RESEARCH ARTICLE

Comparison the changes of mouse intestinal microbiota induced by exercise, high fat diet, and berberine

Guijun Dong[1, †], Wenhao Zong[1, †], Yang He[2], Hexiang Liu[3], Kefeng Li[4, *]

[1]Institute of Physical Education, Quzhou University, Quzhou, Zhejiang, China. [2]Institute of Physical Education, Binzhou University, Binzhou, Shandong, China. [3]Graduate School of Education, Shandong Sport University, Jinan, Shandong, China. [4]Medical School, Quzhou College of Technology, Quzhou, Zhejiang, China.

Received: May 29, 2024; **accepted:** July 26, 2024.

Exercise and berberine are two effective ways to remodel the gut microbiota landscape, but the difference of alterations in the composition properties of microbial ecosystem promoted by exercise and berberine are still largely unclear. To examine the difference between the exercise-induced changes in intestinal flora and those caused by high-fat diets (HFDs) and drugs, the bacterial species in the fecal samples from mice fed with HFDs (60% kcal) and/or doing wheel-running exercise and berberine intervention were characterized. The results showed that exercise increased the richness of intestinal microflora in mice fed with HFDs, whereas berberine treatments could not. HFDs induced an increase in *Firmicutes* and *Proteobacteria* and a decrease in *Bacteroidetes*. A decrease in the abundance of *norank_f__Bacteroidales_S24-7*, *Lachnospiraceae_NK4A136*, *Prevotellaceae_UCG-001*, and *Prevotellaceae_NK3B31* and an increase in the abundance of *Desulfovibrio*, *Lactobacillus*, *Blautia*, and *Lachnospiraceae_UCG-006* induced by HFDs were observed. Exercise led to a significant increase in the abundance of *Bacteroidales_S24-7*, *Prevotellaceae_UCG-001*, and a decrease in the abundance of *norank_f_Lachnospiraceae* and *Ruminiclostridium_9*. *Proteobacteria* exhibited susceptibility to exercise intervention. Additionally, berberine colonized numerous new species in the intestinal tract while exerting a bacteriostasis effect, and the modified microbial ecosystems were different from those of exercise. The results suggested that HFDs disrupted the microbiome profiles in mice, while exercise could rebuild the intestinal microbial ecology, and intestinal microbial diversity was not increased by berberine treatment. The exercise could be benefit to maintain a well-balanced host-microbial symbiotic status and might be a promising therapeutic strategy for intestinal microbiota optimization.

Keywords: berberine; high-fat diet; intestinal microorganisms; *Bacteroidales_S24-7*; *Prevotella*.

[*]**Corresponding author:** Kefeng Li, Medical School, Quzhou College of Technology, Quzhou 324000, Zhejiang, China. Email: qzctlkf@163.com.

[†]These authors contributed equally to this work.

Introduction

Intestinal microbiotas contribute to nutritional and physiological status of the host. These microorganisms play key roles in immune defense, vitamin synthesis, bile salt manipulations, and breakdown of undigested food [1-3]. A health microbiome has a conducive effect on host immunity and metabolism *via* beneficial metabolites that could regulate

intestinal pH and strengthen intestinal barrier by producing short chain fatty acids (SCFAs) including acetate, butyrate, and propionate [4, 5]. Conversely, enteric dysbacteriosis leads to the pronounced appearance of some negative factors such as lipo-polysaccharide (LPS), a systemic inflammatory metabolite derived from Gram-negative bacterial lysis, that can translocate from the intestine into the circulatory system and is strongly linked to initiation and development of some metabolic diseases [6]. Diet is the main driver of intestinal microflora remodeling in the host's daily life. Obesity caused by long-term high-fat diets (HFDs) is regarded as a low-level form of systemic inflammation, which is closely in line with some metabolic diseases [7]. Emerging findings indicate that the enteric dysbacteriosis induced by HFDs drives metabolic inflammation [8].

HFDs are an unhealthy eating pattern that can disrupt the structure of microbial community and decrease microbial diversity [9]. Mice fed with HFDs have a higher *Firmicutes*/*Bacteroidetes* ratio than those fed with regular chow diets [10]. HFDs can significantly increase the abundance of *Actinobacteria* and *Proteobacteria*, which are LPS-carrying proinflammatory bacteria [10, 11]. HFDs can also drive a reduction of *Prevotellaceae* and *Rikenellaceae* (the *Bacteroidetes phylum*) [12]. *Bifidobacterium* reduction is also related with HFDs and may weaken intestinal barrier and facilitate LPS translocation from the intestinal lumen to circulatory system [13]. Exercise can improve obesity-induced metabolic disorders by acting as a positively potent modulator of gut microbiota composition and function. Exercise can diversify intestinal microorganisms, re-build the intestinal microbial ecosystem, and enhance the representation of taxa with beneficial metabolic functions [14]. The diversity of the intestinal flora of professional rugby players is superior to that in controls, and the athletes with a low body mass index (BMI) have a significantly higher *Akkermansia* abundance compared to the high BMI athletes [15]. *Akkermansia* reportedly plays an essential role in promoting intestinal barrier function and regulating obesity and other metabolic diseases [16]. Exercise increases the quantity of *Lactobacillus* in intestinal tract, playing a positive role in reducing LPS production in bacterial wall of gram-negative bacteria and alleviating type II diabetes [17]. Previous study showed that the mouse intestinal bacteria in the group of six-week autorotation exercise mice and the group of control mice were transplanted into the intestines of the aseptic group mice with induced colitis using dextran sulfate sodium. The colitis symptoms in transplantation exercise group were lighter than those in the others, suggesting that the exercise modification of intestinal flora might be an important way of regulating the host's functional status [18]. Furthermore, exercise appeared to decrease systemic inflammation and metabolic dysregulation [19, 20]. Hence, possessing helpful gut microbiota is essential for the host's health. However, much of this exercise-induced alteration remains unknown. Further investigations are needed to reveal the detailed changes in the gut microbiota associated with exercise. Berberine is an isoquinoline-derivative alkaloid with hypoglycemic and weight loss effects and has been traditionally used in Chinese medicine to treat gastrointestinal infections [21, 22]. Evidence showed that, under pathological conditions, berberine could structurally and numerically reverse the gut microbiota changes [23], demonstrating therapeutic potential for metabolic diseases by regulating the metabolic endotoxemia levels [24].

This study investigated whether the mechanisms of exercise altered gut microbiome and alleviated inflammation were the same as the gut-regulating drug, berberine, that affected gut microbiome. The different alterations in the composition properties of microbial ecosystem in mice induced by HFDs and treated with exercise and berberine were explored, and the microbiota alterations were fully elucidated from the phyla to species level based on the 16S ribosomal RNA amplicon sequencing. This study would contribute to revealing the biological mechanism of health promotion by exercise.

Materials and methods

Animal treatments and sample collection

All the procedures of this study were approved by The Animal Care and Use Committee of Shandong Sport University (Jinan, Shandong, China). Two diet patterns were adopted in this study including either regular chow (RC) diets (13.5% kcal) or HFDs (60% kcal) (Beijing Keao Co-operative Feed Co., Ltd., Beijing, China). 44 male C57BL/6J mice aged 12 weeks with body weight of 20.2 ± 2.1 g from Shandong University Laboratory Animal Center (Jinan, Shandong, China) were randomly divided to the control group fed with standard diet (n = 18) and high-fat diet group (n = 26). All mice were kept in cages with one mouse per cage at 23 ± 2℃, 50 ± 20% humidity, 12 h light/dark cycle, and free access to food and water. After 4 weeks of environmental acclimatization, an 8-week dietary intervention was conducted. Briefly, the control group was randomly divided into regular chow diets feeding without exercise (RC_Sed, n = 8) and regular chow diets feeding plus exercise (RC_Ex, n = 8). 2 mice were excluded from the experiment due to the shortest distance of movement. The high-fat diet group was randomly divided into HFDs feeding without exercise (HF_Sed, n = 8), HFDs feeding plus exercise (HF_Ex, n = 8), and HFDs and berberine feeding (HF_Bbr, n = 8) groups. 2 mice were excluded from the experiment because of the shortest distance of movement. The mice were fed with either RC diets or HFDs starting at 12 weeks of age. From 20 – 24 weeks of age, the groups of RC_Ex and HF_Ex received a free-wheel running intervention. A magnetic inductor was used to record the cycle numbers of mice. The previous day's wheel revolutions were documented at 8 am of next day. The mouse that ran less than 4 km per day was excluded. The HF_Bbr group was treated with 150 mg/kg berberine twice a week. All mice were ether anesthetized and sacrificed by cervical dislocation at the end of the experiments. Blood samples were collected *via* heart puncture. The contents of the cecum were collected, and samples from two mice in the same group were combined in a sterile Eppendorf tube to analyze the gut microbial composition.

DNA extraction and polymerase chain reaction (PCR) amplification

The E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract microbial DNA following the manufacturer's instructions. The DNA concentration and quality were examined by using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene was amplified by using GeneAmp 9700 thermocycler (Applied Biosystems, Waltham, MA, USA) with the primers of 338F (5'- ACT CCT ACG GGA GGC AGC AG -3') and 806R (5'- GGA CTA CHV GGG TWT CTA AT -3'). The PCR reaction consisted of 4 µL of 5× FastPfu buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each 5 µM primer, 0.4 µL of FastPfu Polymerase, 0.2 µL of BSA, and 10 ng of template DNA. The PCR program was 95°C for 3 min followed by 27 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, then 72°C for 10 min.

DNA sequencing and sequencing data processing

The PCR products were purified and extracted from a 2% agarose gel using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and then quantified using QuantiFluor™-ST (Promega, Madison, WI, USA) following manufacturer's instructions. The purified PCR products were then sent to Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) for DNA sequencing using Miseq PE300 platform (Illumina, San Diego, CA, USA). A custom Perl script were applied to demultiplex the raw FASTQ format sequence data files followed by applying Fastp (version 0.19.6) (https://github.com/OpenGene/fastp) for quality-filtering and FLASH (version 1.2.7) for data merging with the following criteria: (i) the reads were truncated at each site that received an average quality score of < 20 over a sliding window of 50 bp, and discard truncated reads shorter than 50 bp and reads containing

ambiguous characters; (ii) sequences with more than 10 bp of overlap were merged by overlap, and mismatches were no more than 2 bp; (iii) according to barcodes (exact match) and primers (allowing for two nucleotide mismatches), every sample sequences were separated, and reads containing ambiguous bases were removed. The sequencing reads were filtered by using Quantitative Analysis of Microbial Ecology (QIIME v1.9.1) (http://qiime.org) and subsequently constructing a table containing 16S rRNA sequencing data. UPARSE (version 7.1) (http://drive5.com/uparse/) was used with a 97% similarity threshold to cluster operational taxonomic units (OTUs) with a novel "greedy" algorithm that performed both chimera filtering and OTU clustering. Each 16S rRNA gene sequence in the Silva (SSU128) 16S rRNA database (http://www.arb-silva.de/search/testprime/) was classified and analyzed using the RDP classifier algorithm (http://rdp.cme.msu.edu/) with a confidence threshold of 70%.

Statistical analysis

Majorbio cloud (https://cloud.majorbio.com) was used for bioinformatic analysis of the gut microbiota. Mothur (v1.30.1) (www.mothur.org) was employed to calculate rarefaction curves and alpha diversity indices from OTU information. Similarities in microbial communities across samples were determined by hierarchical clustering and principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities using the Vegan (v2.5-3) software package. Statistical analyses were conducted using SPSS (version 20) (IBM, Armonk, New York, USA). The data were presented as mean ± SD. Two-way ANOVA with Tukey's multiple comparison post-test was used to compare multiple groups. The student's t-test was used to compare the two groups for parametrically distributed data and the Mann-Whitney test for non-parametrically distributed data. Adjustments for multiple tests were estimated using the false discovery rate function. The threshold of significance was $P < 0.05$, while $P < 0.01$ as very significant difference.

Results

Weight loss and hypoglycemic effects by exercise

Moderate running exercise like voluntary wheel running appeared to change the species and functional components of gut microbiota and reduce systemic inflammation, thereby preventing certain metabolic diseases. In this study, voluntary wheel running was exposed to the RC_Ex and HF_Ex mice to characterize the ecology of the exercise-associated gut microbiota. Daily running distance was recorded for RC_Ex and HF_Ex mice group and showed that the distance travelled between the RC_Ex and HF_Ex groups were similar, suggesting that the activity levels of mice were not affected by HFDs (Figure 1A). Three months of HFDs resulted in significant weight gain in the mice. There was a significant difference between the groups of RC_Sed and HF_Sed ($P < 0.001$) (Figure 1B). One month of exercise significantly reduced the mouse's body weight in HF_Ex group compared with HF_Sed ($P = 0.002$). Berberine addition also significantly reduced the mouse's body weight. The glucose tolerance test curve showed that three months of HFD could induce high blood sugar levels, whereas exercise and berberine could significantly reduce the blood sugar levels (Figure 1 C). These results suggested that HFDs could lead to increased body weight and blood sugar levels, while exercise and berberine could reduce body weight and blood sugar.

Exercise, HFDs, and berberine modification of microbial communities

(1) Microbiome diversity

A total of 879,519 high-quality sequences were obtained by 16S rRNA gene sequencing of 20 samples and were classified into 12 phyla, 20 classes, 28 orders, 56 families, 149 genera, 252 species, and 555 OTUs. Comparison of alpha diversity indices showed that the HF_Sed microbial community was significantly less abundant than that of RC_Sed ($P < 0.01$) (Figure 2A). The results also showed that the microbiota richness in the normal chow fed mice (RC_Ex)

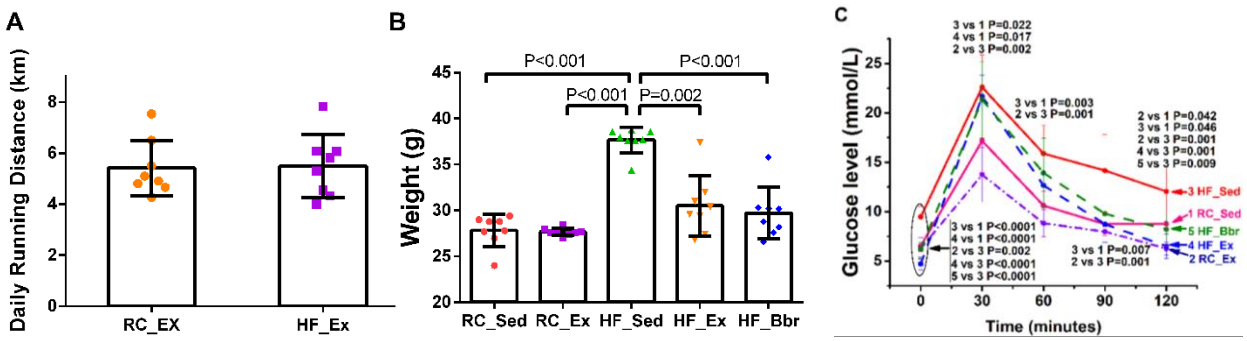


Figure 1. Changes in daily running distance (A), weight (B), and glucose level(C) with HFDs, exercise, and berberine interventions.

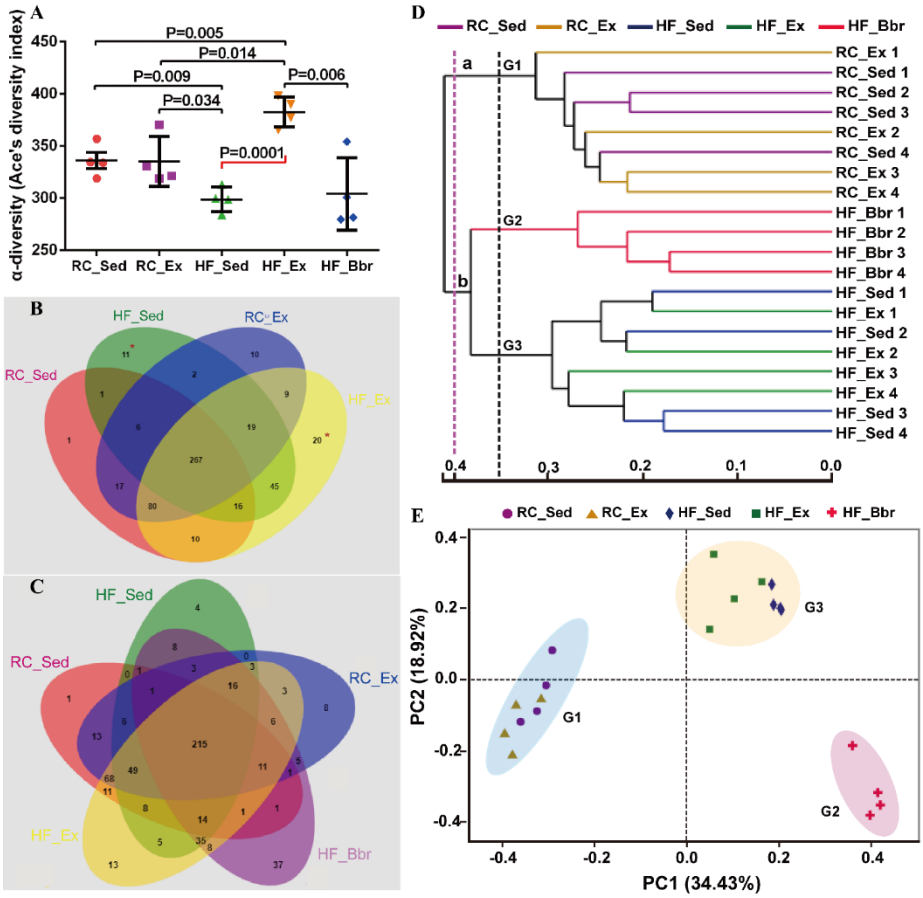


Figure 2. Changes in community richness with HFDs, exercise, and berberine interventions (A). Venn diagrams of unique and shared OTUs (3% distance level) in the different combinations of RC_Sed, HF_Sed, RC_Ex, HF_Ex (B) and RC_Sed, HF_Sed, RC_Ex, HF_Ex, HF_Bbr (C). The relationship of the microbiome shown by hierarchical clustering tree (D). PCoA based on the Bray–Curtis distances on the OTU level (E).

were not affected by exercise compared with that of RC_Sed. However, exercise could enhance the richness of mice fed with HFD (HF_Ex) comparing to that of HF_Sed (P < 0.01). The HF_Bbr group presented a significantly lesser diversity than the HF_Ex group (P < 0.01). Between the HF_Sed and HF_Bbr groups, no significant difference was found. These results suggested that berberine treatments could not improve the microbial richness reduced by HFDs.

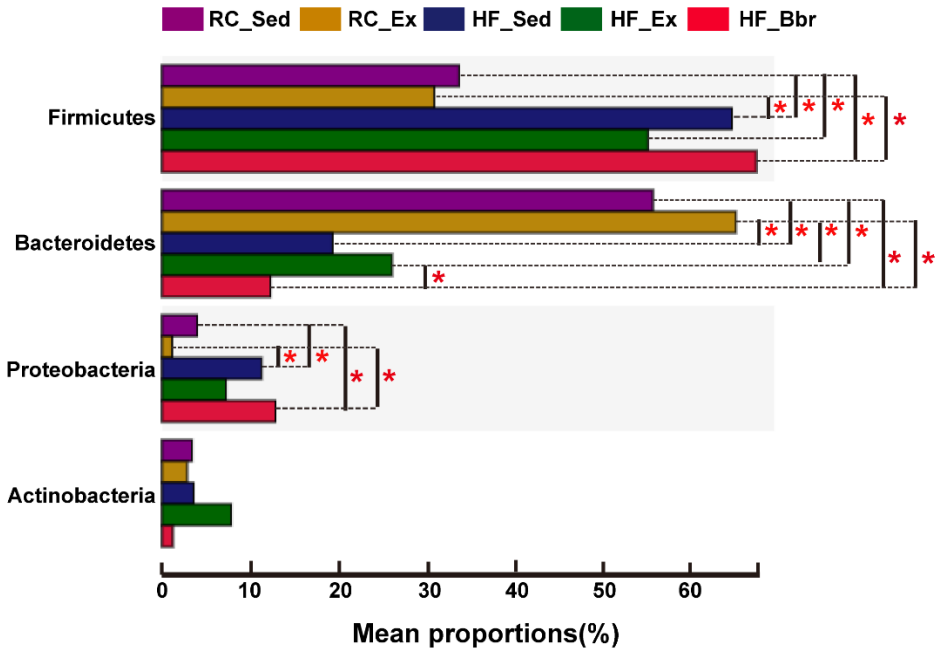


Figure 3. Comparison of dominant phyla (top 4) in the RC_Sed, RC_Ex, HF_Sed, HF_Ex, and HF_Bbr groups. *$P < 0.05$ compared with their respective control.

(2) Unique and shared bacterial taxa among different groups

This research focused on the migration and colonization of alien bacteria in the mice intestine due to HFD and/or exercise intervention. The unique and shared OTUs (3% distance level) among the four groups of RC_Sed, HF_Sed, RC_Ex, HF_Ex (Figure 2B) and five groups of RC_Sed, HF_Sed, RC_Ex, HF_Ex, HF_Bbr (Figure 2C) demonstrated that unique OTUs of 4, 13, and 37 were confined to the HF_Sed, HF_Ex, and HF_Bbr groups, respectively (Figure 2C). Meanwhile, 11 and 20 OTUs were confined to the HF_Sed and HF_Ex groups with 267 core OTUs common to all groups (Figure 2B). The 11 OTUs belong to the following ordersof *Clostridiales* (5 OTUs), *Lactobacillales* (2 OTUs), *Coriobacteriales* (2 OTUs), *Corynebacteriales* (1 OTU), and *Bacteroidales* (1 OTU), while the 20 OTUs belong to the following orders of *Clostridiales* (12 OTUs), *Lactobacillales* (2 OTUs), *Rhizobiales* (1 OTU), *Pseudomonadales* (1 OTU), *Deferribacterales* (1 OTU), *Micrococcales* (1 OTU), *Bacteroidales* (1 OTU), and unclassified_k_norank (1 OTU).

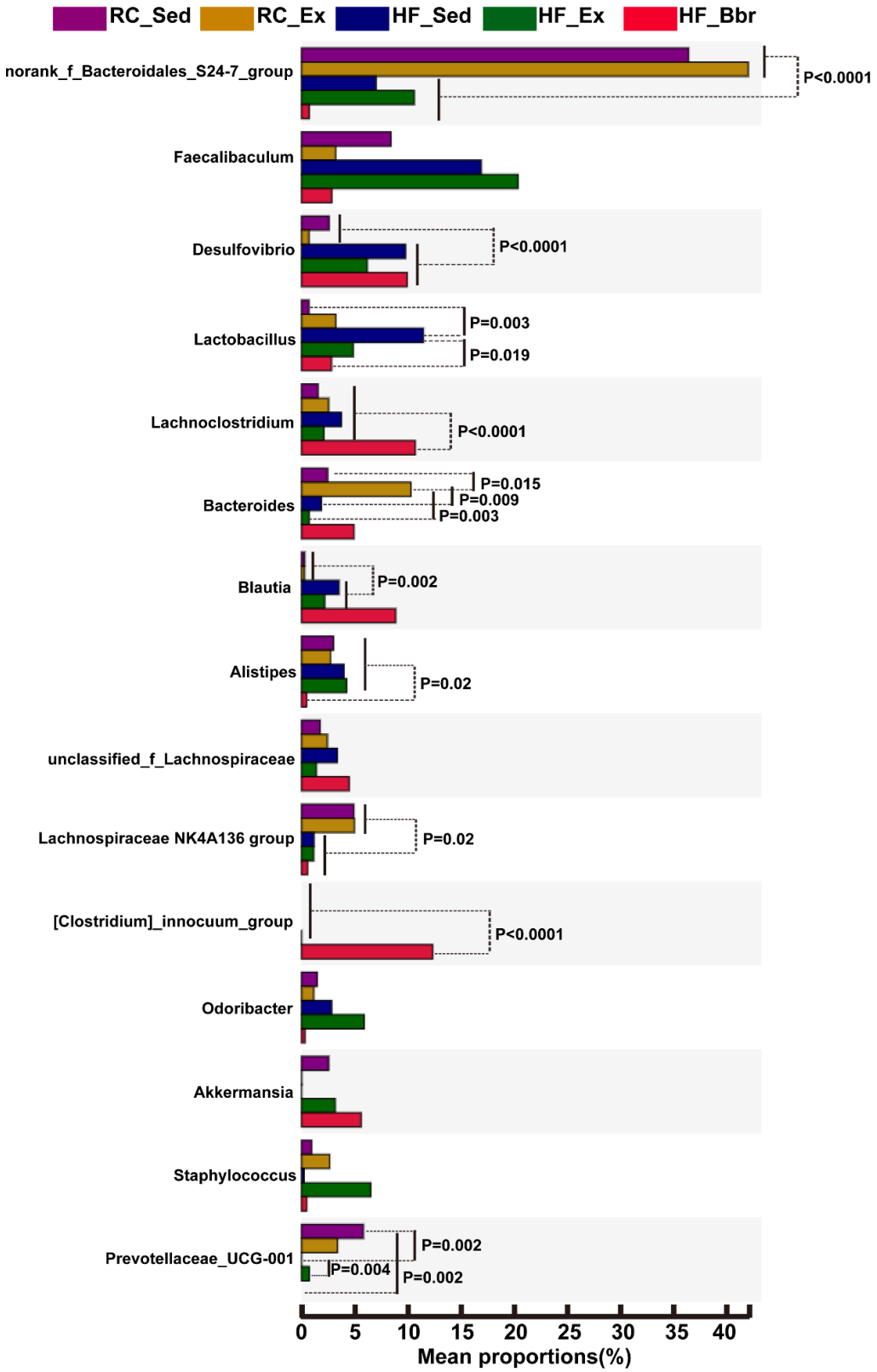
(3) Community structures

Clustering analysis showed that the gut microbiota from the 20 samples could be divided into two groups according to dietary types with gut microbiota of normal chow-fed mice being placed in group a and HFDs fed mice being placed in group b (Figure 2D). Two subgroups of 16 samples could be identified in group b as G2 and G3. All the samples in HF_Bbr were placed in G2, while the others were in G3., The patterns of separation between microbial communities were clearly observed *via* PCoA. The results indicated that the largest separation between microbial communities was of the dietary type (PC 1, 34.43%) (Figure 2E).

(4) Gut microbiota variations

In all groups, differentially abundant levels of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were found. The bacterial composition in HF_Sed was the most distinct. Compared to RC_Sed, the relative abundance of *Firmicutes* and *Proteobacteria* in HF_Sed was significantly higher ($P < 0.05$), while that of *Bacteroidetes* was significantly lower ($P < 0.05$). These results

A.


RC_Sed
RC_Ex
HF_Sed
HF_Ex
HF_Bbr
norank_f_Bacteroidales_S24-7_group
P<0.0001
Faecalibaculum
Desulfovibrio
P<0.0001
Lactobacillus
P=0.003
P=0.019
Lachnoclostridium
P<0.0001
Bacteroides
P=0.015
P=0.009
P=0.003
Blautia
P=0.002
Alistipes
P=0.02
unclassified_f_Lachnospiraceae
Lachnospiraceae NK4A136 group
P=0.02
[Clostridium]_innocuum_group
P<0.0001
Odoribacter
Akkermansia
Staphylococcus
Prevotellaceae_UCG-001
P=0.002
P=0.004
P=0.002
0 5 10 15 20 25 30 35 40
Mean proportions(%)

B.

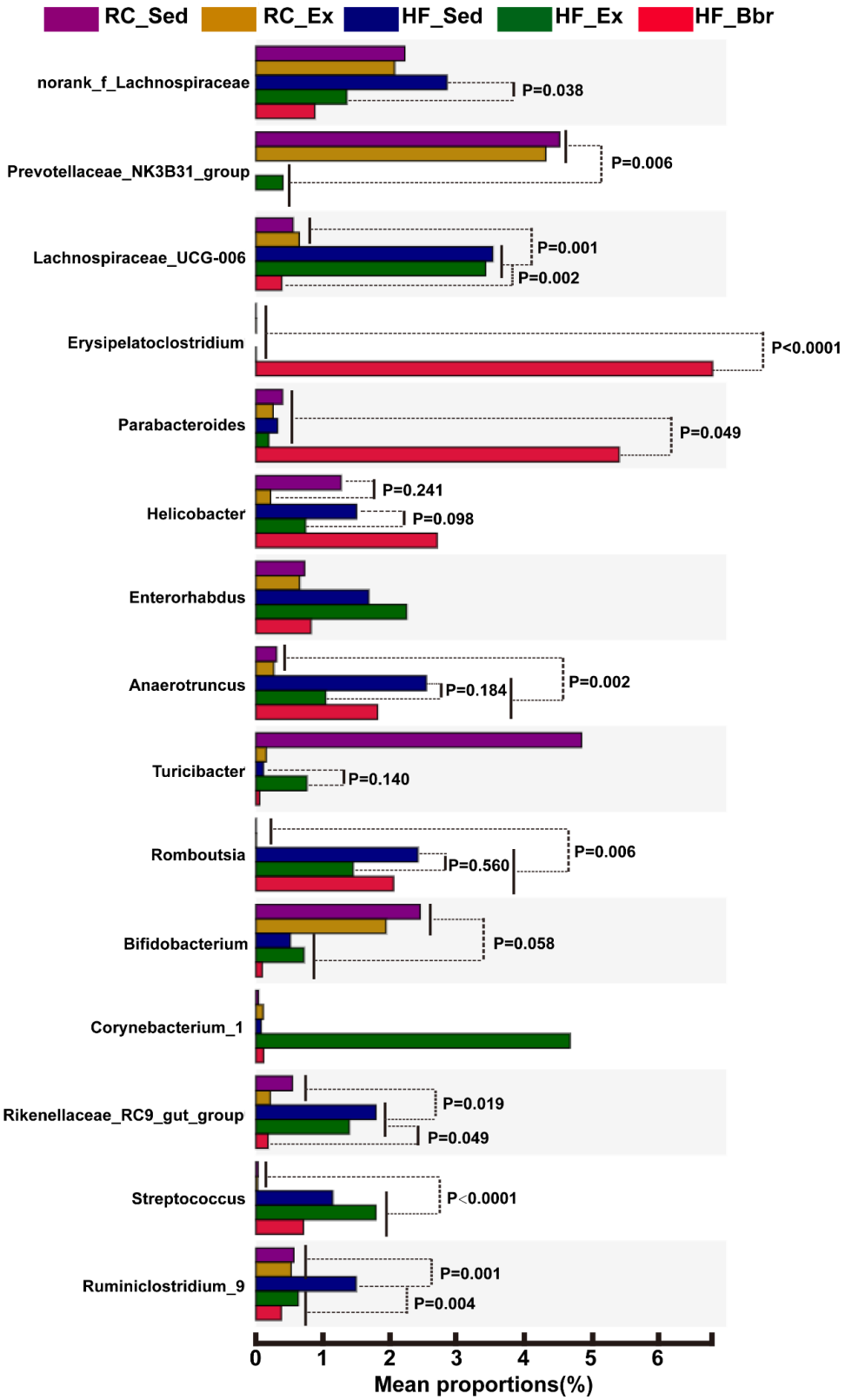


Figure 4. Comparison of the mean relative abundances of the dominant genera (top 1–15 (**A**) and top 16–30 (**B**)) in the RC_Sed, RC_Ex, HF_Sed, HF_Ex, and HF_Bbr groups at the genus level.

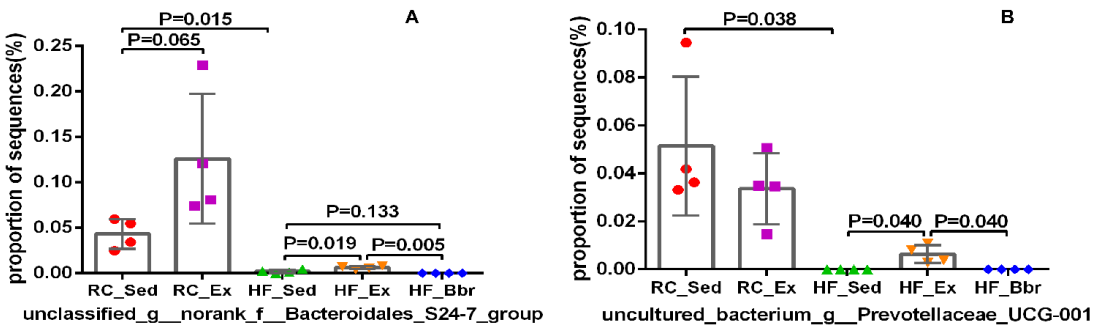


Figure 5. Two species whose relative abundances were significantly affected by HFDs and exercise.

caused by HFDs could be reversed by exercise intervention. A non-significant decrease in *Proteobacteria* in HF_Ex compared to HF_Sed was observed (Figure 3). After berberine treatment, *Firmicutes*, *Bacteroidetes*, and *Ascomycetes* in HF_Bbr group behaved similarly to the HF_Sed group (Figure 3). HF_Bbr group exhibited a significant increased abundant level of *Firmicutes* and *Ascomycetes* ($P < 0.05$) and a significant decreased abundant levels of phylum *Bacteroidetes* ($P < 0.05$). The stratification of microflora populations at the genus level induced by diets demonstrated that HFDs decreased the abundant levels of norank_f_*Bacteroidales*_S24-7_group, *Lachnospiraceae*_NK4A136_group, *Prevotellace-ae*_UCG-001, and *Prevotellaceae*_NK3B31_group, while increased the abundance of *Desulfovibrio*, *Lactobacillus*, *Blautia,* *Lachnospi-raceae*_UCG-006, *Anaerotruncus*, *Romboutsia*, *Rikenellaceae*_RC9_gut_group, *Streptococcus*, and *Ruminiclostridium*_9 (Figure 4). An enhancement in the richness of *Prevotellaceae*_UCG-001 and a reduction in the richness of norank_f_*Lachnospiraceae* and *Ruminiclostridium*_9 could be caused by exercise. *Lachnoclostridium*, *Clostridium_innocuum*_ group, *Erysipelatoclostridium*, and *Parabacteroides* were mainly colonized in the HF_Bbr group. At the species level, two species, uncultured bacterium *Bacteroidales*_S24-7_group (Figure 5A) and *Prevotellaceae*_UCG-001 (Figure 5B), whose relative abundance decreased significantly with HFDs intervention and increased with exercise intervention. In addition, the addition of berberine did not affect the reduction of *Bacteroidales*_S24-7_group and *Prevotellaceae*_UCG-001 induced by HFDs feeding were also noted.

Discussion

Scientific evidence shows that intestinal microbiota is one of indicators and contributors to human health and, therefore, plays significant roles in prevention, diagnosis, and treatment of human diseases. The enteric dysbacteriosis caused by HFDs feeding and unhealthy lifestyle such as the absence of physical activity has many underlying chronic metabolic diseases. Persistent gut micro-biota disturbance may contribute to metabolic inflammation in obesity that is initiated through intestinal barrier disruption and LPS translocation from the gut to the circulation, thereby triggering many innate proinflammatory reactions and leading to disruption in metabolism [8]. In the aspects of prevention and treatment of many metabolic diseases including obesity and hyper-tension, exercise is recognized as a cost-effective lifestyle intervention [25]. The results of this study validated previous report that physical activity not only significantly induced weight loss but reduced the fasting blood insulin level and ameliorated osteoarthritis in which the reshaped gut microbiota might be responsible for these beneficial effects [20]. However, these exercise-induced modifications concerning gut microbiota require further research.

High diversity can provide strong stability for the ecosystem. Thus, microbial diversity is conducive to balancing the intestinal microecology and maintaining normal ecological function [26]. Decreased diversity in gut bacteria accompanied by perturbed intestinal homeostasis and impaired ecological function is intricately linked to disease conditions. Khadka *et al*. found that reduced apparent alpha diversity in the individuals with atopic dermatitis (AD) was correlated with the AD severity [27]. These results were supported by Nylund *et al*. [28]. Likewise, the diversity of microbe was negatively related to the severity of inflammatory bowel disease (IBD), and the disease remission commonly co-occurred with the enhancement of microbial diversity [26]. The treatment of allergic asthma in newborn mice with vancomycin reduced microbial diversity changed the composition of bacterial flora, leading to an increase in the severity of this disease [29]. Furthermore, obese adults had significantly lower alpha diversity compared to non-obese adults [30]. HFDs is an unhealthy eating pattern and a contributor to obesity. In the mice fed HFDs without exercise, a significant decrease in diversity was noted. Exercise did not affect microbiota richness in normal-fed mice but could increase richness in HFD-fed mice. However, intestinal microbial diversity was not increased by berberine treatment. It seems that exercise, in contrast to berberine, can diversify the intestinal microorganisms and readjust the constitution of the bacterial population.

The four main phyla of the gut microbiota were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. At the phyla level, HFDs could result in increased abundant levels of *Firmicutes* and *Proteobacteria* and reduced abundant levels of *Bacteroidetes*, but the abundance of *Actinobacteria* had no change. However, the results of increase or reduction induced by HFDs could be reversed by exercise intervention. Compared to HF_Sed, the abundance of *Firmicutes* and *Proteobacteria* in HF_Ex was lower, and the abundance of *Bacteroidetes* was higher. Similar associations were found in the studies of HFDs affecting the flora at the genus level in comparison with exercise. The inverse role of exercise described was also found in some genera in significant or insignificant amounts, including norank_f__*Bacteroidales*_S24-7_group, *Desulfovibrio*, and *Prevotellaceae*_UCG-001. In some genera, the reverse effect of exercise on the composition of flora did not show a significant difference, which might be related to the limited time of exercise intervention. Additionally, the abundance of *Proteobacteria* was also reduced by exercise intervention, and the alteration of *Proteobacteria* was independent of weight and the diet. This result was the same as the report of Munukka *et al*. [31] and Liu *et al*. [32]. These results supported that *Proteobacteria* might be a responder to exercise training. However, no research on the underlying mechanism of the associations between *Proteobacteria* and exercise has been found. In *Firmicutes*, the increased abundance of *Blautia*, *Lachnospiraceae*_UCG-006, *Ruminiclostridium*_9, *Anaerotruncus*, *Romboutsia*, *Lactobacillus*, and *Streptococcus* was observed after HFDs treatment, but decreased abundance of norank_f__*Lachnospiraceae*, and *Ruminiclostridium*_9 was also observed with exercise intervention. Among them, *Blautia* and *Ruminiclostridium*_9 were identified as HFD-dependent taxa [33]. In the report of Duan *et al*., *Blautia*, *Lachnospiraceae*_UCG-006, and *Romboutsia* were found to be induced by HFDs and highly correlated with nonalcoholic fatty liver disease [34]. *Lactobacillus* was reported to contribute to promoting intestinal lipid absorption and increasing fat mass [35]. In *Bacteroidetes*, the decreased abundance of norank_f__*Bacteroidales*_S24-7_group, *Prevotellaceae*_UCG-001, and *Prevotellaceae*_NK3B31_group was observed after HFDs treatment, but exercise intervention led to an increase in abundance of *Prevotellaceae*_UCG-001. In *Proteobacteria*, the increased abundance of *Desulfovibrio* was observed after HFDs treatment. *Ruminiclostridium* 9 and *Prevotellacee*_ UCG-001 were influenced not only by diet but also by exercise. *Romboutsia* is a genus within the *Peptostreptococcaceae* family, and *Desulfovibrio*

is a genus within the *Desulfovibrionaceae* family. Studies have reported that *Desulfovibrionaceae* and *Peptostreptococcaceae* are bacteria producing endotoxin [36, 37]. Notably, at the species level, HFDs intervention groups exhibited a reduced relative abundant level of uncultured bacterium *Bacteroidales*_S24-7_group and *Prevotellaceae*_UCG-001, and exercise intervention groups showed an increased relative abundant level conversely. *Prevotellaceae*_UCG-001 belongs to *Prevotellaceae*, a family that reportedly produces butyrate, strengthens the functions of intestinal barrier, reduces the levels of endotoxin, and ameliorates the severity of metabolic inflammation [38, 39]. *Prevotellaceae*_UCG-001 and *Bacteroidales*_S24-7_group are suggested to be beneficial SCFAs-producing bacteria. Furthermore, *Bacteroidales*_S24-7 seems to be an exercise responder, which can be enriched with exercise. Similarly, the results of this study showed that *Bacteroidales*_S24-7 was reduced by HFDs feeding, while exercise could recover its loss in obesity. Therefore, this study suggested that exercise could reverse the HFD-induced gut microbiota disorder and exert improved positive health effects.

Berberine, an intestinal-regulating drug, plays reportedly an essential role in the improvement of obesity, diabetes mellitus, atherosclerosis, and other metabolic diseases, and its role in lipid-lowering and insulin resistance is the same as exercise. Berberine exerts its multifunctional effects *via* regulating the gut microbiota [23]. Berberine can revert the effects of the HFD-induced structural changes of gut microbiota and ameliorate the severity of some metabolic disorders caused by HFDs. However, a reduction of microbial diversity was also observed. The results showed that berberine administration could not diversify the gut microbiota and reverse the increase or decrease pattern induced by HFDs. The occurrence of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* in the berberine treated group demonstrated the same trend as that of HFDs treatment. Combined with the low community richness caused by berberine, the emergence of the highest number of unique species implied that berberine could also colonize numerous new species in the intestinal tract while exerting a bacteriostasis effect. Berberine enriched the occurrence of *Lachnoclostridium*, [*Clostridium*]_innocuum _group, *Erysipelatoclostridium*, and *Parabacteroides*. *Lachnoclostridium* reportedly produces trimethylamine and is linked to the promotion of atherosclerosis. Berberine has shown beneficial effects on diabetes-related complications, which is similar to the results of this study, but some transient gastrointestinal adverse effects were also observed. The increased population of [*Clostridium*]_innocuum, which was recently identified as a vancomycin-resistant pathogen, may lead to antibiotic-associated diarrhea in humans. Therefore, the mechanism by which berberine rebuilds the intestinal flora community is different from that of exercise.

Everyone has a unique gut microbiota profile. The establishment of intestinal microflora is influenced by internal and ex-ternal factors, including age, geography, heredity, diet, lifestyle, drugs, and exercise. Even with short-term induction, microorganisms can respond and change rapidly. Long-term stimuli can promote the colonization of some different core microbiota communities, thus facilitating the stratification of microflora populations. The results showed that all the samples were first divided into two camps according to the type of diet (HFDs or RC diets feeding). The PCoA results clearly suggested distinctions between their community composition profiles. Although exercise did not result in significant changes in typical bacterial clusters, alterations of the bacterial taxa at the phyla, genus, and species levels demonstrated the modification that exercise brought to the microbial community.

This study elucidated the differential microbial community profiles related to exercise, HFDs, and berberine, which might shed light on exercise functionality. The results showed that HFDs treatment led to decreased diversity in gut

bacteria, whereas exercise, unlike berberine, could return it to normal status and modulate the bacterial population component. HFDs could result in an increased abundance of *Firmicutes* and *Proteobacteria* and a reduced abundance of *Bacteroidetes*. Interestingly, the results of increase or decrease caused by HFDs could be reversed by exercise intervention. Similar associations were found in the studies of the HFDs affecting the flora at the genus level in comparison with exercise. Notably, some of these reversal effects did not show significant differences, which might be related to the limited time of sports intervention. Furthermore, exercise had limited effects on the stratification of microflora populations. Exercise could improve the proliferation of probiotics but inhibit the proinflammatory microbiota, thereby exerting improved positive health effects. Together, the results suggested that HFDs led to the disruption of the microbiome profiles in obesity, while exercise could rebuild the intestinal microbial ecology in obese individuals, and the mechanism by which berberine acted to regulate the bowel might differ from exercise.

Acknowledgements

This study was funded by the Humanities and Social Science Fund of Ministry of Education of China (Grant No. 23YJAZH026); Special Research Project of Philosophy and Social Science Planning Department of Zhejiang Province (Grant No. 24BMHZ061YB); Quzhou Competitive Science and Technology Research Project (Grant No. 2023K217 and 2023K239).

References

1. Huang ZY, Stabler T, Pei FX, Kraus VB. 2016. Both systemic and local lipopolysaccharide (LPS) burden are associated with knee OA severity and inflammation. Osteoarthritis Cartilage. 24(10):1769-1775.
2. Mokkala K, Houttu N, Cansev T, Laitinen K. 2020. Interactions of dietary fat with the gut microbiota: Evaluation of mechanisms and metabolic consequences. Clin Nutr. 39(4):994-1018.
3. Semenkovich NP, Planer JD, Ahern PP, Griffin NW, Lin CY, Gordon JI. 2016. Impact of the gut microbiota on enhancer accessibility in gut intraepithelial lymphocytes. Proc Natl Acad Sci USA. 113(51):14805-14810.
4. Duncan SH, Louis P, Thomson JM, Flint HJ. 2009. The role of pH in determining the species composition of the human colonic microbiota. Environmental Microbiology. 11(8):2112-2122.
5. McLoughlin RF, Berthon BS, Jensen ME, Baines KJ, Wood LG. 2017. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: A systematic review and meta-analysis. Am J Clin Nutr. 106(3):930-945.
6. Uchiyama K, Naito Y, Takagi T. 2019. Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. Pharmacol Ther. 199:164-172.
7. Hotamisligil GS, Erbay E. 2008. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol. 8(12):923-934.
8. Hersoug LG, Moller P, Loft S. 2016. Gut microbiota-derived lipopolysaccharide uptake and trafficking to adipose tissue: implications for inflammation and obesity. Obes Rev. 17(4):297-312.
9. Malesza IJ, Malesza M, Walkowiak J, Mussin N, Walkowiak D, Aringazina R, *et al*. 2021. High-fat, western-style diet, systemic inflammation, and gut microbiota: A narrative review. Cells. 10(11):3164.
10. Velázquez KT, Enos RT, Bader JE, Sougiannis AT, Carson MS, Chatzistamou I, *et al*. 2019. Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. World J Hepatol. 11(8):619-637.
11. Shin NR, Whon TW, Bae JW. 2015. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33(9):496-503.
12. Sen T, Cawthon CR, Ihde BT, Hajnal A, DiLorenzo PM, de La Serre CB, *et al*. 2017. Diet-driven microbiota dysbiosis is associated with vagal remodeling and obesity. Physiol Behav. 173:305-317.
13. Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, *et al*. 2012. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. Int J Obes (Lond). 36(6):817-825.
14. Campbell SC, Wisniewski PJ. 2017. Exercise is a novel promoter of intestinal health and microbial diversity. Exerc Sport Sci Rev. 45(1):41-47.
15. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, *et al*. 2014. Exercise and associated dietary extremes impact on gut microbial diversity. Gut. 63(12):1913-1920.
16. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, *et al*. 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci USA. 110(22):9066-9071.
17. Li KK, Tian PJ, Wang SD, Lei P, Qu L, Huang JP, *et al*. 2017. Targeting gut microbiota: *Lactobacillus* alleviated type 2 diabetes *via* inhibiting LPS secretion and activating GPR43 pathway. J Funct Foods. 38:561-570.
18. Allen JM, Mailing LJ, Cohrs J, Salmonson C, Fryer JD, Nehra V, *et al*. 2018. Exercise training-induced modification of the gut microbiota persists after microbiota colonization and

attenuates the response to chemically-induced colitis in gnotobiotic mice. Gut Microbes. 9(2):115-130.
19. Codella R, Luzi L, Terruzzi I. 2018. Exercise has the guts: How physical activity may positively modulate gut microbiota in chronic and immune-based diseases. Dig Liver Dis. 50(4):331-341.
20. Li K, Liu A, Zong W, Dai L, Liu Y, Luo R, *et al*. 2021. Moderate exercise ameliorates osteoarthritis by reducing lipopolysaccharides from gut microbiota in mice. Saudi J Biol Sci. 28(1):40-49.
21. Wang Y, Campbell T, Perry B, Beaurepaire C, Qin L. 2011. Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats. Metabolism. 60(2):298-305.
22. Zhang Z, Zhang H, Li B, Meng X, Wang J, Zhang Y, *et al*. 2014. Berberine activates thermogenesis in white and brown adipose tissue. Nat Commun. 5:5493.
23. Habtemariam S. 2020. Berberine pharmacology and the gut microbiota: A hidden therapeutic link. Pharmacol Res. 155:104722.
24. Wong SK, Chin KY, Ima-Nirwana S. 2019. Berberine and musculoskeletal disorders: The therapeutic potential and underlying molecular mechanisms. Phytomedicine. 73:152892.
25. Stefani L, Galanti G. 2017. Physical exercise prescription in metabolic chronic disease. Adv Exp Med Biol. 1005:123-141.
26. Gong D, Gong X, Wang L, Yu X, Dong Q. 2016. Involvement of reduced microbial diversity in inflammatory bowel disease. Gastroenterol Res Pract. 2016:6951091.
27. Khadka VD, Key FM, Romo-Gonzalez C, Martinez-Gayosso A, Campos-Cabrera BL, Geronimo-Gallegos A, *et al*. 2021. The skin microbiome of patients with atopic dermatitis normalizes gradually during treatment. Front Cell Infect Microbiol. 11:720674.
28. Nylund L, Nermes M, Isolauri E, Salminen S, de Vos WM, Satokari R. 2015. Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria. Allergy. 70:241-244.
29. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, *et al*. 2012. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Rep. 13:440-447.
30. Pinart M, Dotsch A, Schlicht K, Laudes M, Bouwman J, Forslund SK, *et al*. 2021. Gut microbiome composition in obese and non-obese persons: A systematic review and meta-analysis. Nutrients. 14(1):12.
31. Munukka E, Ahtiainen JP, Puigbo P, Jalkanen S, Pahkala K, Keskitalo A, *et al*. 2018. Six-week endurance exercise alters gut metagenome that is not reflected in systemic metabolism in over-weight women. Front Microbiol. 9:2323.
32. Liu TW, Park YM, Holscher HD, Padilla J, Scroggins RJ, Welly R, *et al*. 2015. Physical activity differentially affects the cecal microbiota of ovariectomized female rats selectively bred for high and low aerobic capacity. PloS One. 10:e0136150.
33. Zhao Q, Hou D, Fu Y, Xue Y, Guan X, Shen Q. 2021. Adzuki bean alleviates obesity and insulin resistance induced by a high-fat diet and modulates gut microbiota in mice. Nutrients. 13(9):3240.
34. Duan R, Huang K, Guan X, Li S, Xia J, Shen M, *et al*. 2022. Tectorigenin ameliorated high-fat diet-induced nonalcoholic fatty liver disease through anti-inflammation and modulating gut microbiota in mice. Food Chem Toxicol. 164:112948.
35. Zhong W, Wang H, Yang Y, Zhang Y, Lai H, Cheng Y, *et al*. 2022. High-protein diet prevents fat mass increase after dieting by counteracting *Lactobacillus*-enhanced lipid absorption. Nat Metab. 4(12):1713-1731.
36. Schott EM, Farnsworth CW, Grier A, Lillis JA, Soniwala S, Dadourian GH, *et al*. 2018. Targeting the gut microbiome to treat the osteoarthritis of obesity. JCI Insight. 3(8):e95997.
37. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, *et al*. 2010. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J. 4:232-241.
38. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al*. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 56:1761-1772.
39. Chen Y, Liu Y, Wang Y, Chen X, Wang C, Chen X, *et al*. 2022. *Prevotellaceae* produces butyrate to alleviate PD-1/PD-L1 inhibitor-related cardiotoxicity via PPARalpha-CYP4X1 axis in colonic macrophages. J Exp Clin Cancer Res. 41(1):1.