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# Dynamic changes in gut microbiota during the intervention of tea polyphenols to alleviate high-fat diet-induced obesity

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**ABSTRACT:** Tea polyphenols (TPs) could prevent obesity by regulating gut microbiota. However, it is unclear how TPs play this regulatory effect during the development of obesity. To reveal the dynamic changes in gut microbiota, obese mice were induced with a high-fat diet (HFD) and intervened with different doses of TPs for 8 weeks. The results showed that HFD continuously altered gut microbial composition and induced an increase in the *Firmicutes*/*Bacteroidetes* (F/B) ratio, resulting in gut microbial dysbiosis and obesity. TPs intervention improved the increase in F/B ratio and regulated the dysbiosis caused by HFD. This regulatory effect was related to its dose and intervention time, among which the effects of 200 mg/(kg.BW)/d TPs intervention for 8 weeks was the best. Random forest and network analysis results indicated that TPs could continuously increase the relative abundance of prevent-obesity bacteria (such as *Akkermansia*, *Lactobacillaceae*, and *Alistipes*), decrease the relative abundance of promote-obesity bacteria (such as *Lachnospiraceae*, *Oscillospiraceae*, and *Ruminococcaceae*), and enhance the gut microbial network complexity. Consequently, TPs could affect lipid accumulation and prevent obesity development. The study provides new insights into the mechanism of TPs anti-obesity.

**Keywords**: Tea polyphenols; obesity; gut microbiota; dynamic change; co-occurrence network

## 1. Introduction

Obesity has become a prevalent health issue worldwide. It could threaten human health, leading to a variety of diseases including type 2 diabetes, cardiovascular disease, hypertension, and cancer [1]. Therefore, it is of great importance to find ways to alleviate obesity and benefit the public. Over the last decade, increasing evidence has shown that the gut microbiota is a potential factor in the pathophysiology of obesity [2]. Gut microbial dysbiosis can affect lipid metabolism, leading to the occurrence and development of obesity [3]. Therefore, the gut microbiota is considered as a potential target for prevention and treatment of obesity.

Tea polyphenols (TPs) are the main active component in tea, with a good anti-obesity effect. Research on fecal microbiota transplantation has revealed that the anti-obesity effect of TPs was closely related to its regulation of gut microbiota [4]. This regulation mainly includes two aspects. On the one hand, TPs could increase the gut microbial diversity and regulate the abundance of bacteria closely related to obesity, such as

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*Akkermansia*, *Bifidobacterium*, and *Prevotella*, preventing gut microbial dysbiosis [5]. On the other hand, TPs could increase the production of beneficial microbial metabolites such as short chain fatty acids and inhibit bacterial endotoxin, improving host metabolic disorders [6,7].

Although the majority believed that TPs could prevent obesity by regulating gut microbiota, mainly by reducing the *Firmicutes*/*Bacteroidetes* (F/B) ratio [8], there still exist some contradictory findings. For example, according to Janssens et al., long-term green tea supplementation did not alter the human gut microbiota [9]. Another study revealed that Pu-er tea extract could alleviate diet-induced obesity by increasing the abundance of *Firmicutes* and decreasing the abundance of *Bacteroidetes*, i.e. increasing the F/B ratio, after 5 weeks of intervention [10]. These contradictory results might be caused by different treatments used in different experiments, such as TPs types, doses, and intervention times. However, a solid and credible explanation for this issue has not been reported and needs to be verified.

In this study, we focused on the hypothesis that different doses of TPs might have different regulatory effects on gut microbiota in obese mice for different intervention times. Therefore, we evaluated the effects of different doses of TPs on the diversity, composition, and microbial network of gut microbiota in high-fat diet (HFD)-induced obese mice. We also analyzed the effects of TPs intervention on mouse body weight, abdominal fat deposition, and lipid profile. These help to reveal the dynamic effects of TPs intervention on gut microbiota during the development of HFD-induced obesity and its relationship with alleviating obesity. The study provides a new perspective on the role of TPs in preventing obesity development.

## 2. Materials and methods

### *2.1 Materials and reagents*

TPs (purity of 98%) were purchased from Anhui Redstar Pharmaceutical Corp., Ltd (Anhui, China), which contained 50.96% EGCG, 10.44% ECG, 16.02% EGC, and 7.43% EC, analyzed by high-pressure liquid chromatography. Mouse chow was purchased from Jiangsu xietong Pharmaceutical Bioengineering Co., Ltd (Jiangsu, China). All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

### *2.2 Animals and treatment*

Fifty specific pathogen-free (SPF) Kunming male mice (4 weeks, (20 ± 1) g, No. 00298518) were purchased from the College of Biomedicine and Health, Huazhong Agricultural University, Wuhan, China. All animal experiments were approved by the Institutional Animal Care and Use Committee of this university (Permit number SYXK2020-0084). All animal protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals.

All mice were randomized and housed five in a cage at (25 ± 1)℃ and 12/12-h light/dark cycle. After the mice received standard mouse chow and water *ad libitum* for one week, they were divided into five groups, ten mice each. They were as follows: normal control group (NC), received a standard diet (3.6 kcal/g, 12% of energy from fat; 1010009); model control group (MC), received an HFD (5.1 kcal/g, 60% of energy from fat;

XTHF60-1); three doses of TPs groups (TPL, TPM, TPH), received an HFD (5.1 kcal/g, 60% of energy from fat; XTHF60-1) and TPs at a daily dose of 100 mg/(kg.BW), 200 mg/(kg.BW), or 400 mg/(kg.BW), respectively. The dose of TPs was determined based on literature [11], and was safe according to the previous report [12]. After grouping, TPs mice were orally administered 0.2 mL of TPs solution daily, while NC and MC mice received 0.2 mL of distilled water. All mice were given the corresponding treatments for 8 weeks and had free access to food and water during the experimental period.

*2.3 Sample collection*

During the experimental period, the body weight and food consumption of mice were monitored weekly. Fresh fecal samples were collected into sterile tubes at 0, 2, 4, 6, and 8 weeks, and stored quickly at -80℃ for gut microbiota analysis [13]. After 8 weeks of feeding, the mice were euthanized after 12-h fasting. Blood samples were collected by retro-orbital bleeding and centrifuged at 3000 g at 4℃ for 15 min to obtain serum. The liver, adipose tissues around mesentery, epididymis, and kidney were dissected, rinsed with PBS, and weighed. Liver and epididymal adipose tissue were fixed at 4% paraformaldehyde for histological analysis.

*2.4 Serum lipid profile*

The levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (TC) were measured using appropriate commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

*2.5 Histopathological analysis*

The fixed liver and epididymal adipose tissue were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The histomorphology of liver and adipose tissue was observed using a microscope (Nikon E100, Tokyo, Japan), and the size of adipose cells was measured with Image J.

*2.6 Gut microbiota data analysis*

Microbiota genomic DNA was extracted from fecal contents with a TIANamp Stool DNA Kit (TianGen, China). The quality of the DNA samples was monitored on 1% agarose gels. 16S rRNA gene V3-V4 regions were amplified using specific primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA). PCR products were purified with a Universal DNA Purification Kit (TianGen, China), quantified using QuantiFluor™-ST (Promega, Madison, WI, USA), and the purified amplicons were sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, USA) with the assistance of Beijing Novogene Technology Co., Ltd., China, according to the relevant standard procedure.

Paired-end sequencing reads were parsed into sample sets using their individual barcodes; the barcodes and primer sequences were then truncated from the main sequence reads. The reads were merged using FLASH software (Version 1.2.11) to obtain initial rRNA tags. For the Effective Tags obtained previously,

denoise was performed with DADA2 or deblur module in the QIIME2 software (Version QIIME2-202006) to obtain initial ASVs (Amplicon Sequence Variants), and then ASVs with abundance less than 5 were filtered out. Then the Silva database was used to annotate taxonomic information.

*2.7 Statistical analysis*

The data were expressed as means ± standard deviation (SD). Statistical analyses were performed with R (4.3.0) and SPSS 26.0 software. The calculation and plotting of co-occurrence network analysis utilized the "ggClusterNet" package [14]. Chao1 and Shannon applied the R package, “vegan”. Manhattan plot applied the R package, “ggplot2”. Principal coordinate analysis (PCoA) and random forest analysis adopted the R script published by Zhang et al [15]. Differential abundance of the gut microbiota was analyzed using the Kruskal-Wallis or Mann-Whitney U test, with p-values adjusted by FDR. Significance among multiple groups was analyzed using one-way ANOVA by Duncan's post hoc test. For all analyses, values of $P < 0.05$ were considered statistically significant.

## 3. Results and discussion

*3.1 Effect of TPs on the obesity phenotype in HFD-fed mice*

As shown in Fig.1 and Fig.S1, after 8 weeks of HFD feeding, body weight, food efficiency ratio, the weight of epididymal, perirenal, and mesenteric adipose tissues, and ratio of liver weight/body weight in the MC group were significantly higher than those in the NC group. Serum TC, TG, and LDL-C levels in the MC group were also significantly higher than those in the NC group, whereas serum HDL-C level was significantly lower. Histomorphology analysis showed that the MC mice exhibited hepatic steatosis with a large number of fat vacuoles, and its epididymal adipocytes were significantly larger than those in the NC mice. However, compared with the MC group, three doses of TPs significantly reduced the body weight, food efficiency ratio, abdominal fat weight, and ratio of liver weight/body weight in the TPL, TPM, or TPH group, respectively, and significantly improved serum lipid profiles. They also significantly decreased the number of hepatic fat vacuoles and the area of epididymal adipocytes. The results showed that HFD led to fat accumulation and obesity in mice, while TPs intervention alleviated HFD-induced obesity, which was consistent with previous studies [7,11].

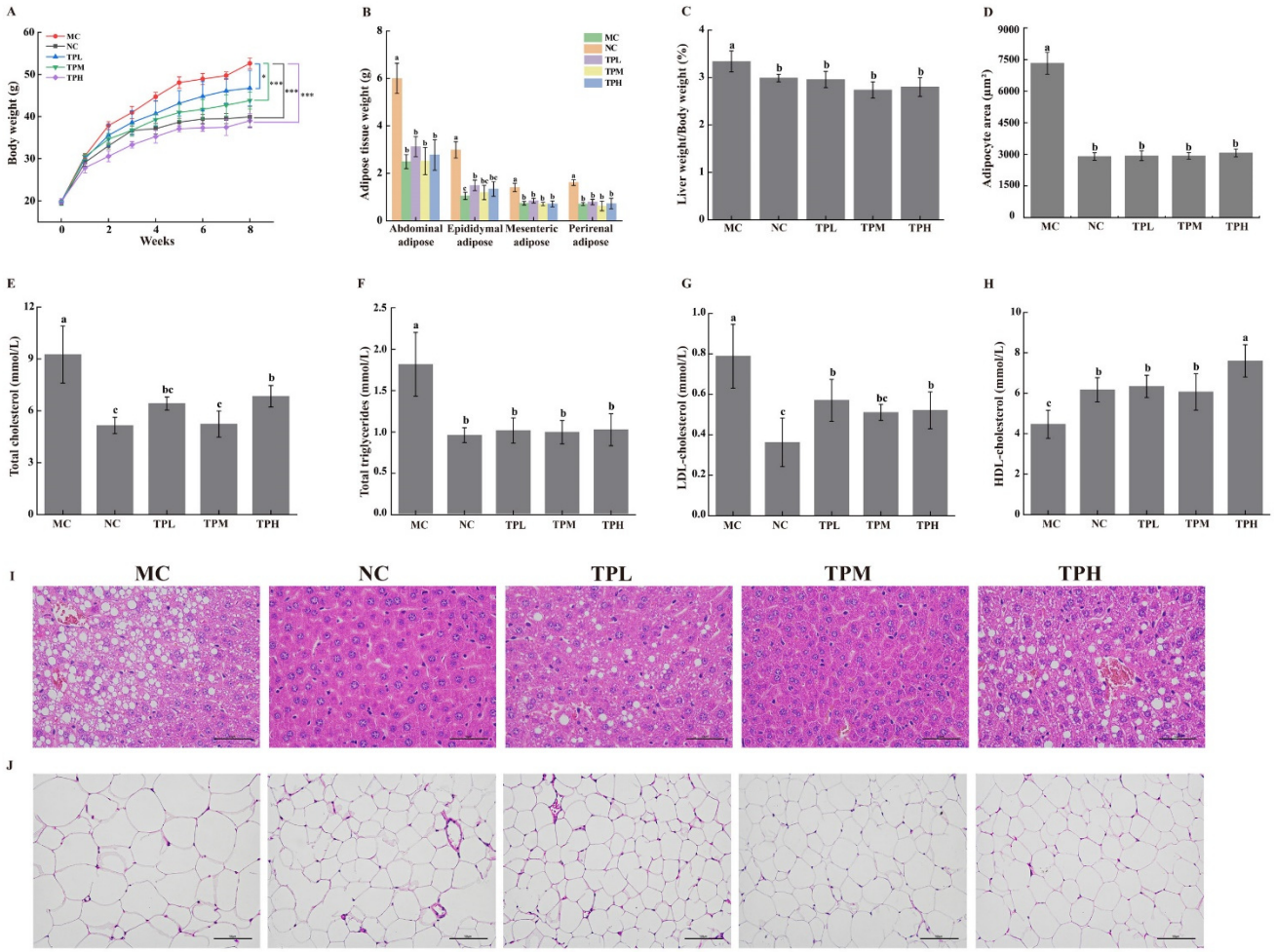


**Fig.1.** Effect of TP on the obesity phenotypes in HFD-fed mice. (A) Changes in body weight during the 8-week feeding; (B) Adipose tissue weight; (C) Epididymal adipocyte area; (D) Liver weight / body weight ratio; (E) Total cholesterol (TC); (F) Triglyceride (TG); (G) Low-density lipoprotein cholesterol (LDL-C); (H) High-density lipoprotein cholesterol (HDL-C); (I) Morphology of hepatic tissue (400×); (J) Morphology of epididymal adipose tissue (200×). Data are expressed as means ± SD. Statistical analysis was performed using one-way ANOVA, followed by Duncan's post hoc test. Different lowercase letters above the bars indicate significant differences at $P < 0.05$. * or *** indicates significant differences at $P < 0.05$ or $P < 0.001$, respectively, compared with the MC group.

### *3.2 Dynamic changes in gut microbial diversity*

Obesity often accompanies gut microbial dysbiosis. However, the stability of the gut microbial ecosystem is closely related to host health. The higher the diversity of gut microbiota, the better the stability of the gut microbial ecosystem, and the stronger the resistance to gut microbial dysbiosis [16]. Therefore, we evaluated the effect of TPs intervention on alpha diversity (Chao1 index and Shannon index) and beta diversity (PCoA) of the gut microbiota in HFD-induced obese mice.

As shown in Fig.2A-B, the Chao1 index and Shannon index in the MC group were invariably lower than those in the NC group during the 8 weeks of HFD feeding, and significant differences were observed after week 4 and week 6, respectively ($P < 0.05$). This indicated that HFD reduced the alpha diversity of gut microbiota and this impact worsened with increasing HFD intake. TPs intervention could effectively alleviate the decrease. The Chao1 index (except for TPH group at the first 2 weeks) and Shannon index in the three TPs groups were higher than those in the MC group during the 8-week intervention, and significant differences

were observed after 8 weeks (except for TPM group after 6 weeks, $P < 0.01$) and after 4 weeks (except for TPL group, $P < 0.01$), respectively.

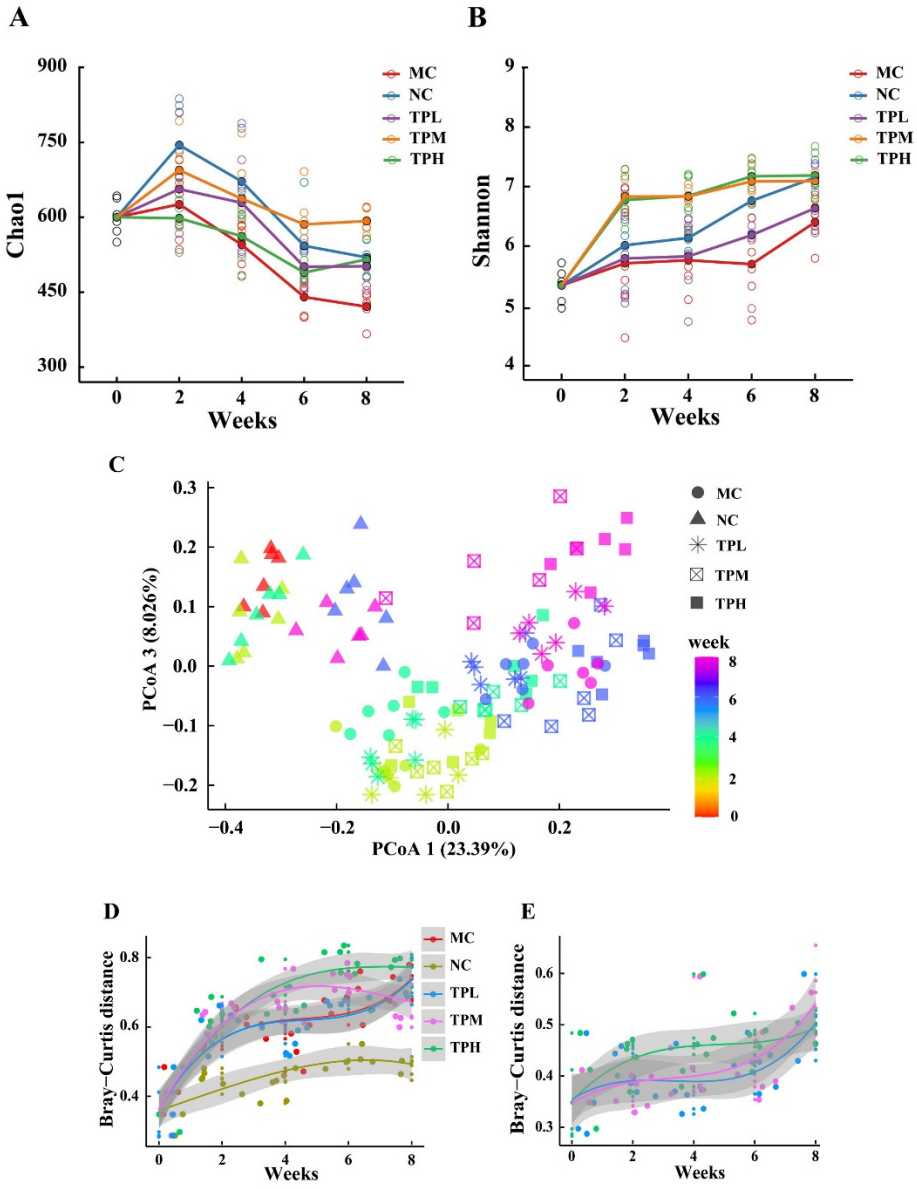


**Fig.2.** Dynamic changes in gut microbial diversity. (A) Chao1 index; (B) Shannon index; (C) Principal coordinate analysis (PCoA); (D) Dynamic changes in Bray-Curtis distance of samples at different times compared with that at week 0; (E) Dynamic changes in Bray-Curtis distance of samples in TP groups compared with that in the MC group.

To evaluate the trend of changes in gut microbial community structure over time, a PCoA plot based on the Bray-Curtis distance was constructed. As shown in Fig. 2C, a predominant central cluster was observed in the NC group, whereas a dispersed cluster was evident in the MC group and the Bray-Curtis distances of samples between each time point and week 0 significantly increased over time ($P < 0.05$). This indicated that the gut microbial community structure was relatively stable, but HFD altered the structure and the variation gradually accumulated with the prolongation of HFD-fed time. TPs intervention regulated the structural change caused by HFD. In all, compared with the Bray-Curtis distance at week 0, the distance in the TPM group or TPH group increased rapidly at the first four weeks, then gradually decreased or leveled off, respectively. On the contrary, the distance in the TPL group continuously increased, resembling the trend of the change in the MC group (Fig.2D). Compared with the structure in the MC group, with the prolongation of

TPs intervention, the structural change in the TPL group or TPM group was small at the first four weeks and then rapidly increased, while the structural change in the TPH group was relatively large at the first four weeks and then leveled off (Fig.2E). These results indicated that the dose and intervention time of TPs played an important role in regulating the HFD-induced changes in gut microbial community structure. At the early stages of intervention, high-dose TPs had a significant impact on the structure. However, the effects of low-dose and medium-dose TPs gradually became dominant after 4 weeks of intervention, with the greatest effect after 8 weeks.

*3.3 Dynamic changes in gut microbial composition*

*Firmicutes* and *Bacteroidetes* are dominant bacterial phyla in the gut [17]. The F/B ratio is crucial to maintaining normal gut homeostasis, although the ratio varies with age [18]. Studies have reported that an increase in the F/B ratio is a potential reason of obesity, and balancing the ratio is important to the prevention or treatment obesity [17]. Therefore, in this study, we evaluated the dynamic effects of TPs on the gut microbial composition and F/B ratio in HFD-induced obese mice during the 8-week intervention.

As shown in Fig.3, from week 0 to week 8, the relative abundance of *Firmicutes* in the MC group continuously increased from 28.09% to 72.77%, mainly attributed to an increase in the relative abundance of *Lachnospiraceae* (except for week 4), *Clostridia_ UCG-014*, and *Oscillospiraceae*. In contrast, the relative abundance of *Bacteroidetes* continuously decreased from 65.77% to 18.86%. Correspondingly, the F/B ratio continued to increase significantly (except for week 4), from (0.43 ± 0.08) (week 0) to (4.62 ± 1.01) (week 8). HFD also inhibited the growth of *Verrucomimicrobia* (except for week 2), mainly *Akkermansiaceae*. This indicated that HFD gradually altered the gut microbial composition, leading to gut microbial dysbiosis. TPs intervention regulated the gut microbial composition and alleviated the increasing trend of F/B ratio. The ratios in the TPM and TPH groups at week 8 were even significantly lower than those at week 6, respectively. TPs intervention also reduced the relative abundance of *Clostridia_UCG-014* and *Lachnospiraceae,* and increased the relative abundance of *Akkermansiaceae* and *Lactobacillaceae*. At week 8, compared with the MC group, the relative abundance of *Akkermansiaceae* in the TPL, TPM, and TPH groups increased by 2.52, 4.27, and 1.26 times, respectively, and the relative abundance of *Lactobacillaceae* increased by 2.02, 2.57, and 0.41 times, respectively. These results suggested that TPs could regulate the gut microbial dysbiosis induced by HFD, and the regulatory effects became increasingly significant over time. Among them, the TPM has the best regulatory effect.

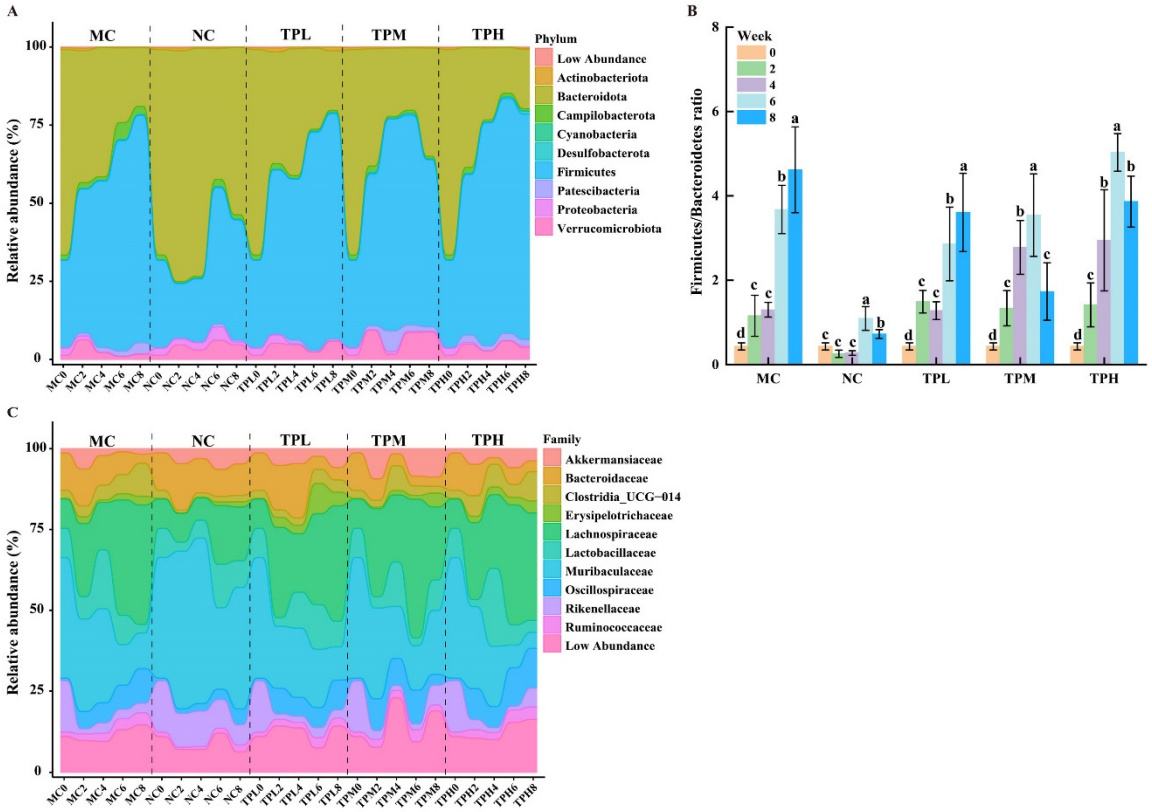


**Fig.3.** Dynamic changes in gut microbiota composition. (A) Taxonomic composition of gut microbiota at the phylum level; (B) *Firmicutes*/*Bacteroidetes* (F/B) ratio, different lowercase letters above the bars indicate significant differences at $P < 0.05$; (C) Taxonomic composition of gut microbiota at the family level.

Previous studies have shown that the abundance of *Lachnospiraceae* is significantly positively correlated with obesity phenotypes [19], which might be due to the fact that a high abundance of *Lachnospiraceae* actively impaired glucose and/or lipid metabolism, leading to metabolic disorder and inflammation [20-21]. Toxic metabolites produced by some members of *Lachnospiraceae* might also cause ecological imbalance of host gut microbiota, inducing host obesity or inflammation [22]. In addition, the increase in the abundance of *Clostridia_UCG-014* might increase blood glucose and promote the development of obesity [23]. In contrast, *Akkermansia* from *Akkermansiaceae* and *Lactobacillus* from *Lactobacillaceae* are probiotics. The abundance of *Akkermansia* is associated with leanness in mice and obesity is often accompanied by a decrease in its abundance [24]. *Lactobacillus* can not only reduce the levels of pro-inflammatory cytokines and inhibit the production of endotoxins [25], but also produce acid to inhibit the growth of commensal *Lachnospiraceae* [26]. The increase in their abundance contributes to weight loss. Therefore, our findings suggested that TPs might prevent obesity caused by HFD by continuously regulating the abundance of gut microbiota related to obesity.

Notably, the relative abundance of *Akkermansiaceae* in the MC group increased transiently from 1.31% (week 0) to 6.26% (week 2) and then dropped back to approximately the initial level. This spike might be due to the short-term protective effect of *Akkermansia* against gut microbial dysbiosis during the first development of obesity [27]. However, with the prolongation of HFD-fed time, the gut microbial diversity continuously decreased in the MC group, and the protective effect gradually disappeared. TPs could maintain this effect by increasing the abundance of *Akkermansia* [28].

### *3.4 Dynamic regulation of TPs on gut microbial composition at the family level*

To reveal the regulatory ability of TPs on gut microbial composition at the family level, we used Manhattan plots to analyze the changes in gut microbial composition and the dynamic regulation of TPs during the 8-week HFD feeding. As shown in Fig.4 and Fig.S2, compared with the NC group, at week 2, the ASVs (including *Lachnospiraceae*, *Oscillospiraceae*, and *Ruminococcaceae*) belonging to *Firmicutes* were significantly enriched in the MC group, while the ASVs (including *Muribaculaceae* and *Rikenellaceae*) belonging to *Bacteroidota* were significantly downregulated. With the prolongation of HFD feeding, this trend gradually weakened. At week 8, the number of regulated ASVs in the MC group significantly decreased, and the gut microbial composition gradually stabilized to new equilibria. However, compared with the MC group, the number of regulated ASVs increased gradually with the prolongation of TPs intervention. At week 8, the ASVs belonging to *Bacteroidaceae*, *Muribaculaceae*, and *Rikenellaceae* in the TPL, TPM, and TPH groups were significantly upregulated, while the ASVs belonging to *Lachnospiraceae*, *Oscillospiraceae*, *Clostridia_UCG-014*, and *Ruminococcaceae* were significantly downregulated. These results indicated that TPs could continue to regulate the changes in gut microbial composition caused by HFD, and the regulatory effect on gut microbial composition gradually became dominant with the prolongation of TPs intervention. This was consistent with previous reports that green TPs could continuously regulate the composition of bacterial species from *Firmicutes* and *Bacteroidota* during a 3-week feeding [29], and it was necessary to continuously consume green tea extract to regulate the gut microbial imbalance caused by the HFD diet for anti-obesity effect [30].

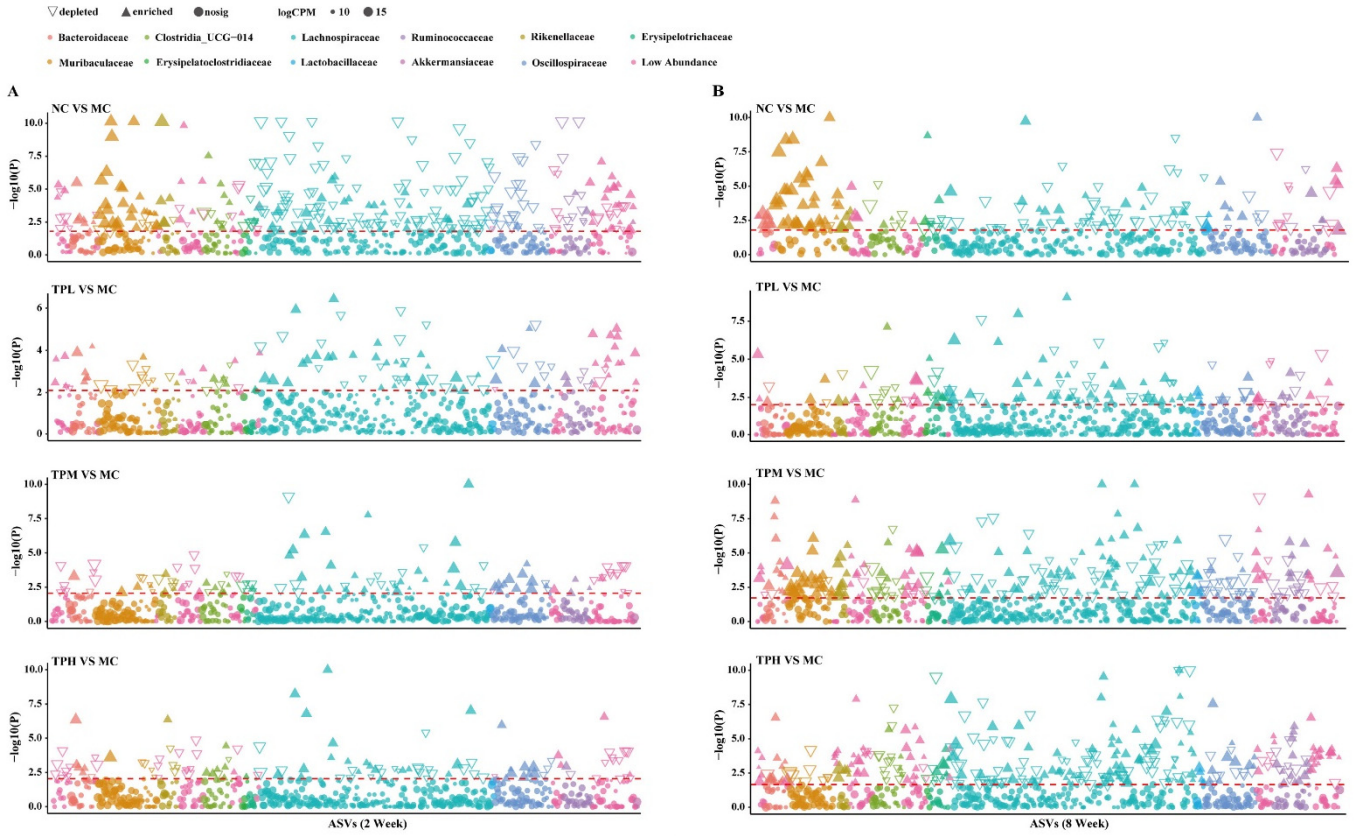


**Fig.4.** Dynamic regulation of TP on HFD-induced gut microbial composition. Manhattan plots of enriched or depleted ASVs in different groups at week 2 (A) and week 8 (B), compared with the MC group. Each dot or triangle represents a

single ASV. Enriched or depleted ASVs are shown with filled or empty triangles, respectively (FDR adjusted $P < 0.05$). ASVs are arranged in taxonomic order and colored according to the family levels. CPM represents counts per million.

### *3.5 Dynamic changes in obesity-related biomarkers*

To explore obesity-related biomarkers in the gut microbiota, we used a random forest model to identify differential gut microbiota causing obesity during the 8-week HFD feeding (Fig.5 and Fig.S3). A total of 44 differential bacterial species at the genus level were identified, which primarily belonged to *Lachnospiraceae*, *Ruminococcaceae*, and *Oscillospiraceae*. TPs intervention significantly regulated the changes in the relative abundance of these differential species. Compared with the MC group, after 8 weeks of intervention, the relative abundance of *Lachnospiraceae* (such as *Acetatifactor*, *Lachnospiraceae_UCG-010*, *Lachnospiraceae_UCG-006*, and *Lachnospiraceae_NK4A136_group*), *Oscillospiraceae* (such as *Colidextribacter*, *Oscillibacter*, and *Intestinimonas*), *Ruminococcaceae* (such as *Incertae_Sedis* and *Harryflintia*), *Enterococcus*, *Clostridia_UCG-014*, *Eubacterium_brachy_group*, *UCG-009*, and *Butyricicoccus* significantly decreased; yet, the relative abundance of *Akkermansia*, *Alistipes*, *Rikenellaceae_RC9_gut_group*, *Turicibacter*, and *Anaerostipes* significantly increased. Among them, the number of bacterial genera with significant changes in abundance was the least in the TPH group. This might be due to the fact that high-dose TPs was reported to transform from antioxidant into "prooxidant" by producing excessive amounts of reactive oxygen species and depleting the oxidant defense [11]. This led to a reduced ability of high-dose TPs to improve intestinal oxidative stress, thereby affecting its regulatory effect on the gut microbiota.

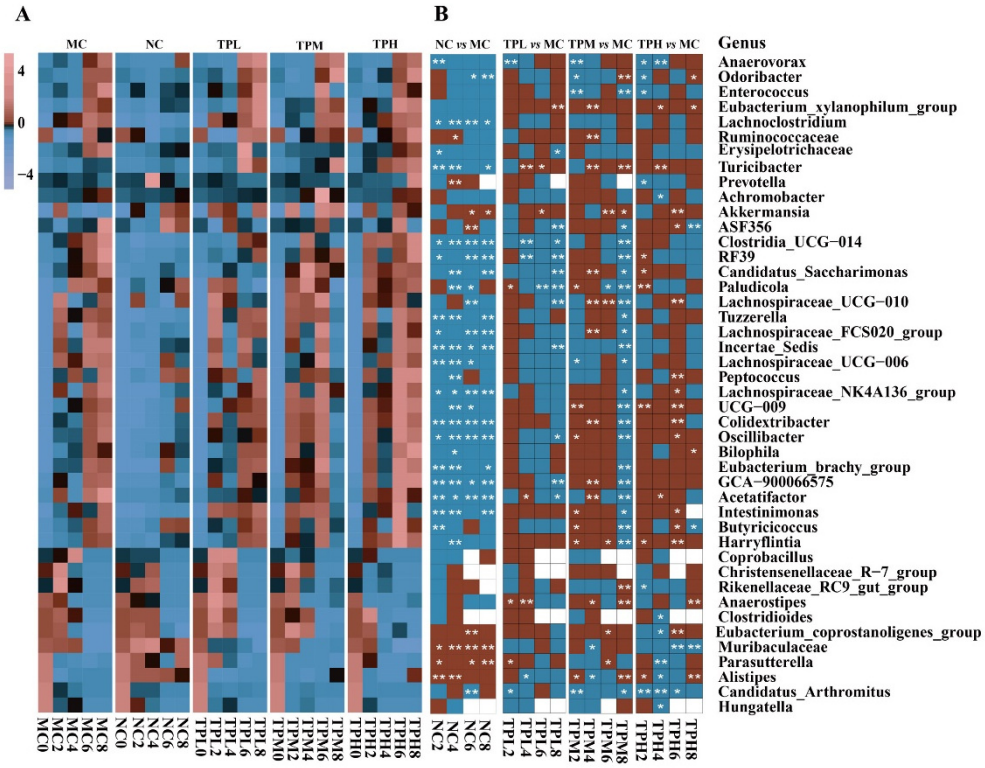


**Fig.5.** Dynamic effect of TP on the relative abundance of obesity-related biomarkers. (A) Heatmap of relative abundance of differential ASVs; (B) Dynamic changes in the relative abundance of differential ASVs in the NC, TPL, TPM, and TPH groups, compared with the MC group. The red, blue, and white represent an increase, a decrease, and no change in the relative abundance of differential ASVs, respectively. * indicates significant differences compared with the MC group, * $P < 0.05$, ** $P < 0.01$.

During TPs intervention, the relative abundance of most bacterial genera from *Lachnospiraceae* increased first and then decreased in three dose groups (Fig.5). The relative abundance of *Colidextribacter*, *Oscillibacter*, *Intestinimona*, *Bilophila*, *Incertae_Sedis*, *Harryflintia*, *Butyricicoccus*, and *Erysipelotrichaceae* also exhibited similar changes, while the relative abundance of *Akkermansia*, *Anaerovorax*, and *Alistipes* (except for TPL group) decreased first and then increased. This might be due to the dominant impact of HFD on gut microbiota at the early stages of TPs intervention, leading to the enrichment of bacteria positively correlated with obesity or inflammation, such as *Oscillibacter*, *Erysipelotrichaceae*, *Ruminococcaceae*, *Lachnospiraceae_UCG_006*, *Colidextribacter*. These enriched bacteria either induced obesity by promoting energy absorption and disrupting lipid metabolism balance [31, 32], or exacerbated obesity by producing endotoxins that disrupted intestinal homeostasis [33]. However, with the prolongation of TPs intervention, the effect of TPs on gut microbiota in HFD-fed mice gradually became dominant, resisting the growth of these bacteria and improving the abundance of bacteria negatively correlated with lipid metabolism and weight gain (such as *Alistipes* and *Akkermansia*) [28, 34]. Therefore, the differences in body weight between the three TPs groups and the MC group became increasingly significant (Fig.1A).

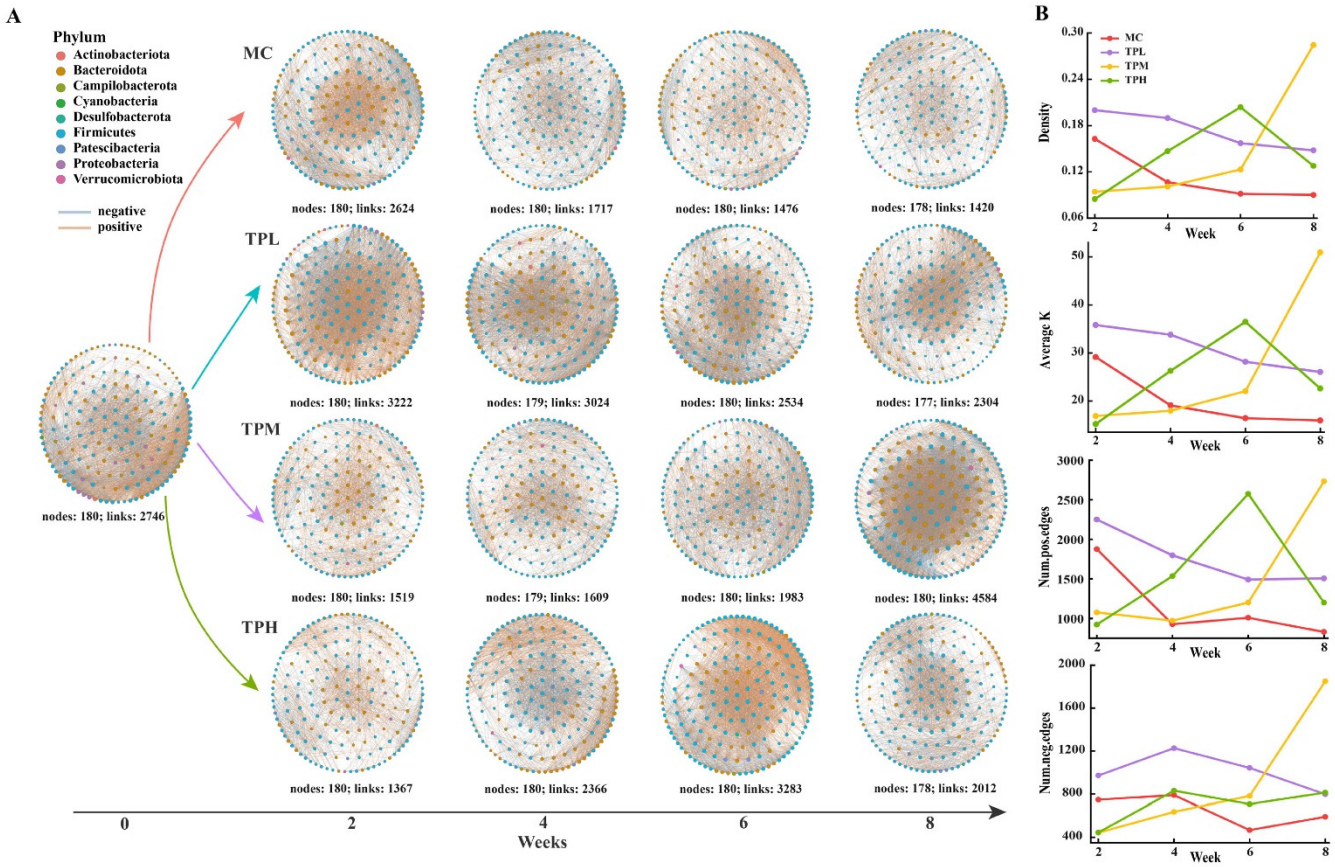


**Fig.6.** Dynamic effect of TP on gut microbiota co-occurrence network in HFD-fed mice. (A) Dynamic changes in gut microbiota co-occurrence network. Each node indicates one ASV. Colors of the nodes indicate the taxonomy species at the phylum level. A brown edge indicates a positive interaction between two individual nodes, while a blue edge indicates a negative interaction. (B) Dynamic changes in network topology, including edge density, average degree (Average K), number of positive edges (Num. pos. edges), and number of negative edges (Num. neg. edges).

### *3.6 Dynamic changes in gut microbial network*

Gut microbial dysbiosis might be driven by differences in bacterial abundance and potential interactions between microbial communities [35]. To explore the potential interactions after TPs intervention, we constructed microbial co-occurrence networks based on the top 180 relative abundance of ASVs in each group and evaluated the impact of their interactions on obesity with network topological calculations. It was reported that the network connectivity and density might be an important feature associated with obesity. The higher the network connectivity and density, the greater the relevance to lean mice, and the stronger the ability to resist the side effects of HFD on gut microbiota [36]. As shown in Fig.6A-B, from week 0 to week 8, the number of links, average degree (average K), and density of the networks in the MC group continuously decreased from 2746, 30.51, 0.170 to 1420, 15.96, 0.090, respectively, indicating that HFD continuously reduced the microbial network complexity, thereby leading to obesity in mice. On the contrary, TPs intervention significantly enhanced the complexity of gut microbial network in HFD mice, and the network complexity in the TPM group and TPH group (except for week 8) gradually increased with the intervention time. Although the trend of variation in the network structure of the TPL group was similar to that of the MC group, it was significantly more complex than that of the MC group ($P < 0.01$). This suggested that TPs might promote gut microbial interactions by establishing more complex relationships to resist HFD-induced side effects and prevent obesity. As mentioned before, the regulatory ability of high-dose TPs on the gut microbiota was weakened at the later stages of intervention, resulting in a decrease in network connectivity at week 8.

Notably, TPs increased positive links more than negative links in the co-occurrence network, indicating that the gut microbiota regulated by TPs tended to be more cooperative or complementary [37]. Although an increase in cooperation within the gut community might lead to a decrease in ecological stability, it could promote overall metabolic efficiency [38], which might contribute to weight loss.

Key nodes identified in co-occurrence networks might be key species that played important roles in maintaining the structure and function of gut microbiota [39]. Regulating these key species could effectively restore the disrupted gut microbial composition to normal levels [40]. To further explore the key gut microbiota regulated by TPs in preventing obesity, we used the values of among-module connectivity (Pi) and within-module connectivity (Zi) of each ASV in the co-occurrence network at week 8 to identify possible keystone taxa. As shown in Fig.7A, a total of 26 key nodes were identified, among which 6, 17, 3, and 2 key nodes were identified in the MC, TPL, TPM, and TPH groups, respectively. ASV4 (*Alistipes*) was identified in the TPL and TPH groups, and ASV205 (unclassified *Lachnospiraceae*) was identified in the MC and TPM groups, respectively.

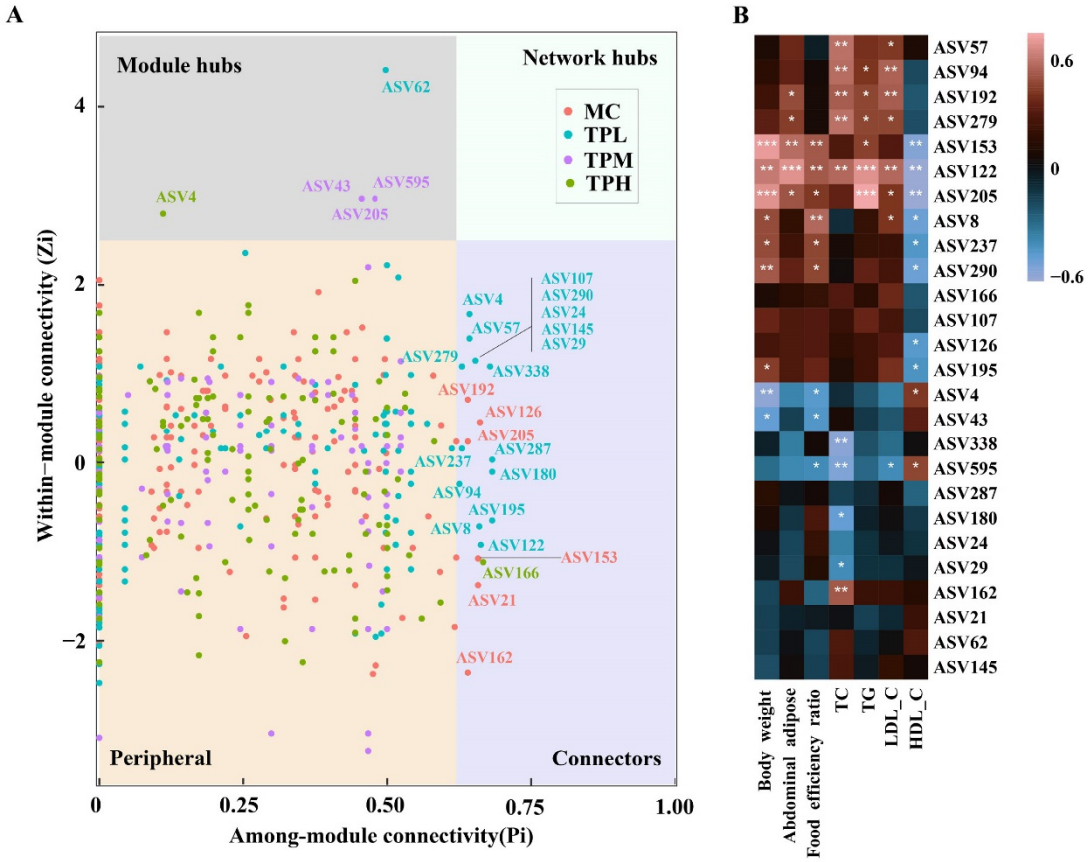


**Fig.7.** Correlation analysis between obesity phenotype and keystone taxa. (A) Classification of nodes in the gut microbiota networks at week 8 to identify potential key ASVs. Each node represents an ASV, and nodes are colored according to different groups. The values of among-module connectivity (*Pi*) and within-module connectivity (*Zi*) of each ASV are used to identify potential keystone ASV, and these nodes are identified into four groups: module hubs ($Zi > 2.5$ and $Pi \leq 0.62$), network hubs ($Zi > 2.5$ and $Pi > 0.62$), connectors ($Zi \leq 2.5$ and $Pi > 0.62$), and peripheral ($Zi \leq 2.5$ and $Pi \leq 0.62$). (B) Correlation analysis between the relative abundance of 26 key ASVs and obesity phenotypes. *, **, or *** indicates significant correlation at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively.

The Spearman correlation analysis was conducted to reveal the relationship between these key nodes and mouse obesity phenotypes (Fig.7B). The results showed that the relative abundance of *Oscillospiraceae* (ASV57, ASV94, ASV122), *Lachnospiraceae* (ASV153, ASV192, ASV195, ASV205, ASV290), *Ruminococcaceae* (ASV237), *Erysipelatoclostridiaceae* (ASV279), and *Muribaculaceae* (ASV8) was significantly positive correlated with body weight, abdominal adipose weight, food efficiency ratio, and serum TC, TG, and LDL-C levels. However, the relative abundance of *Alistipes* (ASV4) and *Lachnospiraceae* (ASV43) was significantly negative correlated with these obesity parameters, and positively correlated with serum HDL-C level. Our above research has shown that TPs reduced the relative abundance of *Oscillospiraceae*, *Lachnospiraceae*, and *Ruminococcaceae* in HFD-fed mice, and increased the relative abundance of *Alistipes*. These findings provided conclusive evidence that TPs prevented obesity caused by HFD by regulating these key ASVs related to obesity.

### *3.7 Cascading relationships among TPs intervention, gut microbiota and obesity*

Obesity is the excessive accumulation of body fat, commonly measured by body mass index (BMI) [41]. To identify the cascading relationships among TPs intervention, gut microbiota, and obesity, we constructed a partial least squares path model (PLS-PM) based on a previous systematic review of the relationship between

TPs and gut microbiota [42]. The PLS-PM analysis indicated that TPs had no significant direct effect on BMI, but TPs significantly positively affected bacterial diversity (0.74) and significantly negatively affected adipose accumulation (including abdominal adipose weight and serum lipid levels, -0.42). Correspondingly, bacterial diversity significantly negatively affected keystone taxa (-0.52), keystone taxa significantly positively affected adipose accumulation (0.39), and adipose accumulation significantly positively correlated with BMI (0.48), respectively (Fig.8). These results suggested that TPs might directly reduce fat accumulation, or indirectly affect fat accumulation by regulating potential keystone taxa to prevent obesity.

In summary, we investigated the dynamic changes in gut microbiota during the intervention of tea polyphenols to alleviate high-fat diet-induced obesity. The results indicated that HFD reduced the gut microbial diversity continuously, altered gut microbial composition, and caused gut microbial dysbiosis, thereby inducing obesity. TPs intervention regulated gut microbial composition, restored gut microbial dysbiosis caused by HFD, and prevented obesity. However, the regulatory effect was related to its dose and intervention time. The effect of HFD on gut microbiota was dominant in the early stages, and the effect of TPs gradually became dominant after 4 weeks of intervention. Among them, the effect of 200 mg/(kg.BW)/d TPs was the best. These findings suggest that long-term intake of moderate TPs can effectively prevent HFD-induced obesity, and the mechanism of action might be regulating key bacteria related to obesity and encouraging fat metabolism. This discovery sets a theoretical foundation for developing and applying TPs in preventing or treating obesity.

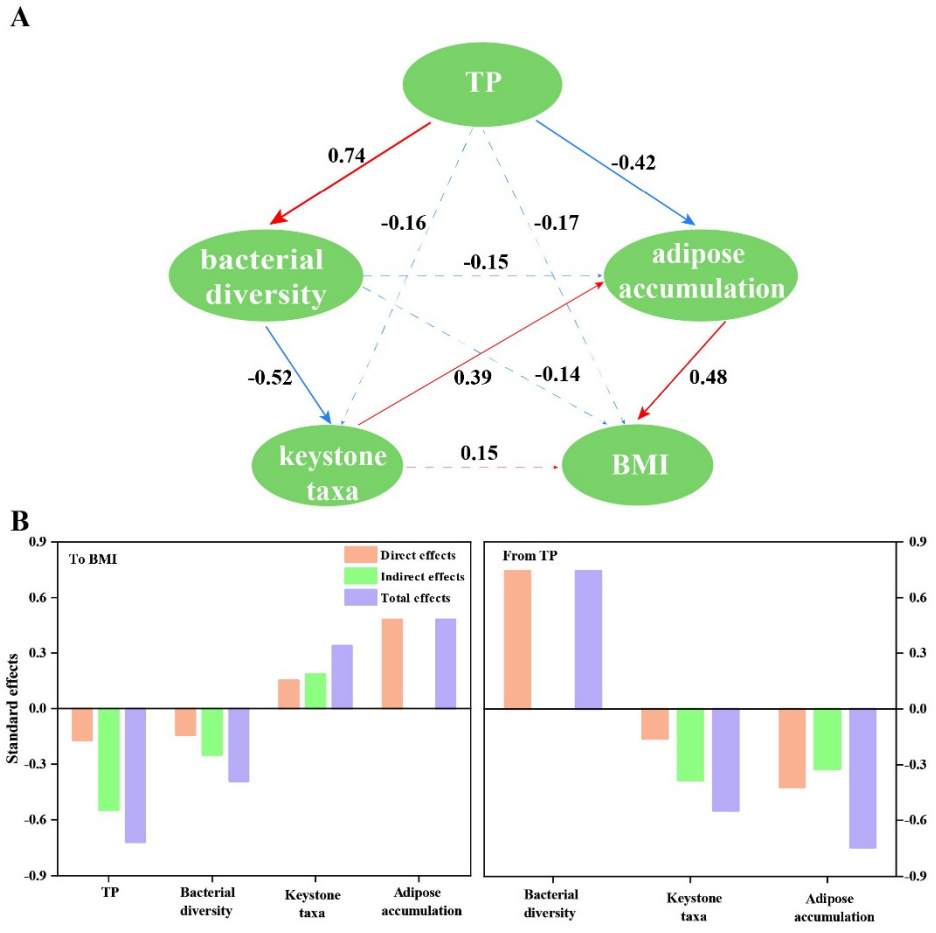

**Fig.8.** Cascading relationships of body mass index (BMI) with TP, bacterial diversity, keystone taxa, and adipose accumulation. (A) Partial least squares path model (PLS-PM) disentangling major pathways of the influences of TP, bacterial diversity (Chao1 index and Shannon index), keystone taxa, and adipose accumulation (including abdominal adipose weight, serum TC, TG, LDL-C, and HDL-C levels) on BMI. After 1000 bootstraps, path coefficients are calculated and represented by the width of the arrow (Value are showed on the arrows). Red and blue arrows mean positive and negative flows of causality, respectively. Solid and dashed lines indicate significant ($P < 0.05$) and nonsignificant ($P > 0.05$) levels, respectively. The GoF statistic is used to evaluate the model, and the GoF value is 0.67. (B) Standardized effects of each factor on mice BMI calculated from the results of PLS-PM analysis. The direct and indirect impacts are added together to form the total effects.

## References


[1] M. Blüher, Obesity: global epidemiology and pathogenesis. Nat. Rev. Endocrinol. 15 (2019) 288-298. https://doi.org/10.1038/s41574-019-0176-8.

[2] G. Muscogiuri, E. Cantone, S. Cassarano, et al., Gut microbiota: a new path to treat obesity. Int. J. Obes. Supp. 9 (2019) 10-19. https://doi.org/10.1038/s41367-019-0011-7.

[3] C. Zhi, J. Huang, J. Wang, et al., Connection between gut microbiome and the development of obesity. Eur. J. Clin. Microbiol. Infect. Dis. 38 (2019) 1987-1998. https://doi.org/10.1007/s10096-019-03623-x.

[4] F. Zhou, Y.L. Li, X. Zhang, et al., Polyphenols from fu brick tea reduce obesity via modulation of gut microbiota and gut microbiota-related intestinal oxidative stress and barrier function. J. Agric. Food Chem. 69 (2021) 14530-14543. https://doi.org/10.1021/acs.jafc.1c04553.

[5] Z. Liu, Q. Chen, C. Zhang, et al., Comparative study of the anti-obesity and gut microbiota modulation effects of green tea phenolics and their oxidation products in high-fat-induced obese mice. Food Chem. 367 (2022) 130735. https://doi.org/10.1016/j.foodchem.2021.130735.

[6] J. Liu, H. Ding, C. Yan, et al., Effect of tea catechins on gut microbiota in high fat diet-induced obese mice. J. Sci. Food Agric. 103 (2023) 2436-2445. https://doi.org/ 10.1002/jsfa.12476.

[7] J.J. Wen, M.Z. Li, C.H. Chen, et al., Tea polyphenol and epigallocatechin gallate ameliorate hyperlipidemia via regulating liver metabolism and remodeling gut microbiota. Food Chem. 404 (2023) 134591. https://doi.org/10.1016/j.foodchem.2022.134591.

[8] X. Zhang, M. Zhang, C.T. Ho, et al., Metagenomics analysis of gut microbiota modulatory effect of green tea polyphenols by high fat diet-induced obesity mice model. J. Funct. Foods. 46 (2018) 268-277. https://doi.org/10.1016/j.jff.2018.05.003.

[9] P.L. Janssens, J. Penders, R. Hursel, et al., Long-term green tea supplementation does not change the human gut microbiota. PLoS One 11 (2016) e0153134. https://doi.org/ 10.1371/journal.pone.0153134.

[10] Y. Xia, D. Tan, R. Akbary, et al., Aqueous raw and ripe Pu-erh tea extracts alleviate obesity and alter cecal microbiota composition and function in diet-induced obese rats. Appl. Microbiol. Biotechnol. 103(4) (2019) 1823-1835. https://doi.org/10.1007/s00253-018-09581-2.

[11] H. Ma, B. Zhang, Y. Hu, et al., Correlation analysis of intestinal redox state with the gut microbiota reveals the positive intervention of tea polyphenols on hyperlipidemia in high fat diet fed mice. J. Agric. Food Chem. 67 (2019) 7325-7335. https://doi.org/10.1021/acs.jafc.9b02211.

[12] R.A. Isbrucker, J.A. Edwards, E. Wolz, et al., Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies. Food Chem. Toxicol. 44 (2006) 636-650. https://doi.org/10.1016/j.fct.2005.11.003.

[13] C. Meng, S. Feng, Z. Hao, et al., Changes in gut microbiota composition with age and correlations with gut inflammation in rats. PLoS One 17 (2022) e0265430. https://doi.org 10.1371/journal.pone.0265430

[14] T. Wen, P. Xie, S. Yang, et al., ggClusterNet: An R package for microbiome network analysis and modularity-based multiple network layouts. iMeta 1 (2022) e32. https://doi.org/10.1002/imt2.32.

[15] J. Zhang, N. Zhang, Y.X. Liu, et al., Root microbiota shift in rice correlates with resident time in the field and developmental stage. Sci. China Life Sci. 61 (2018) 613-621. https://doi.org/10.1007/s11427-018-9284-4.

[16] F. Sommer, J.M. Anderson, R. Bharti, et al., The resilience of the intestinal microbiota influences health and disease. Nat. Rev. Microbiol. 15 (2017) 630-638. https://doi.org/10.1038/nrmicro.2017.58.

[17] S. Stojanov, A. Berlec, B. Štrukelj, The influence of probiotics on the *Firmicutes* /*Bacteroidetes* ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms 8 (2020) 1715. https://doi.org/10.3390/microorganisms8111715.

[18] D. Mariat, O. Firmesse, F. Levenez, et al., The *Firmicutes*/*Bacteroidetes* ratio of the human microbiota changes with age. BMC Microbiol. 9 (2009) 123. https://doi.org/10.1186/1471-2180-9-123.

[19] D. Liu, B. Wen, K. Zhu, et al., Antibiotics-induced perturbations in gut microbial diversity influence metabolic phenotypes in a murine model of high-fat diet-induced obesity. Appl. Microbiol. Biotechnol. 103 (2019) 5269-5283. https://doi.org/10.1007/s00253-019-09764-5.

[20] K. Lippert, L. Kedenko, L. Antonielli, et al., Gut microbiota dysbiosis associated with glucose metabolism disorders and the metabolic syndrome in older adults. Benef. Microbes. 8 (2017) 545-556. https://doi.org/10.3920/BM2016.0184.

[21] M. Vacca, G. Celano, F.M. Calabrese, et al., The controversial role of human gut *Lachnospiraceae*. Microorganisms 8 (2020) 573. https://doi:10.3390/microorganisms8040573.

[22] R. Abdugheni, W.Z. Wang, Y.J. Wang, et al., Metabolite profiling of human-originated *Lachnospiraceae* at the strain level. iMeta 1 (2022) e58. https://doi.org/10.1002/imt2.58.

[23] G. He, T. Chen, L. Huang, et al., *Tremella fuciformis* polysaccharide reduces obesity in high-fat diet-fed mice by modulation of gut microbiota. Front. Microbiol. 13 (2022) 1073350. https://doi.org/10.3389/fmicb.2022.1073350.

[24] M.C. Dao, A. Everard, J. Aron-Wisnewsky, et al., *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut 65 (2016) 426-436. https://doi.org/10.1136/gutjnl-2014-308778.

[25] W. Gu, L. Zhang, T. Han, et al., Dynamic changes in gut microbiome of ulcerative colitis: initial study from animal model. J. Inflamm. Res. 15 (2022) 2631-2647. https://doi.org/10.2147/JIR.S358807.

[26] J. Wu, Y. Chen, H. Yang, et al., Sodium glucose co-transporter 2 (SGLT2) inhibition via dapagliflozin improves diabetic kidney disease (DKD) over time associated with increasing effect on the gut microbiota in db/db mice. Front. Endocrinol. 14 (2023) 1026040. https://doi.org/10.3389/fendo.2023.1026040.

[27] K.V. Higgins, L.N. Woodie, H. Hallowell, et al., Integrative longitudinal analysis of metabolic phenotype and microbiota changes during the development of obesity. Front. Cell. Infect. Microbiol. 11 (2021) 671926. https://doi.org/10.3389/fcimb.2021.671926.

[28] X. Liu, X. Tong, Y. Zou, et al., Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. Nat. Genet. 54 (2022) 52-61. https://doi.org/10.1038/s41588-021-00968-y.

[29] X. Guo, M. Cheng, X. Zhang, et al., Green tea polyphenols reduce obesity in high-fat diet-induced mice by modulating intestinal microbiota composition. Int. J. Food Sci. Tech. 52 (2017) 1723-1730. https://doi.org/10.1111/ijfs.13479.

[30] J. Zhu, R. Cai, Y. Tan, et al., Preventive consumption of green tea modifies the gut microbiota and provides persistent protection from high-fat diet-induced obesity. J. Funct. Foods 64 (2020) 103621. https://doi.org/10.1016/j.jff.2019.103621.

[31] D. Liu, J. Huang, Y. Luo, et al., Fuzhuan brick tea attenuates high-fat diet-induced obesity and associated metabolic disorders by shaping gut microbiota. J. Agric. Food Chem. 67 (2019) 13589-13604. https://doi.org/10.1021/acs.jafc.9b05833.

[32] R. Duan, X. Guan, K. Huang, et al., Flavonoids from whole-grain oat alleviated high-fat diet-induced hyperlipidemia via regulating bile acid metabolism and gut microbiota in mice. J. Agric. Food Chem. 69 (2021) 7629-7640. https://doi.org/10.1021/acs.jafc.1c01813.

[33] R. Xie, Y. Sun, J. Wu, et al., Maternal high fat diet alters gut microbiota of offspring and exacerbates DSS-induced colitis in adulthood. Front. Immunol. 9 (2018) 2608. https://doi.org/10.3389/fimmu.2018.02608.

[34] C. Depommier, A. Everard, C. Druart, et al., Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat. Med. 25 (2019) 1096-1103. https://doi.org/10.1038/s41591-019-0495-2.

[35] L. Chen, V. Collij, M. Jaeger, et al., Gut microbial co-abundance networks show specificity in inflammatory bowel disease and obesity. Nat. Commun. 11 (2020) 4018. https://doi.org/10.1038/s41467-020-17840-y.

[36] L.R. Dugas, B.P. Bernabé, M. Priyadarshini, et al., Decreased microbial co-occurrence network stability and SCFA receptor level correlates with obesity in African-origin women. Sci. Rep. 8 (2018) 17135. https://doi.org/10.1038/s41598-018-35230-9.

[37] X. Qi, Y. Zhang, Y. Zhang, et al., Vitamin B12 produced by *Cetobacterium somerae* improves host resistance against pathogen infection through strengthening the interactions within gut microbiota. Microbiome 11 (2023) 135. https://doi.org/10.1186/s40168-023-01574-2.

[38] K.Z. Coyte, J. Schluter, K.R. Foster, The ecology of the microbiome: Networks, competition, and stability. Science 350 (2015) 663-666. https://www.science.org/doi/10.1126/science.aad2602.

[39] G. Yang, M. Peng, X. Tian, et al., Molecular ecological network analysis reveals the effects of probiotics and florfenicol on intestinal microbiota homeostasis: An example of sea cucumber. Sci. Rep. 7 (2017) 4778. https://doi.org/10.1038/s41598-017-05312-1.

[40] D. Wu, L. Liu, N. Jiao, et al., Targeting keystone species helps restore the dysbiosis of butyrate-producing bacteria in nonalcoholic fatty liver disease. iMeta 1 (2022) e61. https://doi.org/10.1002/imt2.61.

[41] N.V. Dhurandhar, What is obesity? Int. J. Obes. 46 (2022) 1081-1082. https://doi.org/10.1038/s41366-022-01088-1.

[42] T. Chen, C.S. Yang, Biological fates of tea polyphenols and their interactions with microbiota in the gastrointestinal tract: implications on health effects. Crit. Rev. Food Sci. Nutr. 60 (2020) 2691-2709. https://doi.org/10.1080/10408398.2019.1654430.