Article

Discovery Medicine 2025; 37(195): 765–771
https://doi.org/10.24976/Discov.Med.202537195.66

Effects of Aerobic Exercise and Intermittent Fasting on Fecal Microbiome Diversity in Obese Mice

Lingfeng Yuan[1], Yisheng Luan[1], Weihao Hong[1], Bing Zhang[1], Yingzhe Xiong[2,*]

[1]Division of Sports Science and Physical Education, Tsinghua University, 100084 Beijing, China
[2]School of Physical Education, Central China Normal University, 430079 Wuhan, Hubei, China
*Correspondence: xiongyingzhe@ccnu.edu.cn (Yingzhe Xiong)
Published: 20 April 2025

Background: Obesity threatens human health, and interventions to reduce obesity may have important effects on the gut microbiota. This study investigated alterations in gut microbial composition in response to aerobic exercise (AE) and intermittent fasting (IF).

Methods: We randomly divided mice into four groups of seven mice each: normal, obesity, exercise, and fasting. The normal group was fed a Chow Diet, whereas the other three groups were fed a High Fat Diet (HFD). After 13 weeks, the exercise group was subjected to aerobic treadmill running, and the fasting group started IF for 8 weeks. We then analyzed the composition of the fecal microbiome in all mice at the end of 21 weeks.

Results: Our investigation revealed that the HFD significantly influenced *species (s)_Romboutsia ilealis*, *genus (g)_Dubosiella*, and *g_Blautia*. AE predominantly affected *family (f)_Rhizobiaceae* and *g_Rikenella*, indicating its impact on enhancing microbial taxa associated with improved metabolic health profiles. On the other hand, IF prominently altered the abundance of *s_Lactobacillus johnsonii* and *g_Colidextribacter*, which are known for their roles in enhancing glucolipid metabolism and anti-inflammatory activity. Furthermore, the exercise group displayed increased diversity within *f_Rhizobiaceae*, potentially associated with anti-inflammatory benefits. The IF intervention was particularly effective in enriching *s_Lactobacillus johnsonii*, suggesting its pivotal role in regulating metabolic responses influenced by fasting.

Conclusion: The results demonstrated significant beneficial alterations in microbial composition following AE and IF interventions, which supports the use of personalized approaches for obesity management and overall health.

Keywords: fecal microbiome; High Fat Diet; aerobic exercise; intermittent fasting

Introduction

Exploration of the complex ecosystem of fecal microbiota has become a pivotal focus in health sciences, and a study has revealed its profound involvement in multiple facets of host health [1]. A growing body of evidence suggests that lifestyle changes, particularly diet and exercise, can significantly affect the fecal microbiome, thereby potentially mitigating health-related complications, particularly obesity-associated health problems that are prevalent worldwide [2].

Among the array of interventions, aerobic exercise (AE) and intermittent fasting (IF) emerge as prominent strategies with transformative potential. AE, a cornerstone in promoting cardiovascular fitness and weight control, orchestrates a cascade of physiological adaptations conducive to enhanced health [3]. In contrast, the fasting regimen of IF, characterized by cyclic feeding and fasting intervals, showcases promising results in weight management and metabolic improvements, heralding a paradigm shift in health practices [4].

Whereas individual studies have delved into the impact of AE and IF on fecal microbiome diversity, a comprehensive comparative analysis unraveling the nuanced effects of these interventions remains a critical research frontier [5,6]. By elucidating the differential impacts of IF and AE on fecal microbiota diversity in obese mice, this study seeks to unravel the complex tapestry woven between dietary habits, physical activity, fecal microbiota composition, and obesity. Through a deeper exploration of these intricate relationships, we seek to enrich our understanding and tailor innovative, personalized strategies for effective obesity management.

Materials and Methods

Animals and Experimental Design

Wild-type male 3-week-old C57BL/6 mice (n = 28, 16–19 g) were purchased from the Laboratory Animal Research Center of Tsinghua University (Beijing, China). This study was conducted under the oversight and with the approval of the Institutional Animal Care and Use Committee of Tsinghua University (ID: THU-LARC-2023-007).

All the mice were provided with free access to water and food. After adapting for 1 week, mice were randomly divided into four groups of seven mice each: normal, obesity, exercise, and fasting. The normal group was fed a Chow Diet (CD, 12% kcal fat content), whereas the other three groups were fed a High Fat Diet (HFD, 60% kcal fat content).

After 13 weeks, the exercise group was trained on a motorized mouse treadmill at 0° for 8 weeks, 5 days per week, for 45 min/day, after warming up at 5 m/min for 10 min/day. The training pace was 14 m/min in the first week, increased by 0.5 m/min per week, and ended with 17.5 m/min in the eighth week, maintaining the exercise intensity within 50–60% of VO_2max intensity [7]. The fasting group started IF which included free access to HFD every other day and no food on alternate days for 8 weeks [8]. The experimental design is illustrated in Fig. 1.

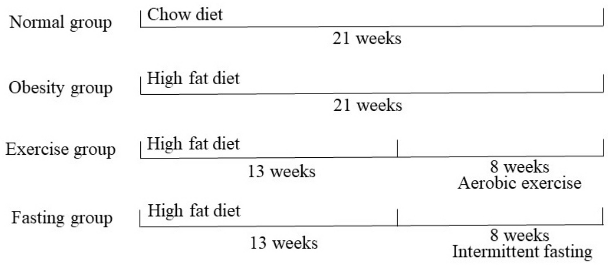


Fig. 1. Experimental design.

Fecal Specimen Collection and Sequencing

At the end of 21 weeks, all mice were fasted for 6 h, then 6–8 pellets of mouse feces samples were newly collected from each mouse and immediately frozen in liquid nitrogen and stored at –80 °C. Mice were euthanized by an overdose of carbon dioxide (SMARTBOX CO_2 Chamber System, EZ systems, Palmer, PA, USA). Total genomic DNA was extracted from the samples using the cetyltrimethylammonium bromide (CTAB) method. All polymerase chain reaction (PCR) reactions were carried out using Phusion® High-Fidelity PCR Master Mix (M0531L, New England Biolabs, Ipswich, MA, USA). Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (20015963, Illumina, San Diego, CA, USA) following the manufacturer's instructions, and index codes were added. Library quality was assessed using a Qubit@ 2.0 Fluorometer (Q32866, Thermo Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer 2100 system (2100 system, Agilent Technologies, Santa Clara, CA, USA). Finally, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

Statistical Analysis

Operational Taxonomic Units (OTUs) clustering was conducted to analyze the fecal microbiome diversity in obese mice. The raw sequencing data were processed using Uparse software (v7.0.1001, Drive5, Tiburon, CA, USA) for quality filtering, trimming, and demultiplexing. Subsequently, sequences were aligned and clustered into OTUs using the MUSCLE algorithm (MEGA6, ASU, Phoenix, AZ, USA). Alpha diversity analysis was performed to calculate diversity metrics including the Chao1 and Shannon indices. Beta diversity analysis, which involves examining differences in microbial composition between samples, was conducted using the Quantitative Insights Into Microbial Ecology (QIIME) platform [9]. Principal Coordinate Analysis (PCoA) was utilized to visualize the beta diversity metrics and assess microbial community dissimilarities. To determine the significance of differences in alpha and beta diversity measures among the experimental groups, statistical tests such as Analysis of Variance (ANOVA) and pairwise distance comparisons were employed. Moreover, the identification of specific bacterial taxa enriched in each group was achieved through Linear Discriminant Analysis Effect Size (LEfSe) analysis, enabling the characterization of key microbial biomarkers associated with different interventions [10]. We considered $p < 0.05$ was statistically significant. The statistical significance and robustness of the results were validated through permutation tests and Monte Carlo simulations, ensuring the reliability and reproducibility of the observed microbial community differences.

Results

Fecal Microbial Composition

To determine whether the sample size was sufficient, species dilution and abundance grade curves were used (Fig. 2A,B). All groups appeared stable, which showed the sample size was sufficient for statistical analysis. The obesity, exercise, and fasting groups were all fed HFD, and we found that the HFD groups displayed decreased species number and abundance compared with the CD group (Fig. 2A,B). The Chao1 and Shannon indices were higher in the CD group than in the HFD groups ($p < 0.01$), indicating a greater richness and diversity of bacterial communities in the CD group than in the HFD group (Fig. 2C,D). Furthermore, the Chao1 index, but not the Shannon index, was significantly lower in the IF group than the obesity group, suggesting that IF could decrease community richness, but not community diversity, in obese mice ($p < 0.01$). Beta diversity analysis of all groups is presented in Fig. 3A–C. The PCoA diagrams indicate that the two groups are clearly separated. The Venn graph shows the overlap of OTUs in the fecal microbiome among samples (Fig. 3D).

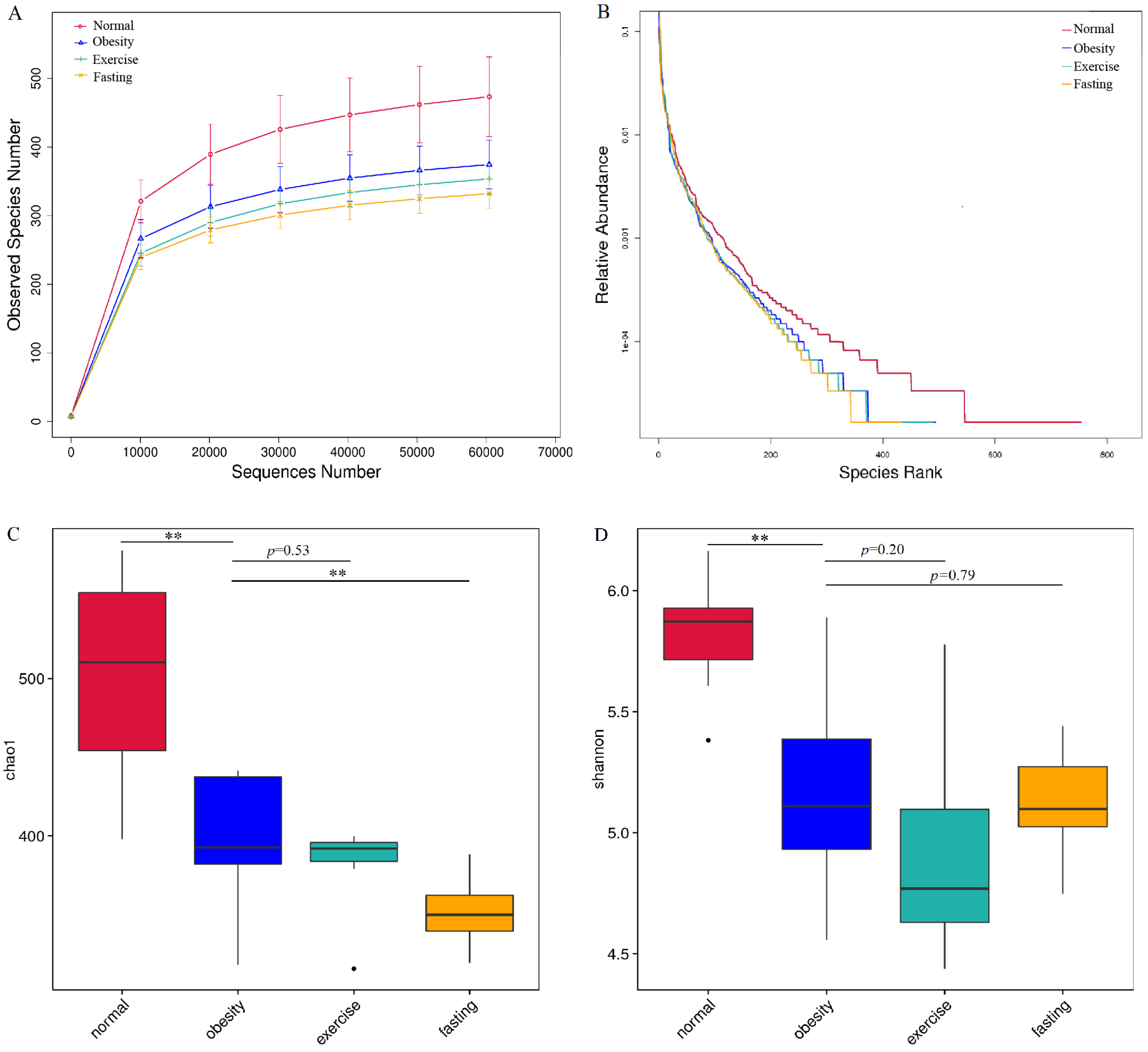


Fig. 2. Alpha diversity analysis. (A) Rarefaction curve, alpha diversity analysis based on species dilution. (B) Rank abundance, abundance grade curves indicate stability in all groups. (C) Chao1 index, higher index values indicate greater bacterial abundance in the Chow Diet (CD) group. (D) Shannon index indicates greater bacterial community abundance in the CD group. ** represents $p < 0.01$.

Fecal Microbial Top Types

To estimate the community abundance, we stacked histograms of the top 10 species in each group at the phylum (p), family (f), genus (g), and species (s) levels (Fig. 4). We focused on the highest bacterial proportions among the four groups. In the normal group, *p_Bacteroidota* (58.32%), *f_Muribaculaceae* (45.28%), *g_Alistipes* (5.10%), and *s_Akkermansia_muciniphila* (1.66%) were detected. In the obesity group, *p_Bacteroidota* (40.92%), *f_Muribaculaceae* (21.45%), *g_Parabacteroides* (9.66%) and *s_Romboutsia_ilealis* (3.40%) were detected. In the exercise group, *p_Bacteroidota* (42.47%), *f_Muribaculaceae* (14.98%), *g_Parabacteroides* (10.53%), and *s_Faecalibaculum_rodentium* (2.58%) were detected. In the fasting group, *p_Bacteroidota* (41.92%), *f_Desulfovibrionaceae* (15.96%), *g_Parabacteroides* (14.98%), and *s_Lactobacillus_johnsonii* (6.90%) were detected.

Biomarker

To identify the specific bacterial taxa related to each group, we identified enriched taxa in the groups using LEfSe. The results showed that there were 26 taxa based on a linear discriminant analysis (LDA) score threshold of >4 (Fig. 5A). Next, we analyzed the taxonomic relationships of these enriched taxa and found that the obesity group was dominated by order *(o)_Peptostreptococcales_Tissierellales*, the exercise

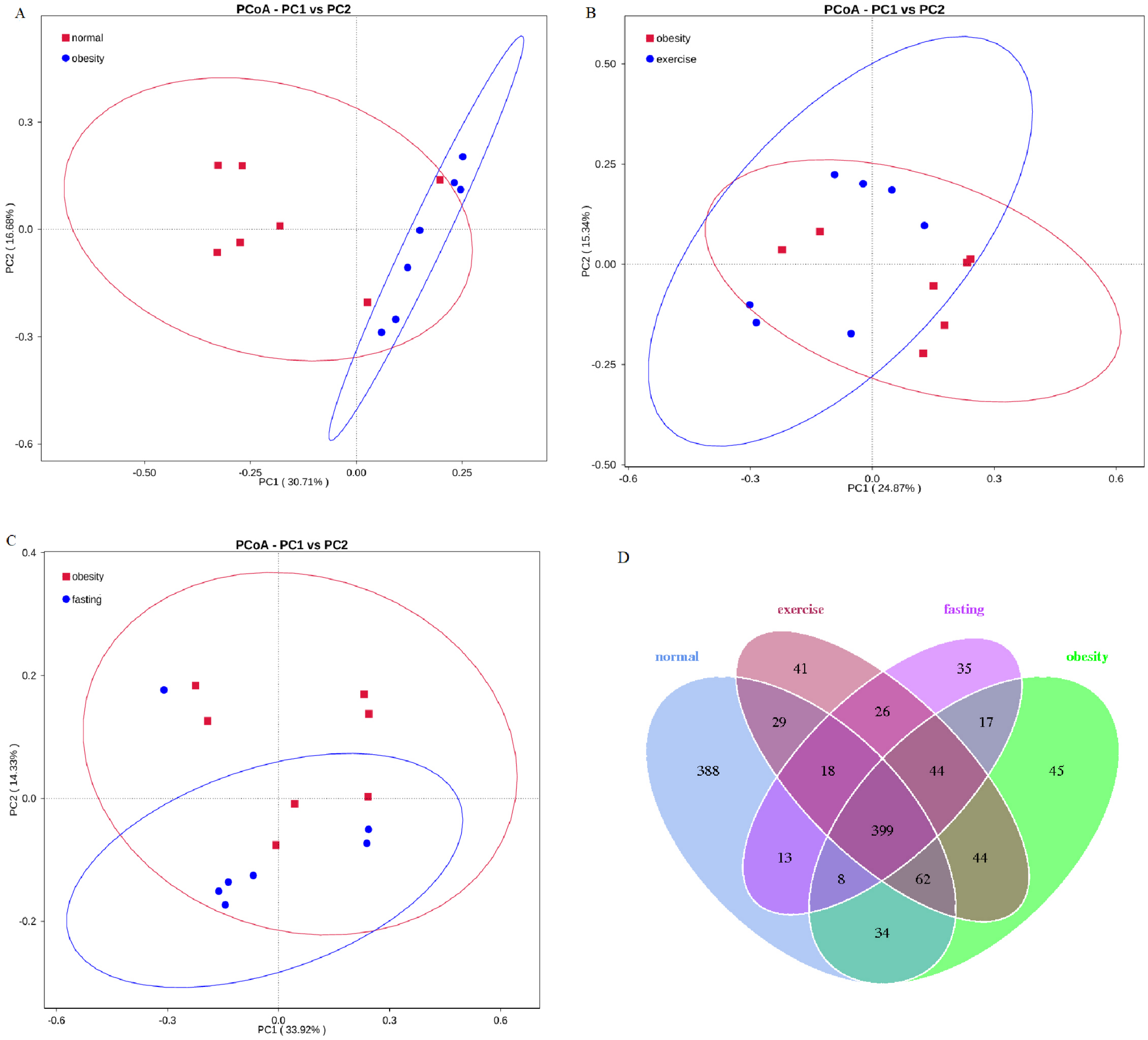


Fig. 3. Beta diversity analysis. (A–C) Principal Coordinates Analysis (PCoA) in different groups as normal vs. obesity, obesity vs. exercise, obesity vs. fasting. (D) Venn graph displays the Operational Taxonomic Units (OTUs) in fecal microbiome among samples.

group was dominated by class *(c)_ Alphaproteobacteria*, and the fasting group was dominated by *o_Lactobacillales* (Fig. 5B). Based on taxonomic relationships in these 24 taxa (Fig. 5C), we clustered the relative abundance of representative taxa in each group (Fig. 5D) and found that *f_Muribaculaceae*, *f_Ruminococcaceae*, *g_Allobaculum*, and *o_Clostridia_UCG_014* were dominant in the normal group and *s_Romboutsia ilealis*, *g_Dubosiella*, and *g_Blautia* were dominant in the obesity group, but they all showed no significant difference between the exercise and fasting groups. In addition, *f_Rhizobiaceae* and *g_Rikenella* were dominant in the exercise group, and *s_Lactobacillus johnsonii* and *g_Colidextribacter* were dominant in the fasting group.

Discussion

In the current study, we observed significant alterations in the fecal microbiome of obese mice following interventions with HFD, AE, and IF. These interventions exerted distinct effects on specific microbial taxa, highlighting their potential roles in obesity-related metabolic disturbances and responses to different interventions. Our findings align with previous research indicating that obesity is a multifaceted condition characterized by chronic low-level inflammation, disrupted lipid metabolism, and insulin resistance. The fecal microbiome emerges as a key player in regulating metabolic processes, inflammation, and hormonal responses in the context of obesity [11,12].

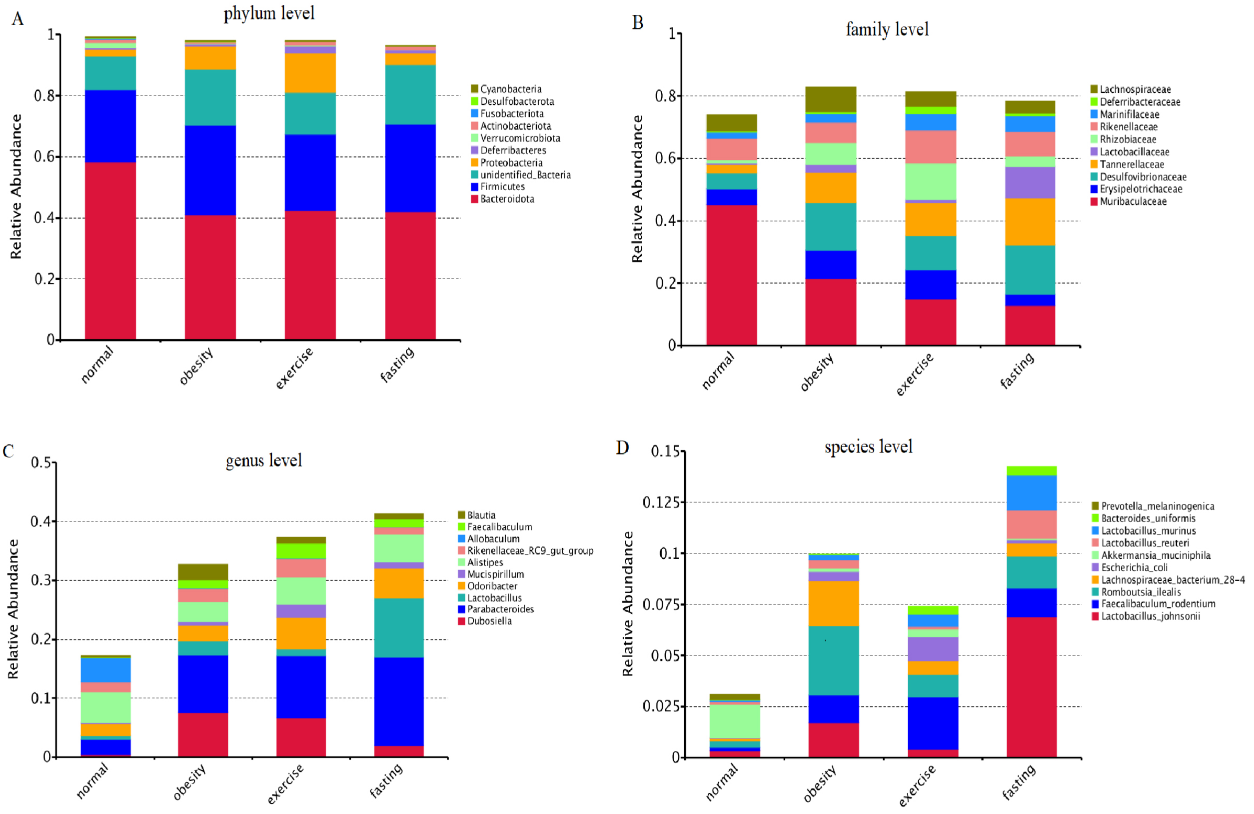
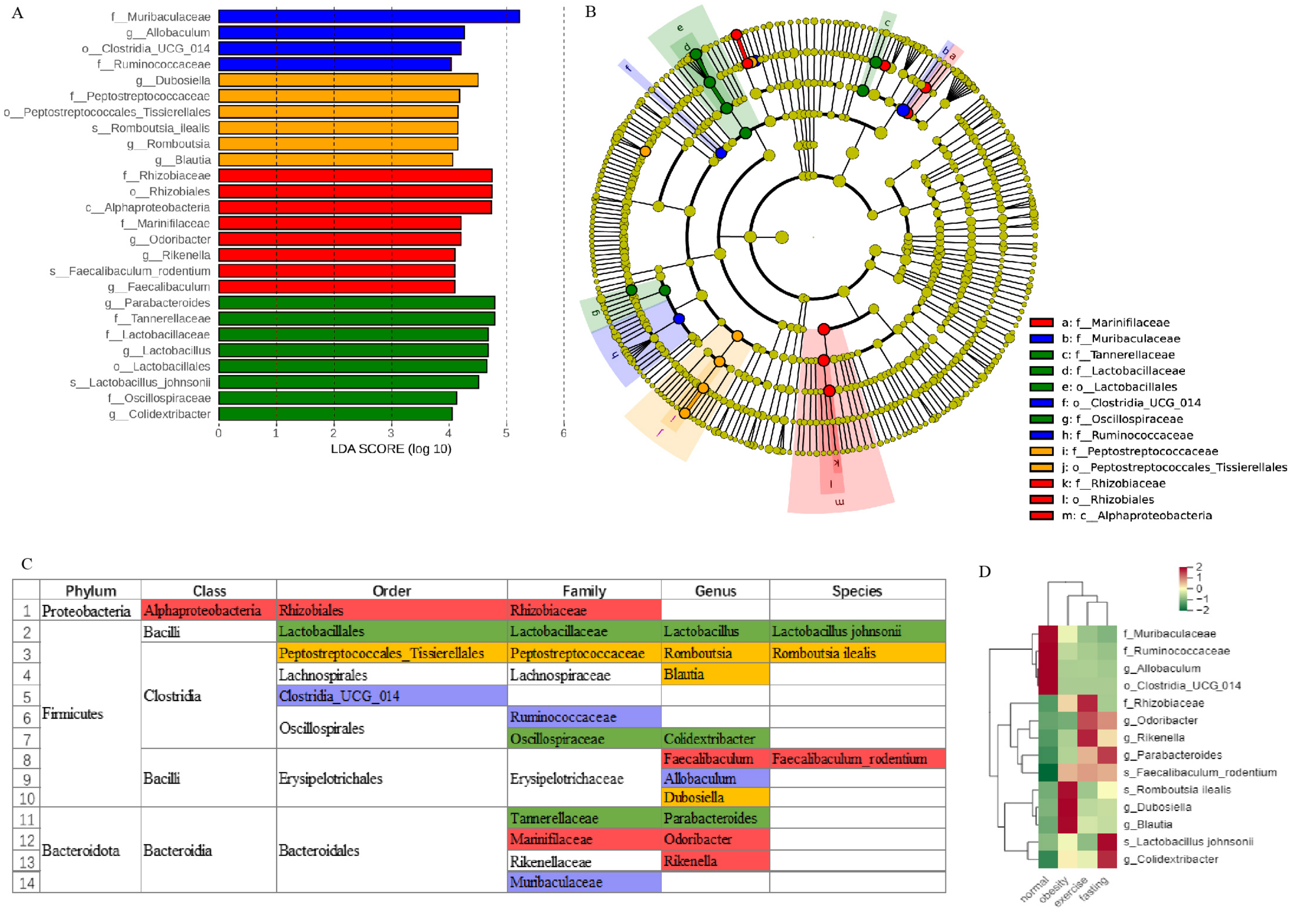


Fig. 4. Relative abundance analysis. Percentages of top species at the phylum (A), family (B), genus (C), and species (D) levels.

Through our analysis, we identified 10 representative taxa that were significantly modulated by HFD, AE, or IF, with implications for inflammatory responses and metabolic outcomes. Specifically, taxa such as *g_Odoribacter* have been associated with anti-inflammatory properties and display negative correlations with insulin resistance [11,12]. Other taxa like *g_Rikenella*, *g_Parabacteroides*, and *g_Dubosiella* have the capacity to produce short-chain fatty acids (SCFAs) that influence host gene expression and inflammatory signaling pathways [13–15]. Conversely, taxa such as *s_Faecalibaculum_rodentium* and *g_Colidextribacter* have been linked to pro-inflammatory activity and blood glucose levels [16]. In addition, *g_Rikenella* and *g_Colidextribacter* have been negatively associated with antioxidant enzymes [17,18]. Moreover, previous studies found that *s_Lactobacillus johnsonii* attenuated diet-induced diabetes and hyperlipidemia, *f_Rhizobiaceae* and *g_Blautia* inhibited the increase in blood glucose, and *s_Romboutsia ilealis* worsened glucose metabolism [19–22].

Our results highlighted the distinct microbial changes associated with different interventions. HFD predominantly impacted *s_Romboutsia ilealis*, *g_Dubosiella* and *g_Blautia,* emphasizing their potential contribution to obesity-related metabolic disturbances. AE influenced *f_Rhizobiaceae* and *g_Rikenella*, suggesting exercise-induced metabolic improvements through inflammation modulation, metabolic regulation, and enhancement of glucose tolerance. IF mainly affected *s_Lactobacillus johnsonii* and *g_Colidextribacter*, possibly implicating them in the regulation of fasting-induced metabolic responses by curbing inflammatory responses, oxidative stress, and glucolipid metabolism. In conclusion, AE and IF have clearly different impacts on microbiota in obese mice.

This study, while illuminating significant connections between AE, IF, and the fecal microbiome diversity in obese mice, presents several limitations that warrant mention. Firstly, the translational potential from murine models to human obesity management remains uncertain, as physiological and microbial responses to interventions like AE and IF could vary significantly across species. Secondly, our study design did not include a group that combined AE and IF interventions, which might have offered insights into synergistic or antagonistic effects between these two strategies. Furthermore, the assessment of microbial changes lacked longitudinal analysis throughout the intervention period, limiting our understanding of the temporal dynamics and early microbial responses to AE and IF. Another limitation is the absence of direct measures of host metabolic



	Phylum	Class	Order	Family	Genus	Species
1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae		
2	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus johnsonii
3		Clostridia	Peptostreptococcales_Tissierellales	Peptostreptococcaceae	Romboutsia	Romboutsia ilealis
4			Lachnospirales	Lachnospiraceae	Blautia	
5			Clostridia_UCG_014			
6			Oscillospirales	Ruminococcaceae		
7				Oscillospiraceae	Colidextribacter	
8		Bacilli	Erysipelotrichales	Erysipelotrichaceae	Faecalibaculum	Faecalibaculum_rodentium
9					Allobaculum	
10					Dubosiella	
11	Bacteroidota	Bacteroidia	Bacteroidales	Tannerellaceae	Parabacteroides	
12				Marinifilaceae	Odoribacter	
13				Rikenellaceae	Rikenella	
14				Muribaculaceae		

Fig. 5. Linear Discriminant Analysis Effect Size (LEfSe) analysis. (A) Linear Discriminant Analysis (LDA) bar plots representing the significantly enriched taxa between groups (the default setting was 4). (B,C) Taxonomic relationships in enriched taxa [purple: normal group; yellow: obesity group; red: exercise group; green: fasting group]. (D) Clustering and heat map of 14 representative taxa in each group. c, class; o, order; f, family; g, genus; s, species.

health, such as insulin sensitivity and inflammatory markers, which would have provided a more comprehensive picture of how changes in the microbiome influence obesity and metabolism. Future studies employing metagenomic sequencing and metabolomic analyses could shed light on the metabolic pathways affected by these interventions and their implications for obesity management.

These findings underscore the intricate relationships among the fecal microbiome, metabolic processes, and interventions aimed at managing obesity. As research in this field advances, a deeper understanding of these interactions could pave the way for personalized approaches to obesity management, centered on modulating the fecal microbiome to improve metabolic health.

Conclusion

In this study, we found significant alterations in fecal microbiome diversity in obese mice in response to AE and IF. The HFD predominantly affected *s_Romboutsia ilealis*, *g_Dubosiella*, and *g_Blautia*, whereas AE influenced *f_Rhizobiaceae* and *g_Rikenella*, and IF mainly affected *s_Lactobacillus johnsonii* and *g_Colidextribacter*. These results highlight the substantial changes in microbial composition following AE and IF interventions, supporting the use of personalized approaches for obesity management and overall health.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

LFY, YSL and YZX contributed to the design and implementation of the research. LFY, YSL, WHH, BZ and YZX contributed to the analysis of the results and to the writing of the manuscript. YSL, WHH and BZ conceived the original and supervised the project. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted under the oversight and with the approval of the Institutional Animal Care and Use Committee of Tsinghua University (ID: THU-LARC-2023-007).

Acknowledgment

Not applicable.

Funding

This work was supported by self-determined research funds of CCNU from the colleges' basic research and operation of MOE (CCNU23XJ033) and the fundamental research funds for the central universities (CCNU24JCPT007).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nature Reviews. Microbiology. 2021; 19: 55–71.

[2] Aron-Wisnewsky J, Warmbrunn MV, Nieuwdorp M, Clément K. Metabolism and Metabolic Disorders and the Microbiome: The Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health-Pathophysiology and Therapeutic Strategies. Gastroenterology. 2021; 160: 573–599.

[3] Carbone S, Del Buono MG, Ozemek C, Lavie CJ. Obesity, risk of diabetes and role of physical activity, exercise training and cardiorespiratory fitness. Progress in Cardiovascular Diseases. 2019; 62: 327–333.

[4] Guo Y, Luo S, Ye Y, Yin S, Fan J, Xia M. Intermittent Fasting Improves Cardiometabolic Risk Factors and Alters Gut Microbiota in Metabolic Syndrome Patients. The Journal of Clinical Endocrinology and Metabolism. 2021; 106: 64–79.

[5] Aragón-Vela J, Solis-Urra P, Ruiz-Ojeda FJ, Álvarez-Mercado AI, Olivares-Arancibia J, Plaza-Diaz J. Impact of Exercise on Gut Microbiota in Obesity. Nutrients. 2021; 13: 3999.

[6] Rong B, Wu Q, Saeed M, Sun C. Gut microbiota-a positive contributor in the process of intermittent fasting-mediated obesity control. Animal Nutrition. 2021; 7: 1283–1295.

[7] Xiong Y, Chen Y, Liu Y, Zhang B. Moderate-Intensity Continuous Training Improves FGF21 and KLB Expression in Obese Mice. Biochemistry. Biokhimiia. 2020; 85: 938–946.

[8] Li G, Xie C, Lu S, Nichols RG, Tian Y, Li L, *et al*. Intermittent Fasting Promotes White Adipose Browning and Decreases Obesity by Shaping the Gut Microbiota. Cell Metabolism. 2017; 26: 672–685.e4.

[9] Shi F, de Fatima Silva F, Liu D, Patel HU, Xu J, Zhang W, *et al*. Salt-inducible kinase inhibition promotes the adipocyte thermogenic program and adipose tissue browning. Molecular Metabolism. 2023; 74: 101753.

[10] Liu W, Sun Z, Ma C, Zhang J, Ma C, Zhao Y, *et al*. Exposure to soil environments during earlier life stages is distinguishable in the gut microbiome of adult mice. Gut Microbes. 2021; 13: 1–13.

[11] Atzeni A, Bastiaanssen TFS, Cryan JF, Tinahones FJ, Vioque J, Corella D, *et al*. Taxonomic and Functional Fecal Microbiota Signatures Associated With Insulin Resistance in Non-Diabetic Subjects With Overweight/Obesity Within the Frame of the PREDIMED-Plus Study. Frontiers in Endocrinology. 2022; 13: 804455.

[12] Yamashita M, Okubo H, Kobuke K, Ohno H, Oki K, Yoneda M, *et al*. Alteration of gut microbiota by a Westernized lifestyle and its correlation with insulin resistance in non-diabetic Japanese men. Journal of Diabetes Investigation. 2019; 10: 1463–1470.

[13] Cui Y, Zhang L, Wang X, Yi Y, Shan Y, Liu B, *et al*. Roles of intestinal Parabacteroides in human health and diseases. FEMS Microbiology Letters. 2022; 369: fnac072.

[14] Lippert K, Kedenko L, Antonielli L, Kedenko I, Gemeier C, Leitner M, *et al*. Gut microbiota dysbiosis associated with glucose metabolism disorders and the metabolic syndrome in older adults. Beneficial Microbes. 2017; 8: 545–556.

[15] Gao S, He Y, Zhang L, Liu L, Qu C, Zheng Z, *et al*. Conjugated linoleic acid ameliorates hepatic steatosis by modulating intestinal permeability and gut microbiota in ob/ob mice. Food & Nutrition Research. 2022; 66.

[16] Yu Z, Yu XF, Kerem G, Ren PG. Perturbation on gut microbiota impedes the onset of obesity in high fat diet-induced mice. Frontiers in Endocrinology. 2022; 13: 795371.

[17] Zhou W, Sailani MR, Contrepois K, Zhou Y, Ahadi S, Leopold SR, *et al*. Longitudinal multi-omics of host-microbe dynamics in prediabetes. Nature. 2019; 569: 663–671.

[18] Ma Y, Zhu L, Ke H, Jiang S, Zeng M. Oyster (Crassostrea gigas) polysaccharide ameliorates obesity in association with modulation of lipid metabolism and gut microbiota in high-fat diet fed mice. International Journal of Biological Macromolecules. 2022; 216: 916–926.

[19] Rodrigues RR, Gurung M, Li Z, García-Jaramillo M, Greer R, Gaulke C, *et al*. Transkingdom interactions between Lactobacilli and hepatic mitochondria attenuate western diet-induced diabetes. Nature Communications. 2021; 12: 101.

[20] Ishii M, Matsumoto Y, Sekimizu K. Bacterial polysaccharides inhibit sucrose-induced hyperglycemia in silkworms. Drug Discoveries & Therapeutics. 2018; 12: 185–188.

[21] Hosomi K, Saito M, Park J, Murakami H, Shibata N, Ando M, *et al*. Oral administration of Blautia wexlerae ameliorates obesity and type 2 diabetes via metabolic remodeling of the gut microbiota. Nature Communications. 2022; 13: 4477.

[22] Wang T, Han J, Dai H, Sun J, Ren J, Wang W, *et al*. Polysaccharides from Lyophyllum decastes reduce obesity by altering gut microbiota and increasing energy expenditure. Carbohydrate Polymers. 2022; 295: 119862.