

Gut microbiota-mediated improvement of metabolic disorders by Qingzhuang tea in high fat diet-fed mice

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ABSTRACT

Qingzhuang tea (QZT) is a dark tea which is widely consumed by Chinese national minority whose staple diets are high fat. It has been previously demonstrated that QZT has anti-inflammatory properties, however its preventive and therapeutic mechanism on metabolic syndromes remains unclear. Particularly, how QZT extract modulates the metabolic syndrome-related gut microbiota composition remains to be elucidated. This study aims to explore the changes in gut microbial communities mediated by a 16-week QZT extract consumption in a diet-induced obese mice model through 16S rRNA sequencing. Our results demonstrate that QZT extract can significantly prevent body weight gain, fat accumulation, and also reduce serum levels of total cholesterol and triglycerides, which is accompanied by a decrease in *Firmicutes-to-Bacteroidetes* ratio and relative abundance of *S24-7* and *Ruminococcaceae*. Overall, our findings suggest QZT supplementation-induced gut microbial changes so that present benefits on mice with metabolic syndrome in high-fat diet-fed mice.

1. Introduction

Metabolic syndrome (MS), a combination of medical disorders including obesity, hyperglycemia, dyslipidemia, and hypertension, can increase the risk of type 2 diabetes mellitus (T2DM) or other related diseases (Ussar et al., 2015; Mahana et al., 2016; Ruby et al., 2017). The accumulated reports reveal that gut microbiota acts an important modulator in the diet and MS development interaction (Ley, Turnbaugh, Klein, & Gordon, 2006; Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Sung et al., 2017). More strikingly, *Erysipelotrichaceae* and *Coriobacteriaceae* have been demonstrated to be positively correlated with MS development (Chen et al., 2018a). Diet, is a significant factor altering the structure and metabolism of the intestinal flora to induce or prevent obesity (Tomas et al., 2016; Guo et al., 2019). Dietary supplementation is considered as one of the most effective strategies for alleviating gut dysbiosis (David et al., 2014; Liu et al., 2016). Emerging data have suggested that functional foods and active ingredients, such as black tea, Oolong tea, and green tea powder (Liu et al., 2016), Fuzhuan tea (Chen et al., 2018a; Chen et al., 2018b), Kuding tea (Chen et al.,

2018a), Pu-erh tea (Lu et al., 2019), and even tea polyphenols (Zhou, Tang, Shen, & Wang, 2018; Huang et al., 2018; Chen et al., 2019), tea polysaccharides (Chen et al., 2018c; Xu et al., 2015; Wu, T., Guo, Liu, Wang, & Zhang, 2016), and tea saponins (Chen, Tai, & Hsiao, 2015; Wang et al., 2017) suppress the development of MS by modulating gut microbiota. However, the effects of Qingzhuang tea on MS by modulating gut microbiota have rarely been reported.

Qingzhuang tea (QZT) is an important Chinese dark tea (Zhang, Zhang, Zhou, Ling, & Wan, 2013; Zheng, Wan, & Bao, 2015; Zhang, Ho, Zhou, Jânio, & Granato, 2019; Zhang, Cao, Granato, Xu, & Ho, 2020), which is mainly produced in the Hubei province. It is a beverage widely consumed by people in high-fat and high-calorie diet areas such as Mongolia, Xinjiang, Qinghai, and Gansu province in China for >100 years. Previous studies have shown that QZT has obvious health effects such as anti-obesity, free radical scavenging, anti-oxidation, and proliferation inhibition of 3T3-L1 pre-adipocytes (Zhang et al., 2013; Zheng et al., 2015). High-maturity raw material accompanying abundant tea polysaccharide and pigment accumulation after QZT fermentation leads to a striking regulation effect on gut microbiota and

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intestinal micro-ecological balance. However, few reports on the effect of QZT on metabolic syndrome by mediating gut microbiota have been available.

This study aims to reveal the metabolic syndrome inhibition effect and the gut microbiota modulatory effect of QZT in high fat diet-induced C57BL/6J mice. Gut microbiota was analyzed using 16S rRNA gene sequencing and the bacteria were characterized by species-level operational taxonomic units (OTUs, at 97% homology cutoff). Furthermore, the correlation of the MS-related indexes and gut microbiota abundance was analyzed. Our results indicated the gut microbiota responded to dietary intervention, and some metabolic disorder-related gut microbiota were identified. Therefore, these findings suggest that QZT can potentially serve as a functional food to prevent obesity in both humans and animals.

2. Materials and methods

2.1. Animal experiments

The animal experiments were approved by the Animal Experiment Center of Anhui Agricultural University (License No. SYXK 2016-007) and the Animal Ethics Committee, and the experimental operation conforms to the guidelines of National Experimental Animal Welfare and Animal Experimental Ethics Requirement. Forty-eight male C57BL/6J mice aged 6–8 weeks were purchased from the Institute of Model Animals of Nanjing University (Nanjing, China), and they were housed in a specific pathogen-free room at 22 ± 2 °C with a relative humidity of 70–75% and 12-h light/12-h dark cycle. The high fat diet (HFD, TP260303 diet with 60% kcal from fat) and its corresponding low-fat diet (LFD, TP26332 diet with 10% kcal from fat) were purchased from Trophic Animal Feed High-tech Co., Ltd (Nantong, China). After one-week acclimation, all the mice were randomly divided into four groups ($n = 12$ /group, four animals per cage). One group was fed with a low-fat diet and distilled water (LFD), the other three groups were supplied with a high-fat diet and respectively with water (HFD), 20 mg/mL of QZT water extract (QZT.L), and 40 mg/mL of QZT water extract (QZT.H). The metabonomic profile analysis of Qingzhu tea was reported in our previous study (Feng et al., 2020), and the contents of main polyphenol compounds in Qingzhu tea infusions were presented in Table S1. All the mice received food and water (or tea infusions) freely for 16 weeks. Tea extracts were prepared with 1.0 g dried QZT powder put in 50 or 25 mL boiling water, brewed for 10 min, and filtered to obtain 1:50 and 1:25 (w/v) QZT infusions. Tea infusions and diet supplied to mice were replaced every day. The weight of mice was recorded weekly, and the fasting blood sugar was successively monitored at the 7th, 12th and 16th week. After 16 weeks' intervention, the mice were sacrificed by cervical dislocation, blood and tissue samples were collected for biochemical analysis, and cecum-contents for gut microbiome analysis. All the samples were frozen immediately in liquid nitrogen and stored at -80 °C.

2.2. Serum biochemical analysis

The contents of triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

After 12-hour overnight fasting, fasting blood glucose (FBG) levels were measured at the 0, 7, 12, and 16 weeks. The oral glucose tolerance test (OGTT) was performed at the 16th week after the mice were administered with glucose solution (2 g/kg.bw). Blood glucose levels in tail blood samples were measured at minute 0, 15, 30, 60, and 120 after glucose treatment. A portable blood glucometer (Roche Co., Ltd., Germany) was used to measure blood glucose levels. The area under the curve (AUC) was calculated by the trapezoid rule.

2.3. Body composition analysis

Three days before the end of the experiment, the body composition of mice was assessed by the Live Mice Analyzer (Minispec, LF90II, Bruker, Germany) based on time-domain nuclear magnetic resonance (TD-NMR), and the data of the proportion of fat, fluid, and lean in the body were obtained.

2.4. Histopathological analysis of liver, epididymal adipose tissue, and cecum

Liver, epididymal fat, and cecum tissues were collected, fixed in paraformaldehyde, and stained with hematoxylin and eosin (H&E) before histopathologic analysis. The obtained slides were then measured using an optical microscope (LEICA DMI3000 B, Leica Biosystems, Wetzlar, Germany).

2.5. Quantification of genes expression

Total RNA of the liver was extracted with the commercial extraction kit (Tiangen Biochemical Technology Co., Ltd., China), and $5 \times$ Prime-script RT Master Mix (Takara, Dalian Bao Bioengineering Co., Ltd., China) was used to obtain the cDNA. The relative expressions of genes involved in lipogenesis and gluconeogenesis in the liver were measured. A SYBR Green premix Ex Taq II solution (Takara, Dalian Bao Bioengineering Co., Ltd., China) was used to amplify the sequences of interest and expression level was calculated using the $2^{-\Delta\Delta Ct}$ methods. In the liver, the target genes included *carnitine palmitoyl transferase* (CPT), *peroxisome proliferator-activated receptor α* (PPAR α), *fatty acids synthase* (FAS), *glucose-6-phosphatase* (G6pase), *phosphoenolpyruvate carboxykinase* (PEPCK), and *acetyl-CoA carboxylase α* (ACC α) (Wang et al., 2017). β -actin was used as a internal reference gene. The primer information was shown in Table S2.

2.6. Gut microbiota analysis

DNA was extracted from the caecum contents, followed by amplification and sequencing of the variable 4 (V4) region in the 16S rRNA using an Illumina MiSeq platform (Beijing Genomics Institute, China), as described in one previous study (He et al., 2016). After trimming and aligning, sequences with $\geq 97\%$ similarity would be grouped into one operational taxonomic unit (OTU), and their locations in a phylogenetic tree would determine the classification of each OTU (Yang, Zhang, Zhang, Huang, & Wang, 2016). Afterwards, community alpha diversity and beta diversity were analyzed to reveal the microbial complexity (Yang et al., 2016). Furthermore, to investigate the functions of the entire bacterial community, PICRUSt (<http://huttenhower.sph.harvard.edu/galaxy>) was used to predict the functions of the microbial community (Langille et al., 2013; Li et al., 2019). The seq. fasta files were used to align OTUs against the GreenGenes database (version 13.5) at a cut-off of 97% identity. The resultant OTUs were normalized, predicted, and categorized according to online PICRUSt protocols using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

2.7. Statistical analysis

All the data were expressed as the mean \pm standard deviation (SD). Statistical significance between groups was evaluated by one-way ANOVA, followed by Tukey test using SPSS Statistics 19.0 (IBM, Chicago, USA). The correlation between relative abundances of OTUs and host biochemical parameters were analyzed by Spearman's correlation. $p < 0.05$ was considered as statistically significant. Finally, the co-occurrence network was visualized using Cytoscape (version 3.5.1).

3. Results

3.1. QZT supplementation suppresses weight gain and fat accumulation in mice

To study the effects of QZT on metabolic disorders, different doses of QZT infusions were prepared and supplied as drinking water to high-fat diet-fed mice. During the 16 weeks' intervention, all the mice grew well. The daily intake of food and water or tea per mice was shown in Fig. S1. As shown in Table 1, in the 16th week, the average body weight of LFD group mice was 34.35 ± 0.83 g, and that of the HFD group mice was 44.75 ± 0.87 g ($p < 0.001$). Body weight and body weight gain of the QZT-supplemented groups were significantly suppressed ($p < 0.01$) (Table 1). The inhibitory effect of high dose of QZT on the body weight and body weight gain was more obvious than that of low dose of QZT. To reveal the impact of QZT on the body composition of mice, small animal nuclear magnetic resonance was used to analyze the percentage of fat, free body fluid, and lean in mice. Compared with the HFD group, the LFD group had lower fat mass, free body fluid, but higher lean content. Intervention with QZT infusion altered the body composition of mice by significantly inhibiting the accumulation of fat and free body fluid, and increasing the lean content. There was no significant difference between the HFD + QZT.H and LFD group (Table S3).

Perirenal adipose tissue (PAT), mesenteric adipose tissue (MAT), and epididymal adipose tissue (EAT) are the main intra-abdominal adipose tissues of mice, which are the white visceral fat attached to the kidney, mesentery, and epididymis, respectively. The effects of QZT on histopathology and weight of the main intra-abdominal adipose tissues and liver in mice were shown in Fig. 1 and Table 1. Compared with those in the LFD group, PAT, MAT, EAT, and liver mass in the HFD group were significantly increased after 16-week intervention ($p < 0.001$) (Fig. 1 and Table 1). Interestingly, QZT substantially attenuated the high fat diet-induced accumulation of PAT, MAT, and EAT ($p < 0.01$), which coincided with a significant reduction in fat deposition in liver ($p < 0.01$) (Table 1). Hematoxylin-eosin (H&E) staining analysis of epididymal fat showed that mice treated with QZT infusion exhibited smaller cell size than HFD group, and that the QZT.H group exhibited more obvious inhibition on adipocyte enlargement induced by high-fat diet (Fig. 1A). Liver H&E staining showed that QZT markedly restored the liver injury caused by high-fat diet, and that the volume and number of fat vacuoles were significantly reduced (Fig. 1D). The size and color

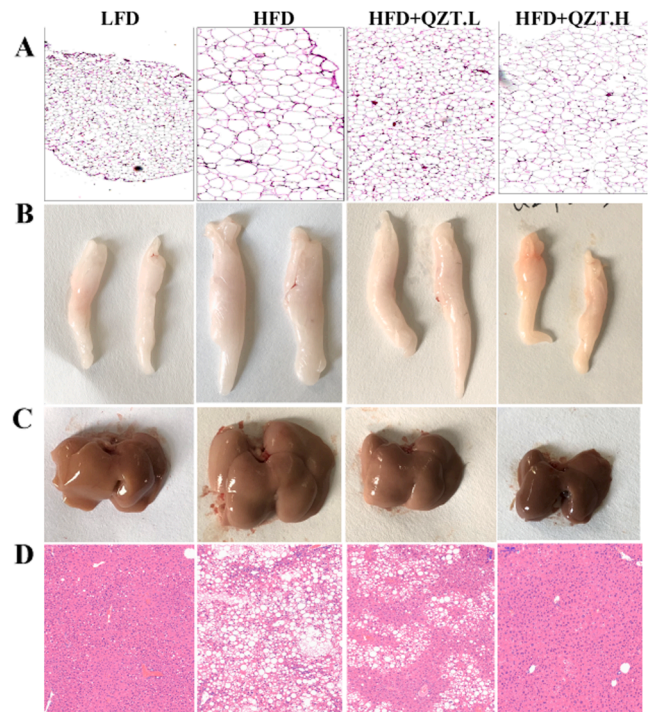


Fig. 1. Effects of QZT on histopathology and weight of intra-abdominal adipose tissue and liver. H&E staining and morphology of epididymal fat (A&B) and liver (C&D).

(from dark to light) of epididymal fat and liver in QZT groups were generally between HFD group (biggest size, lightest color) and LFD group (smallest size, and darkest color) (Fig. 1B-C). The liver index is expressed as the ratio of liver weight to body weight, which reflects the degree of fat deposition in the liver. The liver index in the HFD group (7.2%) was notably higher than that in the LFD group (3.5%), and the intervention of low dose and high dose of QZT infusion resulted in 1.2% and 1.7% reduction in the liver index in HFD group, respectively. These results suggested that QZT reduced body weight and fat deposition in HFD group in a dose-dependent manner.

Table 1

Effects of QZT intervention on tissue weight and serum biochemicals in mice.

| | LFD | HFD | HFD + QZT.L | HFD + QZT.H |
|---|------------------------|-------------------|------------------------|------------------------|
| Tissue weight (g) | | | | |
| Body weight (g) | $31.32 \pm 0.19^{***}$ | 43.52 ± 0.75 | $37.31 \pm 0.47^{**}$ | $33.45 \pm 0.49^{**}$ |
| Liver (g) | $1.08 \pm 0.15^{***}$ | 3.14 ± 0.43 | $2.22 \pm 0.32^{**}$ | $1.72 \pm 0.27^{***}$ |
| Perirenal adipose (g) | $0.43 \pm 0.08^{***}$ | 0.85 ± 0.18 | $0.63 \pm 0.19^{***}$ | $0.46 \pm 0.19^{***}$ |
| Mesenteric adipose (g) | $0.48 \pm 0.10^{***}$ | 0.88 ± 0.23 | $0.52 \pm 0.16^{***}$ | $0.42 \pm 0.12^{***}$ |
| Epididymal adipose (g) | $1.29 \pm 0.23^{***}$ | 2.45 ± 0.36 | 2.21 ± 0.24 | $1.52 \pm 0.30^{***}$ |
| Serum biomarkers | | | | |
| Lipids | | | | |
| Total triglycerides (mmol/L) | $0.69 \pm 0.12^{**}$ | 0.83 ± 0.25 | $0.54 \pm 0.10^{***}$ | $0.50 \pm 0.08^{***}$ |
| Total cholesterol (mmol/L) | $4.85 \pm 0.48^{***}$ | 7.61 ± 1.18 | $5.33 \pm 0.62^{***}$ | $4.82 \pm 0.65^*$ |
| LDL-cholesterol (mmol/L) | $1.58 \pm 0.45^{***}$ | 3.99 ± 0.66 | $2.30 \pm 0.33^{***}$ | $1.80 \pm 0.44^{***}$ |
| HDL-cholesterol (mmol/L) | $1.55 \pm 0.15^{***}$ | 2.27 ± 0.45 | $1.58 \pm 0.11^{***}$ | $1.50 \pm 0.18^{**}$ |
| Alanine aminotransferase (U/L) | $14.66 \pm 1.89^{***}$ | 41.11 ± 3.87 | $16.30 \pm 2.87^{***}$ | $15.66 \pm 2.30^{***}$ |
| Aspartate aminotransaminase (U/L) | $48.89 \pm 3.12^{***}$ | 60.90 ± 5.81 | $46.27 \pm 3.60^{***}$ | $44.35 \pm 3.53^{***}$ |
| Oral glucose tolerance test (OGTT) | | | | |
| Blood glucose at 0 min (mmol/L) | $7.3 \pm 1.03^{***}$ | 9.26 ± 1.08 | $6.26 \pm 0.25^{***}$ | $5.16 \pm 0.47^{***}$ |
| Blood glucose at 15 min (mmol/L) | 19.48 ± 2.00 | 21.92 ± 2.47 | 19.4 ± 1.68 | $16.08 \pm 1.91^{**}$ |
| Blood glucose at 30 min (mmol/L) | 16.54 ± 1.65 | 19.9 ± 1.68 | 19.8 ± 1.66 | $16.12 \pm 2.15^*$ |
| Blood glucose at 60 min (mmol/L) | 14.08 ± 1.38 | 17 ± 1.45 | 15.36 ± 2.29 | $12.8 \pm 1.44^{**}$ |
| Blood glucose at 120 min (mmol/L) | 11.08 ± 1.02 | 12.5 ± 1.02 | 11.58 ± 1.00 | $7.48 \pm 1.20^{***}$ |
| Area under the curve (AUC) | $1685 \pm 105^{**}$ | 1986 ± 151.12 | $1822.05 \pm 117.91^*$ | $1443 \pm 127.69^{**}$ |

Data are expressed as the mean \pm SD (n = 12). Data were analyzed using one-way ANOVA, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ represent different degrees of significant difference.

3.2. QZT supplementation decreases serum lipids in HFD-fed mice

The levels of serum lipids including TG, TC, LDL-C, and HDL-C were shown in Table 1. HFD group exhibited a significant increase in TG, TC, HDL-C, and LDL-C levels ($p < 0.01$ or $p < 0.001$), compared with the LFD group (Table 1). QZT dose-dependently decreased TG, TC, LDL-C and HDL-C levels ($p < 0.001$ or $p < 0.01$ or $p < 0.05$). These results indicated that QZT extract could reduce serum lipids in HFD-fed mice. Furthermore, liver damage induced by high-fat diet was also ameliorated by QZT regarding serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Compared with the LFD group, the HFD group displayed a significant increase in AST and ALT levels, suggesting supplementation of QZT could drastically reduce serum AST and ALT levels ($p < 0.001$), which confirmed that the QZT could effectively suppress lipotoxicity to the liver induced by HFD (Table 1).

Our data also indicated that QZT decreased the blood glucose levels in mice fed with the HFD. The oral glucose tolerance test (OGTT) results showed that the oral glucose tolerance of the HFD group was lower than that of the LFD group, and that the area under the curve (AUC) was significantly increased ($p < 0.01$) (Table 1). The oral glucose tolerance of QZT treatment groups was ameliorated, and the AUC value of QZT treatment groups was significantly lower than that of HFD control group ($p < 0.05$). Especially, high dose of QZT intervention had a comparable effect with LFD on the improvement of glucose tolerance (Table 1).

Previous studies have shown that the expressions of genes involved in hepatic fatty acid and glucose synthesis are changed after HFD treatment (Liu et al., 2016; Yang et al., 2016). In this study, the relative expression levels of *G6Pase*, *ACCa*, *CPT1*, *FAS*, *PEPCK*, and *PPAR α* were quantified by RT-PCR (Fig. 2). Compared with the LFD group, HFD group exhibited an up-regulated *FAS* expression, and the down-regulated expression of *PPAR α* , *CPT*, *ACCa*, *PEPCK*, and *G6Pase*. High dose of QZT significantly increased the expression levels of *PPAR α* , *ACCa*, and *G6Pase*, and decreased *FAS* expression ($p < 0.01$ or $p < 0.05$), thus resulting in gene expression patterns of the above genes similar to those in LFD group. However, the expression levels of these genes in low dose of QZT treatment group showed no significant difference from those of the HFD control group.

3.3. QZT supplementation modulates gut microbial community at different taxonomic levels

In order to investigate the effects of QZT on the gut microbiota in HFD-induced obese mice, we sequenced the V4 hypervariable region of microbial 16S rDNA on the high-throughput sequence platform. In this study. A total of 77,277 clean reads and 416 distinct operational classification units (OTUs) were obtained. The rarefaction index curves (Observe Species, Coverage, Shannon and Simpson) revealed that our

sequencing data covered the vast majority of microbial species (Fig. S2). It has been reported that the dietary intervention had a significant impact on intestinal microbial diversity including the α -diversity (characterized by observed Species, Chao, and ace index) (Tomas et al., 2016; Guo et al., 2019; David et al., 2014; Liu et al., 2016). Our data indicated that long-term high-fat diet significantly reduced the diversity of microbiota (Figs. 3 and S2), suggesting the imbalanced microbiota after HFD treatment), which is in agreement with previous findings (Liu et al., 2016; Lu et al., 2019; Zhou et al., 2018; Yang et al., 2016). The QZT intervention was found to significantly alleviate the reduction in HFD-induced gut microbiota. No significant difference in Simpson index was observed between QZT treatment group and the LFD group ($p > 0.05$). Principal component analysis (PCA) was performed to reveal the influence of QZT intervention on the relative abundance of OTUs. As shown in Fig. 3D, LFD and HFD groups were clearly apart, and high-dose QZT group tended to be close to the LFD group, whereas low-dose QZT group was still more close to the HFD group, indicating that high-fat diet and QZT intervention had a significant impact on the gut microbiota composition, which supported the previous findings (Tomas et al., 2016; Guo et al., 2019).

The functional profiles of OTUs were analyzed using PICRUSt based on the KEGG database. The predicted different KEGG pathways were shown in Fig. S3. Unsurprisingly, Metabolism, Environmental Information Processing, and Genetic Information Processing were successively the top 3 most abundant functional categories (KEGG level-1, Fig. S3.A). Moreover, the abundances of those three categories were higher in HFD group than in LFD group, and different doses of QZT intervention decreased the HFD-induced increase in abundances of these 3 categories (Fig. S3). In the metabolism category, the relative abundance of carbohydrates metabolism-related pathways in the HFD group was significantly higher than in LFD group, whereas relative abundance of cell motility-related pathway was significantly lower in HFD group than in LFD group (in KEGG level-2, Fig. S3.B), and QZT intervention reversed the alteration pattern in HFD group. It should be noted that in KEGG level-3, the relative abundances of the pathways associated with galactose metabolism, pentose and glucuronate interconversions, and starch and sucrose metabolism were higher in HFD group than in LFD group, whereas those of carbon-fixation pathways in prokaryotes and citrate cycle (TCA cycle) were lower in HFD group than in LFD group (Fig. S3.C).

The abundances of the top 10 gut microbiota at the class, order, family, genus, and species levels among groups were shown in Table S4. The effects of QZT treatments on the abundance of gut microbiota at the phylum level and family level were shown in Fig. 3E and 3F, respectively. Taxonomic profiling results suggested that the gut microbiota in mice were mainly composed of *Firmicutes*, *Proteobacteria* and *Bacteroidetes* at the phylum level (Figs. 3E and S4), which was consistent with

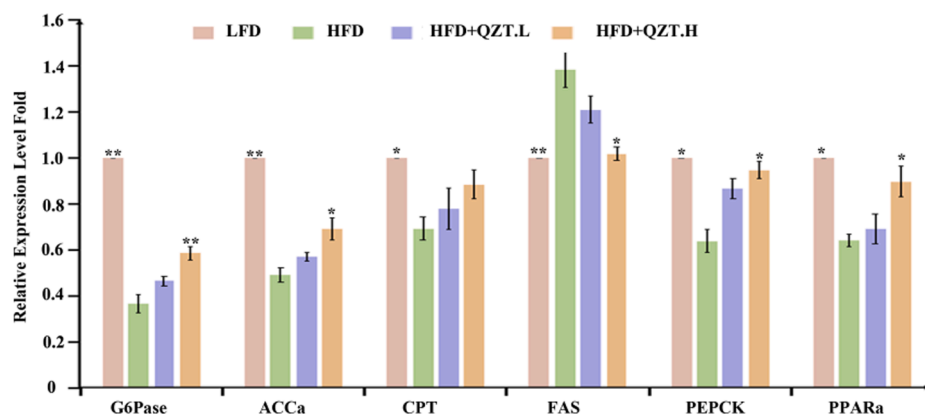


Fig. 2. Genes expression levels in mice hepatic tissue in low-fat diet (LFD) group, high-fat diet (HFD) group, and HFD supplemented with QZT group. One-way ANOVA was performed with. All data were expressed as mean \pm SD. Significant differences were presented at the levels of *, $p < 0.05$. **, $p < 0.01$.

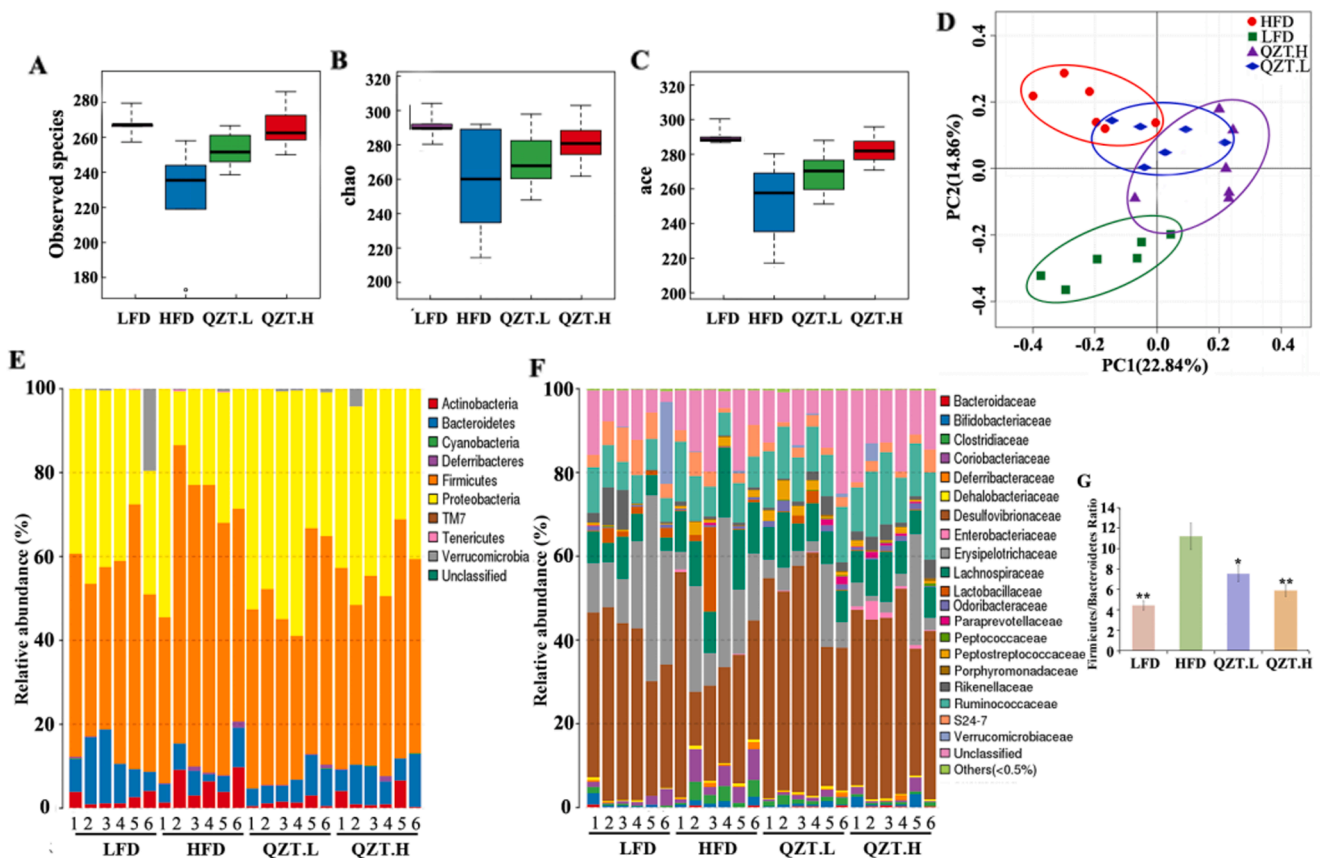


Fig. 3. Overall composition changes of the gut microbiota after 16-week dietary intervention. Observed species (A), Chao (B), and ace (C) indexes as the estimators of the community diversity of gut microbiota. Principal component analysis (PCA) based on OTUs relative abundance (D). Each point represents the composition of gut microbiota in one group. Bacterial taxonomic profiling at the phylum level (E) and family level (F) of gut microbiota. (G) Ratios of *Firmicutes* to *Bacteroidetes* abundance among groups. All data were expressed as mean \pm SD. Significant differences were presented at the levels of *, $p < 0.05$ and **, $p < 0.01$.

previous reports (Liu et al., 2016; Chen et al., 2018b; Zhou et al., 2018; Beaumont et al., 2016). Compared with the LFD group, HFD group exhibited a significant increase in the *Firmicutes* abundance and a significant decrease in the *Proteobacteria* and *Bacteroidetes* abundances, thus resulting in an increase in the ratio of *Firmicutes* to *Bacteroidetes* (F/B) ($p < 0.01$), eventually inducing gut microbiota disorders (Fig. 3G). The increase in the F/B ratio led to a rise in host energy intake, thus increasing the risk of metabolic syndrome (Chen et al., 2018a). Nevertheless, QZT supplementation significantly decreased the F/B ratio to the level of the LFD group ($p > 0.05$). At the family level, significant difference in the abundances of *Desulfovibrionaceae*, *Erysipelotrichaceae*, *Enterobacteriaceae*, *Lactobacillaceae*, *Ruminococcaceae*, and *Coriobacteriaceae* were observed between HFD group and LFD group (Figs. 3F and S4). Our data demonstrated that QZT intervention was found to decrease the relative abundances of *Erysipelotrichaceae* and *Coriobacteriaceae*. These two bacteria were reported to be positively correlated with metabolic syndrome, and the higher abundance of these two bacteria, the higher risk of metabolic syndrome occurrence (Chen et al., 2018a; Chen et al., 2018b; Chen et al., 2018c). Our data also showed that QZT enhanced the relative abundance of *Desulfovibrionaceae*, which was considered as a health-promoting microbial species (Beaumont et al., 2016). The above findings suggested that QZT could inhibit metabolic syndrome by mediating certain gut microbiota.

It was also important to examine the changes of OTUs after QZT treatment since the different bacterial species from the same family may have different responses to the same environmental stress such as high-fat diet (Wang et al., 2015). Bacterial functions and their roles in metabolic diseases are strain-specific. Therefore, Redundancy analysis was conducted to identify the OTUs with relative abundance $> 0.1\%$ at

least in one group, $VIP > 1$, and $p < 0.05$. A total of 94 OTUs were identified, and the relative abundance of resultant 94 OTUs in each treatment group and their taxonomic information were shown in Fig. 4. Of these 94 OTUs, the abundance of 37 OTUs was significantly increased, and that of 57 OTUs was decreased OTUs in the LFD group, compared with those of the HFD group (Fig. 4). QZT.H treatment reversed 40 OTUs abundance change, of which 29 increased and 11 decreased, and QZT.L treatment reversed 38 OTUs abundance change, of which 25 increased and 13 decreased. Of 94 OTUs, 31 OTUs were altered by HFD, were reversed by QZT treatment (Fig. 4). These results indicated that QZT treatments could effectively improve the gut microbiota imbalance induced by HFD.

3.4. OTUs related to metabolic syndrome are identified in HFD-fed mice

Spearman's correlation analysis was conducted to reveal the correlations between the relative abundances of 94 dominant gut microbiota OTUs and parameters of MS. Subsequently, whether these OTUs contributed to alleviating metabolic syndrome in HFD induced mice was further investigated.

As shown in Fig. 5, 44 OTUs were found to exhibit positive or negative correlation with at least one parameter of MS, including liver weight, perirenal fat, intestinal fat, epididymal fat, LDL-C, HDL-C, TG and TC. Of these 44 OTUs, OTU7 and OTU366 belonged to *Erysipelotrichaceae* family; OTU101 and OTU8 belonged to *Coriobacteriaceae* family; 11 OTUs belonged to *Lachnospiraceae*, and 7 OTUs belonged to *Ruminococcaceae*, and *Lachnospiraceae* and *Ruminococcaceae* were also identified in previous study, and they were reported to play an important role in preventing metabolic syndrome, which was consistent

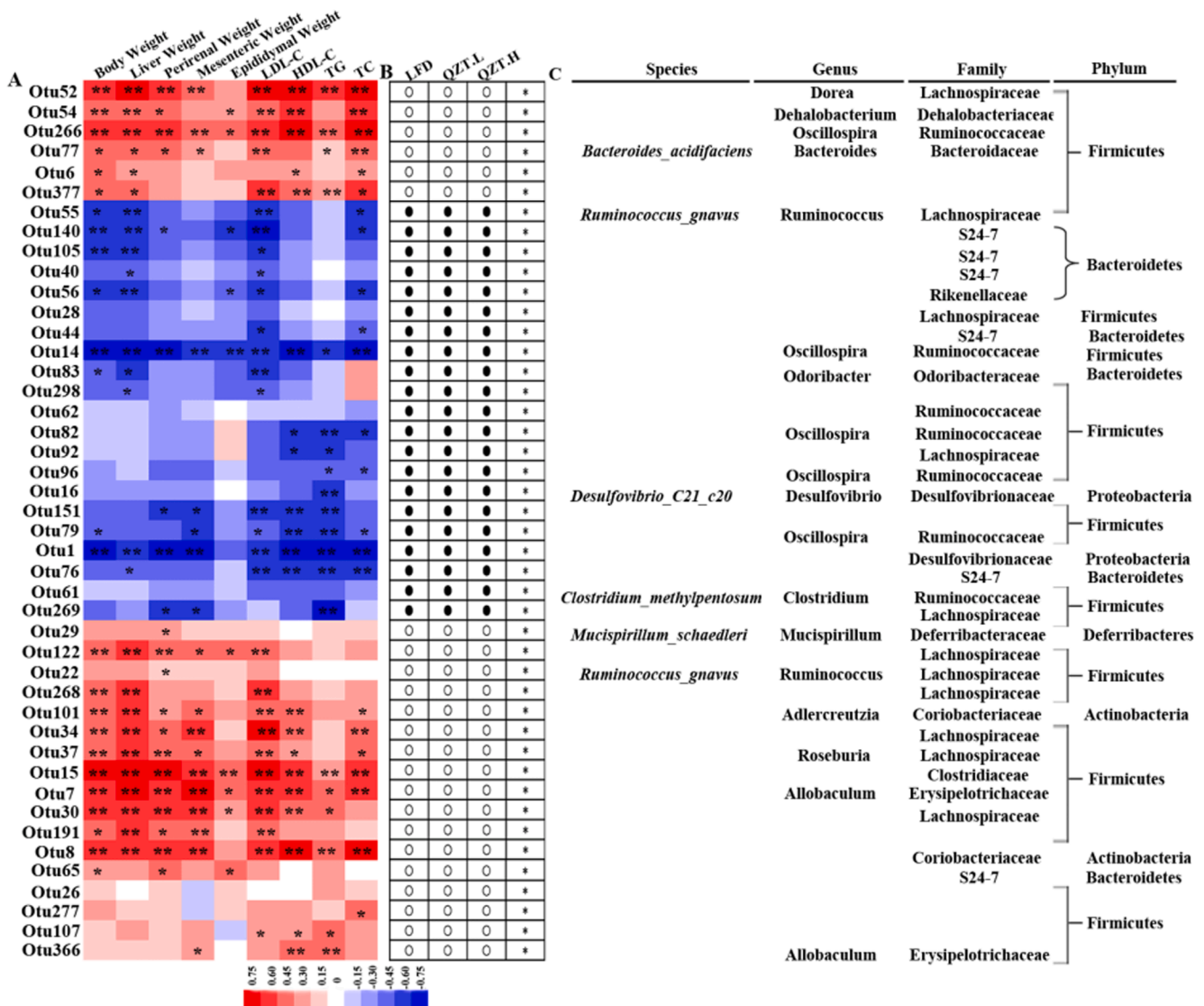


Fig. 5. Spearman's correlation analysis of 44 OTUs and 9 MS-related parameters. (A) Correlation coefficient (R value). Colors of squares represent the R-value of Spearman's correlation. * and ** indicate the significant difference (compared to HFD group) at levels of $p < 0.05$ and $p < 0.01$, respectively; (B) The circles (○) and dots (●) represent higher and lower relative abundance of OTUs in LFD or QZT groups than in HFD group, respectively. (C) OTU information (phylum, family, genus, and species).

with the our studies (Yang et al., 2016; Ziętak, Kovatcheva-Datchary, Markiewicz, Stahlman, & Kozak, 2016). It was worth noting that of 44 metabolic syndrome-related OTUs, 23 OTUs were positively correlated with the metabolic syndrome, and their relative abundances were significantly reduced after QZT supplementation, including *Dehalobacterium* (OTU54), *Dorea* (OTU52), *Mucispirillum* (OTU29), *Oscillospira* (OTU266), *Roseburia* (OTU37), *Allobaculum* (OTU7, OTU366), and *Bacteroides acidifaciens* (OTU77). Conversely, 21 OTUs were negatively associated with metabolic syndrome, and their relative abundance was significantly increased after QZT intervention, including *Oscillospira* (OTU384), *Ruminococcus_gnavus* (OTU55), *Odoribacter* (OTU83), *Desulfovibrio_C21_c20* (OTU16), and *Clostridium_methylpentosum* (OTU61). Our results indicated that QZTs attenuated the HFD-induced intestinal microbial disorders and reconstituted a healthy gut microbial composition similar to that of the LFD group.

To further understand the interactions and connectivity among 94 OTUs, we established networks and identified co-abundance groups (CAGs) using Cytoscape software (Deng et al., 2012). Fig. S5 showed the interaction between the OTUs and their CAGs. In network analysis, the similarity threshold was set as 0.60. A total of 271 OTU pairs with

significant and robust correlations (edges) were identified from 88 nodes (Fig. S5). Specifically, 258 OTU pairs with positive correlations and 13 OTU pairs with negative correlations were identified in the network. The top ten OTU with highest abundance (OTU178, OTU228, OTU33, OTU74, OTU95, OTU64, OTU82, OTU7, OTU104 and OTU15) exhibited no correlation with the parameters of MS. The top 3 co-abundance CAGs were CAG1, CAG2, and CAG3 modules, respectively. CAG1 contained 12 OTUs; CAG2 contained 8 OTUs; and CAG3 contained 13 OTUs (Table S5). Moreover, CAG1 and CAG3 modules were positively correlated with MS parameters, whereas CAG2 module showed a negative correlation. A total of 17 OTUs (OTU 96 in CAG1 module, OTU 268, OTU 34, OTU 30, OTU 191, OTU 7, OTU 101, OTU 22, OTU 52 in CAG2 module, OTU 83, OTU 105, OTU 140, OTU 40, OTU 56, OTU 28, OTU 44, OTU 55 in CAG3 module) were the overlap of the 33 OTUs from CAGs modules and 44 OTU correlated with MS. These 17 OTUs might be responsible for regulating HFD-induced gut microbial disorder.

4. Discussion

A 16-week consumption of QZT infusions prevented body weight

gain, liver steatosis, intra-abdominal fat deposition (perirenal, intestinal and epididymis fat pads) induced by a high-fat diet. Meanwhile, blood biochemical indexes were also reversed in the QZT groups, such as TG, TC, LDL-C, ALT, AST. Increasing studies have been indicated that black tea, oolong tea, and green tea significantly reduce body weight gain and fat accumulation in HFD mice (Liu et al., 2016). Previous reports showed that anti-obesogenic candidates not only prevent fat accumulation, but also regulate oxidative stress, endotoxemia, and inflammation (Yang et al., 2016; Etxeberria et al., 2015). High-dose QZT treatment obviously recovered liver function to the functional level of LFD group. Moreover, tea polysaccharides and polyphenols extracted from black tea or green tea suppressed the obesity development of HFD-fed mice (Liu et al., 2016; Zhou et al., 2018; Huang et al., 2018; Xu et al., 2015; Wu, Guo, Liu, Wang, & Zhang, 2016). Theabrownines, polysaccharides, and polyphenols, and a series of bio-transformed constituents were accumulated during the QZT pile fermentation processes (Feng et al., 2020), and those special constituents jointly contribute to the anti-obesity effect of QZT (Zhang et al., 2013; Zheng et al., 2015; Feng et al., 2020). The consumption of 1: 25(1 g tea power/25 mL water) or 1:50 QZT infusion were equivalent to an adult human with body weight of 65 kg consuming 25.4 g or 11.9 g tea per day, in terms of the Km value of mice (Km = 3) and humans (Km = 37) (Reagan-Shaw, Nihal, & Ahmad, 2008), and the final bodyweight after 16-week intervention of 31 g for QZT.L group, 29 g for QZT.H group, and average daily tea consumption of 3.5 mL per mice (Han et al., 2016). The dosages (1:25 or 1:50) used in this study was lower than the dosage of 1:20 green tea infusion used in previous study for improving insulin resistance in HFD-fed rats (Snoussi et al., 2014), and the dosages of QZT (1:25 or 1:50) in this study, especially the low dosage (1:50) is far lower than the dosage of tea infusion boiled in the teapot consumed in daily life.

The elevated levels of serum AST and ALT might be due to such factors as toxicity, inflammation, hypoxia, and tissue trauma. AST and ALT are regarded as the most sensitive indicators to assess liver function. This study revealed that QZT suppressed the activities of AST and ALT to alleviate the liver inflammation triggered by HFD, which further confirmed the potential liver protective effect of QZT. The liver is the most important organ for lipogenesis, glucogenesis, and energy metabolism (Huang et al., 2013). Moreover, numerous anti-obesogenic candidates were reported to ameliorate MS by regulating the expressions of liver genes mainly involved in energy metabolism (Huang et al., 2018; Suk et al., 2017). *FAS*, as one energy metabolism-related gene, facilitated fatty acid synthesis and TG accumulation through triacylglycerol (Tomas et al., 2016). *PPAR α* , a known regulator for fatty acid oxidation and lipid metabolism, is mainly expressed in the liver, and the upregulation of this gene expression could stimulate lipolysis, thus decreasing TG and LDL-C level in the liver (Tomas et al., 2016). In this work, QZT significantly suppressed the expression levels of *FAS* in the liver and up-regulated the expression of *PPAR α* , *CPT*, *ACC α* , *PEPCK* and *G6Pase*, thus decreasing lipogenesis and glucogenesis in the liver. Therefore, our results suggested that QZT could prevent body weight gain, fat accumulation, and increase plasma lipid level by inhibiting lipogenesis- and glucogenesis-related genes expressions. Recent studies suggested that Kudingcha tea and Fuzhuan brick tea protected against HFD-induced obesity by down-regulating *PPAR γ* , *SREBP-1c* and *FAS* (Liu et al., 2016). Moreover, QZT supplementation showed positive effects on oral glucose tolerance and fasting blood glucose, which were reported to be related to *PPAR α / γ* and *NF- κ B*, the upregulation of whose expressions would reduce pro-inflammatory cytokine concentrations in HFD-fed mice (Tomas et al., 2016).

Gut microbiota are increasingly considered as a vital factor for maintaining body homeostasis and being partially responsible for metabolic disorders, and they are highly associated with several human diseases, such as diabetes and obesity (Chen et al., 2018a; Guo et al., 2019). Caecum has a lower pH and a higher concentration of volatile fatty acids than the distal colon where fecal samples are collected. Moreover, 97% of the 110 observed differentially expressed pathways

were reported to be different between cecal and fecal microbiota in the cecum (Tanca et al., 2017). HFD was found to destroy the biodiversity of intestinal microflora, leading to the disorder of intestinal microflora in the cecum (Chen et al., 2018a). Moreover, QZT supplementation significantly alleviated the intestinal microflora diversity reduction caused by HFD, and significantly increased the ratio of *Firmicute* to *Bacteroides* (F/B), which were likely to be related to increased food energy absorption, low-level inflammation and plasma glucose concentration (Chen et al., 2018a).

It has been reported that HFD-induced gut dysbiosis caused the intestinal integrity damage, and intestinal bacteria-derived endotoxin permeation into the bloodstream, thus resulting in emerging insulin resistance, chronic inflammation, and other symptoms (Yang et al., 2016). For example, *Desulfovibrionaceae* can generate endotoxins (such as LPS) and induce intestinal inflammation (Beaumont et al., 2016) and *Oscillospira* is associated with the degradation of intestinal mucins, thus their reduction was conducive to increasing intestinal integrity (Konikoff & Gophna, 2016). In this study, QZT supplementation might increase intestinal integrity by inhibiting the abundance of these two gut microbiota. The three highest scores CAGs were obtained in our work. OTUs in CAG1 and CAG2 were mainly composed of *Firmicutes*, and OTUs in CAG1 module were negatively correlated with lipid-related parameters, whereas OTUs in CAG2 module were positively correlated with them. OTUs in CAG3 module mainly belonged to *Bacteroidetes*, and were positively correlated with physiological indexes. To be more specific, seven of 12 OTUs in the CAG1 module, OTU96, OTU296, OTU38, OTU41, OTU33, OTU82, and OTU138 belonged to *Oscillospira* genus, *Ruminococcaceae* family, and their relative abundances were detected to reduce in both QZT.H and QZT.L groups. Our results were consistent with previous reports that *Oscillospira* was also decreased in response to green tea polyphenols and dietary fiber supplementation (Chen et al., 2018a; Huang et al., 2018). In addition, this microbiota was reported to be associated with leanness (fat reduction) (Konikoff and Gophna, 2016). In this study, the reduction of *Oscillospira* in QZT groups might account for the increased mucin production, which represented a compensatory mechanism for handling the HFD-induced inflammation. Except OTU 101, almost all the OTUs in the CAG2 module (Otu268, Otu34, Otu30, Otu22, Otu52) belonged to *Lachnospiraceae* family, *Firmicutes* phylum. In the CAG2 module, OTU101 belonging to the *Adlercreutzia* genus of *Coriobacteriaceae* family and OTU7 belonging to *Allobaculum* genus of *Erysipelotrichaceae* family were significantly correlated with all the nine selected lipid-related parameters. It was reported that *Erysipelotrichaceae* family increased the host's energy extraction from food, and the relative abundance of *Erysipelotrichaceae* had a significant correlation with blood lipid in the host. Liu et al. (2016) found a significant correlation between relative abundances of *Erysipelotrichaceae* and *Coriobacteriaceae* and cholesterol metabolism in hamsters' gut. Moreover, the accumulated evidence revealed *Erysipelotrichaceae*, *Coriobacteriaceae* and *Streptococaceae* were the potential targets for preventing metabolic syndrome (Liu et al., 2016; Chen et al., 2018b; Xu et al., 2015). *Lachnospiraceae* family was known to have an anti-inflammatory effect via T-regulatory cell expansion, thus increasing short-chain fatty acid (SCFA) production (Konikoff and Gophna, 2016). SCFAs exerted multiple functions such as lowering the pH of the colon, fighting against inflammation and tumor, and providing nutrients for intestinal epithelial cells (Atarashi et al., 2013). We speculated that in QZT treatment groups, the reduction in OTU abundance led to an increase in the short-chain fatty acids production, thus suppressing lipid metabolism induced by HFD. Of the total 13 OTUs in the CAG3 module, Otu28 and Otu55 belonged to *Firmicutes*, and other 11 OTUs (OTU83, OTU104, OTU178, OTU95, OTU105, OTU140, OTU40, OTU89, OTU56, OTU10, and Otu44) belonged to *Bacteroidetes* phylum, 8 out of 13 OTUs belonging to S24-7 family were negatively correlated with mice lipid-related parameters in agreement with previous reports (Casanova-Martí et al., 2018). In QZT groups, the abundance increase of OTUs (OTU105, OTU140, OTU40, and OTU44) belonging to S24-7 was

observed. We speculated that the increase in *S24-7* abundance in the QZT treatment groups improved intestinal integrity by increasing tight junction proteins (ZO-1, Occludin). The relative abundances of butyrate-producing bacteria including *S24-7* family in the CAG3 module and *Ruminococcaceae* family in the CAG1 module were increased after QZT intervention. Moreover, the *S24-7* family was previously described as a target for polyphenols (Li, Gao, Du, & Mao, 2018). Given that gut microbiota played important roles, and that QZT contained abundant simple and polymerized polyphenols, we speculate that the prevention effects of QZT on MS might be attributed to its abundant polyphenols. Taken together, our data suggest that *S24-7* and *Ruminococcaceae* families are promising targets for the prevention of MS. Additionally, some other bioactive compounds produced in the QZT manufacturing process might enhance the effects of QZT, which remains to be further elucidated.

5. Conclusion

In this study, QZT effectively prevents metabolic syndrome and intestinal microbiota disorder induced by HFD in C57BL/6J mice. QZT attenuated body weight gain and fat deposition in adipose tissues and liver, decreased serum lipids and blood glucose, improved glucose tolerance, suppressed lipogenesis and glucogenesis. Moreover, QZT alleviated the dysbiosis of gut microbial ecology by altering the composition and relative abundance of microbes, which is highly correlated with the prevention of metabolic syndrome. Our results indicate that dietary QZT supplementation can effectively maintain gut microbial community homeostasis and regulate the genes related to lipogenesis and glucogenesis, contributing to a health phenotype of the host.

Ethics statements

The program of the animal experiment is examined and approved by the Animal Experiment Center of Anhui Agricultural University (License No. SYXK 2016-007) and the Animal Ethics Committee, and the experimental operation conforms to the guidelines of the National Experimental Animal Welfare and Animal Experimental Ethics Requirement.

CRediT authorship contribution statement

Lin Feng: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Jie Zhou:** Investigation, Methodology, Resources, Validation, Writing - review & editing. **Liang Zhang:** Visualization, Writing - review & editing, Project administration. **Panpan Liu:** Resources, Validation, Software, Investigation. **Pengcheng Zheng:** Resources, Validation, Software. **Shiwei Gao:** Formal analysis, Validation, Software. **Chuan-kui Song:** Writing - review & editing, Data curation, Resources. **Yongchao Yu:** Data curation, Resources. **Ziming Gong:** Writing - review & editing, Supervision, Project administration, Funding acquisition. **Xiaochun Wan:** Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jff.2021.104366>.

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