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J. Agric. Food Chem., Just Accepted Manuscript • DOI: 10.1021/acs.jafc.8b00296 • Publication Date (Web): 08 Mar 2018

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Fuzhuan brick tea polysaccharides attenuate metabolic syndrome in high-fat diet induced mice in association with modulation in the gut microbiota

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ABSTRACT

An increasing number of evidence suggests that the gut microbiota composition and structure contribute to the pathophysiology of metabolic syndrome (MS), which has been put forward as a new target in the treatment of diet-induced MS. In this work, we aimed to investigate effects of Fuzhuan brick tea polysaccharides (FBTPS) on MS and gut microbiota dysbiosis in high-fat diet (HFD)-fed mice and to further investigate whether its attenuation of MS being related to the modulation of gut microbiota. The results showed that FBTPS intervention could significantly attenuate metabolic syndrome in HFD-induced mice. Based on results of sequencing, FBTPS treatment could increase the phylogenetic diversity of HFD-induced microbiota. FBTPS intervention could significantly restore the HFD-induced increases in relative abundances of Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae. Spearman’s correlation analysis showed that 44 key OTUs were negatively or positively associated with MS. Our results suggested that FBTPS could serve as a novel candidate for prevention of MS in association with the modulation of gut microbiota.

Keywords: Fuzhuan brick tea; polysaccharides; metabolic syndrome; gut microbiota; modulation
INTRODUCTION

Metabolic syndrome (MS), a combination of medical disorders including obesity, hyperglycemia, dyslipidemia, hypertension and insulin resistance, can enhance the risk of developing cardiovascular disease (CVD) and type 2 diabetes (T2D). Due to the obesogenic shifts in nutritional composition, growing caloric intake and lack of exercise, humans are in the midst of worldwide epidemics of MS. Thus, prevention of MS has been a major challenge for modern society. A growing number of evidences suggests that the gut microbiota may be served as an important modulator of the crosstalk between diet and development of MS or related diseases through affecting the extraction of nutrients and energy from diets, energy homeostasis, glucose metabolism, cholesterol metabolism, insulin resistance, non-alcoholic fatty liver and the chronic inflammatory state. Recently and more strikingly, some species or genera of beneficial gut microbiota, such as Akkermansia muciniphila, Bifidobacterium and Lactobacillus, have been demonstrated to be negatively associated with the development of MS. On the contrary, increases in some pro-inflammatory/pathogenic gut microbiota, such as Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae, are consistently associated with the development of obesity, adipose tissue, systemic inflammation and metabolic comorbidities in both humans and rodents. Thus, a potentially viable concept, modulating high-fat diet (HFD) induced gut microbiota dysbiosis as new therapeutic target for the prevention of MS and related diseases, has been presented. A number of potential strategy or candidate based on modulation of gut
microbiota, including prebiotics, probiotics, antibiotics and natural products, has been presented and evaluated for prevention of MS and its related diseases. For example, both *Bifidobacterium* and *Lactobacillus* could differentially attenuate HFD-induced obesity comorbidities partly due to the strain-specific effects on MS-related phylotypes of gut microbiota.\(^\text{13}\) The relative abundance of *Akkermansia muciniphila* was dramatically enhanced and the proportion of Firmicutes/Bacteroidetes (F/B) was significantly decreased after treatment of grape polyphenols, which led to reduction in intestinal and systemic inflammation and improvement in the protection against MS in HFD-induced mice.\(^\text{2}\) Likewise, both water extract and polysaccharides from *Ganoderma lucidum* could prevent obesity-related MS and gut dysbiosis.\(^\text{17}\) In addition, it has been reported that the host-indigestible, fermentable fibers and carbohydrates (regarded as prebiotics) could be definite assets for the potential modulation of gut microbiota by enhancing the growth of specific health-promoting bacteria.\(^\text{18}\) In particular, short-chain fatty acids (SCFAs) such as acetic, propionic and butyric acids are the main products of polysaccharides metabolized by gut microbiota, which not only contribute to gut health as a direct energy source for intestinal epithelial cells, but also enter the systemic circulation and directly regulate features of MS, such as insulin resistance and glucose metabolism.\(^\text{19,20}\) Therefore, the degradation and utilization of polysaccharides by gut microbiota and their health-promoting effects on human health and disease have been widely investigated and reported (reviewed in references\(^\text{18,21-23}\)).

Tea, made from the buds and leaves of *Camellia sinensis* L., is one of the most
The dark tea is post-fermented tea produced by microbial fermentation of heat-processed green tea leaves. According to method of microbial fermentation, dark teas are further classified into Qingzhuan brick tea, Pu-erh tea and Fuzhuan brick tea (FBT), etc. Thereinto, FBT, one kind of dark tea produced in Hunan province of China with the help of fungus Eurotium cristatum, has been demonstrated to have preventive and therapeutic effects on MS. In our recent study, it was found that FBT could significantly attenuate features of the HFD-induced metabolic syndrome in mice, which might be related to the modulation of gut microbiota. However, the bioactive components contributing to the prevention of MS and the potential mechanisms responsible for regulating MS remain unclear. Tea polysaccharides, identified as one of the main active components in tea with a wide range of health-promoting bioactivities, have great potential applications as natural products. Our previous study in vitro demonstrated that polysaccharides from FBT (FBTPS) could pass through the digestive system without being degraded and reach the large intestine safely, where it could be broken down and utilized by gut microbiota. Furthermore, FBTPS could significantly reduce the ratio of F/B, which is expected to result in the decrease of the energy harvest thereby prevention of the risk of MS. Thus, it was speculated that FBTPS might be an important active component of FBT contributing to the prevention of MS and modulation of gut microbiota. The purpose of the present study, therefore, was to investigate whether the intervention of FBTPS to mice could prevent MS and
modulate the gut microbiota dysbiosis in HFD-induced mice. Additionally, the gut microbe phylotypes responding to dietary HFD or FBTPS intervention as well as whether they were associated with MS in HFD-induced mice were analyzed, aiming to provide potential insights into mechanisms of the gut microbiota with prevention of MS.

MATERIALS AND METHODS

Preparation of FBTPS. FBTPS was prepared according to our reported method. Briefly, the dried sample of FBT (provided by Hunan Yiyang Tea Processing Factory Co., Ltd., Yiyang, Hunan, China) was treated with aqueous ethanol solution (85%, v/v) three times at 70 °C for 8 h to remove small molecule substances. The resulting residue was dried and used for extraction of FBTPS by distilled water (10 mL/g, v/w) three times at 70 °C for 3 h. After filtration, the extracts were precipitated with triple volumes of dehydrated ethanol and kept at 4 °C overnight. After centrifugation at 3040 g for 15 min, the precipitates were dissolved in distilled water, removed dissociative protein by Sevag method, dialyzed with distilled water and lyophilized, affording FBTPS. As reported, the average molecular weight of FBTPS was 828 kD. The contents of natural carbohydrate, uronic acid and protein were 55.20 ± 3.07, 37.78 ± 1.26 and 2.93 ± 0.06%, respectively, indicating that polysaccharide was the main composition of FBTPS. Moreover, FBTPS was composed of mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose and arabinose in a molar ratio of 3.66:1.69:12.11:1.41:28.17:21.97:19.15:11.83.

Animals and Experimental Design. All animal experiments were approved and
carried out in accordance with the Guidelines of the Ethical Committee of Experimental Animal Center of Nanjing Agricultural University and the National Guidelines for Experimental Animal Welfare (MOST of People’s Republic of China, 2006). Forty male C57BL/6 mice (6 weeks of age, animal number NO.201608240) were purchased from Comparative Medicine Centre of Yangzhou University (Yangzhou, Jiangsu, China) and housed in Animal Center of Nanjing Agricultural University (SYXK<Jiangsu>201190037) under controlled light conditions (12 h light-dark cycle) with *ad libitum* access to food and water. After one week acclimatization, the mice were randomly divided into 5 treatment groups (8 mice per group) and fed with (1) normal-chow diet (ND, 10% calories from fat, MD12031) with administration of water as healthy control, (2) HFD (45% calories from fat, MD12032) with administration of water as model control, (3) HFD plus daily administration of 200 mg/kg/day FBTPS (HFD-L group), (4) HFD plus daily administration of 400 mg/kg/day FBTPS (HFD-M group) or (5) HFD plus daily administration of 800 mg/kg/day FBTPS (HFD-H group) by intragastric gavage for 8 weeks. The diets were obtained from Jiangsu Medicience Ltd. (Yangzhou, Jiangsu, China) and their compositions are showed in Table S1 (Supplemental material). During the experiment, food intake and body weight were recorded weekly.

**Histology Analysis.** Histology analysis was carried out according to our reported method. In brief, the liver and epididymal fat were immediately fixed in formaldehyde solution (12%) for 24 h after washing with saline. After dehydration with 30, 50, 70, 80, 90, 95 and 100% ethanol, respectively, all the samples were
embedded in paraffin, cut into 5 µm thick microsections and stained with hematoxylin and eosin. Then, the sections were observed by light microscopy, and the size of epididymal adipocyte was evaluated by Image J software.

**Biochemical Analysis.** The contents of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in serum were measured by commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**RNA Preparation and qRT-PCR Analysis.** The total RNA of liver was extracted by MiniBEST Universal RNA Extraction Kit (TaKaRa Bio. Inc., Dalian, China). The concentration and integrity of RNA were evaluated by Nanodrop 2000 (Thermo Fisher Scientific Inc., USA). cDNA was prepared by reverse transcription of RNA by PrimeScript RT Master Mix (TaKaRa Bio. Inc.). Quantitative real-time reverse-transcription PCR (qRT-PCR) was performed with SYBR Green Master Mix and QuantStudio 6 Flex (Thermo Fisher Scientific Inc.) according to the protocol of manufacturer. All samples were performed in duplicate in a single plate and relative quantification was calculated by the $2^{\Delta\Delta C_t}$ method. The primer sequences for the targeted mouse genes, including carnitine palmitoyl transterase-1 (CPT-1), peroxisome proliferator-activated receptor α (PPARα), fatty acid synthase (FAS), peroxisome proliferator-activated receptor γ (PPARγ), sterol regulatory element-binding protein 1c (SREBP-1c) and liver X receptor (LXRα), are presented in Table S2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as housekeeping gene.
**Gut microbiota Analysis.** DNA of fecal sample was extracted by QiAamp DNA stool Mini Kit (NO.51504, Qiagen, Germany). The resulting DNA was amplified with barcoded specific bacterial primers targeting the variable region 4 (V4) of 16S rRNA gene using primers with the Primer F: Illumina adapter sequence 1 + AYTGGGYDTAAAGNG and Primer R: Illumina adapter sequence 2 + TACNVGGGTATCTAATCC. For each fecal sample, the sequencing and bioinformatics was carried out by Genesky Biotechnologies Inc. (Shanghai, China) on the Illumina MiSeq platform to generate 2 × 250 bp paired-end reads. The remaining unique reads were clustered into operational taxonomic units (OTUs) based on Ribosomal Database Project (RDP) by UPARSE with 97% similarity cutoff after the raw reads were quality filtered and merged by fastx, Cutadapt, Usearch and FLASH. Mothur was used to calculate rarefaction analysis, the community richness index and alpha diversities including Shannon and Simpson indexes. Principal component analysis (PCA) and hierarchical cluster analysis by euclidean distance matrix and ward.D2 method were performed according to the abundances of OTUs by R package (V3.4.0).

**Statistical analysis.** All values are expressed as the mean ± standard deviation (SD) or box-and-whisker plots. Statistical significances between groups were evaluated by one-way ANOVA procedure followed by Tukey test using SPSS 22 software (IBM, USA). Differences in relative abundances of OTUs were calculated using Tukey's honest significant difference (HSD) test by R package (V3.4.0). Canoco for Windows 4.5 (Microcomputer Power, USA) was used for redundancy analysis (RDA), which
was assessed by Monte Carlo permutation procedure (MCPP) with 499 random permutations and P values of 0.002. The correlations between relative abundances of OTUs and host parameters of MS were analyzed by Spearman’s correlation (SPSS 22, IBM). Furthermore, adjusted $p$-values obtained based on false discovery rate (FDR) were used to evaluate differences in this work. A value at $p < 0.05$ was considered significant difference.

RESULTS

FBTPS Attenuated Features of HFD-induced MS. To investigate the effects of FBTPS on the development of MS after administration of HFD, the male C57BL/6 mice ($n = 8$ per group) received intervention including HFD and HFD supplemented with 200, 400 or 800 mg/kg/day of FBTPS for 8 weeks. Compared with ND group, HFD treatment for 8 weeks led to significant increases in body weight gain, adipose tissue weight (epididymal and perirenal fat), epididymal adipocyte size, liver weight as well as hepatic lipid deposition (Figure 1a-1h). As expected, dietary supplementation with FBTPS could significantly prevent body weight gain, accumulation of fat, increase in epididymal adipocyte size and hepatic lipid deposition in a dose-dependent manner. Furthermore, the levels of TC and LDL-C in serum were significantly elevated after HFD intervention (Figure 2a and 2c) compared with those of ND group. As expected, FBTPS, especially high dosage of FBTPS, could significantly reduce these levels. However, the levels of serum TG and HDL-C were not significantly affected by treatment of FBTPS (Figure 2b and 2d). The results suggested that FBTPS could attenuate features of MS in HFD-fed mice. Food intake
was not significantly different for any of the groups between HFD intervention groups (Figure S1), indicating that the prevention of MS by FBTPS was not due to the decrease in food consumption.\textsuperscript{17}

**FBTPS Regulated Expression of Lipid Metabolism Related Genes.** Previous studies have shown that hepatic fatty acid synthesis and oxidation might be regulated by genes expression after HFD treatment.\textsuperscript{35-37} Therefore, the effects of FBTPS on gene expression of fat metabolism-related genes in liver including CPT-1, PPAR\textsubscript{α}, PPAR\textsubscript{γ}, SREBP-1c, FAS and LXR\textsubscript{α} were investigated by qRT-PCR, and the results are shown in Figure 3. Compared with ND group, significantly higher expression of FAS, LXR\textsubscript{α}, PPAR\textsubscript{γ} and SREBP-1c and while lower expression of CPT-1 and PPAR\textsubscript{α} were observed in mice fed with HFD. Compared with HFD group, FBTPS treatment could lead to the down-regulation of LXR\textsubscript{α}, FAS, SREBP-1c and PPAR\textsubscript{γ} and up-regulation of CPT-1. In particular, the expression levels of CPT-1, PPAR\textsubscript{γ}, SREBP-1c, FAS and LXR\textsubscript{α} in treatment with high dosage of FBTPS exhibited no difference with those of ND group. However, FBTPS intervention had limited effect on expression of PPAR\textsubscript{α}.

**Overall Structural Changes of Gut Microbiota in Response to HFD and FBTPS.** An increasing number of works using germ-free animals and microbiota transplant shows that development of MS and associated diseases are strongly implicated in the gut microbiota.\textsuperscript{15,38,39} Therefore, the effects of HFD and FBTPS intervention on gut microbiota were investigated by pyrosequencing V4 of bacterial 16S rRNA genes. The average sequence number were all more than 44,397 to afford 3,208 distinct
OTUs from 40 samples (n = 8 for each group) at a 97% similarity level. The results from the Rarefaction and Shannon index curves suggested that most of the diversity and new phylotypes were covered (Figure S2). The changes of gut microbiota community diversity in response to the treatments with HFD and FBTPS were evaluated using Shannon and Simpson indexes. Similar to the previous findings,\textsuperscript{14} the diet was found to be a key factor in changing phylogenetic diversity. In this work, a significant increase in Simpson index and decrease in Shannon index, indicating the lower microbiota community diversity, were found in HFD group (Figure 4a). Nevertheless, the microbiota phylogenetic diversity was significantly restored by treatment with high dosage of FBTPS, which showed no difference with that of ND group.

Beta-diversity analyses including PCA and hierarchical cluster analysis were carried out to further provide an overview of the effects of HFD and FBTPS treatments on the composition and structure of gut microbiota. PCA revealed a clear separation between the ND and HFD groups (Figure 4b). As expected, the composition of gut microbiota exhibited a obvious response to FBTPS intervention. Furthermore, PC1, which explained 59.45% of total variance, showed that FBTPS intervention shifted the gut microbiota composition of HFD group towards to that of ND group in dose-dependent manner. Notably, the HFD-H group had kept away from HFD group and was close to ND group as seen in PC1, indicating that FBTPS could reverse HFD-disrupted gut microbiota back to health status. On the other hand, the results of hierarchical cluster analysis also confirmed significant separation between
HFD and ND groups (Figure 4c). As expected, shift from HFD group to ND group after FBTPS intervention was also observed. This is consistent with the result of PCA. Obviously, some mice samples from HFD-M and HFD-H groups got close to ND group, which demonstrated the similarity in gut microbiota composition between these mice from HFD-M and HFD-H groups with those of ND group. Taxonomic analysis suggested that the gut microbiota of mice at the phylum level was mainly dominated by the members of Firmicutes and Bacteroidetes, which is the same as other reports.\textsuperscript{17,40} An increase in relative abundance of Firmicutes and a decrease in relative abundance of Bacteroidetes were noted for HFD treatment (Figure 4d and 4e), which led to a significant increase in the ratio of F/B compared to that of ND group. However, FBTPS intervention exhibited limited effects on the relative abundances of Firmicutes and Bacteroidetes and the ratio of F/B.

After supplementation with HFD, significant decreased relative abundances of Porphyromonadaceae, Rikenellaceae, Lactobacillaceae and Bdellovibrionaceae and significant increased relative abundances of Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae were observed compared with ND group at family level (Figure 5). FBTPS intervention could significantly reverse the HFD-induced increases in the relative abundances of Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae. However, FBTPS exhibited limited effect on HFD-induced decreased gut microbiota such as Porphyromonadaceae, Rikenellaceae, Lactobacillaceae and Bdellovibrionaceae.

It is necessary to evaluate the changes of gut microbiota in OTUs levels after
FBTPS treatment, due to the fact that the different bacterial species in the same family have different responses to HFD or FBTPS. Therefore, RDA was performed based on OTUs with more than 0.1% relative abundance at least in one group to identify the specific bacterial phylotypes of gut microbiota responding to HFD or FBTPS intervention. Mice treated with HFD exhibited changes in 122 OTUs (69 increased and 53 decreased) compared with those of the ND group (Figure S4a). As shown in Figure S4b-S4d, the treatments of HFD-L, HFD-M and HFD-H altered 58 (33 increased and 25 decreased), 50 (33 increased and 17 decreased) and 54 (31 increased and 23 decreased) OTUs in comparison with HFD group, respectively. As a result, a total of 95 distinct OTUs were significantly altered responding to FBTPS intervention (Figure 6 and Supplementary Data 1). Thereinto, 27, 23 and 30 of the 95 OTUs that altered by HFD treatment were reversed in HFD-L, HFD-M and HFD-H groups, respectively, resulting in significant reversion of 46 OTUs in total.

The correlations between the relative abundances of dominant gut microbiota in OTUs levels and parameters of MS were carried out by Spearman’s correlation analysis to identify the OTUs that might contribute to attenuating MS in HFD-induced mice. As shown in Figure 7, it was found that 44 of the 46 OTUs were negatively or positively associated with at least one parameter of MS, including weight body gain, liver weight, epididymal fat, perirenal fat, TG, TC, LDL-C and HDL-C. Thereinto, OTU0021, OTU0345, OTU0316, OTU0197, OTU0493, OTU0363 and OTU0249 belonged to Erysipelotrichaceae, OTU0260 belonged to Coriobacteriaceae and OTU0406 belonged to Streptococcaceae were significantly positively correlated with
MS, which are in accord with the results mentioned above. Furthermore, 9 OTUs that represented Lachnospiraceae, 8 OTUs that represented Porphyromonadaceae and 10 OTUs that represented Ruminococcaceae might also play an important role in prevention of MS, supporting the prior findings.\textsuperscript{14,40,41} Notably, 26 of the 44 OTUs which were positively correlated with parameters of MS were significantly reduced by FBTPS treatment, including \textit{Acetatifactor} (OTU0208), \textit{Anaerobacterium} (OTU0408), \textit{Clostridium}\textsubscript{IV} (OTU0267 and OTU0288), \textit{Coprobacter} (OTU0009), \textit{Flavonifractor} (OTU0389), \textit{Leuconostoc} (OTU0216), \textit{Olsenella} (OTU0260), \textit{Oscillibacter} (OTU0042 and OTU0300) and \textit{Streptococcus} (OTU0406). On the contrary, 18 of the 44 OTUs, negatively correlated with parameters of MS, were significantly enhanced by FBTPS treatment, including \textit{Alistipes} (OTU0054 and OTU0218), \textit{Anaerobacterium} (OTU0408), \textit{Bacteroides} (OTU0391), \textit{Eisenbergiella} (OTU0029 and OTU0254), \textit{Erysipelotrichaceae}\textsubscript{incertae\_sedis} (OTU0361) and \textit{Odoribacter} (OTU0008 and OTU0078). Taken all together, the results demonstrated that FBTPS intervention could modulates the HFD-induced gut microbiota, resulting in a healthy gut microbiota composition similar to that of ND group.

\textbf{DISCUSSION}

MS, characterized by central obesity, hypertension, hyperlipidemia, insulin resistance and hyperglycemia, has been regarded as one of the urgent worldwide public health problems of this century, which enhances the risk of developing CVD and T2D.\textsuperscript{2,13,42} In recent years, tea consumption as potentially viable nutritional strategy for the prevention of MS has been demonstrated by using animal models and human studies,
thereby possible reducing the risk of T2D and CVD.\textsuperscript{35} Previous studies have showed that both FBT and tea polysaccharides could attenuate MS,\textsuperscript{29,43} and the abundant phenolic compounds in tea, specifically catechins, are considered to be the main components contributing to the putative beneficial metabolic effects of tea.\textsuperscript{44} An increasing number of evidences suggested that the human gut microbiota plays a fundamental role in the metabolic capacity for processing nutrients and host health,\textsuperscript{45} and previous work showed that Fuzhuan brick, green, Oolong and black teas could modulate the gut microbiota in HFD-induced obese mice or rat.\textsuperscript{27,44} Nevertheless, there was no report about the effects of FBTPS on MS and gut microbiota and possible mechanisms of FBTPS-mediated attenuation of MS. Thus, the effects of FBTPS on the HFD-induced MS and gut microbiota dysbiosis were investigated in this work. As expected, FBTPS could significantly suppress the HFD-induced body weight gain, adipose tissue weight, adipocyte size, liver weight as well as hepatic lipid deposition. Serum biochemical parameters, including TC, TG, LDL-C and HDL-C, could also clearly reflect the status of lipid adsorption and metabolism.\textsuperscript{36} The present results showed that FBTPS could reduce the levels of TC and LDL-C. However, FBTPS exhibited limited effects on TG and HDL-C levels, which should be further investigated. Wu \textit{et al.} reported that tea polysaccharides from black tea could significantly decrease the body weight, fat weight, fat cell size and Lee’s index, and improve the biochemical profile in rats fed with HFD.\textsuperscript{46} Likewise, the effect of green tea polysaccharides on HFD-induced obesity was investigated, and the significantly suppressive effects on body weight gain and fat weight and improvement of blood
lipid and antioxidant levels were observed in HFD-induced rats. Interestingly, the HFD-induced MS was improved by FBTPS in a dose-dependent manner from 200 to 800 mg/kg/day. The data suggested that FBTPS was effective for control of MS parameters, making it a promising candidate for prevention of HFD-induced MS.

The liver is central to lipogenesis and lipids metabolism. It has been reported that some anti-obesogenic candidates could regulate the gene expression levels related to fatty acid synthesis and lipid metabolism in liver, which resulted in prevention of obesity and serum lipid levels. LXRα, a member of the nuclear receptor family, has a critical role in hepatic lipogenesis, which is mainly involved in the regulation of cholesterol and lipid metabolism. Recent studies have shown that SREBP-1c, a central transcription factor to dietary regulation of most hepatic lipogenic genes, is related to the encoding enzymes catalyzing various steps in the synthetic pathways of fatty acid and TG, such as FAS. PPARγ, which is another well-known nuclear receptor, has been proven to play a major role in regulating the uptake of free fatty acid and hepatic steatosis, and down-regulation of PPARγ expression might lead to the suppression of fatty acid synthesis and stimulation of lipolysis in liver. In the present study, the expression levels of LXRα, FAS, SREBP-1c and PPARγ related to lipogenesis and accumulation of fat were significantly reduced by FBTPS. Furthermore, FBTPS treatment could significantly increase the expression level of CPT-1, which is related to lipid catabolism. Up-regulation of CPT-1 expression could result in the transfer of the acyl group of a long-chain fatty acyl-CoA from coenzyme A to L-carnitine, which might stimulate the β-oxidation of fatty acids.
Therefore, the results showed that alleviation of MS by FBTPS might be involved in decrease in fat lipogenesis and accumulation as well as enhancement of fatty acid catabolism and oxidation. Similarly, alleviation of HFD-induced insulin resistance and nonalcoholic steatohepatitis by Pu-erh tea extract has been reported to be involved in down-regulation of the mRNA expression levels related to lipogenesis and accumulation of fat in the liver of HFD-fed mice. 

Accumulating evidences have suggested that gut microbiota might be a potential target for treatment of MS. Furthermore, numerous reports indicated that the prevention of MS by polysaccharides was related to the modulation of gut microbiota. In the present work, HFD treatment led to profound alteration in gut microbiota structure and composition based on results of PCA and hierarchical cluster analysis, which is consistent with previous reports. Nevertheless, an obvious amelioration of HFD-induced gut microbiota back to health status was observed for FBTPS intervention, especially for high dosage of FBTPS, suggesting superior gut microbiota modulation by FBTPS. Low diversity has been associated with several metabolic-disorders or immune-disorders. For example, it has been reported that gut microbiota diversity in obese individuals or mice was lower than that of lean hosts, and the measure of alpha diversity was negatively associated with obesity-related phenotypes, specifically abdominal adiposity. Our results showed that higher dosage of FBTPS could result in higher diversity of gut microbiota, confirming the importance of gut microbiota diversity for prevention of MS. Based on the results of alpha and beta-diversity analyses, FBTPS modulated gut microbiota in a
dose-dependent manner, which is in agreement with the dose-dependent manner of
MS amelioration, indicating a strong association between the modulation of gut
microbiota and syndrome amelioration alleviation. Besides decrease in gut
microbiota community diversity and alteration in the overall gut microbiota structure,
a significant increase in ratio of F/B was also observed after HFD treatment. An
increasing number of findings showed significant difference in the ratio of F/B
between the obesity and lean humans or mice. Furthermore, increase in the ratio
of F/B can result in enhancement of energy harvest, which is believed to be associated
with obesity. Thus, some researchers found that some potential candidates for
prevention of MS could reduce the ratio of F/B, which might play an important role in
amelioration of MS. However, it was found that supplementation with FBTPS had
limited effects on the relative abundances of Firmicutes and Bacteroides or the ratio
of F/B.

The early work showed that a bloom of Erysipelotrichaceae was observed in
diet-induced obese animals and individuals. It has also been reported that
Erysipelotrichaceae could lead to enhancement of energy extraction from the diet,
which is associated with lipidemic profiles in the host. Martínez et al. found that the
abundances of several bacterial taxa from Coriobacteriaceae and Erysipelotrichaceae
displayed significantly high relationship with cholesterol metabolites in hamsters
model. In our study, the relative abundance of Erysipelotrichaceae increased from
1.65 ± 1.09% to 26.19 ± 10.63% after HFD treatment, and then decreased from 26.19
± 10.63% to 8.76 ± 4.39% after high dosage of FBTPS intervention. Furthermore,
OTUs (OTU0021, OTU0316, OTU0345, OTU0197, OTU0493, OTU0363 and OTU0249) within family of Erysipelotrichaceae in OTUs level all showed high positive association with parameters of MS. Similarly, the enrichment of Coriobacteriaceae and Streptococcaceae at family level has been reported in HFD-induced MS in several studies.\textsuperscript{8,53,62,63} It has been demonstrated that the relative abundance of Coriobacteriaceae had a high positive relation with plasma cholesterol level in hamsters model (r = 0.84, P = 0.0002).\textsuperscript{64} Moreover, the hepatic glycogen, glucose, TG and the metabolism of xenobiotics have been reported to have strong association with the abundance of Coriobacteriaceae.\textsuperscript{65} Some strains within the family of Streptococcaceae have been reported to induce mild inflammation in humans.\textsuperscript{57} Furthermore, high abundances of Streptococcaceae family in gut microbiota are known to be related to the risks of metabolic disorders, diabetes and colon cancer.\textsuperscript{63} In the current study, the HFD-induced increases in abundances of Coriobacteriaceae and Streptococcaceae were significantly reversed to those of ND group. Both OTU0260 within the family of Coriobacteriaceae and OTU0406 within the family of Streptococcaceae were also significantly positively correlated with MS. At the same time, some researchers reported that decreases in relative abundance of Erysipelotrichaceae, Coriobacteriaceae or Streptococcaceae by some potential candidate or strategy for prevention of MS might play an important role in amelioration of MS, which might be potentially therapeutic targets for prevention of MS.\textsuperscript{8,61,66,67} Therefore, the alleviation of MS by FBTPS intervention might be also owed to the reversion of relative abundances of Erysipelotrichaceae,
Spearman’s correlation between the 46 key OTUs reversed by FBTPS and parameters of MS was carried out to identify the potentially beneficial phylotypes of gut microbiota. As results, 44 key OTUs, which were significantly associated with at least one parameter of MS, mainly belonged to Erysipelotrichaceae (8 OTUs), Lachnospiraceae (9 OTUs), Porphyromonadaceae (8 OTUs) and Ruminococcaceae (10 OTUs). Besides Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae, the families of Lachnospiraceae,\cite{44} Porphyromonadaceae\cite{68} and Ruminococcaceae\cite{54} had also been reported to be closely related to MS in previous works. Notably, the bloom of Erysipelotrichaceae in HFD group was mainly caused by the presence of OTU0021 whose abundance increased from 0.04 ± 0.04% to 23.27 ± 10.62%, and the relative abundance decreased to 6.21 ± 4.21% after intervention with high dosage of FBTPS. Furthermore, OTU0021 had significantly correlation with weight body gain, liver weight, epididymal fat, perirenal fat, TC, LDL-C and HDL-C, indicating that OTU0021 might play a central role in amelioration of MS by FBTPS. Gene sequencing data showed that OTU0021 belonged to \textit{Faecalibacterium rodentium}, however, limited information is available for it. Furthermore, FBTPS intervention could significantly enhance the relative abundances of 18 OTUs which were negatively correlated with MS. Thereinto, \textit{Alistipes},\cite{44} \textit{Bacteroides}\cite{17} and \textit{Odoribacter}\cite{69} have been reported to be negatively correlated with MS. On the other hand, FBTPS intervention could significantly reduce the relative abundances of 26 OTUs which were positively correlated with MS. Thereinto, \textit{Clostridium IV}\cite{70}, \textit{Flavonifractor},\cite{71}
Leuconostoc, Oscillibacter and Streptococcus have been reported to be positively correlated with MS. Although it is difficult to arrive at the cause-effect relationships among these associations in this work, our data suggested that FBTPS-induced modulation in the gut microbiota was highly associated with MS in obese mice and these changes might be an important mechanism for FBTPS mediating its beneficial metabolic effects. Therefore, the possible mechanism of MS prevention by FBTPS is that indigestible FBTPS reach the large intestine and then modulate the gut microbiota.

In conclusion, it was found that HFD-induced body weight gain, epididymal and perirenal fat accumulation, epididymal adipocyte size and the levels of TC and LDL-C in serum of mice were significantly restored by oral administration of FBTPS. The effects were associated with alleviation of liver weight, hepatic lipid deposition and expression of lipid metabolism-related genes. In addition, FBTPS intervention could increase microbiota phylogenetic diversity and reduce the relative abundances of Erysipelotrichaceae, Coriobacteriaceae and Streptococcaceae. The present results further suggested that 44 key OTUs that were negatively or positively associated with MS might play a key role in prevention of MS, leading us to propose that prevention of MS by FBTPS may be significantly related to modulation of the gut microbiota.

ASSOCIATED CONTENT

Supporting information

Tables for compositions of experimental diets, target genes and primers sequence.

Figures for food intake of mice, alpha diversity analysis of sample, and biplot of the
RDA based on the OTUs abundance between HFD and (a) ND, (b) HFD-L, (c) HFD-M and (d) HFD-H groups, respectively.

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**Funding**

The study was supported by the National Key Research and Development Program of China (2017YFD0400800), a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions and Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX17_0632).

**Notes**

The authors declare no competing financial interest.

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Figure Captions

Figure 1. Effects of FBTPS administration on (a) body weight, (b) body weight gain, (c) epididymal fat, (d) perirenal fat, (e) epididymal adipocyte microsections, (f) epididymal adipocyte size evaluated by Image J software, (g) liver weight and (h) liver microsections stained with H&E, the arrows indicate small lipid droplets. Values are means ± SD for n=8, and One-way ANOVA procedure followed by Tukey test was used to evaluate the statistical significance. The different letters represent significant differences between different groups (p < 0.05).

Figure 2. Effects of FBTPS on serum biochemical variables including TC (a), TG (b), LDL-C (c) and HDL-C (d). Values are means ± SD for n=8, and One-way ANOVA procedure followed by Tukey test was used to evaluate the statistical significance. The different letters represent significant differences between different groups (p < 0.05).

Figure 3. Effects of FBTPS on the relative expression of CPT1 (a), LXRα (b), FAS (c), SREBP-1c (d), PPARα (e) and PPARγ (f) in liver. Values are means ± SD for n=8, and One-way ANOVA procedure followed by Tukey test was used to evaluate the statistical significance. The different letters represent significant differences between different groups (p < 0.05).

Figure 4. FBTPS modulate the HFD-disrupted gut microbiota composition. a, comparison of alpha diversity accessed by Shannon and InvSimpson indexes; b, PCA of gut microbiota based on OUT relative abundance; c, hierarchical cluster analysis by euclidean distance matrix and ward.D2 method; d, bacterial taxonomic profiling at the phylum level of gut microbiota; e, the relative abundances of Firmicutes,
Bacteroidetes, and the ratio of Firmicutes to Bacteroidetes. One-way ANOVA procedure followed by Tukey test was used to evaluate the statistical significance. The different letters represent significant differences between different groups ($p < 0.05$).

**Figure 5.** Bacterial taxonomic profiling at the family level of gut microbiota. a, bubble chart of relative bacterial composition at the family level; b, comparative analysis of 20 most relative abundance of gut microbiota at the family level. One-way ANOVA procedure followed by Tukey test was used to evaluate the statistical significance. The different letters represent significant differences between different groups ($p < 0.05$).

**Figure 6.** The gut microbiota composition was altered by HFD or FBTPS intervention according to RDA results. a, Heatmap shows the relative abundance (log10 transformed) of 95 OTUs, the color of the squares indicated the relative abundance of the OTUs; b, the changing direction of OTUs induced by HFD or FBTPS intervention. The circles (○) and dots (●) represent the less and more relative abundances of OTUs in ND or FBTPS-fed groups compared with the HFD group, respectively. The asterisk (*) indicates OTUs in ND group altered by HFD intervention were reversed by FBTPS treatment; c, the OTUs represent bacterial taxa information (phylum, family, genus and species) modulated by FBTPS. Differences in relative abundance of OTUs were calculated using Tukey's HSD test. A value of $p < 0.05$ was considered significant.

**Figure 7.** 46 OTUs reversed by FBTPS intervention were significantly correlated with parameters of MS. a, the changing direction of OTUs induced by HFD and
FBTPS treatment. The circles (●) and dots (○) represent the less and more relative abundances of OTUs in ND or FBTPS-fed groups compared with the HFD group, respectively; b, the results of Spearman’s correlation between OTUs and parameters of metabolic syndrome. Colors of squares represent R-value of Spearman’s correlation. * and ** indicate the associations significant (p < 0.05 and p < 0.01, respectively); c, the OTUs represent bacterial taxa information (phylum, family, genus and species).
Figure 1.
Figure 2.
Figure 3.
Figure 4.
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Figure 7.
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Dysbiosis to Health

PC1-percent variation explained 59.45%
PC2-percent variation explained 11.31%