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Basic nutritional investigation

Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet

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ABSTRACT

Objective: Gut microbiota plays a crucial role in host energy homeostasis, which is affected by both high-fat diets (HFDs) and high-sucrose diets (HCDs). Probiotics treatment can effectively modulate intestinal microbiota. However, it remains unclear whether probiotics can effectively improve HFD- and HCD-induced microbiota dysbiosis.

Methods: Mice were fed either an HFD, HCD, or normal diet for 13 wk and administered probiotics during the last 4 wk of the diet. Fecal and cecal samples were collected and analyzed by high-throughput 16S ribosomal RNA sequencing.

Results: Body weight increased more in the HFD group compared with the HCD group. Probiotics supplementation slowed weight gain in both the HFD and HCD groups. Both the HFD and HCD reduced microbial diversity, abundance of butyric acid–producing bacteria, and some other beneficial bacteria, including *Lactobacillus, Clostridium sensu stricto, Prevotella,* and *Alloprevotella,* but increased conditional pathogenic bacteria, such as *Bacteroides, Alistipes,* and *Anaerotruncus.* Probiotics markedly restored the proportions of bacteria affected in the HFD and HCD groups and increased the abundance of microbiota negatively associated with obesity, including *Bifidobacterium, Lactococcus*, and *Akkermansi*a. In addition, *Oscillibacter, Escherichia/Shigella, Acinetobacter,* and *Blautia* significantly increased in the HCD group; *Allobaculum, Olsenella,* and *Ruminococcus* were significantly changed in the HFD group. HCD-induced microbiota dysbiosis was more susceptible to probiotics treatment compared with the HFD.

Conclusions: Probiotics treatment can mitigate diet-induced obesity partly through modulating intestinal microbiota, especially in HCD-induced obesity.

Introduction

The prevalence of obesity has been dramatically increasing globally over decades [1]. Obesity is caused by a complex interaction of genetic and environmental factors and is also suggested to be the result of a long-term imbalance between energy intake and expenditure [2]. Calorie-rich foods that are high in fat or carbohydrates combined with sedentary lifestyles are the most common factors underlying the global obesity epidemic [3]. High-fat diets (HFDs) increase adipose tissue and induce metabolic and cardiovascular disorders (such as atherosclerosis, type 2 diabetes, stroke, and hypertension [4,5]), especially in those who are genetically susceptible. High-carbohydrate diets (HCDs), especially those that are high in sucrose (high-sucrose diets, which belong to HCDs), also increase intraabdominal fat accumulation, which in turn causes metabolic abnormalities and leads to obesity [6]. HFDs and HCDs can result in two different types of obesity classifications. HFD-induced obesity is related to hyperphagia and a compensation in fat metabolism, whereas HCD-induced obesity only manifests a metabolic pattern in muscle, which favors carbohydrate metabolism over fat oxidation [7]. These findings suggest that diet composition reflects specific phenotypes and mechanisms that underlie the different types of

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obesity. Here we aimed to clarify whether there are relevant measures to correct the damage to health caused by HFDs or HCDs.

Accumulating evidence indicates that the gut microbiota is a crucial environmental factor that contributes to obesity through affecting host energy harvest and storage [8,9]. Clinical studies have found that gut microbiota dysbiosis is closely related to obesity, mostly manifesting as a decrease in bacterial richness and diversity [10,11]. Distinct microbial interaction, metabolites related to lean body composition, and abnormal composition of gut microbiota result in obesity and metabolic disease [12]. Animal studies also verified that gut microbiota influence adiposity and weight gain through altering host gene expression, metabolic pathways, inflammatory signaling, and the gut-brain axis [13,14]. Transplantation of fecal microbiota from obese donors into germ-free mice increases fat mass [15]. In addition, transmission of fecal material from mice that have undergone a Roux-en-Y gastric bypass operation to germ-free control mice successfully rescued the obesity phenotype. These findings further suggest a causal association of gut microbes and different metabolic outcomes [16].

At present, the main approaches to treat obesity include diet regulation, exercise, surgical treatment, and drug modulation. Among these, surgical treatment, especially sleeve gastrectomy, can significantly reduce patient weight but consequently alter gut microbiota. However, as an invasive treatment, sleeve gastrectomy also has complications such as portal thrombosis, stenosis, bleeding, and leak, which can be difficult to avoid [17]. In addition, medical treatment of obesity varies widely in drug efficacy and side effect profiles and is only applied to a few patients because of concerns about safety, efficacy, and health insurance coverage [18]. As a future direction microbiota intervention could confer a positive safety profile and high levels of effectiveness without side effects and complications caused by drugs and surgery.

The consumption of probiotics has been heralded as a means to promote digestive health and reverse dysbiosis to restore gut mucosal homeostasis [19]. A meta-analysis of randomized controlled experiments found that administration of probiotics significantly reduced body weight, body mass index, and fat percentage compared with placebo [20]. In addition, alterations in different *Lactobacilli* and *Bifidobacteria* strains also indicate an antiobesity effect in animal models of dietary-induced obesity [21, 22]. Although these studies have identified the positive effects of probiotics on reducing obesity, few studies have comprehensively investigated the impact of probiotics on gut microbiota in dietary-induced obesity. Therefore in this study we established HCD and HFD animal models and used 16S ribosomal RNA (rRNA) amplicon Illumina sequencing to assess the impact of probiotics on gut microbiota. Our study assessed the response of gut microbiota to length and type of diet as well as microbiota susceptibility to probiotics.

Materials and methods

Animals and study design

Eighty female C57 BL/6 J mice were obtained from Shanghai SLAC Laboratory Animal Limited Liability Company and housed in cages at 22 ± 2°C and 55 ± 15% humidity with free access to normal diet (Ralston Purina, St. Louis, MO,USA) under a 12-h light/dark cycle at Tongji University. At age 6 wk, mice were separated into three groups: HFD group (n = 25) fed a HFD consisting of 20% kcal protein, 20% kcal carbohydrate, and 60% kcal fat with 5.24 kcal/g (D12492; FBSH Biotechnology Co. Ltd.); HCD group (n = 25) fed a HCD consisting of 20% kcal protein, 70% kcal sucrose, and 10% kcal fat with 4 kcal/g (TD.98090; FBSH Biotechnology); and a normal group (n = 30) fed a normal diet (ND) consisting of 20.6% kcal protein, 67.4% kcal carbohydrate, and 12% kcal fat with 3.6 kcal/g for 13 wk, respectively. The ingredients and proportions of the HCD, HFD, and normal diet are shown in Table 1. At age 15 wk, 15 mice in each group were intragastrically administered an encapsulated probiotics preparation (Shanghai Xinyi Pharmaceutical, Shanghai, China) daily for 30 d. The encapsulated probiotics preparation contained *Lactobacillus acidophilus, Bifidobacterium longum*, and *Enterococcus faecalis* (1:1:1) at a daily dose of 2.0×10^7 colony-forming unit as the total amount for all three bacteria for each mouse. Body weight and food intake were measured weekly, and fecal samples were collected from each cage at ages 6, 9, and 19 wk; fecal and liver samples were collected after sacrifice (Fig. 1). All collected samples were immediately frozen in liquid nitrogen. Care and treatment of the experimental mice followed the protocols of the Institutional Animal Care and Use Committee.

Table 1
Composition of experimental diets

	HFD	HCD	ND
Fat			
Lard	245	0	0
Soybean oil	25	50	40
Carbohydrate			
Dextrin	125	20	0
Cornstarch	0	20	498
Sucrose	68.8	645	100
Protein			
Casein	200	200	200
Vitamin mix	10	10	10
Mineral mix	10	35	35
Total weight (g)	1000	1000	1000
Energy density (kcal/g)	5.24	4	3.6
% Macronutrient (kcal)			
Fat	60	10	12
Carbohydrate	20	70	67.4
Protein	20	20	20.6

HCD, high-sucrose diet; HFD, high-fat diet; ND, normal diet.
Ingredients expressed by weight (g).

Liver histology

Liver tissues were fixed with 10% buffered neutral formalin, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, viewed with a Nikon Eclipse CI microscope (Nikon, Tokyo, Japan), and photographed at a final magnification of × 200.

Fecal DNA extraction, polymerase chain reaction amplification, and sequence analysis of 16S rRNA

Total DNA was extracted from the fecal and cecal samples using the method described previously [23]. Universal primers for 16S rRNA (341F and 806R) containing inducers and sequencing adaptors were employed to synthesize the V3-V4 gene regions. Polymerase chain reaction amplification was performed using a 10X polymerase mix (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instruction. Polymerase chain reaction product purification was performed using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified by a NanoDrop (Thermo Scientific, USA). Sequencing libraries were quantified using Qubit and then pooled to obtain a sufficient concentration. Paired-end reads were concatenated into longer tags based on the 3′ overlapping regions by paired-end assembler for illumina sequences [24]. Low-quality sequencing reads were discarded based on the following criteria: 1) tags with average quality scores <20; 2) tags with more than three ambiguous N bases; and 3) tags that were <220 or >500 nt.

Sequence analysis

The raw data of 16S rRNA gene sequencing was organized into operational taxonomic units at 97% identity using UPARSE [25]. Taxonomy was analyzed by the Ribosomal Database Project and used as a reference database. The α- and β-diversity indices were detected in the rarefied operational taxonomic unit data using the Qiime program. The α-diversity represented the analysis of diversity data, including Chao 1, good coverage, Simpson index, and Shannon index [26]. The Wilcoxon test in R was employed to compare the α-diversity index. The microbiota structure between groups was measured using the β-diversity index. The weighted and unweighted Unifrac distances underwent a principal coordinate analysis (PCoA). Analyses of similarities were processed by the R package "ade4." Differential abundance of the taxa with a prevalence ≥10% was analyzed by Wilcoxon rank sum test for phylum, order, class, family, and genus levels. The false discovery rate (FDR) was analyzed by the Benjamini-Hochberg methods for bacterial multiple comparisons. Microorganism features were analyzed using the linear discriminant analysis effect size method (http://huttenhower.sph.harvard.edu/lefse/) with 0.05 α cutoff and 2.0 effect size cutoff. Phylogenetic investigation of communities by reconstruction of unobserved states was employed to detect the functional categories of the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog.

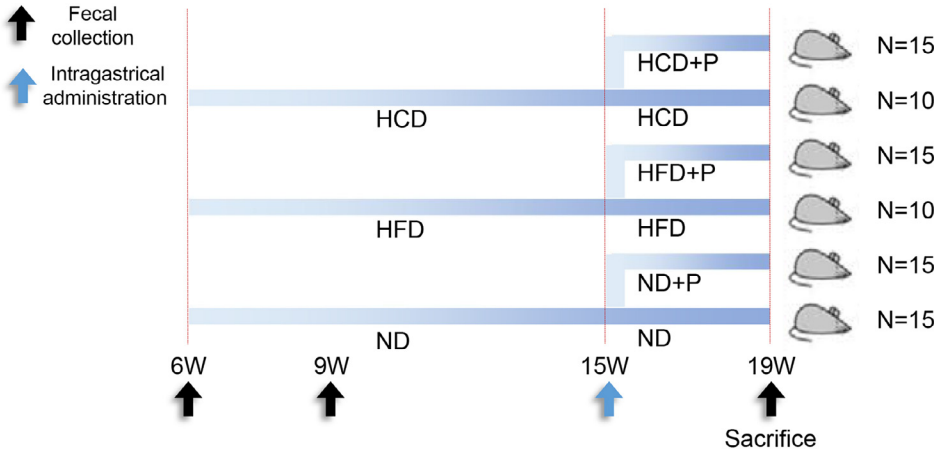

Fig. 1. Diagram of dietary and probiotics intervention in mice. Female C57 BL/6 J mice were fed HCD, HFD, or ND with or without a probiotics gavage and sacrifice at 19 wk. Their feces was collected at 6, 9, and 19 wk (arrows). HCD, high-sucrose diet; HFD, high-fat diet; ND, normal diet; P, probiotics; W, weeks.

Data access

All 16S sequencing data was submitted to the National Center for Biotechnology Information Sequence Read Archive (accession number SRP154250).

Statistical analysis

Student's *t* test (SPSS Software Version 22.0, IBM Corp., Armonk, NY, USA) or the Mann-Whitney *U* test (GraphPad Prism Version 7.00, GraphPad Software, La Jolla, CA, USA) was employed to analyze all quantitative data. Microbiota-related figures were created by R software. Metabolic pathway correlations were examined to evaluate the association between fecal microbiome and microbial metabolism. $P < 0.05$ indicated statistical significance. Multiple comparisons were corrected by Bonferroni adjustment for FDR.

Results

Effect of HFD, HCD, and probiotics on body weight

After 13 wk, the average body weight of the mice was 37.12 ± 1.35 g and 26.62 ± 0.95 g in HFD and HCD groups, respectively, which was significantly greater ($P < 0.05$) compared with the ND group (22.83 ± 0.30 g). Before probiotics treatment (15 wk), the groups of HFD or HCD fed with or without probiotics had no statistical difference in body weight (all $P > 0.05$). After probiotics intervention, body weight gain in the HFD, HCD and ND groups slowed, especially in the HCD group ($P < 0.05$; Fig. 2A, B). To measure the extent to which probiotics slow down body weight gain, we used another parameter, weight gain ratio (weight gain ratio = [body weight (19 wk) − body weight (15 wk)] / body weight (15 wk) × 100%). The weight gain ratio of HFD group and HFD + probiotics (P) group was 11.34% and 5.97%, respectively. The weight gain ratio of HCD group and HCD + P group was 8.17% and 4.89%, respectively.

Mice that remained on the HFD consumed more than both those on the HCD (21.52 ± 0.40 g/wk versus 19.94 ± 0.52 g/wk, $P = 0.02$) and ND (21.52 ± 0.40 g/wk versus 20.23 ± 0.34 g/wk, $P = 0.02$) when data were collapsed across weighing (Fig. 2C). However, energy intake was lower in the ND group compared with the HCD group (72.84 ± 1.23 kcal/wk versus 79.75 ± 2.10 kcal/wk, $P = 0.0091$) and HFD (72.84 ± 1.23 kcal/wk versus 112.80 ± 2.11 kcal/wk, $P < 0.0001$; Fig. 2D). Histologic sections from livers of HFD and HCD groups indicated the presence of innumerous fat vesicles compared with the ND group, which characterizes hepatic steatosis. Nevertheless, treatment with probiotics reduced this fat accumulation in the liver parenchyma (Fig. 2E).

HFD or HCD significantly changes gut microbiota

The α-diversity of the gut microbiota, including Chao index (491.47 ± 17.27 versus 332.67 ± 7.27, $P < 0.0001$) and Simpson index (0.96 ± 0.002 versus 0.95 ± 0.004, $P = 0.02$), decreased significantly after 13 wk in the HFD group compared with the ND group. The diversity indices of the HCD group had a downward trend, especially the Shannon index (5.75 ± 0.13 versus 5.42 ± 0.076; $P = 0.04$), which also decreased significantly compared with the ND group. These results indicate that high-calorie diets significantly reduce gut microbiota diversity in mice.

The abundance of Bacteroidetes decreased in the HCD group (65.5% versus 59.0%) but increased in the HFD group (65.5% versus 70.6%) compared with the ND group. However, Firmicutes increased in the HCD group (31.9% versus 36.8%) but decreased in the HFD group (31.9% versus 23.6%), although these changes were not significant (FDR > 0.05). Proteobacteria increased in both the HCD (0.8% versus 2.4%, FDR = 0.01) and HFD (0.8% versus 2.5%, FDR = 0.004) groups compared with the ND group. At genus levels, the abundance of beneficial bacteria like *Clostridium sensu stricto* (HCD, 0.1% versus 0.001%; HFD, 0.1% versus 0 %), *Lactobacillus* (HCD, 10% versus 1.4%; HFD, 10.1% versus 2.6%), *Prevotella* (HCD, 8.1% versus 0.8%; HFD, 8.1% versus 0.2%), *Alloprevotella* (HCD, 2.7 versus 1.5%), *Ruminococcus* (HFD, 0.06% versus 0%), *Allobaculum* (HFD, 0.01% versus 0%), and *Olsenella* (HFD, 0.09% versus 0.03%) decreased significantly (all FDR < 0.05) in both high-calorie diet groups compared with the ND group. Obesity negatively correlated with bacteria *Akkermansia* (HCD, 1.4% versus 0.02%, FDR = 0.002), and some butyric acid–producing bacteria (including *Faecalibacterium* [HFD, 0.01% versus 0%] and *Bifidobacterium* [HFD, 0.001% versus 0%]) decreased as well, although these changes were not significant (FDR > 0.05). In addition, some conditional pathogenic bacteria, including *Bacteroides* (HCD, 1.6% versus 11.4%; HFD, 1.6% versus 6.7%), *Alistipes* (HCD, 4.4% versus 8.0%; HFD, 4.4% versus 7.4%), *Anaerotruncus* (HCD, 0.02% versus 0.3%; HFD, 0.02% versus 0.03%), *Oscillibacter* (HCD, 0.5% versus 1.2%), *Blautia* (HCD, 0% versus 0.07%), *Escherichia/Shigella* (HCD, 0.02% versus 0.1%), and *Acinetobacter* (HCD, 0% versus 0.01%) increased significantly (all FDR < 0.05) in the HCD and HFD groups, as did the obesity-related bacteria *Dorea* (HCD, 0.04% versus 0.09%, FDR > 0.05). Finally, we used bubble charts to reflect the abundant changes of these bacteria in response to the HCD and HFD at ages 6, 9, and 19 wk (Fig. 3A). Unweighted PCoA analysis indicated a clear separation ($r = 0.877$, $P = 0.001$) between the three different long-term diets, and the

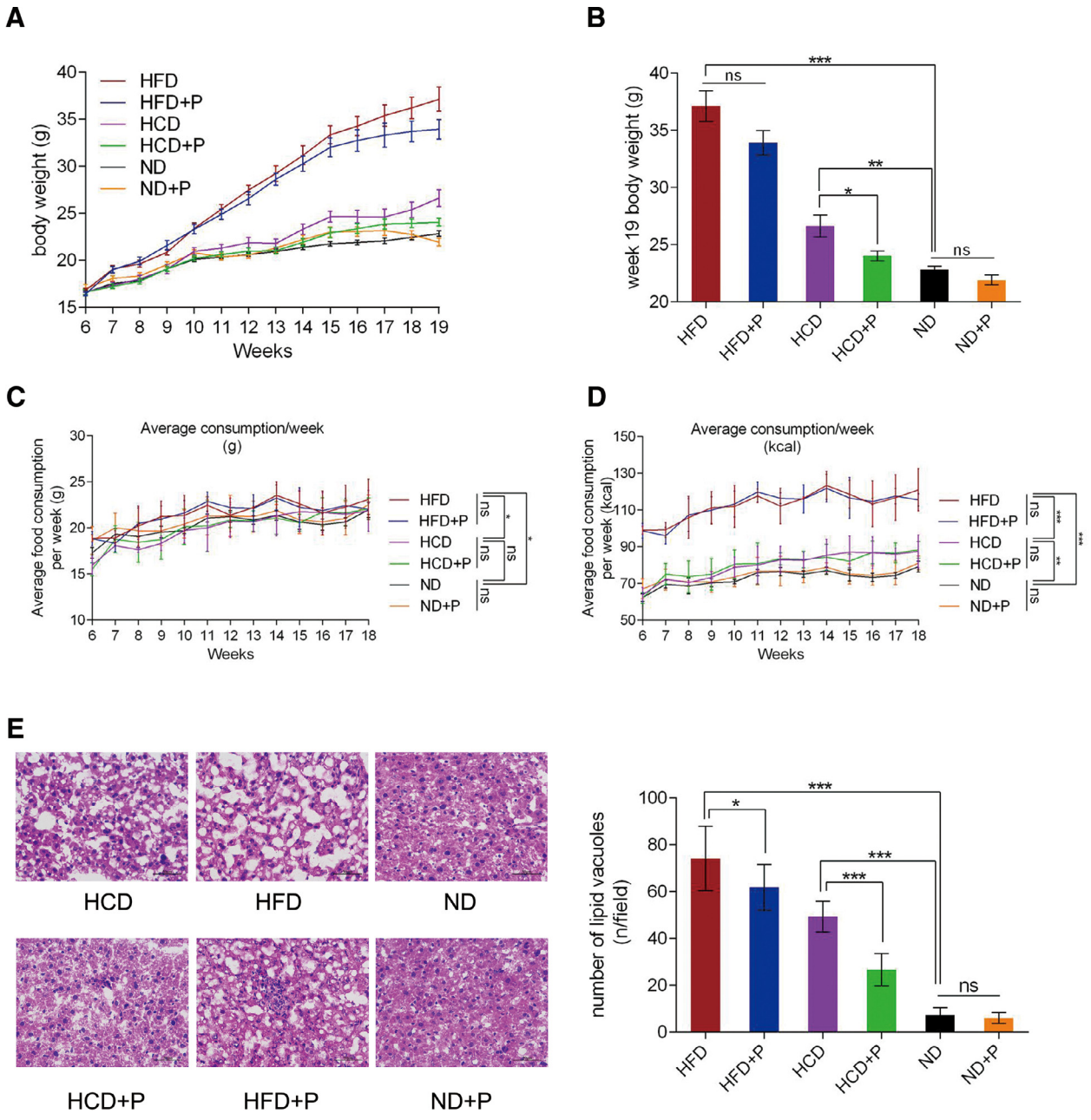

Fig. 2. Effect of diets and probiotics on body weight, average food consumption per week, and hepatic steatosis. (A) Body weights of the C57 BL/6 J mice fed with HFD (red), HCD (purple), or ND (black) for 13 wk was recorded. Some mice from each group (HFD + P: blue; HCD + P: green; and ND + P: orange) received probiotics at age 15 wk. (B) The average body weight of each group at age 19 wk was calculated. (C) The average food consumption in grams and (D) in kcal of each group per week. (E) Representative liver tissue-staining images and liver lipid accumulation in mice (scale bars: 50 μm). Original magnification × 400. Each value is expressed as the mean ± SEM. *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively. HCD, high-sucrose diet; HFD, high-fat diet; ND, normal diet; ns, non-significant; P, probiotics; SEM, standard error of the mean.

HFD group had a better tendency toward separation (Fig. 3B). A heatmap based on the unweighted UniFrac distance was constructed; three clusters existed in all samples, and they respectively represented the HCD, HFD, and ND groups (Fig. 3C). To further analyze the pattern of the intestinal microbiota, we employed linear discriminant analysis coupled with effect size analysis (linear discriminant analysis effect size) and confirmed that *Bacteroides* and *Alistipes* were key microbiota in the HCD group, whereas *Barnesiella* and *Alloprevotella* were considered the dominant microbiota in the HFD group. We further identified *Lactobacillus* and *Prevotella* as key microbiota in the ND group (Fig. 3D).

Meanwhile, microbiota alterations in the cecal samples were assessed from 10 HCD mice, 10 HFD mice, and 15 ND mice. The α- and β-diversity of the microbiota in the cecal samples also had significant reduction and separation between both high-calorie diets and the ND group (Supplemental Fig. 1A–C). We then compared microbial differences between diet groups and found that the change in cecal microbes was similar to diet-mediated fecal microbial. It is worth noting that Bacteroidetes (HCD, 50.3% versus 32.3%; HFD, 50.3% versus 41.6%) significantly decreased in both high-calorie diets groups, whereas Firmicutes significantly increased in the HCD (46.6 versus 61.4%; all FDR < 0.05), which was consistent with the obesity-related lower ratio of Bacteroidetes to Firmicutes reported in a

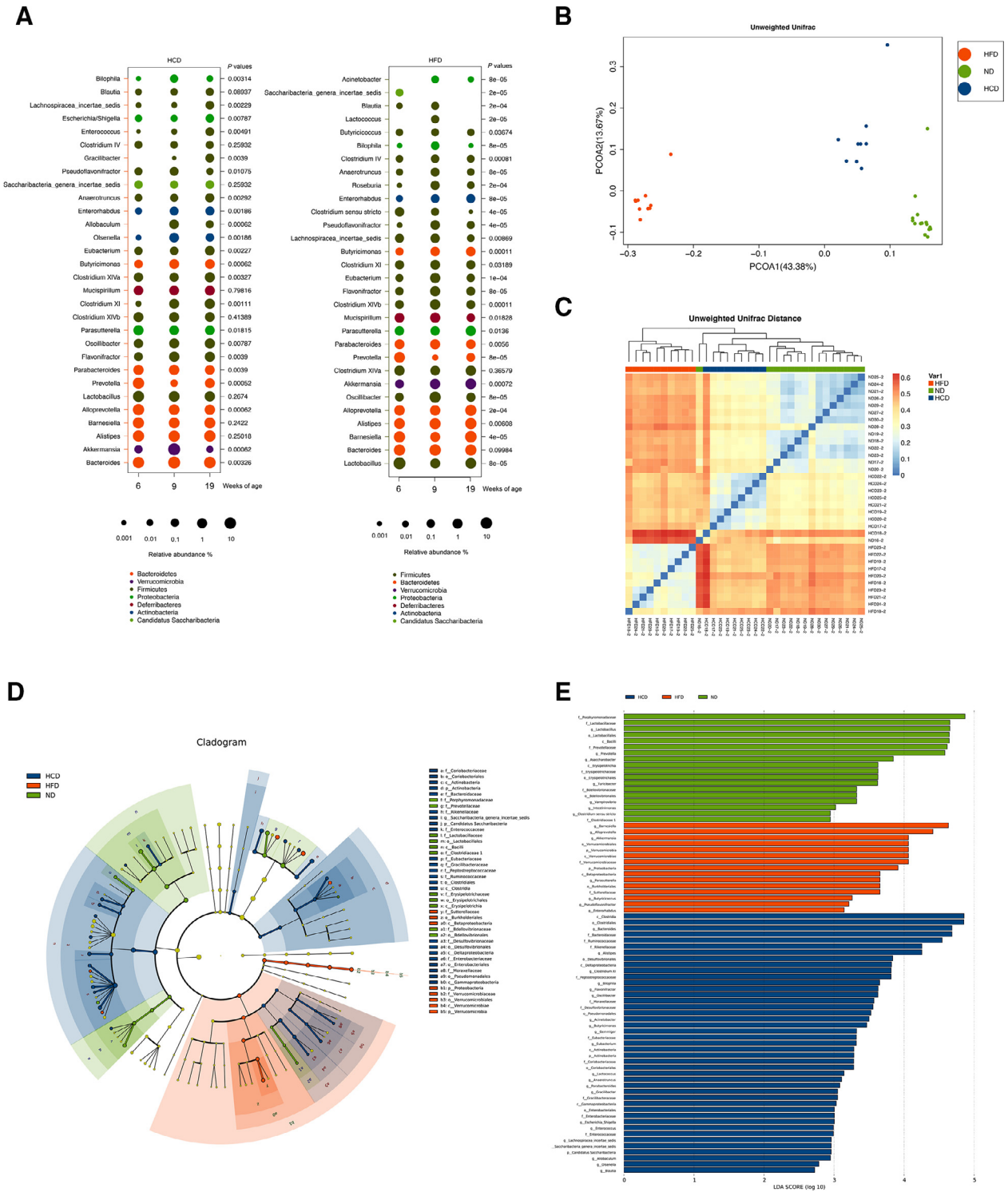

Fig. 3. Comparison of gut microbiota among the HCD, HFD, and ND groups. (A) Top 30 prevalent bacterial genera were identified in the HCD and HFD groups at ages 6, 9, and 19 wk, with relative abundance denoted by circle size and colors representing different phylum. (B) Plot of unweighted UniFrac principal coordinates were scored on the relative abundance of OTU (97% similarity level). Each dot denotes a sample. Blue dots represent the HCD group, red dots represent the HFD group, and green dots represent the ND group. (C) Heatmap calculated from the unweighted UniFrac distance of the fecal samples of the three groups. (D) Cladogram of the LDA scores showing the differentially abundant genera. (E) Histogram of the LDA scores represents the differentially abundant genera. HCD, high-sucrose diet; HFD, high-fat diet; ND, normal diet; LDA, linear discriminant analysis; OTU, operational taxonomic units.

previous study [27]. In addition, we found that some conditional pathogenic bacteria, including *Dorea* (HCD, 0.03% versus 1.2%) and *Fusobacterium* (HCD, 0% versus 0.04%), increased significantly (all FDR < 0.05) in the cecal samples of the HCD group (Supplemental Fig. 1D).

Probiotics improve gut microbiota in HFD- and HCD-fed mice

To investigate the impact of probiotics on the gut microbiota in response to high-calorie diets, we administered probiotic treatment to

15 HCD mice, 15 HFD mice, and 15 ND mice for 4 wk and compared the gut microbiota among the groups. Probiotic treatment did not significantly affect α-diversity in either the HFD group or the HCD group. Contrary to our expectations, probiotics treatment did not reduce the ratio of Firmicutes to Bacteroidetes in the gut microbiome. At genus levels, the abundance of *Escherichia/Shigella* (HCD, 0.1% versus 0.07%), *Oscillibacter* (HCD, 1.2% versus 0.5%), and *Acinetobacter* (HCD, 0.01% versus 0%; HFD, 0.004% versus 0.001%), all of which increased in the long-term HCD in our study, decreased in both the HFD and HCD groups after probiotics intervention. Interestingly, *Oscillibacter* (HFD, 0.3% versus 3.0%) continued to significantly increase (all FDR < 0.05) in the HFD group. Moreover, the original increased abundance of *Alistipes, Anaerotruncus*, and *Bacteroides* in the high-calorie diet groups was also neutralized by probiotics. The original decreased abundance of *Allobaculum* (HCD, 0.06% versus 0.1%; HFD, 0% versus 0.02%), *Alloprevotella* (HCD, 1.5% versus 4.8%), *Lactobacillus* (HCD, 1.4% versus 9.4%), and *Clostridium sensu stricto* (HFD, 0% versus 0.03%) also significantly increased after probiotic treatment. Some other probiotics, including *Bifidobacterium* (HCD, 0% versus 0.4%; HFD, 0% versus 0.01%) and *Lactococcus* (HCD, 0.03% versus 0.1%; HFD, 0 versus 0.09%), also increased significantly (all FDR < 0.05). The bacteria negatively correlated with obesity, *Akkermansia*, decreased significantly in the HCD group, but the abundance no longer decreased (there was a slight increase, 0.01% versus 0.04%) in the HCD group after probiotic intervention. Further, the probiotics offset the original decreasing trend of *Olsenella* in the HFD group and even induced a significant increase in the HCD group (0.1% versus 0.6%, FDR = 0.008). Changes in the abundance of these bacteria with dietary and probiotic interventions over time are shown in bubble charts (Fig. 4A). These results indicate that *Oscillibacter, Lactobacillus, Escherichia/Shigella* (HCD), and *Clostridium sensu stricto* (HFD), are susceptible to probiotic intervention. The unweighted PCoA analysis identified a significant difference (HCD, $r = 0.226$, $P = 0.01$; HFD, $r = 0.887$, $P = 0.001$) between probiotic intervention and non-intervention in the HFD and HCD groups, respectively (Fig. 4B). Heatmaps also clearly identified the notable differences in gut microbial community clustering between probiotic intervention and non-intervention in the HFD and HCD groups (Fig. 4C). Together, these results indicate that probiotics can reverse the effect of HFD- and HCD-induced changes in microbial composition and that the HCD-induced altered gut microbiota in mice is more sensitive to the effects of probiotics compared with HFD-induced microbiota.

Network construction and function prediction

We performed network analysis of the different diets and probiotics intervention groups using Cytoscape to evaluate the relationships among the microbes. A network consisting of 27 nodes and 191 correlations was displayed between the HCD and ND groups (average correlation coefficient of 0.8). In the network, HCD and ND had complex microbial community relationships. The reduction of *Akkermansia* in HCD had the best positive correlation with the reduced *Prevotella* ($r = 0.84$, $P < 0.0001$), which also displayed the strongest inhibitory relationship to the surrounding microbes in the ND cluster, especially with *Anaerotruncus* ($r = -0.92$, $P < 0.0001$; Fig. 5A). After probiotic intervention in HCD, the original relationship was broken, resulting in a stronger positive correlation network dominated by several probiotics, including *Bifidobacterium* and *Lactococcus* ($r = 0.74$, $P < 0.0001$), accompanied by the negative correlation caused by the reduction in conditional pathogens (*Escherichia/Shigella* and *Lactobacillus*, $r = -0.64$, $P < 0.0001$; Fig. 5C). Similarly, microbial communities between HFD group and ND group also revealed strong negative correlations with abundance changes (*Allobaculum* and *Bacteroides*, $r = -0.83$, $P < 0.0001$; *Anaerotruncus* and *Prevotella*, $r = -0.77$, $P < 0.0001$). After probiotics intervention, the positive correlation between beneficial bacteria predominated (*Clostridium sensu stricto* and *Lactococcus*, $r = 0.94$, $P < 0.0001$; Fig. 5B, D).

To understand the correlation between microbiota community structure differences and metabolic differences in response to the diets and probiotics intervention, we conducted a KEGG analysis at the L3 category and selected 11 metabolic pathways associated with obesity and nutrient metabolism from 267 metabolic pathways: adipocytokine signaling pathway, butanoate metabolism, carbohydrate digestion and absorption, carbohydrate metabolism, citrate cycle, energy metabolism, fatty acid biosynthesis, fatty acid metabolism, lipid metabolism, glycolysis/gluconeogenesis and starch and sucrose metabolism. We found that various enriched conditional pathogens (*Anaerotruncus, Alistipes,* and *Oscillibacter*) were positively associated with obesity- and nutrient-related metabolic pathways in the HCD group, but the correlation between the bacteria and metabolic pathways in the HFD and ND groups was less (Supplemental Fig. 2A). After probiotic intervention in HCD and HFD group, the diversity of gut microbe obesity-related metabolic pathways was higher, helping to avoid the loss of certain metabolic functions. The HCD group after probiotic intervention suffered more from the influence of the dominant group *(Alloprevotella),* whereas the HFD group after probiotic intervention was more homogeneous in its metabolism, which was dominated by multimicrobes (Supplemental Fig. 2B, C).

Discussion

High-calorie diets, including HCD and HFD, are used to mimic the Westernized diet pattern, and the ND mimicked a healthy diet pattern. The HFD group (60% fat composition) had significantly higher body weight compared with the HCD (70% sucrose composition). As previously reported, a decrease in HCD intake may be related to bad taste, anorexigenic effects, and high difficulty of forage [28]. In addition, a diet high in both sucrose and fat has been reported to cause glucose intolerance and insulin resistance, which reveals that dietary sucrose itself has specific effects according to the amount of fat in the diet [29,30]. Therefore we concluded that the amount of fat and calorie intake were positively correlated with body weight, whereas calorie intake may alter gut microbiota. Administration of probiotics had been reported to significantly reduce body weight in high-calorie–fed mice [31]. This effect of probiotics may explain the recovery of weight-reducing microbiota in the gut as a consequence of increased metabolic rate and energy consumption, maintained nutrient balance, reduced lipid synthesis, and decreased fat accumulation, which relieve inflammatory reactions caused by high-calorie diets [32]. We found that the two high-calorie diet groups with probiotic intervention had a lower weight gain ratio and degree of hepatic steatosis than the non-intervention group and to some extent supported the effect of probiotics on slowing down weight gain. Because we only intervened with probiotics for 1 mo, we hypothesize that an extended intervention would result in a more significant decrease in obesity as determined by body weight.

Beneficial bacteria, including *Allobaculum, Alloprevotella, Bifidobacterium, Clostridium sensu stricto, Faecalibacterium, Lactobacillus, Olsenella, Prevotella*, and *Ruminococcus*, which can produce short-chain fatty acids (SCFAs), significantly decreased after high-calorie diets, indicating a capacity for providing energy for intestinal cells and protecting the gut barrier. Similar alterations were also found in other studies [33–35]. Previous studies have already suggested a crucial role of SCFAs in the human gastrointestinal system, especially in positively modulating host fat mass storage [36–38]. The relative abundance of *Ruminococcus* was greatly reduced, indicating impaired utilization of plant polysaccharides [39]. Application of Bifidobacteria in the gut, either as a directly ingested probiotic or indirectly with bifidogenic

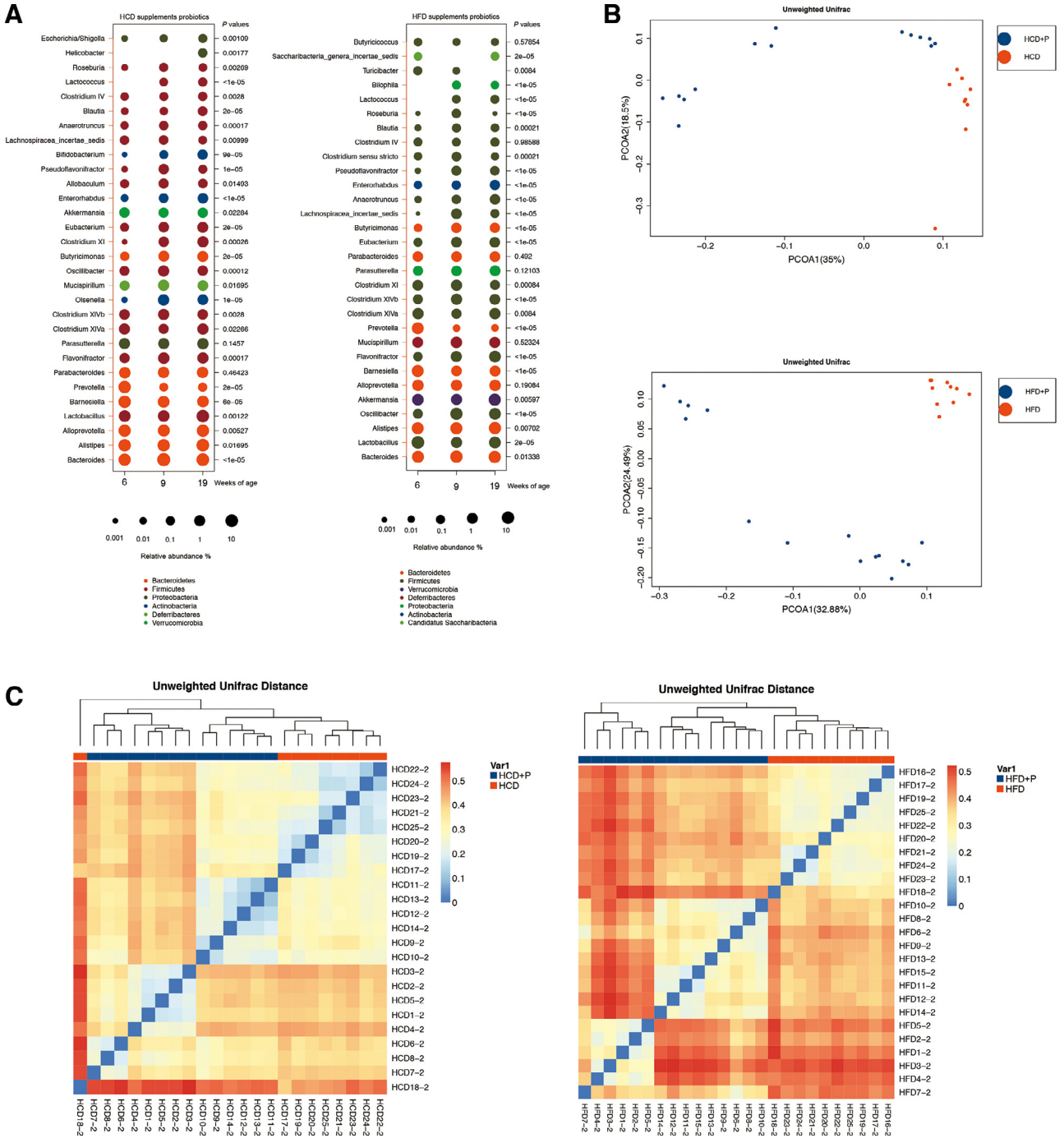

Fig. 4. Comparison of gut microbiota between probiotics intervention and non-intervention in HCD- and HFD-fed mice. (A) Top 30 prevalent bacterial genera identified in the HCD group or HFD group administered probiotics at ages 6, 9, and 19 wk, with relative abundance denoted by circle size and colors representing different phylum. (B) Plot of unweighted UniFrac PCoA scores based on the relative abundance of OTU (97% similarity level). Blue circles in two charts represent the HCD or HFD group with probiotics intervention; red circles represent the HCD or HFD group without probiotics intervention. (C) Heatmap of unweighted UniFrac distance of all the samples between probiotics intervention and non-intervention in the HCD and HFD groups. HCD, high-sucrose diet; HFD, high-fat diet; ND, normal diet; P, probiotics; OTU, operational taxonomic units; PCoA, principal coordinate analysis.

prebiotics, can decrease inflammation and improve glucose tolerance [40,41]. In addition, higher levels of Bifidobacteria have also been found to be associated with reduced gut leakiness, limiting the translocation of lipopolysaccharide to serum [42]. Changes in *Akkermansia* were most noticeable with the high-calorie diets. These changes were inversely associated with body weight in rodents and have recently been found to reverse high-fat-diet–induced metabolic disorders, such as fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance in humans [43]. *Akkermansia* can increase the gut endocannabinoid level that controls inflammation, gut barrier, and gut peptide secretion [43]. In addition, conditional pathogenic bacteria or potentially harmful bacteria with proinflammatory effects, such as *Alistipes, Acinetobacter, Anaerotruncus, Bacteroides, Blautia, Dorea, Escherichia/Shigella*, and *Oscillibacter*, all increased

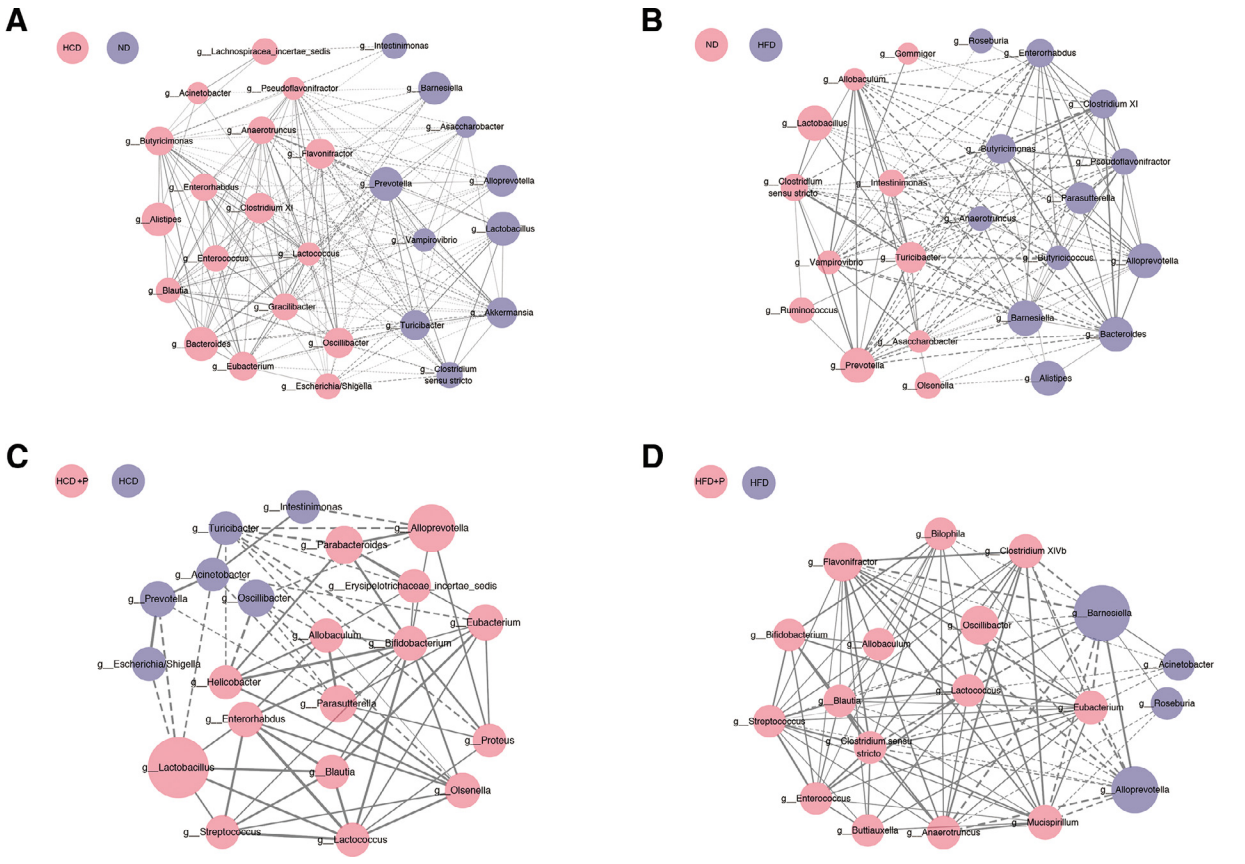

Fig. 5. Relationships among the microbes by network construction of Cytoscape analysis. Network analysis of (A) HCD and ND groups; (B) HFD and ND groups; and probiotics intervention and non-intervention in the (C) HCD group and (D) HFD group were performed. Solid lines represent positive correlations and dotted lines indicate negative correlations. Line thickness represents the correlation strength. Node size is determined by the thickness of all lines on the node. HFD, high-fat diet; ND, normal diet; P, probiotics.

significantly in our study, indicating that a high-calorie diet may destroy the balance of the gut microecology and activate intestinal pathogenic bacteria in response to inflammation [44]. A study of melatonin-mediated weight reduction found that melatonin not only reduced low-grade inflammation, liver steatosis, and insulin resistance in HFD-fed mice but also increased the *Akkermansia* level and reduced obesity-related bacteria, including *Alistipes* and *Anaerotruncus*, which was consistent with our findings [45].

Phylum Bacteroidetes was significantly reduced in the cecal samples from both the HCD and HFD groups. These Bacteroidetes included anaerobic, gram-negative, common bacteria that exist in the surrounding environment, intestine, and skin [46]. The prevalence of phylum Firmicutes bacteria tended to increase in the high-sucrose groups but not in the high-fat groups. Similar alterations have been reported in animals fed Western diets, leptin-mutated obese mice, and obese humans [47–49]. In addition, in a study of patients with perioperative colorectal cancer, application of the same probiotics significantly reduced *Fusobacterium* [50]. This finding is similar to the offset effect of probiotics on *Fusobacterium* in the cecal samples presented here.

To assess if the beneficial bacteria could mitigate the dysbiosis of microbiota induced by diet in the gut, we applied probiotics to modulate the gut microenvironment. Early clinical and animal experiments confirmed that probiotics alleviate high-calorie-diet–induced obesity and associated intestinal microbiota dysbiosis [31]. Consistent with the findings of previous studies, the probiotic intervention increased the abundance of *Bifidobacterium, Lactobacillus*, and *Lactococcus* in addition to some butyrate-producing bacterium, such as *Olsenella, Allobaculum,* and *Clostridium sensu stricto*. These findings indicate that probiotics can improve the composition of gut microbiota and recover the level of SCFA [51,52]. Probiotic treatment reduced key phylotypes within *Escherichia/Shigella, Oscillibacter*, and *Acinetobacter*, which either implicates them in the development of obesity-related metabolic disorders or categorizes them as proinflammatory bacterial species [53–55]. Similar to the research results of Liang et al. [31], *Ruminococcus* and *Faecalibacterium*, which have been reported as beneficial bacteria related to improving malnutrition and promoting health recovery, stop declining after probiotic treatment, although both were significantly reduced in the HFD group.

HCDs and HFDs have different effects on gut microbiota. Consistent with the previous study, we found that an HFD is more likely to reduce gut microbiota diversity, whereas an HCD alters the bacterial composition related to obesity [28,56]. Some beneficial bacterium (*Allobaculum, Bifidobacterium, Olsenella, Faecalibacterium,* and *Ruminococcus*) decreased in the HFD group. In contrast, *Oscillibacter* and *Escherichia/Shigella,* as well as some obesity-related bacteria (*Acinetobacter, Blautia*, and *Dorea*), significantly increased only in the HCD group [57]. After probiotics treatment, the genus *Allobaculum, Lactococcus*, and *Bifidobacterium* significantly increased in both the HCD and HFD groups. However, compared with the HFD group, the HCD group was more susceptible to probiotic regulation. *Lactobacillus, Akkermansia*, and *Olsenella* increased, whereas *Oscillibacter* and *Escherichia/Shigella* decreased only in the HCD after probiotics treatment. Our findings strongly indicate that dietary sucrose has a greater impact on the obesity-related intestinal microbiota than fat. Indeed, there was a

difference in body weight between the mice receiving the two high-calorie diets in this study. Thus calorie intake may be key for altering intestinal microbiota. Caloric restriction by 20% to 50% is one strategy to extend lifespan. In short-lived animals, such as rodents, caloric restriction increases lifespan by 50%, improves general health, and decreases aging-related diseases [58]. A 2-year clinical trial in healthy and non-obese individuals revealed further evidence that persistent metabolic decline decreased oxidative stress, which supports the theory that oxidative damage is associated with mammalian aging [59]. Collectively, our data indicate that restricting calorie intake can maintain healthy intestinal homeostasis, reduce obesity, and promote health.

Conclusions

This study indicated that high-calorie diets contribute to obesity partly by changing gut intestinal microbiota. Moreover, probiotics can improve gut microbiota dysbiosis induced by high-calorie diets through increasing the beneficial bacteria and reducing proinflammatory bacteria. In addition, an HCD changes the composition of the gut microbiota and is more susceptible to probiotic treatment compared with an HFD. Further studies will investigate the precise mechanisms by which high-calorie diets or probiotics alter intestinal microbiota.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2018.10.002.

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