Research Paper

Effect of piperine on the mitigation of obesity associated with gut microbiota alteration

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\textbf{A B S T R A C T}

An obese mouse model induced by high-fat diet (HFD) feeding was used to reveal the role of piperine in modulating gut microbiota (GM). Piperine was administrated at 20 and 40 mg/kg body weight every day. As a result, piperine at 40 mg/kg significantly decreased body weight, liver weight, perirenal fat weight, and lowered serum triglycerides, total cholesterol, low-density lipoprotein cholesterol, and glucose levels in HFD-fed mice. Additionally, piperine significantly attenuated fatty liver and modulated hepatic mRNA expressions of SREBP-1c, SREBP2, and HMGCR. In perirenal fat, FAS, C/EBP\textalpha, MCP1, and IL-6 expressions were significantly downregulated by piperine. 16S rRNA sequencing revealed that piperine elevated GM diversity. The relative abundance of Muribaculaceae and Ruminococcaceae were significantly elevated, while Dubosiesta and Enterorhabdus genera were suppressed by piperine. The Pearson correlation analysis showed that the altered phylotypes were highly correlated with obesity phenotypes. These findings suggest that piperine modulates energy homeostasis and inflammation to alleviate obesity associated with GM regulation.

1. Introduction

Obesity results from complex interactions among inherited, physiological and environmental factors (Jackson et al., 2020). In the past four decades, due to the overconsumption of energy-dense food combined with a sedentary lifestyle, obesity has tripled, leading to an epidemic that affects both children and adults. It is worrisome that 7% of the children and adolescents had obesity in 2016 compared with less than 1% in 1975 (NCD Risk Factor Collaboration, 2017). By 2025, about 1 billion adults will have obesity (Loos and Yeo, 2022). The prevalence raised a growing concern about economic burden worldwide of related chronic diseases, including cardiovascular disease, diabetes, nonalcoholic fatty liver disease, musculoskeletal disorders, and certain cancers (Formica et al., 2020). However, obesity is largely preventable.

Gut microbiota (GM) is an enormous microbial community that plays a vital role in lifestyle-related disorders (Chen et al., 2021). GM is considered an endocrine organ that highly involved in the host regulation of nutrient handling and energy homeostasis, and thus has significant contributions to obesity (Gomes et al., 2018). GM dysbiosis may contribute to the development of metabolic disorders and chronic inflammation present in obesity (Gomes et al., 2018; Li et al., 2017). Therefore, the modulation of GM has been proposed as a potential strategy to prevent obesity and associated metabolic disorders (Cani et al., 2019).

Piperine, one of the most widely used spices, is the main bioactive component in \textit{Piper} species (Jwa et al., 2012). Previous studies have found the beneficial effects of piperine on metabolic disorders, and multiple mechanisms appear to be involved. It was reported that piperine protected against hepatic steatosis and insulin resistance possibly via suppression of LXR\textalpha-mediated lipogenesis (Jwa et al., 2012), and also by activation of adiponectin-AMPK signaling (Choi et al., 2013). The metabolic inflammation was inhibited by piperine, which helped to ameliorate insulin resistance (Liu et al., 2020). Surprisingly, piperine was found to upregulate the metabolic rate of resting muscle to attenuate obesity and diabetes (Nogara et al., 2016). It was recently reported that piperine attenuated liver fibrosis by activating Nrf2 cascade and subsequently suppressing TGF-\beta1/Smad axis (Shu et al., 2021).

However, whether piperine can improve metabolic disorders by
altering GM has not been investigated so far. In this study, for the first time, the role of piperine in GM regulation and the correlation with obesity-induced metabolic dysfunction were revealed using an HFD-fed mice model.

2. Material and methods

2.1. Animal study

Male 5-week-old C57BL/6 mice (20.36 ± 0.83 g, Shanghai Shilaize Laboratory Animal Co, Ltd, Shanghai, China) were randomly divided into four groups (n = 10) and treated daily. The mice in the normal group were fed with a control diet (10% kcal from fat, D12450J, Research Diets, New Brunswick, NJ, USA) and intragastrically (i.g.) treated with 5% lecithin (Macklin Biochemical, Shanghai, China) at 10 mL/kg body weight (Normal). The mice in the model group were fed with an HFD (60% kcal from fat, D12492, Research Diets) and treated with 5% lecithin (Model). The mice in the low-dose piperine (C17H19NO3, purity ≥98%, Macklin Biochemical) group were fed with an HFD and treated with 20 mg/kg piperine suspended in 5% lecithin (PIP 20). The mice in the high-dose piperine group were fed with an HFD and treated with 40 mg/kg piperine suspended in 5% lecithin (PIP 40). The low and high doses were determined according to literature ([Aswar et al., 2015; Liu et al., 2020]). Food intake was measured daily, and body weight was recorded every other day. The weight gained through the experimental period was divided by cumulative dietary intake to calculate the food efficiency ratio.

At the end of 10 weeks of treatment, the mice were fasted overnight and anesthetized by inhalation of isoflurane, and the blood was collected via the retro-orbital sinus. In each group, cecal samples (n = 6) were randomly collected and immediately stored in liquid nitrogen for GM analysis. The liver and perirenal fat were isolated by surgery, and the tissue was collected and anaesthetized by inhalation of isoflurane, and the blood was quickly weighted. A small portion of the tissue was collected for histological evaluation. The liver and adipose samples were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. The tissue sections were sliced and stained with haematoxylin and eosin (HE, Leica, Ben- sheim, Germany) using standard methods. The histological images were acquired with the use of Nikon E801i microscopy (Nikon, Tokyo, Japan).

2.2. Quantitative RT-PCR analysis

Trizol (Biouniquer Technology Co., Ltd, Nanjing, China) was used to extract total RNA from liver or perirenal adipose. A commercial kit (R333-01, Vazyme Biotech, Nanjing, China) was used to convert the RNA from each sample into cDNA. The quantitative RT-PCR was performed on the LightCycler 96 instrument (Roche, Basel, Switzerland) using Taq Pro Universal SYBR qPCR Master Mix (Q712-02, Vazyme Biotech) according to the manufacturer’s instructions. The mRNA levels of fatty acid synthase (FAS), sterol regulatory element-binding protein 1c (SREBP-1c), sterol regulatory element-binding protein 2 (SREBP-2), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), cholesterol 7α-hydroxylase (CYP7A1), and low-density lipoprotein receptor (LDLR) in the liver were measured. For the perirenal fat, the mRNA expressions of FAS, CCAAT/enhancement-binding protein-α (C/EBPα), monocyte chemotactant protein-1 (MCP1), F4/80, and interleukin-6 (IL-6) were measured. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The 2-ΔΔCt method was used to analyze the relative changes in gene expression. The primers sequences were shown in Table S1.

2.3. Histological evaluation

The liver and adipose samples were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. The tissue sections were sliced and stained with haematoxylin and eosin (HE, Leica, Ben-sheim, Germany) using standard methods. The histological images were acquired with the use of Nikon E801i microscopy (Nikon, Tokyo, Japan).

2.4. Biochemical analysis

The serum levels of triacylglycerol (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose were measured by an automatic hematology analyzer (BS-240VET, Mindray, Shenzhen, China).

2.5. GM analysis

The 16S rRNA gene sequencing was performed by Gene Denovo Biotechnology Co. Ltd (Guangzhou, China) for identification, classification, and quantification of GM in different treatment groups. The bacterial DNA extraction, target region amplification and sequencing, quality control and clustering, and taxonomy annotation were conducted as previously reported ([Zhang et al., 2021]).

To analyze the microbiota structure generally, the Chao1 richness index was obtained by the QIIME software package (University of Colorado, Boulder, CO, USA, version 1.9.1), and rank abundance curves were plotted in R project ggplot2 package (version 2.2.1). Principal coordinates analysis (PCoA) was performed to present differences of the gut microbial communities among groups. The stacked bar plot of the community composition on the phylum, family and genus level was visualized using R project ggplot2 package (version 2.2.1). Pearson correlations between the gut microbial composition and host parameters were calculated in R project psych package (version 1.8.4) and the heatmaps were plotted using “pheatmap” package (version 1.0.12) in R project.
2.6. Statistical analysis

Data were presented as mean ± SD. GraphPad Prism 8 (San Diego, CA, USA) was employed to create histograms, and determine the statistical significance between groups by one-way ANOVA with the post-hoc Tukey test. GM analysis was performed using Omicsmart platform (http://www.omicsmart.com). The p values < 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of piperine on obesity-related features in HFD-fed mice

HFD-fed mice gained significantly more body weight than the normal group (Fig. 1A). However, compared with the model group, the piperine-treated groups showed a significant reduction in body weight gain in a dose-dependent manner. Additionally, piperine treatment decreased energy intake and food efficiency ratio dose-dependently (Fig. 1B and C), which explained a less body weight gain.

Furthermore, the elevated serum TG, TC, LDL-C, HDL-C, and glucose levels by HFD feeding have been lowered to some extent in low- or high-dose (20 or 40 mg/kg) piperine treated groups. Particularly, the serum TG, LDL-C, and glucose of the high-dose group were at the same levels as those in the normal group (Fig. 1D-H).

3.2. Effect of piperine on fatty liver in HFD-fed mice

To evaluate the effect of the intervention of piperine on HFD-induced liver injury, the histological change, the serum AST and ALT levels, and the mRNA levels of the hepatic lipid metabolism key regulators, SREBP-1c, SREBP-2, HMGR, CYP7A1, and LDLR were determined. As shown in Fig. 2A, prominent fat accumulation within hepatocytes was observed in the model group, suggesting the progression of fatty liver induced by long-term HFD feeding. However, piperine dose-dependently alleviated fat deposition in the liver tissue. In addition, the HFD feeding caused a 37% increase of liver weight, which was significantly lowered by high-dose piperine treatment (Fig. 2B). Although serum AST did not show significant differences among different groups, ALT was significantly elevated in the model group, indicating hepatic damage caused by fat deposition (Fig. 2C and D). Compared with the model group, serum ALT was lower in the high-dose piperine group, but the differences were not significant.

Lipid regulators in the liver were largely altered by HFD and piperine treatment. SREBP-1c (p = 0.35) and SREBP-2 (p = 0.19) were upregulated by HFD feeding, but SREBP-1c was dramatically decreased by piperine at 20 or 40 mg/kg, and SREBP-2 was significantly decreased by 40 mg/kg of piperine treatment (Fig. 2E and F). Hepatic HMGR mRNA level was also highly elevated by HFD, but remarkably downregulated by piperine at both doses (Fig. 2G). The mRNA levels of the cholesterol regulators, CYP7A1 and LDLR were all significantly upregulated by high-dose piperine treatment (Fig. 2H and I). The results showed that piperine attenuated HFD-induced fatty liver in mice, probably by regulating lipid and cholesterol signaling.

3.3. Effect of piperine on perirenal fat in HFD-fed mice

Perirenal adipose tissue was dissected to represent the visceral white adipose tissue. HE staining showed that the cell vacuole was increased by HFD. The enlargement was mitigated by piperine treatment (Fig. 3A). Additionally, the weight of perirenal fat was increased evidently in the model group, but reduced by high-dose piperine treatment (Fig. 3B).

To explore the mechanism underlying piperine’s effect on the adipose tissue, mRNA expressions of fat deposition regulators FAS and C/EBPα, and inflammation regulators MCP1, F4/80, and IL-6 of perirenal fat were measured. As a result, all of them were significantly upregulated in the model group, and downregulated in the high-dose piperine group. Additionally, C/EBPα and MCP1 were also downregulated in the low-dose group (Fig. 3C-G).

3.4. Effect of piperine on GM profiles in HFD-fed mice

The effects of piperine on GM were investigated by the sequencing of 16S rRNA. According to the Venn diagram, the investigated groups had overlapped and unique operational taxonomic units (OTUs). Notably, high-dose piperine treatment resulted in the most unique OTUs (Fig. 4A). Consistently, the values of Chao1 richness were significantly increased by high-dose piperine treatment compared with the model group (Fig. 4B).

In a rank abundance plot, species richness is indicated by the number of different species, and species evenness is reflected in the slope of the line that best fits the graph (Sharma et al., 2021). Therefore, the rank abundance plot visualizes both the species richness and evenness. As evidently shown in Fig. 4C, the high-dose piperine group had the most diverse GM among the four investigated groups.

Fig. 1. The effect of piperine on the obesity-related features in high-fat diet-fed mice. (A) Body weight gain. (B) Energy intake. (C) Food efficiency ratio (the body weight gain divided by cumulative dietary intake). (D) Serum triacylglycerol (TG). (E) Serum total cholesterol (TC). (F) Serum low-density lipoprotein cholesterol (LDL-C). (G) Serum high-density lipoprotein cholesterol (HDL-C). (H) Serum glucose. Mean ± SD (n = 10); *p < 0.05, **p < 0.01, ***p < 0.001 versus Normal; $p < 0.05, ###p < 0.001, ####p < 0.0001, Model versus Normal; *p < 0.05, **p < 0.01, ***p < 0.001 versus Model; $ p < 0.05, PIP 40 (piperine at 40 mg/kg) versus PIP 20 (piperine at 20 mg/kg).
For the beta diversity, the PCoA plot showed that the clusters of GM in the model group appeared to be distinct from those in the normal group, whereas the clusters in low- and high-dose piperine groups were between those in the normal and model groups (Fig. 4D).

As a whole, piperine modulated the GM of HFD-fed mice, especially at high-dose, by which the mice had the most diverse GM.

3.5. Effect of piperine on GM composition at different levels in HFD-fed mice

In the present study, GM phylotypes at the phylum, family, and genus levels were analyzed for different groups, to evaluate the effect of piperine on GM composition of mice fed an HFD. As shown in Fig. 5A, GM in the normal group was mainly composed of Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria, and Actinobacteria at the phylum level. All these 5 major phylotypes were evidently altered by HFD. The abundance of Firmicutes and Proteobacteria were significantly elevated, while those of Bacteroidetes, Actinobacteria, and Verrucomicrobia were significantly decreased. Piperine showed no obvious modulatory effect on either of the 5 major phyla (Fig. 5B-F), or the Patescibacteria phylum (Fig. 5G). It is worth noting that the HFD-elevated Tenericutes abundance was decreased by piperine in a dose-dependent manner (Fig. 5H). Cyanobacteria, a very low abundance phylotype, was significantly decreased by HFD (Fig. 5I).
Epsilonbacteraeota and Deferribacteres were not significantly altered by either HFD or piperine treatment (Fig. 5 J and K).

It seems that HFD feeding had a great impact on the abundance of all the major phyla, but piperine had little influence. However, at the family level, the effects of piperine surfaced (Fig. 6 A). Fig. 6 B-F showed the abundance of the Firmicutes members in each group. The Lachnospiraceae family was elevated about 2-fold by HFD feeding, and low- or high-dose piperine did not reverse the elevation (Fig. 6 B). The abundance of the Ruminococcaceae family showed no differences among normal, model, and low-dose piperine groups, but was significantly elevated in the high-dose group (Fig. 6 C). Conversely, the Erysipelotrichaceae family was decreased by high-dose piperine (Fig. 6 D). The abundance of Lactobacillaceae family and Peptococcaceae family appeared to be elevated by HFD feeding in some individual mice, and the average abundance of both families were lower in piperine treatment groups compared with the model group. However, the differences were not significant (Fig. 6 E and F). The Bacteroidetes member, Muribaculaceae accounted for 14.92 ± 3.79% of total bacteria in the normal group, but was decreased to 2.78 ± 1.99% by HFD feeding. However, high-dose piperine restored the abundance to 8.38 ± 2.93% (Fig. 6 G). The growth of the Verrucomicrobia member, the Akkermansia family was fully inhibited in the HFD-fed mice, but piperine administration restored it in some individual animals (Fig. 6 H). Piperine did not show the opposite effect to HFD on the families Desulfovibrionaceae, Atopobiacae, and Bifidobacteriaceae (Fig. 6 I-K).

At the genus level, *Akkermansia* was the most dominant in the normal group, while *Lachnospiraceae_NK4A136_group* was the most abundant in all the other three groups (Fig. 7 A), indicating that HFD could strongly inhibit *Akkermansia* (Fig. 7 B), but stimulate *Lachnospiraceae_NK4A136_group* (Fig. 7 C). Among the top 10 genera (shown in Fig. 7 B-K), high-dose piperine significantly decreased the abundance of the Erysipelotrichaceae family member *Dubosiella* (Fig. 7 H), but elevated the abundance of *Intestinimonas* (Fig. 7 I), which belongs to the Ruminococcaceae family. We also found that high-dose piperine highly elevated the abundance of the other 3 genera that belong to the Ruminococcaceae family, including *Ruminiclostridium, Ruminococca ease_UCG-014*, and *Oscillibacter* (Fig. 7 L–N). The Eggerthellaceae family member, *Enterorhabdus* was elevated in HFD-fed mice, but reduced by high-dose piperine treatment (Fig. 7 O).

Overall, piperine effectively modulated GM composition in HFD-fed mice, which was especially obvious at the high dose.

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Fig. 3. Effects of piperine on perirenal fat of high-fat diet-fed mice. (A) Histology examined by HE staining. (B) Perirenal fat weight. mRNA levels of (C) FAS, (D) C/EBPα, (E) MCP1, (F) F4/80, and (G) IL-6. Mean ± SD (n = 10); #p < 0.05, ##p < 0.01, ###p < 0.001 Model versus Normal; *p < 0.05, **p < 0.01, ****p < 0.0001 versus Model; $p < 0.05, PIP 40 (piperine at 40 mg/kg) versus PIP 20 (piperine at 20 mg/kg).
Additionally, piperine treatment resulted in obvious improvement of the serum TG, and a much lower food efficiency compared with the model group. The alteration in lipid and glucose metabolism in HFD-fed mice, evidenced by remarkable body weight gain, abnormal serum indicators, fatty liver, and visceral fat deposition, which largely imitated human obesity.

### Discussion

Besides body weight gain, obesity is associated with local fat accumulation, insulin resistance, chronic inflammation, and progressive alteration in lipid and glucose metabolism. In this study, 10-week HFD feeding effectively resulted in obesity, evidenced by remarkable body weight gain, abnormal serum indicators, fatty liver, and visceral fat deposition, which largely imitated human obesity.

Piperine lowered body weight gain with a decreased energy intake and a much lower food efficiency compared with the model group. Additionally, piperine treatment resulted in obvious improvement of the elevated serum indicators in HFD-fed mice. Furthermore, the serum TG, LDL-C, and glucose were lowered to similar levels to those in the normal mice.

The classical role of HDL-C is to deliver cholesterol to the liver to excrete, so it has been regarded as “good cholesterol” for decades. However, this function depends more on the number of HDL particles, as well as the protein and lipid composition, than on the HDL-C concentration (März et al., 2017). Therefore, the HDL-C concentration is not always inversely associated with heart disease. Recently, a U-shape relationship between HDL-C and cardiovascular disease was found, which means an extremely high HDL-C is also associated with elevated cardiovascular risk (Feng et al., 2020). In the current study, HFD-feeding highly increased serum HDL-C, but piperine significantly lowered it at both low and high doses (Fig. 1G). According to the recent literature, the lowering of HDL-C level of HFD-fed mice by piperine might be beneficial.

The HFD-induced fatty liver was ameliorated by piperine dose-dependently. Moreover, key regulators of hepatic lipid metabolism were investigated. As a result, SREBP-1c and SREBP-2 were dramatically lowered by piperine. Evidently, the highly increased hepatic HMGCR mRNA level in HFD-fed mice was remarkably decreased by the piperine treatment. CYP7A1 encodes the rate-limiting enzyme that catalyzes the conversion of cholesterol to bile acids. Hepatic CYP7A1 expression was decreased by HFD feeding and elevated by high-dose piperine treatment. Thus, piperine might reduce serum cholesterol level by promoting the synthesis of bile acids. The hepatic mRNA level of LDLR, which helps to bind serum low-density lipoprotein, was also significantly upregulated by high-dose piperine, contributing to a lowered serum LDL-C level.

Many enzymes are involved in the complex process of lipid metabolism. FAS catalyzes the synthesis of saturated fatty acids and subsequently increases the TG levels in the tissue. CEBP/α is the master transcription factor that regulates preadipocytes differentiation. In our study, HFD feeding elevated FAS and C/EBPα expression in the perirenal adipose tissue, while they were downregulated by 40 mg/kg piperine. Thus, piperine might downregulate fat synthesis and deposition in visceral adipose tissue.

Obesity is often accompanied by low-grade metabolic inflammation. In obesity, M1-like macrophages are recruited into visceral adipose tissue and secrete a great amount of pro-inflammatory cytokines (Liu et al., 2020). MCP-1 is a key chemokine involved in the migration and accumulation of macrophages in obesity. In the current study, the serum MCP-1 level was significantly increased by HFD feeding and decreased by piperine, indicating that piperine may ameliorate the inflammatory state.

### 3.6 The correlation of piperine-altered GM phylotypes with host parameters

The potential association between GM and host parameters was measured by the Pearson correlation analysis. At the genus level, GM is associated with HFD-related indexes including body weight, organ weight, serum indicators, and gene expressions of hepatic or perirenal fat regulators or biomarkers (Fig. 8). It is worth noting that several Ruminococcaceae family members, like *Intestimonas, Ruminiclostridium, Ruminococcus_UCG-014* and *Oscillibacter* showed a negative correlation with body weight, liver weight, and fat weight and the other obesity-related features, while these bacteria exhibited a positive correlation with regulators that promote lipid metabolism, like hepatic CYP7A1 and LDLR mRNA levels. Since the abundance of these bacteria were significantly elevated by high-dose piperine treatment (Fig. 7I, 7L-7N), it was proposed that the mitigating effect of piperine on HFD-induced obesity was associated with the alteration.

Meanwhile, the Erysipelotrichaceae members *Fae calibaculum* and *Dubosiella*, the Lactobacillaceae member *Lactobacillus*, and the Egerthellaceae member *Enterorhabdus* were positively correlated with obesity-related parameters, and negatively correlated with hepatic CYP7A1 and LDLR expressions, indicating that they might be significant genera for the development of HFD-induced obesity. Since piperine downregulated *Dubosiella* (Fig. 7H) and *Enterorhabdus* (Fig. 7O) abundance, it can be inferred that piperine exhibited its anti-obesity effect associated with *Dubosiella* and *Enterorhabdus* suppression.

### 4. Discussion

Besides body weight gain, obesity is associated with local fat accumulation, insulin resistance, chronic inflammation, and progressive alteration in lipid and glucose metabolism. In this study, 10-week HFD feeding effectively resulted in obesity, evidenced by remarkable body weight gain, abnormal serum indicators, fatty liver, and visceral fat deposition, which largely imitated human obesity.

Piperine lowered body weight gain with a decreased energy intake and a much lower food efficiency compared with the model group. Additionally, piperine treatment resulted in obvious improvement of the elevated serum indicators in HFD-fed mice. Furthermore, the serum TG, LDL-C, and glucose were lowered to similar levels to those in the normal mice.
infiltration of monocytes and macrophages (Deshmane et al., 2009). F4/80 is the major marker of macrophages (dos Anjos Cassado, 2017). IL-6 is a cytokine that plays multiple roles in immune responses and inflammation (Tanaka et al., 2014). To investigate the intervention of piperine on the inflammation of adipose tissue, the mRNA levels of MCP1, F4/80, and IL-6 were measured. The mRNA levels of MCP1, F4/80, and IL-6 were vigorously increased by HFD feeding, and greatly lowered by piperine, especially at 40 mg/kg. Our data suggested that piperine might attenuate the inflammatory state in the visceral adipose tissue, and may be used to lower obesity-relevant metabolic inflammation.

Taken together, piperine effectively ameliorated obesity induced by HFD feeding in mice.

GM is considered to play a critical role in the development of obesity. Interestingly, we found that GM was modulated by piperine. Comparing GM composition among groups at different levels could provide key

Fig. 5. Specific phyla of gut microbiota modulated by piperine intervention in high-fat diet-fed mice. (A) The proportion of gut microbial communities at phylum level. The relative abundance of (B) Firmicutes, (C) Bacteroidetes, (D) Verrucomicrobia, (E) Proteobacteria, (F) Actinobacteria, (G) Tenericutes, (I) Cyanobacteria, (J) Epsilonbacteraeota, and (K) Deferribacteres. Mean ± SD (n = 6); #p < 0.05, ##p < 0.01, ####p < 0.0001 Model versus Normal; **p < 0.01, ***p < 0.001 versus Model. PIP 20 (piperine at 20 mg/kg). PIP 40 (piperine at 40 mg/kg).
information on the effects of piperine.

At the phylum level, HFD exhibited an extensive effect on different phyla, and piperine treatment did not alter the major phyla with the exception of Tenericutes. Tenericutes displayed a high level of correlation with obesity phenotypes (Fig. S1). Tenericutes is represented by a single class, Mollicutes, which has been shown to be increased in the HFD-fed mice and can cause ulcerative colitis in humans (Allen et al., 2015; Wirostko et al., 1990). Although Tenericutes had a very low abundance, the role of Tenericutes related to the beneficial effects of piperine on obesity should be pursued in future studies.

At the family level, Muribaculaceae (previously known as S24-7), a dominant Bacteroidetes phylum member, was remarkably decreased by HFD, but largely restored by piperine treatment (Fig. 6G). Muribaculaceae is found almost exclusively in the guts of homeothermic...
Fig. 7. Specific genera of gut microbiota modulated by piperine intervention in high-fat diet-fed mice. (A) The proportion of gut microbial communities at the genus level. The relative abundance of (B) Akkermansia, (C) Lachnospiraceae_NK4A136_group, (D) Blautia, (E) Ruminiclostridium_9, (F) Coriobacteriaceae_UCG-002, (G) Faecalibaculum, (H) Dubosiella, (I) Intestinimonas, (J) Lactobacillus, (K) Bifidobacterium, (L) Ruminiclostridium, (M) Ruminococcaceae_UCG-014, (N) Oscillibacter, and (O) Enterorhabdus. Mean ± SD (n = 6); #p < 0.05, ##p < 0.01, ####p < 0.0001 Model versus Normal; *p < 0.05, **p < 0.01 versus Model. PIP 20 (piperine at 20 mg/kg). PIP 40 (piperine at 40 mg/kg).
were found to be increased by piperine. Coincidentally, all of the 4
play a vital role in prevention of obesity by piperine. Since few species in the Muribaculaceae are classified, the correlation
analysis at the genus level could not be done. However, at the family level, Muribaculaceae was significantly negatively correlated with body
level, Muribaculaceae was significantly negatively correlated with body weight gain but negatively correlated with fecal
composition. The abundance of Muribaculaceae and Ruminococcaceae
were suppressed. Intriguingly, piperine-altered phylotypes were highly correlated with the phenotypes of obesity. Therefore, we concluded that
piperine might be associated with the inhibition of the growth of
Dubosiella and Enterorhabdus.

5. Conclusion

Piperine significantly mitigated HFD-induced obesity in mice, evidenced by the lowered body weight gain, ameliorated serum indicators, the attenuation of fatty liver, and the decrease of visceral fat deposition. At the same time, piperine elevated GM diversity and altered GM composition. The abundance of Muribaculaceae and Ruminococcaceae families were elevated, and the Dubosiella and Enterorhabdus genera were suppressed. Intriguingly, piperine-altered phylotypes were highly correlated with the phenotypes of obesity. Therefore, we concluded that piperine’s anti-obesity effect might partly attributed to GM regulation. Our findings reveal a new mechanism that may underlie the anti-obesity action of piperine.

CRediT authorship contribution statement

Jianlin He: Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing – review & editing. Qingqing Le: Resources, Methodology, Visualization, Data curation. Yufeng Wei: Conceptualization, Writing – review & editing. Longhe Yang: Resources, Methodology, Data curation. Bing Cai: Conceptualization, Resources, Project administration. Yuansen Liu: Resources, Methodology, Data curation. Bihong Hong: Conceptualization, Resources, Project administration, Writing – review & editing.

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 data

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