the liver, plasma, and intestinal contents, and detected protein expression of bile acid transporter receptors bile salt export pump (BSEP), apical sodium-dependent bile acid transporter (ASBT), multidrug resistance-associated protein 2 (MRP2) and intestinal tight junction proteins zonula occludens-1 (ZO-1), Occludin and Claudin-5 during aging. The content of TC, TG and LDL-C in plasma gradually increased during aging, the *Lactobacillus*, *Bacteroides*, *Bacillus*, *Ruminococcus*, *Blautia*, and *Streptococcus* which can produce bacterial enzymes bile salt hydrolase (BSH) were increased. The concentration of total BAs in intestinal contents and plasma was increased, especially the content of unconjugated BAs, and the ratio of unconjugated BAs to conjugated BAs was increased in liver, plasma and intestinal contents. The expression of BSEP and MRP2 in the liver and ASBT in the intestinal were significantly decreased. Meanwhile, the expression of ZO-1, Occludin, and Claudin-5 were decreased. The presented data reveals that the changes of BA profile mediated by gut microbiota was closely related to aging, and highlights that the prevention and treatment of aging-related disorders, especially disorders of lipid metabolism by targeting gut microbiota-regulated BAs metabolism would become a promising therapy for the prevention and treatment of aging.

1. Introduction

Aging is a progressive, systemic and multifaceted process of degradation in the later stage of life. In recent years, many aging-related diseases such as cardiovascular diseases and Alzheimer's disease have been found closely related to gut microbiota [1–3]. However, the relationship between gut microbiota and aging has not been studied clearly. Recently, Barcena et al. [4] found that gut microbiota transplantation could increase the life span of progeroid mice, indicating the connection between aging and gut microbiota providing a strategy of age-related diseases intervention based on gut microbiota.

BAs are synthesized from cholesterol in hepatocyte and pass into the small intestine where they promote the intestinal absorption of dietary lipids before reabsorption into the liver by enterohepatic circulation or excretion in the feces. BAs play an important role in promoting lipid digestion and absorption, regulating cholesterol balance. In the liver, hepatic enzymes generate unconjugated and conjugated primary BAs [5]. In the intestine, BAs restrict bacterial proliferation, whereas bacterial bile salt hydrolase (BSH) and 7 α -dehydroxylase deconjugate BAs and convert primary BAs into secondary BAs, respectively [6]. Meanwhile, some hydrophobic bile acids, for example deoxycholic acid (DCA), also have a direct inhibitory effect on the growth of bacteria and play an important role on regulating the structure and composition of gut microbiome [7]. Accordingly, gut microbiota and BA profile are closely related to aging, but the characteristics of gut microbiota-regulated BAs metabolism in the aging process have not been reported.

In our current study, we performed a systemic investigation on BA pool profiles in liver, plasma and intestinal contents as well as the composition of gut microbiota in the aging process. Moreover, we evaluated the expression of bile acid transporter and tight junction protein that affect the hepatoenteric circulation of BAs respectively. Our results showed that aging caused gut microbiota disorders and comprehensively dysregulated BAs homeostasis. Therefore, our current study highlights that gut microbiota is a potential target for prevention and treatment of aging-related disorders, especially disorders of lipid metabolism by restoring BAs homeostasis.

2. Materials And Methods

2.1 Animals and experimental design

Specific pathogen free (SPF) C57BL/6JNarl male mice (25 ± 5 g) of 3–4 weeks old were purchased from Guangdong Medical Laboratory Animal Center. The mice were housed in a temperature-controlled room at $24 \pm 2^\circ\text{C}$, with a humidity of 60% – 70%, 12 h of light and darkness alternated, standard solid food and water were provided during the experiment. The study was reviewed and approved by the Animal Ethical Committee of Guangdong pharmaceutical University (SPF2017079).

The mice were raised to 4-month-old, 12-month-old and 18-month-old respectively with 8 animals in each group. Feces were collected in SPF feeding environment one day before mice were sacrificed. Overnight fasted mice were sacrificed after anesthesia with 1% pentobarbital sodium solution by intraperitoneal injection. Samples were collected and immediately frozen at -80°C for further analysis.

2.2 Pathological examination of liver and intestine

The tissues were fixed in 4% paraformaldehyde. Paraffin-embedded samples were processed to obtain 4 μm thick sections. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy. Images were captured by using the PerkinElmer Automated Quantitative Pathology System (PerkinElmer, Inc.).

The liver and jejunum with a length of 1.5 cm was fixed in 2% glutaraldehyde solution for JEM1010 (JEOL) electron microscope.

2.3 Biochemical indexes measurement

The concentrations of triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma were analyzed using kits (Shanghai Rongsheng Biopharmaceutical Co., Ltd., according to the manufacturers protocols.

2.4 Protein Extraction and Western Blotting

The detailed procedure has been described in our previous report [8]. Primary antibodies included rabbit anti-ZO-1, rabbit anti-claudin-5, rabbit anti-ASBT, rabbit anti-BSEP, rabbit anti-MRP2, and mouse anti-GAPDH. Second antibodies included HRP-donkey anti-mouse IgG and HRP-donkey anti-rabbit IgG.

2.5 BA extraction and quantification

50 μ L plasma samples or 20 mg ($\pm$ 1 mg) of liver or intestinal content samples added internal standard with a steel ball were homogenate, then were shaken at 2,500 rpm for 10 min, and refrigerated at -20 °C for 10 min. Then the samples were centrifuged at 12,000 rpm for 10 min. Subsequently, the supernatant was reconstituted with 100 μ L 50% methanol water resolution then was transferred into a vial subjected for LC-MS analysis.

An ACQUITY UPLC HSS T3 column(1.8 μ m 2.1 $\times$ 100 mm; Waters, USA)was used at 40°C column temperature. The injection volume was 3 μ L in both positive and negative ion mode. The mobile phase consisted of 0.1% formic acid and 5 mM ammonium acetate (Merck, Germany) in water as solvent A and 0.1% formic acid in acetonitrile (Merck, Germany) as solvent B in gradient elution mode (0 ~ 0.5 min, 95% A; 0.5 ~ 4.5 min, A 60%; 4.5 ~ 7.5 min, A 50%; A 7.5 ~ 10 min, 20%; 10 ~ 12 min, 90% A). The flow rate of the mobile phase was 0.4 mL $\cdot$ min⁻¹.

The mass spectrometric parameters were as follows: Electrospray ionized-ion source (ESI), multi-reaction scanning (MRM), ionized model; EPI scanning range; M/z: 50–640; Scanning speed: 1000 da/s; Curtain Gas: 35 PSI; Ion Source Gas 1:40 PSI; Ion Source Gas 2: 40 PSI; Temperature: 550 °C.

2.6 16S rDNA sequencing

Fecal bacterial DNA extraction, 16S rDNA gene amplification and subsequent 16S rDNA gene analysis were performed by Gene Denovo Biotechnology Company (Guangzhou, China). The experimental procedures were performed as previously described [9, 10]. All α diversity indexes were calculated in QIIME [11] (version 1.9.1; <http://qiime.org>). Shannon rarefaction curves, Operational taxonomic units (OTU) rarefaction curve and rank abundance curves were plotted in R project ggplot2 package [12] (v2.2.1).

2.7 Statistical Analysis

Data are shown as means $\pm$ SD unless otherwise noted. All the bar plots in this study were generated with Prism 6.0 (GraphPad, La Jolla, CA, USA). Differences between groups were calculated by Mann-Whitney U test or Kruskal-Wallis H test

using SPSS 24.0 (IBM, SPSS, USA). In addition, the *P* value was adjusted using Benjamini-Hochberg to control the multiple testing false discovery rate in the analysis of bile acids and the differential bacteria. Statistical differences were considered significant at a *P* < 0.05.

3. Results

3.1 Increased blood lipids and abnormal liver function during aging

The body weight of mice increased from 3 month to 12 month, and reached the peak at 12 month. After that, the food intake gradually decreased, and the body weight also decreased (Fig. 1A). TC, TG and LDL-C gradually increased significantly in plasma during aging (Fig. 1B). AST and ALT content also increased during aging (Fig. 1B).

3.2 Aging causes structural abnormalities of hepatic and the small intestine

The results showed the central hepatic vein dilated and blood extravasated, the cytoplasm of hepatocytes becomes loose and inflammatory cells increased from H&E staining, partial cytoplasmic cavitation occurs in hepatocytes. Moreover, a few collagen fibers were observed in jejunum under electron microscope, the microvilli were shorter at 18 month compared with 4 month, the mitochondria were swollen, and phagocytes of different shapes can be viewed in jejunum electron microscopy. As an important digestive organ of the body, the intestinal tract is the primary place for alimentation. Inevitably, changes in intestinal tissue morphology will lead to intestinal functional abnormalities, which will affect the formation of intestinal mucosal barrier and intestinal bacterial translocation (Fig. 1C-D).

3.3 Aging alters BAs profile in liver, intestinal contents and plasma

A targeted metabolomics method of BAs was established to detect the BAs in liver, intestinal contents and plasma as shown in Fig. 2. With the increase of age, the content of total BA increased significantly in intestinal contents and plasma, especially the content of unconjugated BAs. Compared with the 4 month-old, CA in the liver of the 18 month-old was significantly increased (*P* < 0.05), while the conjugated BA such as TDCA and GDCA were significantly increased (*P* < 0.05). The levels of DCA and LCA increased significantly with age in plasma (*P* < 0.05), and TDCA, TUDCA, TCA and T- β MCA increased significantly (*P* < 0.05). The proportion of unconjugated BAs to conjugated BAs increased in liver, plasma and

intestinal contents, among which there were significant differences in intestinal contents with 1.19 fold and 2.83 fold in plasma respectively (Fig. 2).

3.4 Aging alters gut microbiota composition

Gut microbiota composition were distinct at different ages according to Principle coordinate analysis (PCoA) (Fig. 3A). The proportion of different bacteria differ widely. *Bacteroides* and *Firmicutes* were the two most common phylum, the proportion of *Bacteroidetes* increased and then declined, while *Firmicutes* declined and then increased. And we found the abundance of *Proteobacteria* and *Cyanobacteria* in 18 month-old mice increased, while the *Verrucomicrobia* decreased which consistent with previous studies in progeroid mice [4], as shown in Fig. 3B.

In LEfSe analysis, the abundance of *Akkermansia*, *Verrucomicrobia* and *Ruminococcaceae_UCG* were significant greater, while *Clostridium* was less in the 4-month-group compared to 18-month-group (Fig. 3C). These bacteria are closely related to health. *Lactobacillus*, *Bacteroides*, *Bacillus*, *Ruminococcus*, *Blautia*, *Enterococcus*, *Clostridium*, *Lactococcus*, *Streptococcus*, *Bifidobacterium* are the main bacterium that produces BSH, which can hydrolyze and bind bile salts to form unconjugated BAs [13]. We found that the above bacteria increased during aging (Fig. 3D). The correlation between BAs and gut microbiota also showed that most of the bacteria producing BSH were in direct proportion to unconjugated BAs (Fig. 3E).

3.5 Aging alters expression of bile acid transporter receptors and intestinal tight junction proteins

Bile acid transporters and intestinal mucosal barriers also affect the enterohepatic circulation of BAs. With aging, the bile acid transporter BSEP and MRP2, the intestinal tight junction proteins ZO-1, occludin and claudin-5 significantly decreased, indicating that the intestinal mucosal barrier function of bile acid weakened during the aging process (Fig. 3F-G).

4. Discussion

Our results showed that plasma levels of TC, TG, LDL-C and ALT significantly increased with age, which was consistent with our previous research reports [44].

Gut microbiota plays a vital role in regulating host metabolism, and its composition changes throughout the life cycle [14]. The aging of the body is usually accompanied by decreasing in the diversity of gut microbiota, and increasing in proteolytic bacteria. Specifically, the *Bifidobacteria*, *Bacteroidetes*, *Enterobacteria*, *Firmicides* and *Streptococcus* decrease significantly [15]. In the study, the diversity of gut microbiota decreased during aging, and PCoA analysis for different ages achieved a good separation. Mariat et al. [16] proposed that the most significant change feature of gut microbiota in the elderly was the *Firmicobacteria/Bacteroidetes* ratio, it was associated with metabolic diseases [17]. The study found that the content of *Bacteroidetes* showed a trend of increasing and then decreasing, while *Firmicutes*

showed a opposite trend. It was also found that the content of *Proteobacteria* increased significantly with the aging which can make the intestinal wall more permeable, allowing toxins to enter the bloodstream, causing a variety of adverse reactions, such as inflammatory bowel disease, obesity, diabetes, etc [18]. These results suggest that the imbalance of gut microbiota plays an important role in the occurrence and development of metabolic disorders.

Compared with 4 month-old mice, the content of *Akkermansia* and *Bifidobacterium* in 18 month-old mice decreased relatively, while *Lactobacillus*, *Bacteroides* and *Ruminococcaceae_ucg-014* related to intestinal diseases were increased, which was consistent with previous reports [19]. Our results showed that the relative abundance of *Bifidobacterium* increases in 12 month-old mice and decreases in 18 month-old mice, which was consistent with the evidence of the decrease of *Bifidobacterium* in elderly mice and humans [20]. The abundance of *Akkermansia* decreases significantly during aging, *Akkermansia* may play a key role in maintaining the barrier function of the digestive tract, controlling the metabolism and fat storage [21]. Gut microbiota has an important influence on the production of secondary conjugated bile acids and BSH converts conjugated BAs into unconjugated BAs [22]. The activity of BSH is mainly attributed to *Lactobacillus* and *Bifidobacteria* [23]. Our results showed that the relative abundance of major bacteria producing BSH, including *Lactobacillus*, *Bacteroides*, *Bacillus*, *Ruminococcus*, *Blautia*, *Enterococcus*, *Clostridium*, *Lactococcus* and *Streptococcus*, increased in the aging mice, indicating that gut microbiota changed and the activity of BSH enzyme increased which promoted the deconjugated BA in the small intestine, resulting in the content of unconjugated BAs increased during aging.

The biological functions of BAs are diverse and related to their concentration and tissue distribution [24]. Our results shows that the total concentration of BAs remain relatively constant in the liver, while in plasma and intestine contents are increased during aging, suggested that the reduction of BAs reabsorption in the small intestine leads to an increase in intestine contents and plasma that consistent with Ma previously reported [25]. The beneficial or adverse effects of BAs are also related to the degree of hydrophobicity or hydrophilicity [26]. We found the proportion of unconjugated BAs to conjugated BAs increased with 1.19 fold in intestine contents and 2.83 fold in plasma during aging, resulting in increasing of hydrophobicity of BAs which can lead to DNA damage, apoptosis and necrosis, and increasing the occurrence of age-related diseases because of cytotoxicity and the production of reactive oxygen species [27]. Furthermore, it is well known that the concentration of secondary BAs in feces is associated with the incidence of colon cancer [28]. The present study found that the major secondary BAs such as UDCA and LCA increased with age in intestine contents.

Meanwhile, BAs also function as signaling molecules regulating glucose, lipid and energy metabolism through BA receptors such as nuclear farnesoid X receptor (FXR) and membrane Takeda G protein receptor 5 (TGR5) [29]. Intestine-specific Fxr-null mice are resistant to diet-induced steatohepatitis, obesity and insulin resistance [30]. The present study found that higher levels of T- β -MCA, TUDCA and other conjugated BAs in intestine contents of elderly mice may lead to decreasing FXR activity. Therefore, the increased conjugated BAs level in elderly mice presented beneficial effects, which may indicate that BAs composition and compensate for disease susceptibility are initiated during aging [34]. However, compare

with 4-month-old mice, the ratio of unconjugated BAs to conjugated BAs was increased significantly, and unconjugated BAs such as CA, CDCA, DCA, LCA and UDCA in plasma, liver and intestine contents were generally increased in 18-month-old mice. CA and DCA was increased by 1.88 fold and 2.46 fold in liver respectively. CA, CDCA, DCA and LCA in plasma were increased by 2.71 fold, 5.46 fold, 1.82 fold and 3.16 fold respectively. CDCA and LCA in intestine contents increased 1.45 fold and 1.19 fold respectively. The above unconjugated BAs are effective endogenous ligand of FXR, which can activate the intestinal FXR-FGF15 signaling pathway and cause cholesterol metabolism disorder. Ileum FXR signaling induced by ampicillin was reduced by oral CA rather than TCA, indicating the importance of unconjugated BAs for FXR activation [31]. In our study, the unconjugated BAs were increased in the intestine contents might due to BSH activity improved which may further activate the FXR-FGF15 signaling axis in the intestine and liver, causing the increase of TC and LDL-C in plasma and liver.

As is known to us all, 95% BAs is effectively absorbed in the ileum, hepatocytes release BAs into the gallbladder through BSEP and MRP2. Therefore, BAs in the gallbladder is higher than that in hepatocytes [32]. The ASBT is responsible for the reabsorption of unconjugated BAs at the distal ileum [33]. In the study, the expression of BSEP and MRP2 in the liver was decreased significantly, and ASBT controlling the reabsorption in the intestinal was also significantly decreased with aging. The decrease of bile acid transport and intestinal reabsorption and the increase of bile salt accumulation in hepatocytes may lead to liver injury, which is consistent with the pathological results.

Meanwhile, intestinal barrier function is closely related to aging [34]. The physical changes of intestinal epithelial mucosal barrier play an important role in the migration of gut microbiota [35]. Man reported that the imbalance of gut microbiota caused by aging is often accompanied by the increase of intestinal permeability, which result in gut microbiota and their metabolites entering the blood circulation and causing systemic inflammation [36]. Our previous research found that the levels of TNF- α , IL-6 and COX-2 in the serum and small intestine of elderly mice increased significantly [37]. The results of this study also showed that during the aging process, the expression of intestinal tight junction proteins ZO-1, occludin and claudin-5 decreased, resulting in a weakened intestinal mechanical barrier and increased intestinal wall permeability, which was consistent with intestinal pathological results.

In summary, the study reveals the gut microbiota-regulated BA metabolism features in the aging mice, highlighting the potential of targeting gut microbiota to regulate the BA metabolic axis to prevent or treat age-related diseases. But the different BA profile in mice and humans alter signaling via bile acid receptors, so the transfer of results from mice to humans should be treated with caution.

Declarations

Author Contributions Statement

GJ, RX and LD were responsible for the conception and design of the study; LD, CC, ZY, WL,YY and YL for the data collection, analysis, image processing and writing the manuscript. All authors read and approved the final manuscript.

Data availability statement

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

Conflict of interest

The authors declare that they have no competing interests.

Funding declaration

This study was supported by a grant from the National Natural Science Foundation of China (Grant No. 81530102, 2018; No. 81202619, 2020), and a research project with the Guangdong Province Natural Science Fund (Grant No. 2020A1515010245).

References

1. Kazemian, N., Mahmoudi, M., Halperin, F., Wu J. C., Pakpour S. (2020) Gut microbiota and cardiovascular disease: opportunities and challenges. *Microbiome*. 8(1), 36.
2. MahmoudianDehkordi, S., Arnold, M., Nho, K., Ahmad, S., Jia, W. et al. (2019) Altered bile acid profile associates with cognitive impairment in Alzheimer's disease-An emerging role for gut microbiome. *Alzheimers Dement*, 15(1), 76–92.
3. Nho, K., Kueider-Paisley, A., MahmoudianDehkordi, S., Arnold, M., Risacher, S. L. et al. (2019) Altered bile acid profile in mild cognitive impairment and Alzheimer's disease: Relationship to neuroimaging and CSF biomarkers. *Alzheimers Dement*, 15(2), 232–244.
4. Bárcena, C., Valdés-Mas, R., Mayoral, P., Garabaya, C., Durand, S. et al. (2019) Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nat Med*, 25(8), 1234–1242.
5. de Aguiar Vallim, T. Q., Tarling, E. J., Edwards, P. A. (2013) Pleiotropic roles of bile acids in metabolism. *Cell Metab*, 17(5), 657–69.
6. Wahlström, A., Sayin, S. I., Marschall, H. U., Bäckhed, F. (2016) Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab*, 24(1), 41–50.
7. Sousa, T., Castro, R. E., Pinto, S. N., Coutinho, A., Lucas, S. D. et al. (2015) Deoxycholic acid modulates cell death signaling through changes in mitochondrial membrane properties. *J Lipid Res*, 56(11), 2158–71.
8. Lei, Z., Yang, L., Yang, Y., Yang, J., Niu, Z. et al. (2020) Activation of Wnt/ β -catenin pathway causes insulin resistance and increases lipogenesis in HepG2 cells via regulation of endoplasmic reticulum stress. *Biochem Biophys Res Commun*, 526(3), 764–771.
9. Yang, Y., Yang, F., Huang, M., Wu, H., Yang, C. et al. (2019) Fatty liver and alteration of the gut microbiome induced by diallyl disulfide. *Int J Mol Med*, 44(5), 1908–1920.

10. Yang, Y., Lei, Z., Huang, L., Yang, F., Zhang, J. (2017) Antitumor ability of berberine accompanied by modulation of gut microbiome in sarcoma-180 tumor-bearing mice. *International Journal of Pharmacology*, 14(4), 460–470.
11. Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*, 7(5), 335–6.
12. Cedric, & Ginestet. (2011). Ggplot2: elegant graphics for data analysis. *Journal of the Royal Statistical Society: Series A (Statistics in Society)*.
13. Song, Z., Cai, Y., Lao, X., Wang, X., Lin, X. et al. (2019) Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome*, 7(1), 9.
14. Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R. et al. (2016) Gut Microbiota and Extreme Longevity. *Curr Biol*, 26(11), 1480–5.
15. Bischoff, S. C. (2016) Microbiota and aging. *Curr Opin Clin Nutr Metab Care*, 19(1), 26–30.
16. Mariat, D., Firmesse, O., Levenez, F., Guimarães V., Sokol, H. et al. (2009) The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol*, 9(undefined), 123.
17. Cani, P. D., Delzenne, N. M. (2009) The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des*, 15(13), 1546–58.
18. Fransen, F., van Beek, A. A., Borghuis, T., Aidy, S. E., Hugenholtz, F. et al. (2017) Aged Gut Microbiota Contributes to Systemical Inflammation after Transfer to Germ-Free Mice. *Front Immunol*, 8(undefined), 1385.
19. Wang, F., Yu, T., Huang, G., Cai, D., Liang, X. et al. (2015) Gut Microbiota Community and Its Assembly Associated with Age and Diet in Chinese Centenarians. *J Microbiol Biotechnol*, 25(8), 1195–204.
20. Ni, Y., Yang, X., Zheng, L., Wang, Z., Wu, L. et al. (2019) *Lactobacillus* and *Bifidobacterium* Improves Physiological Function and Cognitive Ability in Aged Mice by the Regulation of Gut Microbiota. *Mol Nutr Food Res*, 63(22), e1900603.
21. Sakamoto, M., Takagaki, A., Matsumoto, K., Kato, Y., Goto, K. et al. (2009) *Butyricimonas synergistica* gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov., butyric acid-producing bacteria in the family 'Porphyromonadaceae' isolated from rat faeces. *Int J Syst Evol Microbiol*, 59(null), 1748–53.
22. Jia, W., Xie, G., Jia, W. (2018) Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol*, 15(2), 111–128.
23. Huang, F., Zheng, X., Ma, X., Jiang, R., Zhou, W. et al. (2019) Theabrownin from Pu-erh tea attenuates hypercholesterolemia via modulation of gut microbiota and bile acid metabolism. *Nat Commun*, 10(1), 4971.
24. de Aguiar Vallim, T. Q., Tarling, E. J., Edwards, P. A. (2013) Pleiotropic roles of bile acids in metabolism. *Cell Metab*, 17(5), 657–69.

25. Ma, J., Hong, Y., Zheng, N., Xie, G., Lyu, Y. et al.(2020). Gut microbiota remodeling reverses aging-associated inflammation and dysregulation of systemic bile acid homeostasis in mice sex-specifically. *Gut Microbes*, 11(5), 1450–1474.
26. Fu, Z. D., Csanaky, I. L., Klaassen, C. D. (2012) Gender-divergent profile of bile acid homeostasis during aging of mice. *PLoS One*, 7(3), e32551.
27. Yui, S., Kanamoto, R., Saeki, T. (2009) Biphasic regulation of cell death and survival by hydrophobic bile acids in HCT116 cells. *Nutr Cancer*, 61(3), 374–80.
28. Hill M. J. (1983) Bile, bacteria and bowel cancer. *Gut*, 24(10), 871–5.
29. Wang, H., Venkatesh, M., Li, H., Goetz, R., Mukherjee, S. et al. (2011) Pregnane X receptor activation induces FGF19-dependent tumor aggressiveness in humans and mice. *J Clin Invest*, 121(8), 3220–32.
30. Li, F., Jiang, C., Krausz, K. W., Li, Y., Albert, I. et al. (2013) Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun*, 4(undefined), 2384.
31. Kuribayashi, H., Miyata, M., Yamakawa, H., Yoshinari, K., Yamazoe, Y. (2012) Enterobacteria-mediated deconjugation of taurocholic acid enhances ileal farnesoid X receptor signaling. *Eur J Pharmacol*, 697(null), 132–8.
32. Martinez-Augustin, O., Sanchez de Medina, F. (2008) Intestinal bile acid physiology and pathophysiology. *World J Gastroenterol*, 14(37), 5630–40.
33. Dietschy, J. M., Turley, S. n D. (2002) Control of cholesterol turnover in the mouse. *J Biol Chem*, 277(6), 3801–4.
34. Kühn, F., Adiliaghdam, F., Cavallaro, P. M., Hamarneh, S. R., Tsurumi, A. et al. (2020) Intestinal alkaline phosphatase targets the gut barrier to prevent aging. *JCI Insight*, 5(6), undefined.
35. Yeung, C. Y., Chiang Chiau, J. S., Chan, W. T., Jiang, C. B., Cheng, M. L. et al. (2013) In vitro prevention of salmonella lipopolysaccharide-induced damages in epithelial barrier function by various lactobacillus strains. *Gastroenterol Res Pract*, 2013(undefined), 973209.
36. Man, A. L., Bertelli, E., Rentini, S., Regoli, M., Briars, G. et al. (2015) Age-associated modifications of intestinal permeability and innate immunity in human small intestine. *Clin Sci (Lond)*, 129(7), 515–27.
37. Luo, D., Li, J., Chen, K., Yin, Y., Guo, J. (2019) Study on metabolic trajectory of liver aging and the effect of fufang zhenzhu tiaozhi on aging mice. *Frontiers in Pharmacology*, 10, 926.

Figures

Figure 1

(A-C) Food intake and body weight, biochemical indexes and Histological changes in mice (100 ×). (A) Food intake and body weight. (B) TG, TC, LDL-C content and AST, ALT levels in plasma. (C) liver, jejunum and colon pathology H&E staining. (D) liver, jejunum and colon electron microscope.

All data were expressed as the means $\pm$ SD (n = 6-8 per group). * $P < 0.05$, ** $P < 0.01$ compared with 4-month-old group, # $P < 0.05$, ## $P < 0.01$ compared with 12-month-old group.

Figure 2

(A-C) BA profile in the liver, plasma, and intestine content. Total bile acids, ratios of conjugated to unconjugated, and concentrations of individual BAs in liver (A), plasma (B) and intestine content (C). Values are means $\pm$ SD (n = 6- 8 per group). * $p < 0.05$, ** $p < 0.01$ compared with 4-month-old group, # $P < 0.05$, ## $P < 0.01$ compared with 12-month-old group.

Figure 3

Aging induces gut microbiota disbalance and alters expression of bile acid transporter receptors and intestinal tight junction proteins. (A) PCoA plot based on the OTU matrix of mice gut microbiota in different age. (B) Bacterial classification at the phylum level. (C) Significant differences in bacterial abundance. Significant differences were identified by LefSe analysis as a $P < 0.05$. The threshold logarithmic LDA score was 2.0. (D) Relative abundance of BSH-producing microbe at genus level. (E) Spearman correlations of the relative abundance of differential microbial species selected by the LDA effect size method and the concentrations of BAs among the samples from 4-month-old group and 18-month-old group. (F) Expression of bile acid transporter receptors BSEP, MRP2. (G) Expression of intestinal proteins ZO-1, claudin-5, occludin and ASBT. Values are means $\pm$ SD ($n = 3$ per group). $*p < 0.05$, $**p < 0.01$ compared with 4-month-old group, $\#P < 0.05$, $##P < 0.01$ compared with 12-month-old group.

Figure 4

Gut microbiota-regulated BA metabolism features in the aging process in mice. 18-month-old mice had dramatic changes in gut microbiota composition and BA profiles compared with 4-month-old. The *Lactobacillus*, *Bacteroides*, *Bacillus*, *Ruminococcus*, *Blautia* and *Streptococcus* which can produce BSH were significantly increased. Meanwhile, the ratio of unconjugated BAs to conjugated BAs was increased, and the concentrations of unconjugated BAs such as CA, CDCA, DCA, LCA and UDCA in plasma, liver and intestine content were generally increased. The expression of BSEP, MRP2 and ASBT were significantly decreased, ZO-1, occludin and claudin-5 decreased. In summary, the changes of BA profile mediated by gut microbiota was closely related to aging-related disorders, especially disorders of lipid metabolism. Red and blue represent increased and decreased gene expression and BAs levels in 18-month-old mice when compared with 4-month-old mice, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [file.docx](#)