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Applied nutritional investigation

Fucoidan and galactooligosaccharides ameliorate high-fat diet–induced dyslipidemia in rats by modulating the gut microbiota and bile acid metabolism

Qichao Chen B.S. [a,1], Min Liu B.S. [b,1], Pengyu Zhang B.S. [a], Shujun Fan Ph.D. [c], Jinli Huang B.S. [a], Shunying Yu B.S. [b], Caihua Zhang Ph.D. [d], Huajun Li Ph.D. [a,*]

[a] Department of Microecology, College of Basic Medical Sciences, Dalian Medical University, Dalian, PR China
[b] Dalian Medical University, Dalian, PR China
[c] Department of Pathology, College of Basic Medical Sciences, Dalian Medical University, Dalian, PR China
[d] Department of Pathophysiology, College of Basic Medical Sciences, Dalian Medical University, Dalian, PR China

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ABSTRACT

Objectives: Dyslipidemia is an important risk factor for cardiovascular diseases. Fucoidan (FUC) is a polysaccharide extracted from brown marine algae with various biological activities. Galactooligosaccharides (GOS) are important prebiotics that exert benefits on the intestinal microbiota. The aim of this study was to investigate the effects of FUC and GOS on dyslipidemia in rats by modulating the gut microbiota and bile acid metabolism.

Methods: Twenty-four male inbred Sprague-Dawley (SD) rats aged 8 wk were fed a normal or high-fat diet (HFD) for 8 wk. During the feeding period, rats were gavaged with normal saline solution, FUC solution (100 mg/kg),or GOS solution (800 mg/kg), or a combination of both once daily. Serum biochemical parameters were determined, and the gut microbiota were analyzed via 16S rRNA gene sequencing. Bile salt hydrolase (BSH) activity in the small intestinal contents was also analyzed. The effects of FUC and GOS on *Lactobacillus casei* DM8121 were analyzed in vitro.

Results: In rats, GOS and FUC supplementation significantly improved serum total cholesterol, low-density lipoprotein cholesterol, lipopolysaccharide, serum total bile acid, hepatic tissue steatosis, aortic arch injury, gut microbiota, serum high-density lipoprotein cholesterol, cholesterol 7-alpha hydroxylase expression in the liver, and BSH activity in the small intestinal contents. In an in vitro experiment, GOS and FUC supplementation significantly increased *L. casei* DM8121's BSH activity.

Conclusions: In rats, FUC and GOS supplementation improved serum dyslipidemia, gut microbiota, BSH activity, and bile acid metabolism–related pathways. In vitro, GOS and FUC supplementation increased *L. casei* DM8121's BSH activity.

Introduction

Dyslipidemia refers to abnormal lipid levels in the blood, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerides (TGs), and total cholesterol (TC) [1]. Furthermore, LDL-C and HDL-C levels are considered biological predictors of cardiovascular disease [2]. A prospective experiment showed that cardiovascular events will increase by 23% annually in China between 2010 and 2030 owing to blood pressure, TC, diabetes, and active smoking [3].

Bile acids (BAs) are primarily synthesized from cholesterol in the liver and are the chief components of bile. BA metabolism involves a complex series of reactions. First, cholesterol is converted to conjugated BAs via the "classic" and "alternative" pathways in the liver [4]. Next, the conjugated BAs are converted into unconjugated BAs under the action of bile salt hydrolase (BSH) in the small intestine [5]. BSH enzymes convert conjugated BAs into unconjugated BAs by cleaving the amino acid side chains of glycine- and taurine-conjugated BAs [6,7]. Most BSH enzymes are produced by gram-positive bacteria, but

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* Corresponding author: Tel.: +86 0411 86110305; Fax: +86 0411 86110282.
E-mail address: lhjcmu@hotmail.com (H. Li).

[1] QC and ML contributed equally to this work.

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some are expressed by gram-negative bacteria [6]. BSHs have been characterized from species of *Enterobacter, Enterococcus, Bacteroides, Clostridium, Lactobacillus, Bifidobacterium*, and *Listeria* [8–13]. A study showed that BSH-producing *L. plantarum* strains significantly reduced LDL-C and serum TGs in Sprague-Dawley (SD) rats [8]. In addition, BAs have lipid-digestive functions and have emerged as novel metabolic modulators [14]. For example, tauro-beta-muricholic acid affects intestinal farnesoid X receptor (FXR) activity [15]. Farnesoid X receptors play important roles in metabolizing sugars and lipids in the host [16]. Therefore, BSH enzymes play important roles in host lipid metabolism.

The gut microbiota consists of trillions of symbiotic prokaryotes, and the bacterial density gradually increases from the stomach to the colon [17]. Two phyla dominate the gut microbiota: *Bacteroidetes* and *Firmicutes*. The number of other bacteria (*Clostridium* and *Proteobacteria*) is relatively few [18]. The gut microbiota participates in the host's nutrient metabolism [19], responding to environmental factors and affecting the host's metabolic and immune activities [20]. Gut microbiota genes outnumber human genes by at least two orders of magnitude, and these gene products deeply affect the host's metabolism [21].

As dietary supplements, prebiotics cannot be digested and assimilated by the host but can selectively promote beneficial bacterial growth [22]. Prebiotics can enhance body health by promoting beneficial bacterial growth, inhibiting harmful bacterial growth, and inducing the gut microbiota to produce special small molecules [23]. More importantly, prebiotics play important roles in lipid metabolism [24]. Galactooligosaccharides (GOS) are an important class of dietary prebiotics [25]. Several studies have shown that GOS can modulate the gut microbiota and relieve dyslipidemia in mice and rats [26,27]. Fucoidan (FUC) is a natural bioactive ingredient in functional foods. FUC extracted from *Miyeokgui* showed antitumor activity against HeLa (cervical cancer), PC-3 (prostate cancer), A549 (alveolar carcinoma), and HepG2 (hepatocellular carcinoma) cells in vitro [28]. Recent experiments demonstrated that orally administering FUC extracted from *Laminaria japonica* modulated lipid metabolism in C57BL/6 mice [29]. In a previous study, FUC extracted from *Undaria pinnatifida* alleviated dyslipidemia in BALB/c mice [30].

In the present study, we investigated the effects of FUC and GOS on dyslipidemia in SD rats by modulating the gut microbiota and bile acid metabolism.

Material and methods

Animals and diets

Twenty-four male inbred SD rats aged 8 wk were obtained from Dalian Medical University. Dyslipidemia was induced in the SD rats by feeding them a high-fat diet (80.3% basal diet, 10% lard, 4% cholesterol, 5% sucrose, 0.2% propylthiouracil, and 0.5% bile salts). FUC was extracted from *Undaria pinnatifida* (Dalian Haibao Biotechnology Co., Ltd., Dalian, China). FUC contains 27.8% sulfate and 20.3% fucose and weighs ~309,000 Da. FUC's major framework is α-1,3-linked-L-fucose-4-sulfate. The GOS were produced from lactose (Quantum Hi-Tech Bio Co., Ltd., Jiangmen, China) and contained trisaccharides (37.7%), tetrasaccharides (21.7%), disaccharides (18.58%), and pentose (12.1%). GOS molecular weights range from 300 to 2000 Da.

After 1 wk of adaptation, the rats were randomly divided into the normal (NFD), high-fat diet (HFD), FUC, and GOS groups, with six per group. GOS and FUC were each dissolved in normal saline (0.9% sodium chloride). The NFD and HFD groups were orally gavaged with normal saline solution once daily for 8 wk; the FUC group was orally gavaged with FUC solution (100 mg/kg) once daily for 8 wk, and the GOS group was orally gavaged with GOS solution (800 mg/kg) once daily for 8 wk. The NFD group was fed a normal diet. The HFD, FUC, and GOS groups were fed HFDs. All rats had access to water and food during the experiment. The Animal Ethics Committee of Dalian Medical University approved the protocols.

All rats were sacrificed after being anesthetized in week 8. Blood samples were collected and centrifuged at 3000*g* for 15 min to obtain serum. The cecal contents, small intestinal contents, cecal segments, small intestinal segments, large liver lobes, and serum were stored at −80°C. The aortic arches and small liver lobes were fixed in 10% formaldehyde.

Biochemical analysis

Lipid-related indicators included TC, TG, LDL-C, and total bile acid (TBA). TC was measured via the glycerol cholesterol oxidase-peroxidase method. TG was measured via the glycerol phosphate oxidase-peroxidase method. LDL-C was measured by the direct method (Nanjing Jiancheng Bioengineering Institute, China). TBA, lipopolysaccharide (LPS), and HDL-C were measured using enzyme-linked immunosorbent assay (Shanghai Langton Biological Technology Co., Ltd., China).

Histological analysis of the livers and aortic arches

After fixation in 10% formaldehyde, paraffin-embedded liver tissue and aortic arches were sectioned (5 μm) and used for hematoxylin and eosin (HE) staining. Sections were photographed at 100 × magnification using optical microscopy (Nikon Instruments Co., Ltd., Japan).

DNA extraction and high-throughput 16S rRNA gene sequencing

Genomic DNA was isolated from the frozen cecal contents using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Doraville, GA, USA). Polymerase chain reaction (PCR) was performed on the V3-V4 hypervariable region of the 16S rRNA gene using universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR amplicons were sequenced using Illumina MiSeq at Shanghai Personal Biotechnology Co., Ltd. Sequence samples with >97% similarity in their 16S rRNA gene sequences were defined as one operational taxonomic unit (OTU). Alpha diversities (Shannon and Simpson) and richness (ACE and Chao1) were obtained via QIIME analysis of the OTUs from each sample. Beta diversity was determined using OTUs from each sample. Gut microbiota compositions of the groups at different levels (phylum and genus) were analyzed using MUSCLE software. Mothur software and Metastats statistical algorithms were used to compare the relative bacterial abundances at the phylum and genus levels [31], and significant differences in taxonomic compositions were analyzed via the Kruskal–Wallis test. Linear discriminant analysis (LDA) used nonparametric Kruskal–Wallis and Wilcoxon rank-sum tests to screen key community members.

Analysis of BSH activity in the intestinal contents

Intestinal contents (0.5 g) were dissolved in 1 mL phosphate-buffered saline and 5 mL extract buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCL, and 1 mM $MgSO_4$). After vortexing, the bacteria were lysed using an ultrasonic disrupter (95V, 18s intervals, 5s disruption, 60 times), then centrifuged at 14 000*g* for 30 min at 4°C to obtain the supernatants (crude enzyme). Protein concentrations of the supernatants were measured per the manufacturer's instructions (Beyotime Biotechnology Institute, China). Samples of known concentrations were added sequentially according to the reaction, and every sample included a blank control. The products were centrifuged at 14 000*g* for 10 min. The amino acid concentrations in the supernatants were measured using ninhydrin colorimetry. The BSH enzymatic activity of the deconjugated taurocolic acid was expressed as the number of amino acids dissociated by 1 mg of crude enzyme in 1 min [16]. The relationship between the bacteria and BSH enzyme activity was examined using Spearman's rank correlation test.

RT-PCR analysis

Total RNA was isolated from the frozen livers using the E.Z.N.A. Total RNA Kit (Omega Bio-Tek). Reverse transcriptase PCR was performed per the Prime Script II 1st Stand cDNA Synthesis Kit. Supplementary Table 1 lists the primer sequences. For β-actin and cholesterol 7-alpha hydroxylase (CYP7A1), each cycle consisted of 10 s at 98°C, 30 s at 55°C, and 6 s at 72°C. PCR products after 30 cycles were electrophoresed in 2% agarose gel. The results were analyzed using Quantity One image analysis software (version 4.6.1, Bio-Rad, Hercules, CA, USA).

Western blot analysis

Frozen liver tissue was homogenized using a glass homogenizer with a tissue protein extraction kit per the manufacturer's instructions (KeyGEN Biotech Co., Ltd., Jiangsu, China). The samples were centrifuged at 15 000*g* for 10 min at 4°C to obtain the supernatants. Protein concentration was measured per the manufacturer's instructions (Beyotime Biotechnology Institute, China). Primary antibodies (CYP7A1, glyceraldehyde 3-phophate dehydrogenase) and horseradish peroxidase were purchased from Boster Biotechnology Co., Ltd., China.

Analysis of L. casei *DM8121 growth and BSH enzyme activity in vitro*

L. casei DM8121 was obtained from Dalian Medical University. The strain was incubated in aseptic de Man Rogosa and Sharpe (MRS) medium (Beijing Aoboxing Bio-tech Co., Ltd., China) three times for experimental use. Special sugar-free MRS supplemented with 0.5% (w/v) sodium salt of tauro-deoxycholic acid (Sigma,

Table 1
Alpha diversity of gut microbiota in different groups

Group	NFD	HFD	FUC	GOS
Simpson	0.97 ± 0.01	0.93 ± 0.03	0.96 ± 0.02	0.96 ± 0.01
Chao1	835.46 ± 89.11	872.44 ± 136.41	967.82 ± 84.74	850.31 ± 157.31
ACE	952.22 ± 89.19	907.46 ± 150.24	997.82 ± 58.89	897.99 ± 153.27
Shannon	7.38 ± 0.19	6.41 ± 0.23*	7.10 ± 1.12	6.68 ± 0.61

FUC, fucoidan; GOS, galactooligosaccharide; HFD, high-fat diet; NFD, normal fat diet.
Results are presented as the means ± SD.
*$P < 0.05$ vs the NFD group.

Ronkonkoma, NY, USA) was autoclaved at 121°C for 15 min and stored at 4°C until use. Glucose (20 g/L), FUC (20 g/L), and GOS (20 g/L) were dissolved in special sugar-free MRS broth to obtain three distinct MRS broths (M-GLU, M-FUC, and M-GOS). DM8121 was inoculated into the M-FUC, M-GOS, and M-GLU broths with an inoculum size of 1% and incubated anaerobically at 37°C for 24 h. Colony-forming units (CFUs) were determined from the samples at different time points (6, 12, 18, and 24 h). Growth rates were calculated for the CFUs. The DM8121 strain's BSH enzyme activity was analyzed in the broths as previously described [16].

Statistical analysis

All values are expressed as the mean ± SD; all statistical analyses were performed using SPSS version 20 (IBM, Armonk, NY, USA). The biochemistry results were compared using one-way analysis of variance. The gut microbiota was analyzed using the Kruskal–Wallis test. The relationship between bacteria and BSH enzyme activity was examined via Spearman's rank correlation test. $P < 0.05$ was considered statistically significant.

Results

Effects of FUC and GOS on serum lipids and TBA

HDL-C was significantly increased in the GOS and FUC groups compared with the HFD group ($P < 0.05$; Fig. 1A). TC, LDL-C, and TBA were significantly increased ($P < 0.05$) in the HFD group compared with the NFD group (Fig. 1B, D, E). TC and LDL-C were significantly increased ($P < 0.05$) in the GOS and FUC groups compared with the NFD group (Fig. 1B, E). However, the GOS and FUC groups showed significant decreases ($P < 0.05$) in TC, LDL-C, and TBA compared with the HFD group (Fig. 1B, D, E). TG did not differ among groups (Fig. 1C).

Effects of FUC and GOS on serum LPS

LPS was significantly increased ($P < 0.05$) in the HFD group compared with the NFD group and significantly decreased ($P < 0.05$) in the GOS and FUC groups compared with the HFD group (Fig. 1F).

Effects of FUC and GOS on alpha and beta diversity of the cecal microbiota

The Venn diagram shows that 3277 OTUs were common to all four groups: 1487 were exclusive to the NFD group, 1384 were exclusive to the HFD group, 1811 were exclusive to the FUC group, and 1462 were exclusive to the GOS group. Interestingly, 483 OTUs were shared between the NFD and HFD groups, 691 OTUs were shared between the NFD and FUC groups, and 557 OTUs were shared between the NFD and FUC groups. Only 324 OTUs were shared among all four groups (Fig. 2A). No significant differences were found in the observed species between groups (Fig. 2B). The Simpson and ACE indices revealed no significant difference ($P > 0.05$) between the HFD and NFD groups, but the

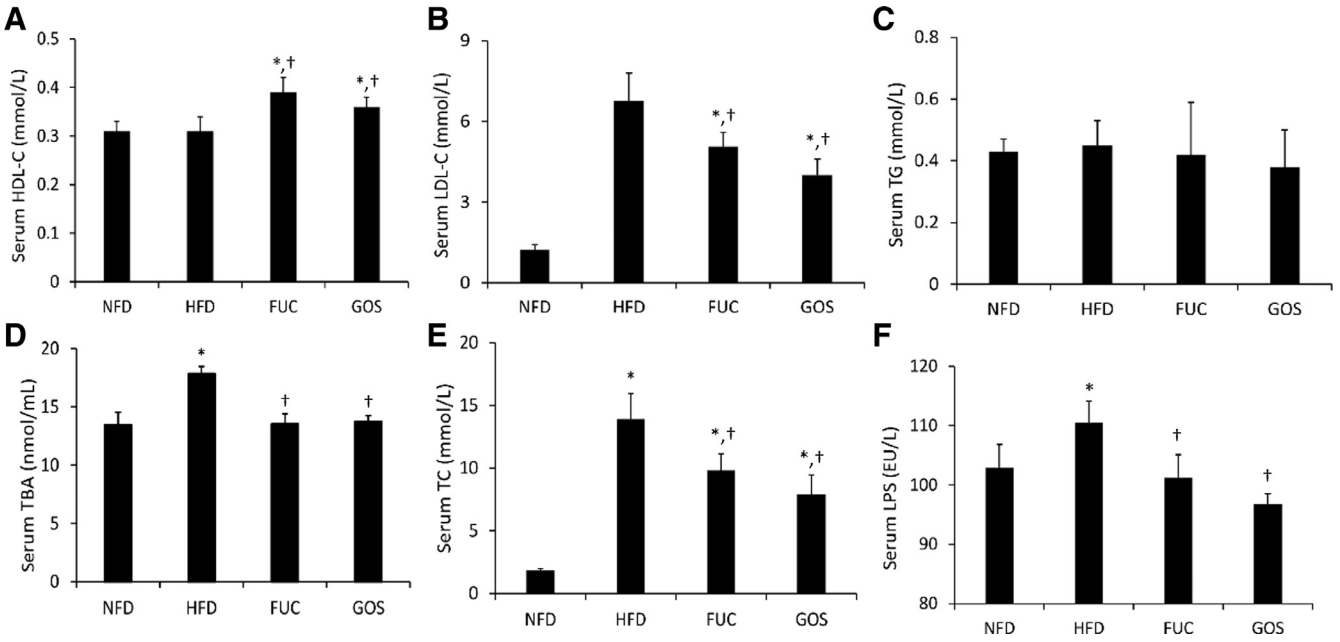

Fig. 1. **(A)** HDL-C in serum, (**B**) LDL-C in serum, (**C**) TG in serum, (**D**) TBA in serum, (**E**) TC in serum, and (**F**) LPS in serum. Values are means ± SD. *$P < 0.05$ vs NFD group. †$P < 0.05$ vs HFD group. The data were submitted to analysis of variance followed by one-way ANOVA test. ANOVA, analysis of variance; FUC, fucoidan; GOS, galactoogligosaccharide; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharide; NFD, normal fat diet; TBA total bile acid; TC, total cholesterol; TG triacylglyceride.

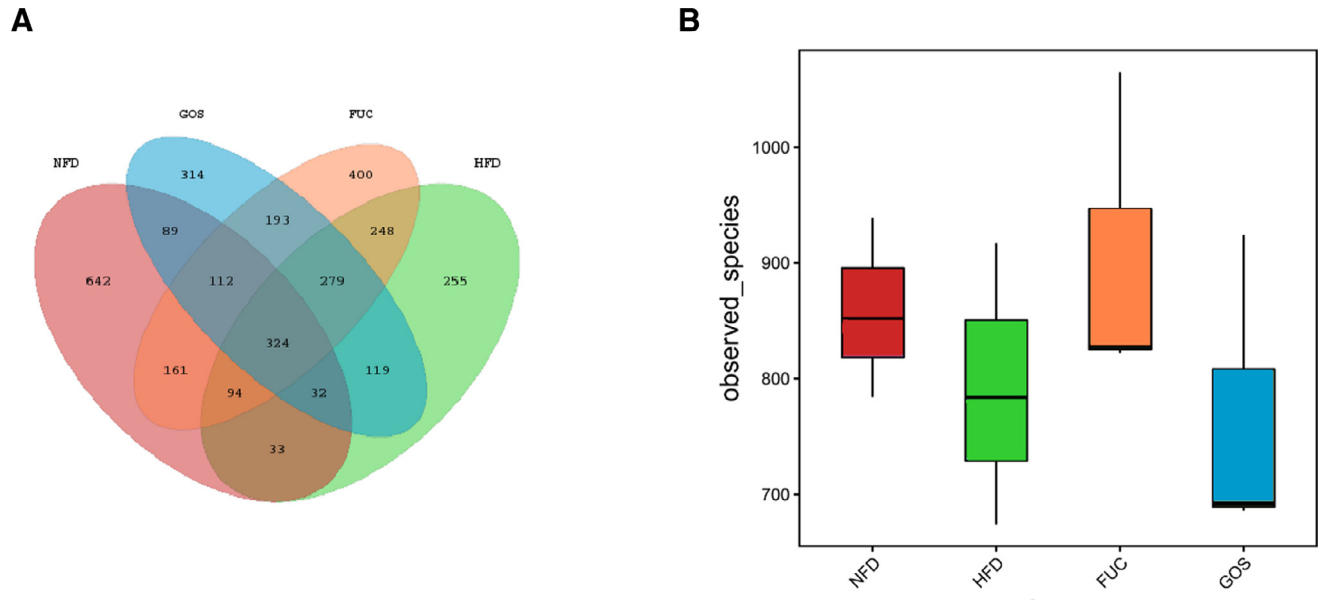

Fig. 2. **(A)** Venn diagram of bacterial OTUs in different groups. (**B**) Observed species in different groups. Sequence samples with a similarity >97% in different 16S rRNA gene sequences were defined as one OTU. FUC, fucoidan; GOS, galactoogligosaccharide; NFD, normal fat diet; OTU, operational taxonomic unit.

Shannon index revealed a significant decrease in the HFD group compared with the NFD group ($P < 0.05$). The Chao1, Simpson, ACE, and Shannon indices showed no significant differences between the FUC and HFD groups ($P > 0.05$) or between the GOS and HFD groups (all $P > 0.05$). The gut community composition structure at the genus level was determined from the principal component analysis (PCA) score plot (Fig. 3A). The dendrogram revealed two main clusters: HFD1, HFD2, HFD3, FUC1, FUC2, FUC3, GOS1, GOS2, and GOS3 formed one cluster, whereas NFD1, NFD2, and NFD3 formed the other cluster (Fig. 3B). Per Adonis test (Supplementary Fig. 3), the natural distribution of the gut community composition and structure differed significantly among groups ($P < 0.001$).

Effects of FUC and GOS on cecal microbiota by LDA analysis

The LDA analysis used the nonparametric Kruskal–Wallis and Wilcoxon rank sum tests to screen key community members. At the phylum level, the NFD and HFD groups yielded significantly increased relative abundances of *Actinobacteria*, the FUC group yielded a significantly increased relative abundance of *Proteobacteria*, and the GOS group revealed a significantly increased relative

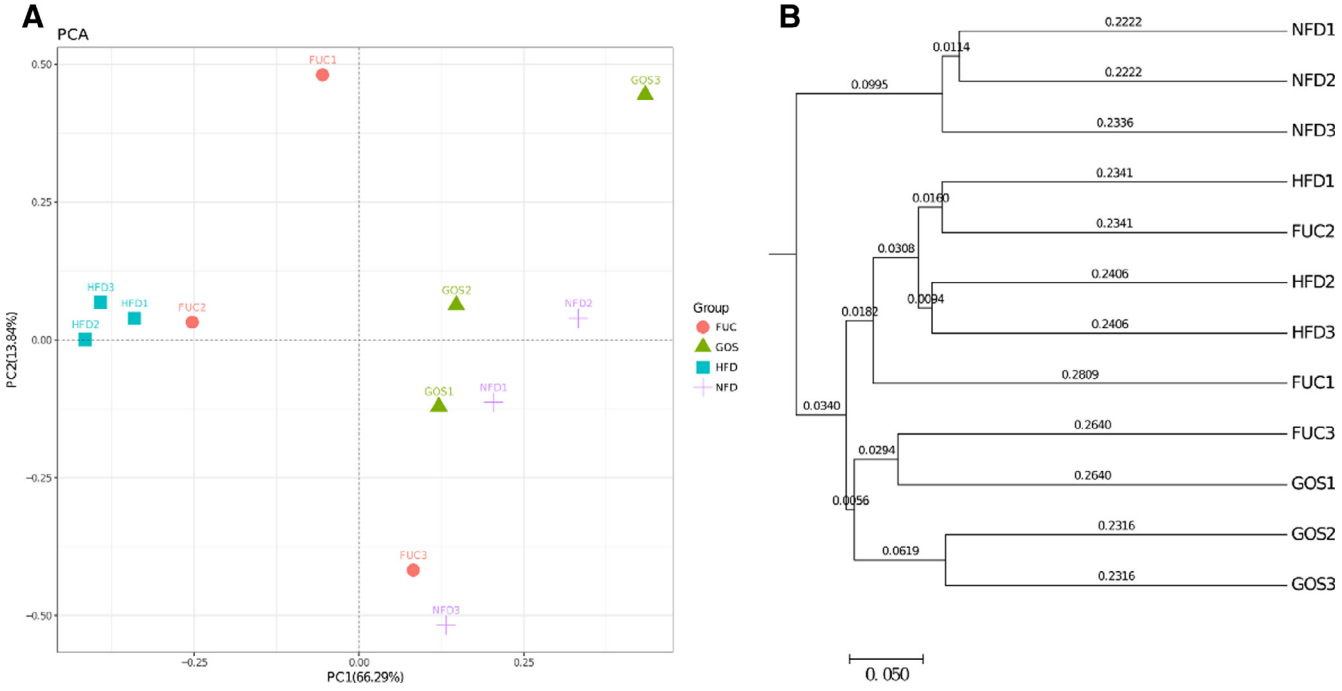

Fig. 3. **(A)** PCA score plot of gut microbiota at the genus level. (**B**) Response of the gut microbiota structure to FUC or GOS treatment. FUC, fucoidan; GOS, galactoogligosaccharide; NFD, normal fat diet; PCA, principal component analysis.

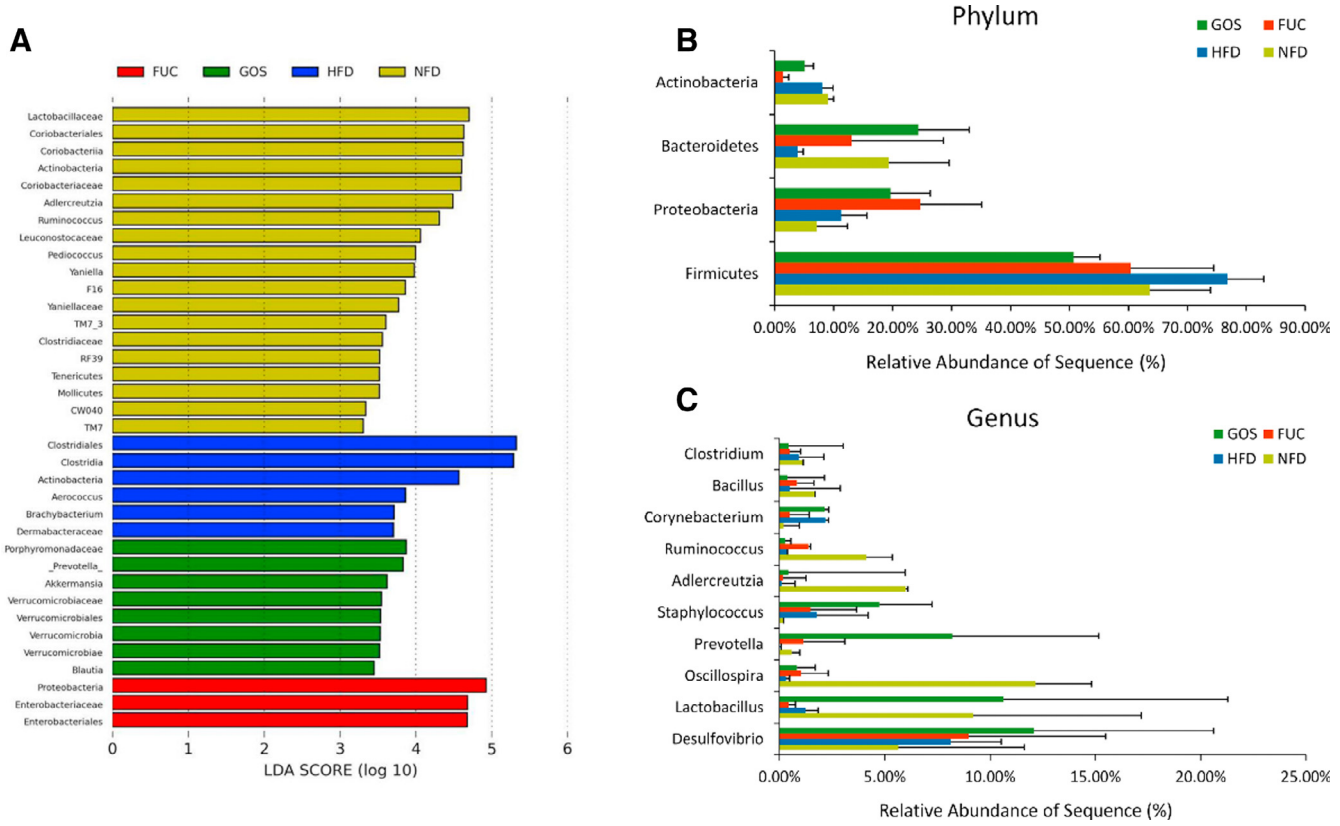

Fig. 4. (**A**) LDA comparison of gut microbiota in each group. (**B**) Taxonomic cardiogram derived from LDA analysis of 16S rRNA gene sequences. Relative abundance of gut microbiota at the phylum level. (**C**) Relative abundance of gut microbiota at the genus level. Red areas indicate FUC-enriched taxa; green areas indicate GOS-enriched taxa; blue areas indicate HFD-enriched taxa; whereas yellow areas indicate NFD-enriched taxa. FUC, fucoidan; GOS, galactoogligosaccharide; HFD, high-fat diet; LDA, linear discriminant analysis; NFD, normal fat diet.

abundance of *Verrucomicrobia* (all $P < 0.05$). At the genus level, the NFD group yielded significantly increased relative abundances of *Adlercreutzia, Yaniella,* and *Ruminococcus*, and the HFD group had significantly increased relative abundances of *Clostridia, Aerococcus,* and *Brachybacterium*. The FUC group showed a significantly increased relative abundance of *Enterobacter*. Finally, the GOS group had significantly increased relative abundances of *Akkermansia, Ruminococcu,s* and *Blautia* (all $P < 0.05$; Fig. 4A).

Effects of FUC and GOS on the taxonomic levels of the cecal microbiota

The OTU classification and status identification results revealed each group's specific composition at the different classification levels. At the phylum level in the HFD group, the relative abundances of *Firmicutes* and *Proteobacteria* increased, whereas the relative abundances of *Bacteroidetes* and *Actinobacteria* decreased compared with those in the NFD group. In the GOS and FUC groups, the relative abundances of *Firmicutes* and *Actinobacteria* decreased, whereas those of *Bacteroidetes* and *Proteobacteria* increased compared with the HFD group (Fig. 4B). At the genus level, the relative abundances of *Corynebacterium, Staphylococcus,* and *Desulfovibrio* in the HFD group increased, whereas those of *Lactobacillus, Oscillospira, Prevotella, Adlercreutzia, Ruminococcus, Bacillus,* and *Clostridium* decreased compared with NFD group. In the FUC group, the relative abundances of *Clostridium, Corynebacterium, Staphylococcus,* and *Lactobacillus* decreased, whereas those of *Bacillus, Ruminococcus, Adlercreutzia, Prevotella, Oscillospira,* and *Desulfovibrio* increased compared with the HFD group. In the GOS group, the relative abundances of *Clostridium, Bacillus,* and *Ruminococcus* decreased, whereas those of *Adlercreutzia, Staphylococcus, Prevotella, Oscillospira, Lactobacillus,* and *Desulfovibrio* increased compared with the HFD group (Fig. 4C). The Metastats statistical algorithm in Mothur was used to compare the relative bacterial abundances at the phylum and genus levels; significant differences in taxonomic composition were analyzed using the Kruskal–Wallis test. At the phylum level, *Cyanobacteria* was significantly decreased ($P < 0.05$) in the HFD group compared with the NFD group. Compared with the HFD group, *Actinobacteria* was significantly decreased ($P < 0.05$) in the FUC group, whereas *Verrucomicrobia* and *Bacteroidetes* were significantly increased ($P < 0.05$), and *Firmicutes* was significantly decreased ($P < 0.05$) in the GOS group (Figs. 5 and 6). At the genus level, compared with the NFD group, *Ruminococcus, Adlercreutzia, Coprococcus*, and *Yaniella* were significantly decreased ($P < 0.05$) and *Aerococcus* and *Brevibacterium* were significantly increased in the HFD group ($P < 0.05$). Compared with the HFD group, the relative abundance of *Aerococcus* in the FUC group was significantly decreased, and *Blautia* and *Akkermansia* were significantly increased in the GOS group compared with the HFD group ($P < 0.05$; Fig. 6).

Effects of FUC and GOS on liver and aortic arch histology

In the HFD group, smooth muscle fascicle arrangement in the aortic arch was disordered and showed foam cell infiltration. However, FUC- and GOS-supplemented rats showed improved aortic arches with less disorder in the smooth muscle cells and less foam cell infiltration than the HFD group (Fig. 7A). Hepatic tissue in HFD-fed rats showed more steatosis than in the NFD group, but the GOS

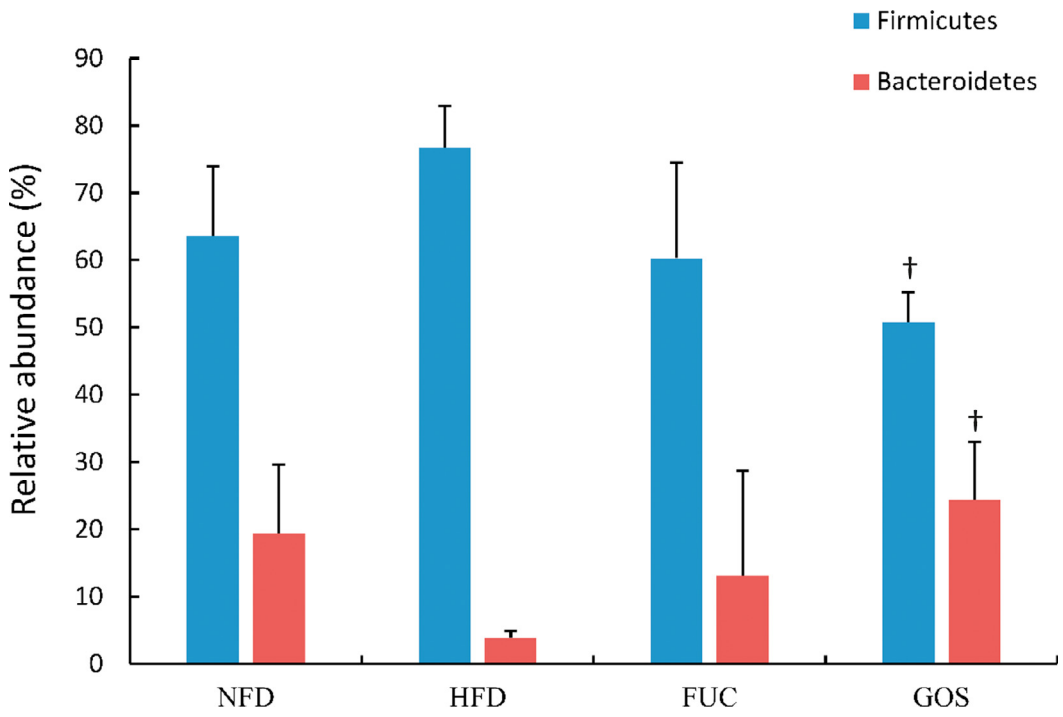

Fig. 5. The relative abundance of *Firmicutes* and *Bacteroidetes* between the groups. $^{\dagger}P < 0.05$ vs the HFD group. The data were submitted to analysis of variance followed by one-way ANOVA test (Tukey test). ANOVA, analysis of variance; FUC, fucoidan; GOS, galactoogligosaccharide; HFD, high-fat diet; NFD, normal fat diet.

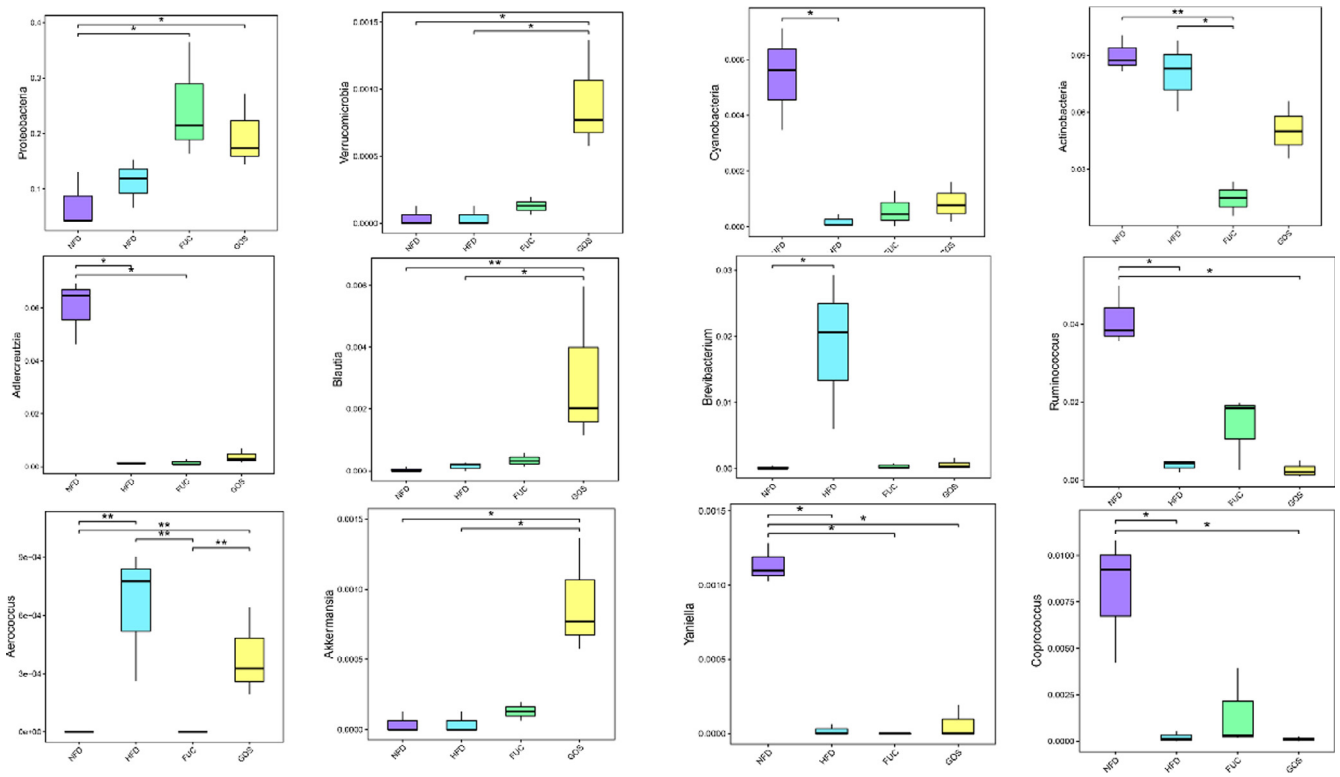

Fig. 6. The relative abundance of the bacteria with the significant differences between the groups. The results are presented as the box charts. * $P < 0.05$. $^{\dagger}P < 0.01$. The data were analyzed by Kruskal-Wallis test. FUC, fucoidan; GOS, galactoogligosaccharide; HFD, high-fat diet; NFD, normal fat diet.

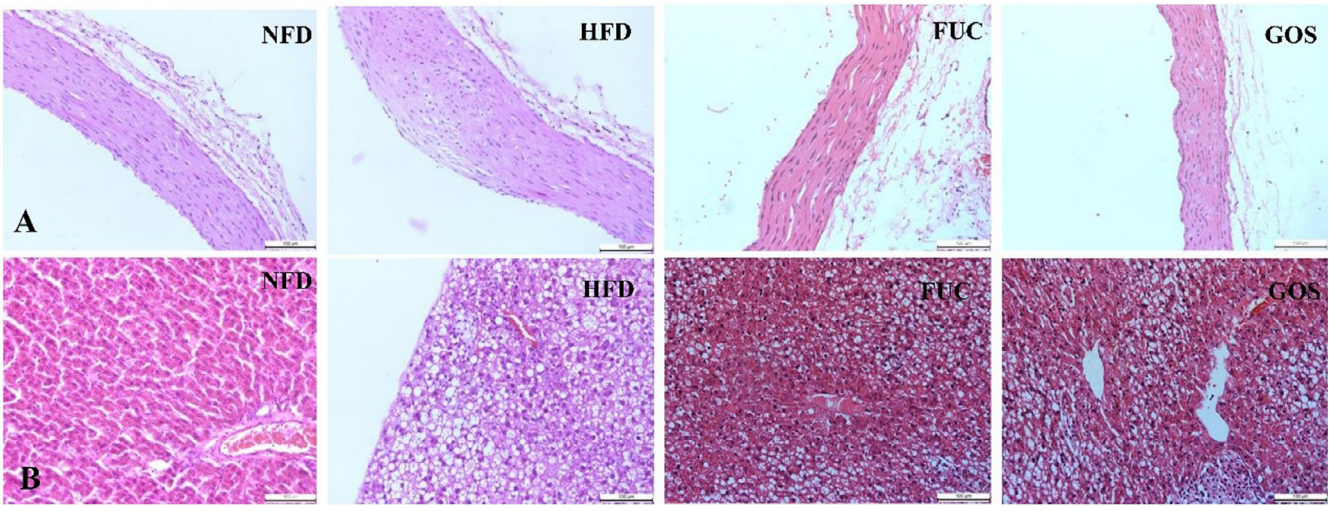

Fig. 7. **(A)** Representative images of hematoxylin and eosin-stained aortic arch. (**B**) Representative images of hematoxylin and eosin-stained hepatic tissue. Representative images are at 100 × magnification. FUC, fucoidan; GOS, galactoogligosaccharide; HFD, high-fat diet; NFD, normal fat diet.

and FUC groups showed improved hepatic tissue with less steatosis than in the HFD group (Fig. 7B).

Effects of FUC and GOS on BA metabolism–related pathways

The HFD group yielded significantly decreased mRNA and protein expressions of CYP7A1 in the liver ($P < 0.05$) compared with the NFD group; however, GOS and FUC supplementation increased these expressions in the liver ($P < 0.05$) compared with those of the HFD group (Figs. 8A–D).

Effects of FUC and GOS on BSH activity in the ileal contents

BSH activity in the HFD group's ileal contents was significantly decreased ($P < 0.05$) compared with that of the NFD group, whereas in the GOS and FUC groups, BSH activity was significantly increased ($P < 0.05$) compared with the HFD group (Fig. 8E).

Correlation between the gut microbiota and BSH activity

Spearman analysis showed that *Adlercreutzia* and *Oscillospira* were positively correlated with BSH enzyme activity, whereas *Aerococcus* and *Brevibacterium* were negatively correlated with BSH enzyme activity (Table 2).

Effects of FUC and GOS on L. casei *DM8121 growth and BSH enzyme activity in vitro*

L. casei DM8121 grew in M-GOS, M-FUC, and M-GLU broths. No significant differences were found in CFU values between the groups in vitro (Supplementary Table 2). However, the 24-h

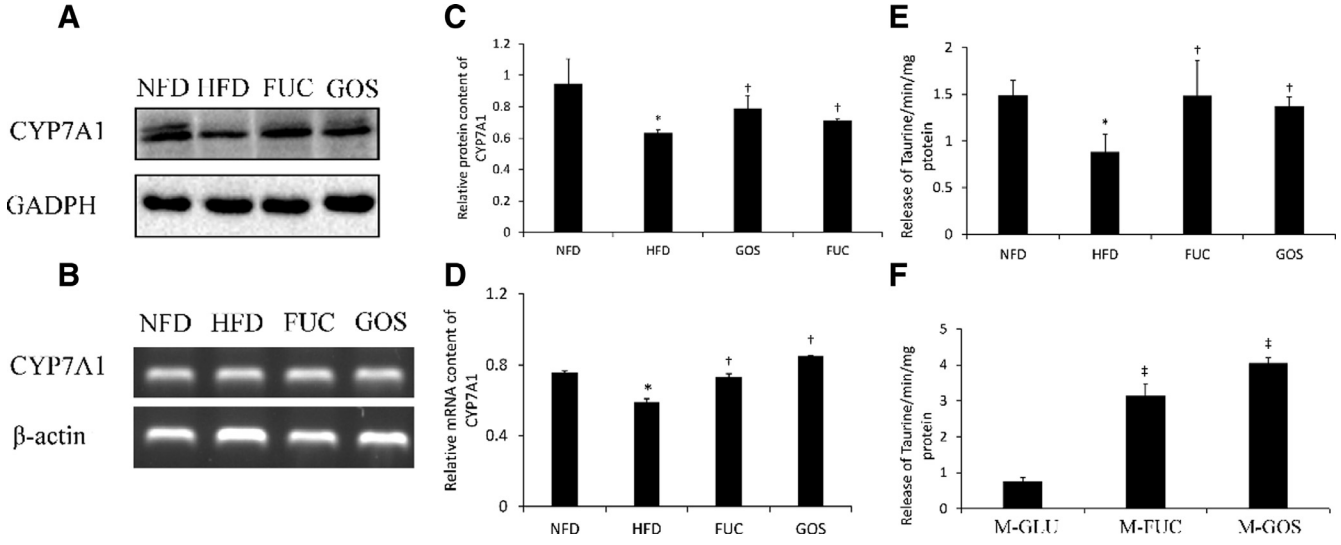

Fig. 8. **(A)** Protein of hepatic CYP7A1 in different groups (CYP7A1; GADPH). (**B**) mRNA of hepatic CYP7A1 in different groups. (**C**) Relative protein content of hepatic CYP7A1. (**D**) Relative mRNA expression of hepatic CYP7A1. (**E**) Ninhydrin assay showing the release of taurine from conjugated bile acids as an index of BSH activity in intestine contents. Values are means ± SD. The data were submitted to analysis of variance followed by one-way ANOVA test. (**F**) Ninhydrin assay showing the release of taurine from conjugated bile acids as an index of BSH activity in *Lactobacillus casei* DM8121 in vitro. *$P < 0.05$ vs the NFD group. †$P < 0.05$ vs the HFD group. ‡$P < 0.05$ vs the M-GLU group. Values are means ± SD. The data were submitted to analysis of variance followed by one-way ANOVA test. ANOVA, analysis of variance; CYP7A1, cholesterol 7α-hydroxylase; GADPH, glyceraldehyde-3-phosphate dehydrogenase; FUC, fucoidan; GOS, galactoogligosaccharide; HFD, high-fat diet; M-GLU,; NFD, normal fat diet.

Table 2
Correlation between bacteria and BSH enzyme activity

	Correlation coefficient	*P*-value
Adlercreutzia	+0.705*	0.043
Aerococcus	−0.728*	0.007
Brevibacterium	−0.602*	0.038
Oscillospira	+0.685*	0.014

+, positive correlation between bacteria and BSH enzyme. −, negative correlation between bacteria and BSH enzyme; BSH, bile salt hydrolase.
Data (N = 12) were analyzed by Spearman test.
*$P < 0.05$.

growth rates of *L. casei* DM8121 in M-GOS and M-FUC were significantly decreased compared with those in M-GLU (Supplementary Fig. 2). BSH activity in the M-GOS and M-FUC groups were significantly increased ($P < 0.05$) compared with that in the M-GLU group (Fig. 8F).

Discussion

Dyslipidemia includes hypertriglyceridemia, hypercholesterolemia, low HDL-C levels and combined hyperlipidemia [32]. Per the International Atherosclerosis Society, healthy diets and lifestyles can reduce non−HDL-C levels and other risk factors [33]. Therefore, prebiotics with hypolipidemic effects are gaining attention. Here, we induced dyslipidemia in SD rats by feeding them a long-term HFD. After gavage treatment, both FUC and GOS improved the rats' serum lipid levels. GOS is a common prebiotic, which can regulate lipid metabolism. One study showed that trans-GOS positively affected TC and TG in overweight adults [34]. GOS also decreased serum TC in infants by regulating their microbiota [35]. Another study showed that FUC extracted from *Cladosiphon okamuranus* alleviated HFD-induced dyslipidemia in *ApoE* (shl) mice [36].

The gut microbiota modulates the host's lipid metabolism mainly via the three mechanisms:

1. Short-chain fatty acids (SCFAs) produced by the bacteria can induce peptide YY release, which suppresses gut motility and lipid absorption [37]. Propionic acid in SCFAs can inhibit cholesterol synthesis in the liver [38].
2. LPS is a cell wall component of gram-negative bacteria, which leads to chronic inflammation. Macrophages play important roles in lipid metabolism. Chronic inflammation stimulates macrophages in the body; thus, chronic inflammation indirectly affects lipid metabolism [39].
3. Enzymes produced by the intestinal microbiota metabolize host BAs, and BA regulates the rate-limiting enzyme of bile acid synthesis in the liver (CYP7A1), which converts cholesterol into BA; thus, BA metabolism affects lipid metabolism [40].

In this experiment, HFD-fed rats showed significantly increased LPS compared with the NFD group, whereas the FUC and GOS groups showed significantly decreased LPS compared with the HFD group. Serum LPS and chronic inflammation were positively correlated in humans [41]. The gut microbiota was analyzed for alpha diversity, beta diversity, and compositions of different bacterial levels. The gut microbiota's increased richness and diversity contribute to intestinal environmental stability, and low gut microbiota richness is common in elderly and obese patients [42]. A recent study showed that FUC increased bacterial community diversity in rats with breast cancer [43]. In this study, HFD-fed rats showed significantly decreased community diversity compared with NFD-fed rats, but the FUC and GOS groups did not differ in community richness or diversity compared with the HFD group. We also analyzed the similarities in research data via PCA and denaturing gradient gel electrophoresis (Supplementary Fig. 1) and illustrated the differences in multidimensional data on a two-dimensional map. The PCA map showed naturally distributed significant differences in the gut community composition and structure among groups. This result showed that both GOS and FUC could restore the gut microbiota dysbiosis induced by the HFD.

Recent studies showed that long-term HFDs increased the relative abundance of *Firmicutes* but reduced the relative abundances of *Bacteroidetes* and *Lactobacillus* [44]. The present study suggested that the relative abundance of *Firmicutes* increased and the relative abundance of *Bacteroidetes* decreased in HFD-fed rats. As expected, GOS-fed rats showed significant increases in *Bacteroidetes* and significant decreases in *Firmicutes* compared with the HFD group. Interestingly, FUC also showed an increase in *Bacteroidetes* and a decrease in *Firmicutes* compared with the HFD group, but the effect was not significant. At the genus level, the relative abundances of *Coprococcus, Yaniella, Ruminococcu,s* and *Adlercreutzia* were significantly decreased in the HFD group. *Coprococcus* is a common gram-positive bacterium in human intestinal tracts. Studies have shown that *Coprococcus* produces SCFAs, such as acetic and butyric acid, which benefit the intestinal environment [45,46]. *Ruminococcus* and *Adlercreutzia* are common species in the intestinal flora and play important roles in maintaining the intestinal environment [47,48]. Interestingly, the relative abundances of *Corynebacterium, Aerococcus,* and *Brevibacterium* increased in the HFD group but decreased in the FUC group. *Corynebacteria* are gram-positive aerobic bacteria, which are mostly conditional pathogens, but some species, such as *C. diphtheria*, can induce serious diseases. A study showed that *Corynebacterium* content in the nasopharynx is closely related to a low anti-diphtheria toxin immune response [49,50]. *Aerococcus* is a gram-positive bacterium that is significantly increased in obese mice, and some species can cause urethral inflammation [51,52]. *Brevibacterium* spp. are gram-positive bacteria, and some can induce endocarditis [53]. GOS-fed mice showed significant increases in *Akkermansia* and *Blautia*, and these bacteria can produce SCFAs and are shown to be decreased in obese patients [54]. In general, HFDs disrupt rat gut microbiota, but GOS and FUC can effectively restore them.

BSH is produced by gut microbes that can convert conjugated BA into non-conjugated BAs [6,7,55]. BSH alters the BA composition, thus playing an important role in host lipid metabolism and energy harvesting [40]. In this study, HFD-fed rats showed significantly decreased BSH enzyme activity. However, BSH activity in the GOS and FUC groups was significantly increased compared with the HFD group. Recent studies showed that resveratrol significantly improved BSH activity in mouse feces [56]. GOS and FUC exert similar effects. In this study, *Adlercreutzia* and *Oscillospira* were positively correlated with BSH enzyme activity. *Aerococcus* and *Brevibacterium* were negatively correlated with BSH enzyme activity. LDA analysis revealed that the NFD group contained more *Clostridium* than did the HFD group. In the FUC group, the gut microbiota showed a marked increase in *Enterobacter*. Some studies have reported the BSH activities of *Enterobacter* and *Clostridium* [57]. The in vitro experiments in this study showed that the BSH enzyme activity of *L. casei* DM8221 cultured with FUC and GOS was significantly increased compared with that of *L. casei* DM8121 cultured with glucose. In summary, FUC and GOS can significantly increase intestinal BSH enzyme activity by modulating the gut microbiota.

The liver plays an important role in lipid metabolism. Experiments have shown that steatosis in the liver seriously affects liver function and related gene expressions [58]. In this study, liver steatosis in the GOS and FUC groups was significantly reduced compared with that of the HFD group. Experiments showed that low

doses of GOS improved hepatic steatosis in an HFD-fed mouse model [59]. FUC exhibited protective effects in rats with non-alcoholic fatty liver disease [60]. CYP7A1 is a BA synthesis rate-limiting enzyme of the "classical" pathway; its content directly affects lipid metabolism [61]. Experiments showed that CYP7A1 expression in rats with dyslipidemia was two- to threefold lower than that in control rats [62]. In this study, CYP7A1 mRNA and protein expressions in the liver were significantly increased in the GOS and FUC groups compared with the HFD group. Experiments showed that increased CYP7A1 expression can reduce serum cholesterol levels [63]. Therefore, GOS and FUC may alleviate dyslipidemia by increasing CYP7A1 expression in the liver.

Conclusions

GOS and FUC improved blood lipids in SD rats with dyslipidemia, reduced steatosis in the liver and aortic arch, enhanced intestinal BSH enzyme activity by regulating the gut microbiota, increased CYP7A1 expression in the liver, and decreased serum LPS levels. Therefore, FUC and GOS are potential natural bioactive ingredients for alleviating dyslipidemia.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.03.001.

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