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Original Article

Exploring the Effects of Water-Soluble Dietary Fiber on Intestinal Flora of Obese Mice Based on 16S rRNA Gene Sequencing and Metabolomics

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Abstract:

Background: Obesity represents a global health challenge, linked to a variety of chronic diseases. The gut microbiota plays a pivotal role in the onset and progression of obesity, and water-soluble dietary fibers are thought to influence obesity by altering the gut microbial composition. This study investigates the effects of water-soluble dietary fiber on the gut microbiota of obese mice.

Methods: Employing 16S rRNA gene sequencing and metabolomics analysis, this research established an obese mouse model and administered a diet enriched with water-soluble dietary fiber. The composition changes in the gut microbiota were analyzed using 16S rRNA gene sequencing techniques. Metabolomic analysis was utilized to assess the impact of water-soluble dietary fiber on metabolic pathways in mice.

Results: The results demonstrated that water-soluble dietary fiber supplementation significantly altered the gut microbiota composition in obese mice, notably increasing the abundance of beneficial bacteria such as Bifidobacteria and Lactobacilli. Metabolomic analysis revealed that water-soluble dietary fiber affected the metabolic activities of the gut microbiota, especially in lipid metabolism and the production of short-chain fatty acids. These alterations were associated with the obesity status of the mice, suggesting that water-soluble dietary fiber may alleviate obesity by modulating the gut microbiota.

Conclusion: This study confirms that water-soluble dietary fiber can effectively regulate the gut microbiota composition in obese mice and positively influence their metabolic pathways. These findings provide scientific evidence for the potential application of water-soluble dietary fiber in obesity treatment and pave the way for future gut microbiota-mediated obesity intervention strategies.

Keywords : Obesity, Gut Microbiota, water-soluble dietary fiber, 16S rRNA Gene Sequencing, Metabolomics.

1. Introduction

Obesity is a major global public health issue, primarily caused by an excess of energy intake over energy expenditure, leading to excessive or abnormal fat accumulation. This condition can trigger or exacerbate a range of related diseases such as Type II diabetes, cardiovascular diseases, non-alcoholic fatty liver, hypertension, and hyperlipidemia, thereby reducing quality of life and increasing mortality risk[1] . With the economic development of countries worldwide and the continuous improvement in living standards, the prevalence of obesity is showing an increasing

trend year by year[2] . The gut microbiome, with its vast array of cells and genes, provides numerous enzymatic systems and metabolic pathways not inherent to the host[3] . It directly or indirectly influences many vital physiological functions, such as digestion and metabolism, intestinal barrier protection, defense against pathogen infection, and regulation of host development and immunity[4] .

Water-soluble dietary fibers, known for their ability to lower glycemic response to foods, can effectively help obese individuals control blood sugar levels through long-term intervention[5] . Increasing research indicates a close relationship between obesity and the composition and function of the gut microbiome[6] . The gut microbiota, as the most complex microbial community in the human body, not only participates in food digestion and nutrient absorption but also affects the host's energy metabolism and immune function[7] . For instance, studies have shown significant differences in the gut microbiota composition between obese individuals and those of normal weight, which may be related to increased efficiency in energy harvesting[8] . Water-soluble dietary fiber, as an important non-digestible food component, plays a crucial role in regulating the gut microbiota. Not digested in the small intestine, water-soluble dietary fibers reach the colon where they are fermented by gut microbes, producing metabolites such as short-chain fatty acids.

These metabolites are believed to confer multiple health benefits to the host, including improving intestinal barrier function, modulating immune responses, and influencing energy metabolism[9] . Therefore, water-soluble dietary fibers, by modulating the composition and activity of the gut microbiota, may hold potential value in preventing and treating obesity and its related diseases[10] . Regarding the regulatory effect of water-soluble dietary fibers on the gut microbiota, existing studies have shown that specific types of water-soluble dietary fibers can promote the growth of beneficial bacterial groups such as Bifidobacteria and Lactobacilli, while inhibiting certain harmful groups[11] . However, the specific mechanisms by which water-soluble dietary fibers modulate the gut microbiota in obese mice are not yet fully understood[12] . Water-soluble dietary fiber, warrant particular attention for their potential regulatory effects on the gut microbiota[13] .Moreover, literature suggests that dietary interventions can directly act on the gut microbiota, mediating the host's physiological metabolism by altering the composition and function of the microbiota, which in turn profoundly affects the dietary intervention's impact on metabolic functions and internal balance[14] .Water-soluble dietary fiber water-soluble dietary fibers can improve diabetes by modulating the gut microbiota. However, the regulatory mechanisms of water-soluble dietary fibers on the gut microbiota in obese mice are not yet clear.

Based on this, our study aims to verify whether water-soluble dietary fiber dietary fibers can positively impact the obesity condition in mice by modulating their gut microbiota composition. We will employ 16S rRNA gene sequencing and metabolomics methods to deeply explore the effects of water-soluble dietary fiber on the gut microbiota of obese mice and their potential mechanisms. This research will not only aid in understanding the mechanisms of dietary fiber in regulating the gut microbiota but also provide new perspectives and strategies for nutritional intervention in obesity.

2.Methods

2.1 Construction of the Obese Mouse Model

Animal modeling and materials: Thirty inbred SPF-grade C57BL/6L mice, aged 3 weeks with a body mass of (17.25 ± 2.56) g, were provided by Hunan SJA Laboratory Animal Co., Ltd. The mice were genetically homogeneous, with stable heredity and uniform responses. They were free of zoonotic pathogens, virulent infectious disease agents, and common infectious pathogens, excluding those that could significantly interfere with this experiment. The mice were kept in excellent living conditions with a relative humidity of 40% and a temperature of 21-23°C, with free access to water and food. The water-soluble dietary fiber (inulin 83.3%, xylo-oligosaccharides 16.7%) of Nutrasumma. The experimental groups included: Control group (normal diet) fed with high-fat diet for 2 months. Non-obese (N) group, showing no significant weight gain after high-fat feeding. Obese (F) group. Water-soluble dietary fiber (FS) group, given 0.1g of water-soluble dietary fiber plus 0.1 mL of saline, gavage for 6 weeks. For the FC group, 0.1 mL of saline per animal, gavage for 6 weeks[15] .Groups of mice were fed either a high-fat purified diet or a regular diet. Mouse feces were collected for 16S rRNA Analysis and Metabolomics.

2.2 16S rRNA Analysis of Gut Microbiota

RNA quantification was performed using a NanoDrop2000 UV-Vis spectrophotometer. The heat block was preheated to 85°C for denaturation. After denaturation, the samples were placed in the heat block for 10 minutes, followed by cooling on ice for 10 minutes. 1μL of 0.1% EB was added to the tissue RNA denaturation system, mixed, and then loaded into the gel wells. 1×MOPS buffer solution was used as the electrophoresis medium, with a voltage of 60V. Electrophoresis was stopped when the bromophenol blue indicator band in each sample reached 2/3 of the gel. Images were captured using the Bio-Rad Gel Doc200 gel imaging system. Roche RNA reverse transcription kit and Gene Amp2400 PCR instrument were used for the reaction. cDNA quantification was also performed using the NanoDrop 2000 UV-Vis spectrophotometer. Universal bacterial primers 27-F-AGAGTTTGATCCTGGCTCAG and 1492-R-GGTTACCTTGTTACGACTT were used for the full-gene amplification of bacterial 16S rRNA. The PCR products of the gut microbiota from each group of mice were ligated into vectors, transformed into E. coli, and positive clones were screened. Recombinant plasmids were extracted to construct a 16S rRNA gene library. The positive clone plasmid DNA was amplified for the 16S rRNA fragment by PCR, and the PCR products were subjected to single enzyme digestion. The digestion products were detected by 4.0% agarose gel electrophoresis. By analyzing the polymorphism of the restriction fragments of each clone obtained by electrophoresis, clones with a similarity of >98% in their restriction patterns were classified into the same operational taxonomic unit (OTU). Thus, the types and numbers of gut microbiota corresponding to each group of mice were statistically analyzed.

2.2.1 OTU Analysis

The original sequencing data were assembled and filtered to obtain valid data. Based on these data, OTUs (Operational Taxonomic Units) clustering, species annotation, and abundance analysis were performed, followed by a T-test to identify differences in community structure between samples. 16S rDNA gut microbiota structure spectrum sequencing analysis was based on the Illumina HiSeq sequencing platform, using the Paired-End method to construct small fragment libraries for sequencing. The original sequencing sequences were filtered and paired-end assembled to obtain optimized sequences (Tags). These sequences were clustered into OTUs, and their species classification was determined based on the composition of OTU sequences.

2.2.2 Alpha and Beta Diversity of Mouse Gut Microbiota

Based on the OTU analysis results, further analyses were conducted, including alpha diversity (Alpha diversity), beta diversity (Beta diversity), and significant species difference analysis, to explore differences between samples. Rarefaction curves, one of the methods in Alpha and Beta diversity analysis, measure whether the data volume of the tested samples is sufficient to reflect the species diversity in the samples. Beta diversity analysis was performed using QIIME software, comparing the degree of similarity in species diversity among different samples. ANOSIM analysis, a type of Beta diversity analysis, calculates the distance of Beta diversity between samples using four different algorithms. Unweighted unifrac and Jaccard are unweighted algorithms, while Weighted unifrac and Bray-Curtis are weighted algorithms. Unweighted algorithms reflect the presence or absence of species, while weighted algorithms consider not only the presence or absence of species but also their abundance. RDP classifier Bayesian algorithm was used for taxonomic analysis of OTUs at 97% similarity level, and the community composition of each sample was statistically analyzed at various classification levels. Each set of experimental data was represented as mean ± standard deviation (x), with $P<0.05$ indicating statistical significance.

2.2.3 Lefse (Linear Discriminant Analysis (LDA) Effect Size) Analysis

Lefse (Linear Discriminant Analysis (LDA) Effect Size) analysis was used to identify statistically significant marker species between different groups, with an LDA score >4 set as the screening criterion. KEGG analysis was completed using PICRUSt software. Primers used for amplification targeted the bacterial 16S rDNA (V3+ V4) region: 338 F: 5' - ACTCCTACGGGAGGCAGCA - 3', 806 R: 5' - GGACTACHVGGGTWTCTAAT - 3'. DNA extraction, PCR amplification, and sequencing were collaboratively completed by Biomarker Technologies Company."

2.2.4 Metabolomics

In the on-site fecal collection method, fresh feces were collected by lifting the mice out of the cage by grasping the end of their tails with one hand. The feces were collected into 1.5 mL Eppendorf tubes. Mice that did not defecate were placed individually in an empty cage for 30 minutes, followed by a second attempt at on-site collection. In all three cases, fecal collection was successful on the second attempt. The collection of feces accumulated over 24 hours was conducted daily between 10:00 and 11:00 AM. Feces were directly collected from the cage floor using small tweezers, pooled by cage, and placed on ice. Once thawed, metabolites from the stored samples were extracted and labeled for LC-MS analysis[16] . For the extraction of fecal metabolites, the feces were solvent-extracted with water and acetonitrile, as previously described. Analysis was performed using an Agilent 1290 UPLC connected to an Agilent electrospray ionization time-of-flight mass spectrometer. The resulting LC-MS data were processed using IsoMS data analysis[17] .

2.2.5 Statistics

Statistical calculations were undertaken utilizing SPSS software (IBM, USA). The measurement data were represented as the definition of mean ± standard deviation. Multiple groups were done through a one-way analysis of variance followed by Tukey's post hoc test. A P-value < 0.05 was considered significant.

3.Results

3.1The Impact of Water-soluble dietary fiber on the Gut Microbiota Species Composition in Obese Mice

At the phylum level, the abundance of microbial communities showed minimal variation across all groups, with Firmicutes being the predominant phylum. However, at the genus level, significant alterations in microbial abundance were observed. Compared to the control group, the Obese (F) group exhibited a marked increase in the abundance of the pathogenic bacterium Helicobacter. In the Non-obese (N) group, which did not exhibit significant weight gain following a high-fat diet, there was an increase in Helicobacter abundance, although not to the extent observed in the Obese (F) group, and an elevation in the abundance of Lachnospiraceae was also noted. In the group fed with water-soluble dietary fiber water-soluble dietary fiber (FS group), a decrease in the abundance of the pathogenic Helicobacter was observed, alongside a significant increase in beneficial genera, including Lachnospiraceae, Lachnospiraceae_NK4A136_group, and Ileibacterium (Figure 1). These findings indicate that a high-fat diet can enhance the abundance of harmful bacteria in the gut microbiota of mice, albeit with individual variations. Mice that did not become obese despite a high-fat diet exhibited some degree of regulation over their gut microbiota. Feeding water-soluble dietary fiber reduced the abundance of harmful bacteria and increased that of beneficial bacteria in obese mice. Notably, the abundance of Lachnospiraceae was high in both groups, suggesting that Lachnospiraceae may play a key role in the regulation of obesity.

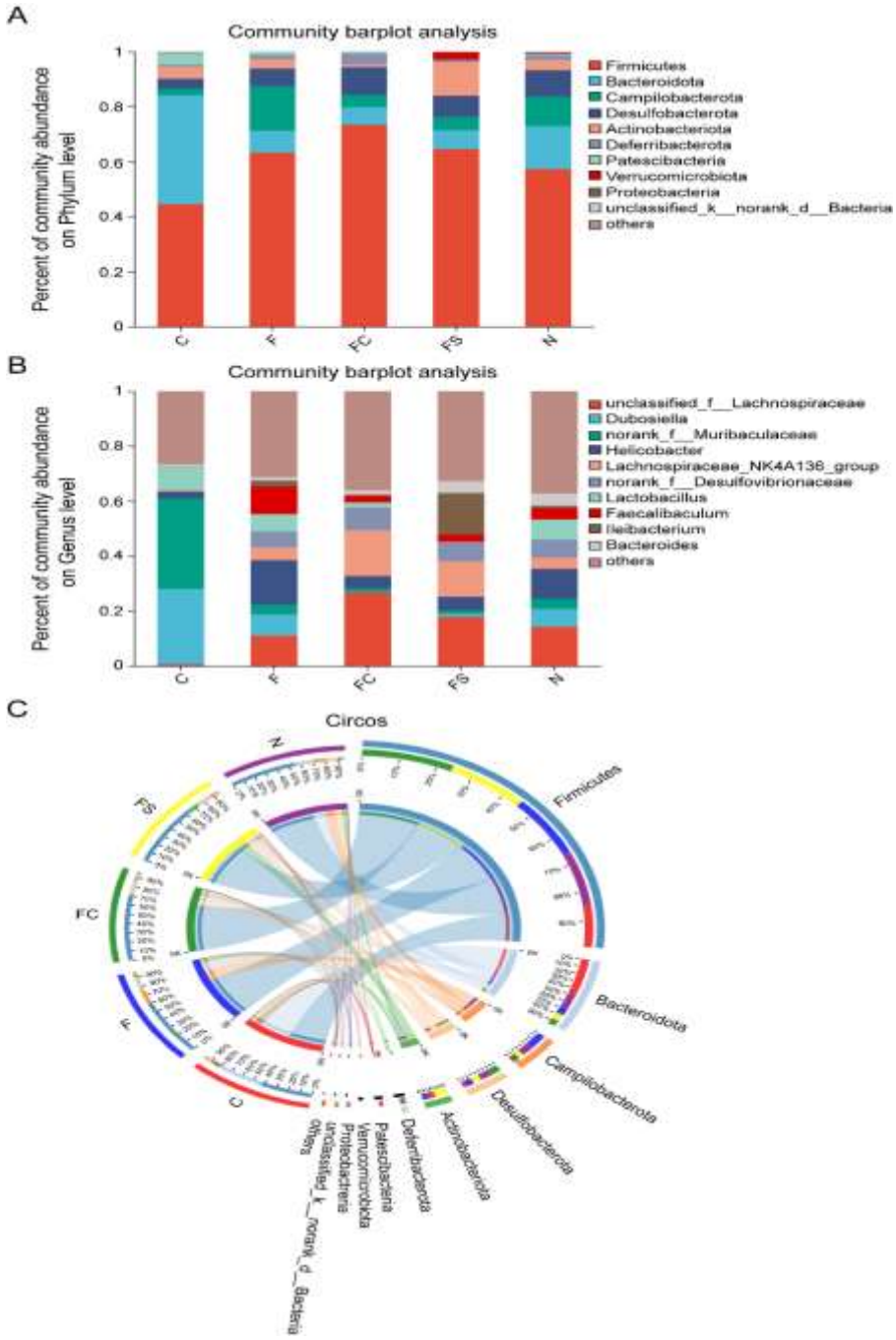

Figure 1. The Impact of water-soluble dietary fiber on the Gut Microbiota Species Composition in Obese Mice (A-B). Relative abundance statistics of microbial groups in each group (A: at the phylum level; B: at the genus level). These bar graphs represent the relative abundance of species at different taxonomic levels. The larger the proportion of a color/category in the graph, the higher the richness; (C). Genus-level phylogenetic tree, starting from the central point, each branch represents an evolutionary event, with species further from the center being more closely related in evolutionary terms.

3.2The Effect of Water-soluble dietary fiber on the Diversity of Gut Microbiota in Obese Mice

Subsequently, we compared the microbial diversity between the two groups and found that when compared to the control group, both the Non-obese (N) group, which did not exhibit significant weight gain after a high-fat diet, and the Obese (F) group showed reduced levels of Chao1, ACE, Simpson, and Shannon indices, with the reduction being more pronounced in the Obese (F) group. In contrast, the group fed with water-soluble dietary fiber (FS

group) demonstrated an increase in microbial α-diversity indices (Figure 2. A, B, C, D). Principal Coordinates Analysis (PCoA) and Metric Multidimensional Scaling (MMDS) revealed that the composition of the gut microbiota in the Non-obese (N) and Obese (F) groups was not significantly different. However, a notable alteration in the gut microbiota composition was observed in obese mice after being fed water-soluble dietary fiber (Figure 2. E, F). These results indicate that a high-fat diet can disrupt the α-diversity of the microbiota, but this disruption can be ameliorated by feeding water-soluble dietary fiber, suggesting its potential in rectifying gut microbiota dysbiosis in obese mice.

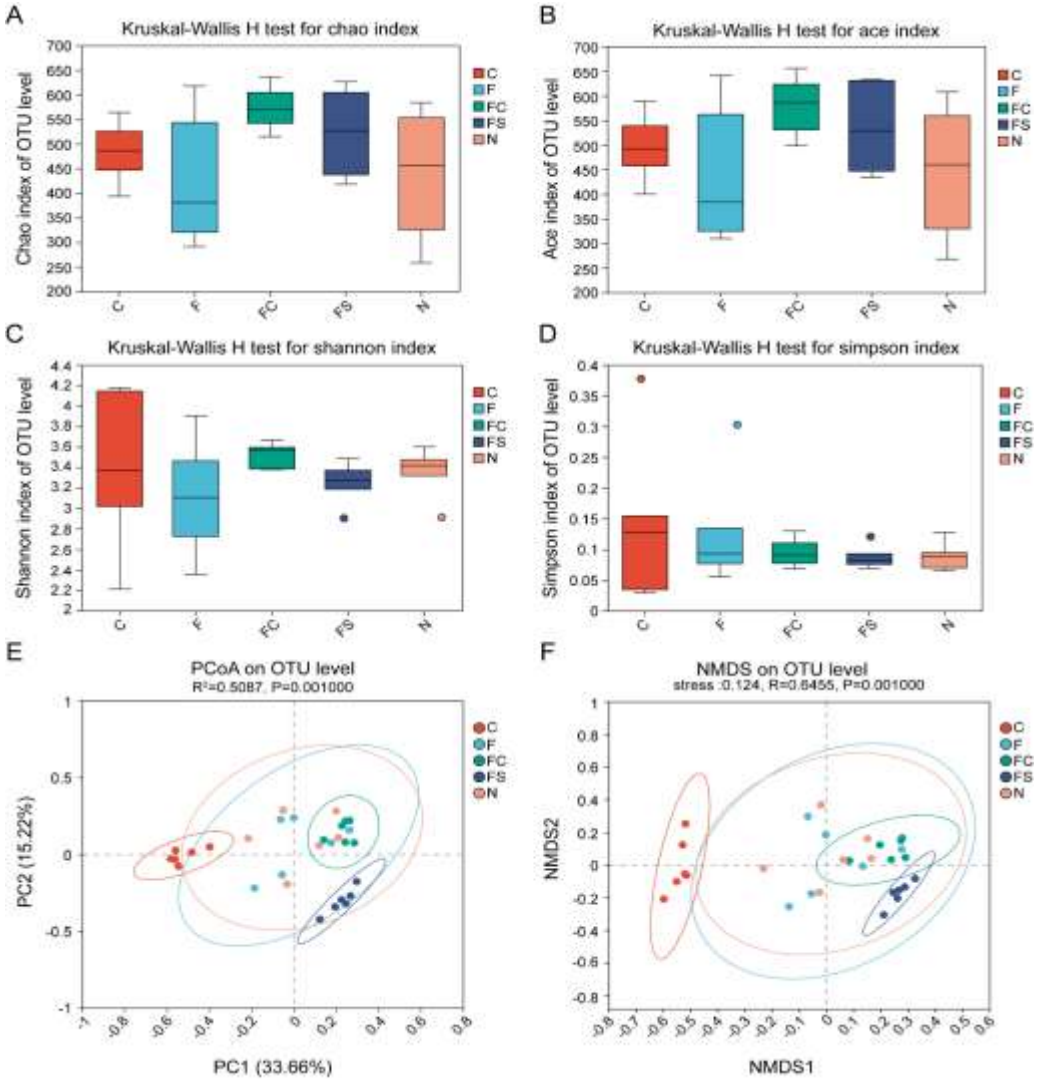

Figure 2. The Effect of water-soluble dietary fiber on the Diversity of Gut Microbiota in Obese Mice (A-D). Microbial α-diversity indices statistics among groups (A: Chao1; B: ACE; C: Shannon; D: Simpson) (E). β-diversity of gut microbiota among groups based on PCoA analysis (left: based on Weighted Unifrac distance; right: based on Unweighted Unifrac distance); (F). β-diversity of gut microbiota among groups based on MMDS analysis (left: based on Weighted Unifrac distance; right: based on Unweighted Unifrac distance).

3.3Water-soluble dietary fiber's Influence on Gut Microbiota Biomarkers and Functional Analysis in Obese Mice

Following this, further analysis of the dominant bacterial groups revealed that LEfSe analysis (LDA >4) revealed that at the genus level, the dominant species within the gut microbiota of the five groups predominantly comprised Lactobacillus, *Faecalibaculum*, *Lachnospiraceae*, *Ileibacterium*, and *Bacteroides* (Figure 3). Compared to the

control group, both the Non-obese (N) group and the Obese (F) group exhibited a notable reduction in the diversity and abundance of dominant bacterial species. This reduction was more pronounced in the Obese (F) group than in the Non-obese (N) group. In the water-soluble dietary fiber group (FS), there was a significant rebound in the diversity and abundance of dominant bacterial species. Notably, the Non-obese (N) group showed the highest abundance of Bacteroides, while the water-soluble dietary fiber group (FS) exhibited the highest abundance of *Ileibacterium*, indicating distinct dominant bacterial species in these two groups. These results suggest the presence of individual variations among mice, with those not gaining significant weight after a high-fat diet being able to regulate their gut microbiota differently from the effects observed post water-soluble dietary fiber intervention. Bacteroides plays a significant role in the gut microbiota of mice resistant to obesity, while *Ileibacterium* may play a crucial role in the improvement of gut microbiota in obese mice following water-soluble dietary fiber supplementation.

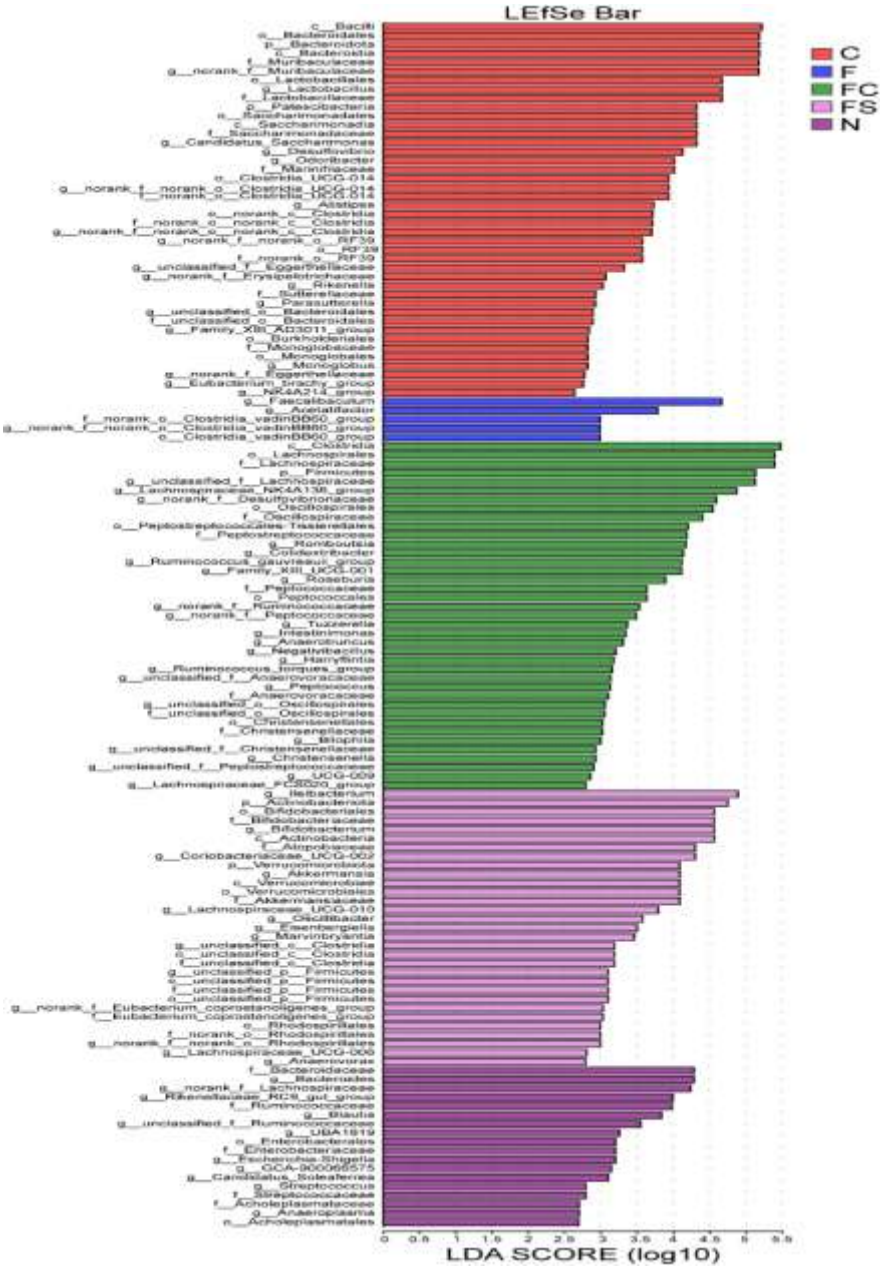

Figure 3. Water-soluble dietary fiber 's Influence on Gut Microbiota Biomarkers and Functional Analysis in Obese Mice Microbial biomarkers selected based on LEfSe, Anosim validation of community differences between groups, T-test analysis of species differences between groups (at the genus level), microbial biomarkers selected based on LEfSe.

3.4The Effect of Water-soluble dietary fiber on Differential Fecal Metabolites in Obese Mice

In comparing the metabolomic profiles between the two groups, we observed significant differences in fecal metabolome composition between the Non-obese (N) group and the Obese (F) group, in comparison to the control group (C). Despite certain similarities between these two groups, the differences were more pronounced. Additionally, the water-soluble dietary fiber group (FS) displayed distinct differences in comparison to the Physiological saline group (FC) (Figure 4.A). This indicates that non-obese mice, despite not gaining significant weight post high-fat diet, can regulate their overall metabolism to a certain extent, and water-soluble dietary fiber has a significant impact on the overall metabolism of obese mice. Compared to the control group (C), both the Non-obese (N) and Obese (F) groups showed substantial changes in fecal metabolites, with marked differences between them. Similarly, there were significant differences in fecal metabolites between the Physiological saline group (FC) and the water-soluble dietary fiber group (FS) (Figure 4.B). Supplementation with water-soluble dietary fiber led to an increase in the metabolism of compounds such as 6-keto PGE1, 20-carboxy-LTB4, 20-Carboxy-leukotriene B4, Ganoderic acid J, 16-alpha-Hydroxyandrosterone, N-Acetyllactosamine, and a decrease in the metabolism of Lacto-N-biose I, Butralin, Urobilin, Taurocholic acid 3-sulfate, Guanine. These findings demonstrate that both the inherent metabolic regulation in non-obese mice following a high-fat diet and the supplementation with water-soluble dietary fiber can effectively reverse the metabolic dysregulation induced by a high-fat diet in obese mice.

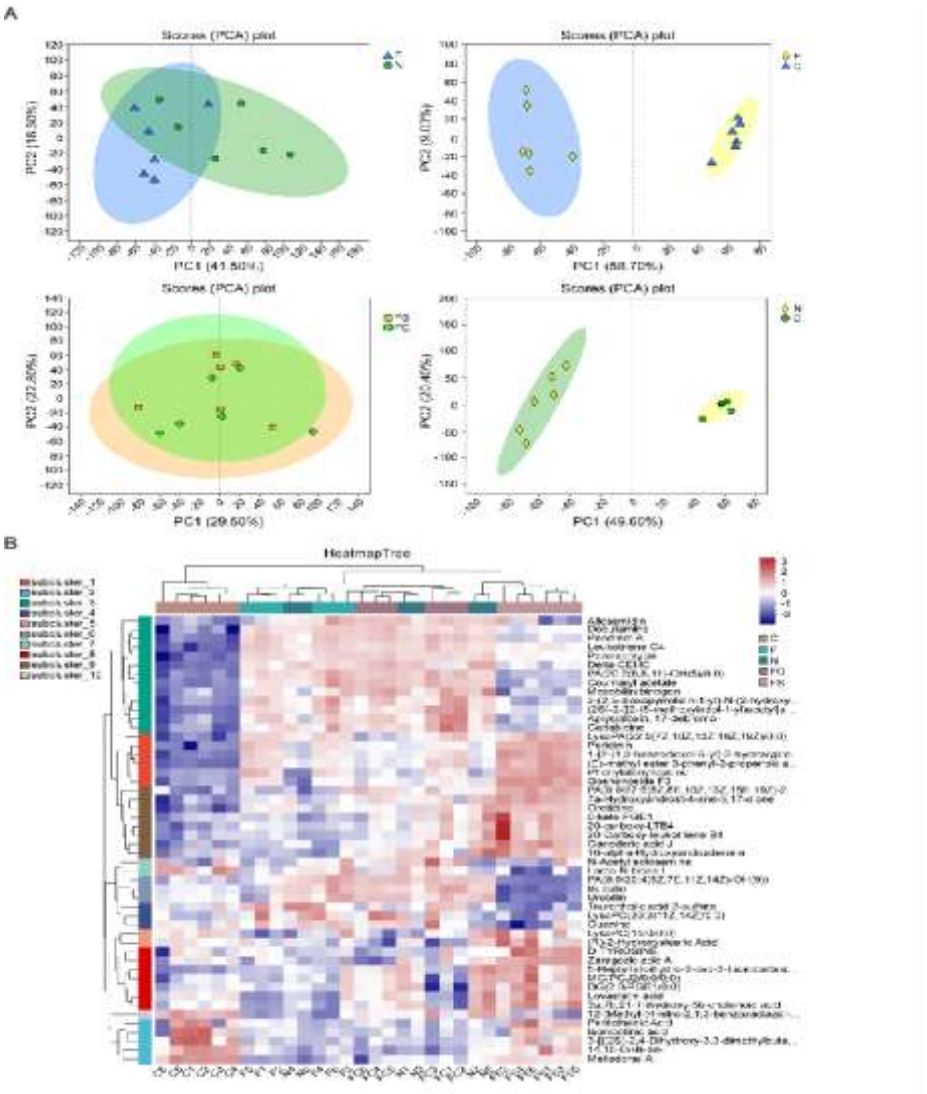

Figure 4. The Effect of water-soluble dietary fiber on Differential Fecal Metabolites in Obese Mice (A). Principal component analysis (PCA) scoring of fecal metabolomic characteristics in each group,

(B). clustering analysis of differential metabolites, with red indicating high levels and blue indicating low levels.

3.5Analysis of Differential Metabolic Pathways in Obese Mice Influenced by Water-soluble dietary fiber

Comparative analysis of the metabolomic KEGG pathways between the groups revealed distinct differences. Compared to the control group (C), the Obese (F) group showed an enrichment of differential metabolites in pathways such as glycerophospholipid metabolism, the biosynthesis of propyl, piperidine, and pyridine alkaloids, lysine degradation, and histidine metabolism. In contrast, the Non-obese (N) group, which did not exhibit significant weight gain after a high-fat diet, showed an enrichment in different pathways including protein digestion and absorption, sphingolipid signaling, arachidonic acid metabolism, and the biosynthesis of plant secondary metabolites, compared to the Obese (F) group. Furthermore, compared to the Physiological saline group (FC), the water-soluble dietary fiber group (FS) demonstrated an enrichment in the biosynthesis of propyl, piperidine, and pyridine alkaloids, carbon metabolism, arachidonic acid metabolism, and glycerophospholipid metabolism (Figure 5). The metabolic pathways enriched in each group were distinct, indicating that non-obese mice post high-fat diet can regulate metabolic imbalances, and supplementation with water-soluble dietary fiber can reverse metabolic pathway dysregulation caused by obesity. The similarities in the metabolic regulation pathways between these two groups suggest that the mechanisms underlying their ameliorative effects on obesity may be similar.

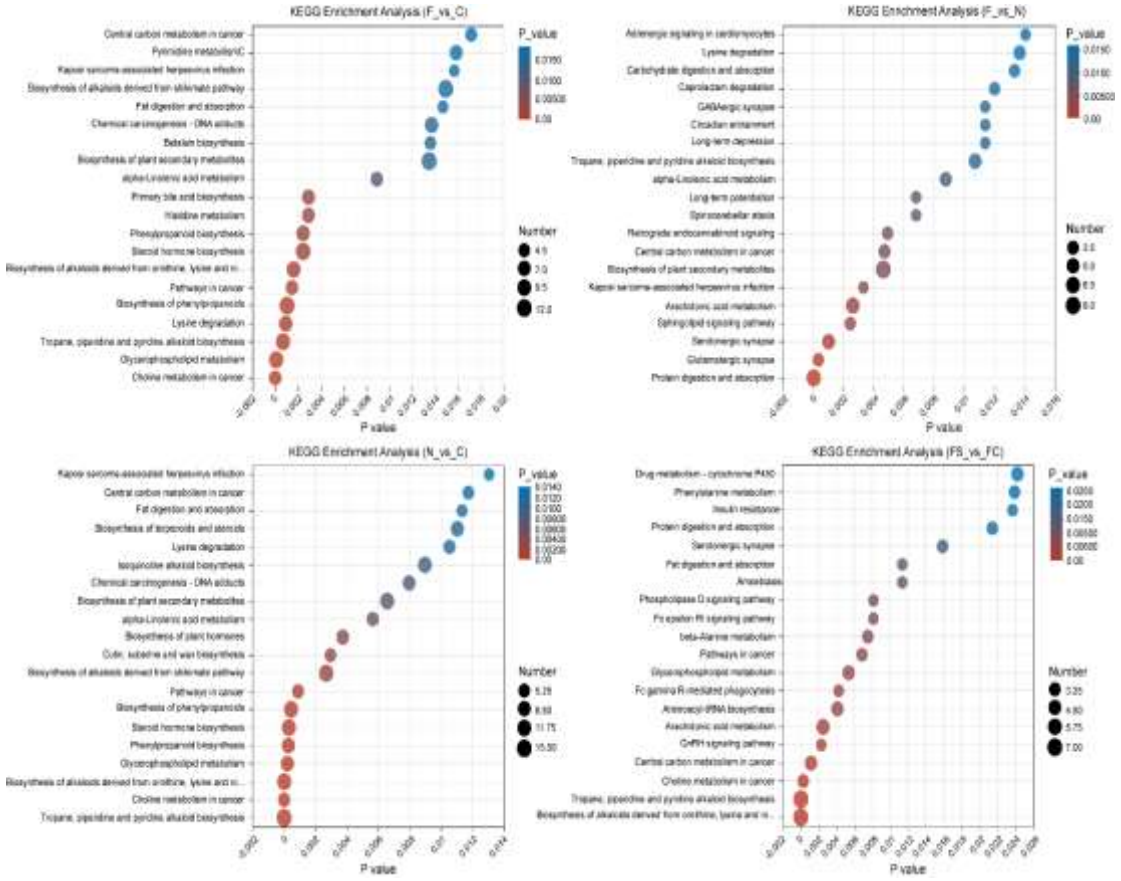

Figure 5. Analysis of Differential Metabolic Pathways in Obese Mice Influenced by watersoluble dietary f iber

Functional pathways of differential metabolites predict ed by the KEGG database, with smaller p values indicating greater differences.

3.6Joint Analysis of Gut Microbiota and Metabolomics in Obese Mice Affected by Water-soluble dietary fiber

In our final analysis, using heatmaps for joint

analysis of the gut microbiota and metabolome, we observed distinct correlations in the Non-obese (N) group, which did not show significant weight gain after a high-fat diet, compared to the control group (C). Specifically, Erysipelotrichaceae showed a strong positive correlation with PE (24:0/22:0), N-Nervonoyl Glutamine, and Dihydro-3-coumaric acid, and a strong negative correlation with Phenylpyruvic acid, Oxyglutinosone, Valnemulin, and Tryptophyl-Gamma-glutamate. In contrast, in the water-soluble dietary fiber group (FS) compared to the Physiological saline group (FC), Lachnospiraceae exhibited a strong positive correlation with Aplysiatoxin, 17-debromoCadabicine, Taurocholic acid 3-sulfate, and (2S)-2-[[2-(5-methoxyindol-1-yl) acetyl] amino]-4-methylpentanoic acid. Ileibacterium showed a strong positive correlation with Phenylalanylleucine and a strong negative correlation with Penitrem A, Butralin, and PA (20:3(6,8,11)-OH(5)/8:0) (Figure 6). These results indicate that both the Non-obese (N) group and the water-soluble dietary fiber group (FS) experienced changes in the structure/composition of their gut microbiota, significantly altering the fecal metabolic phenotype, albeit in different ways.

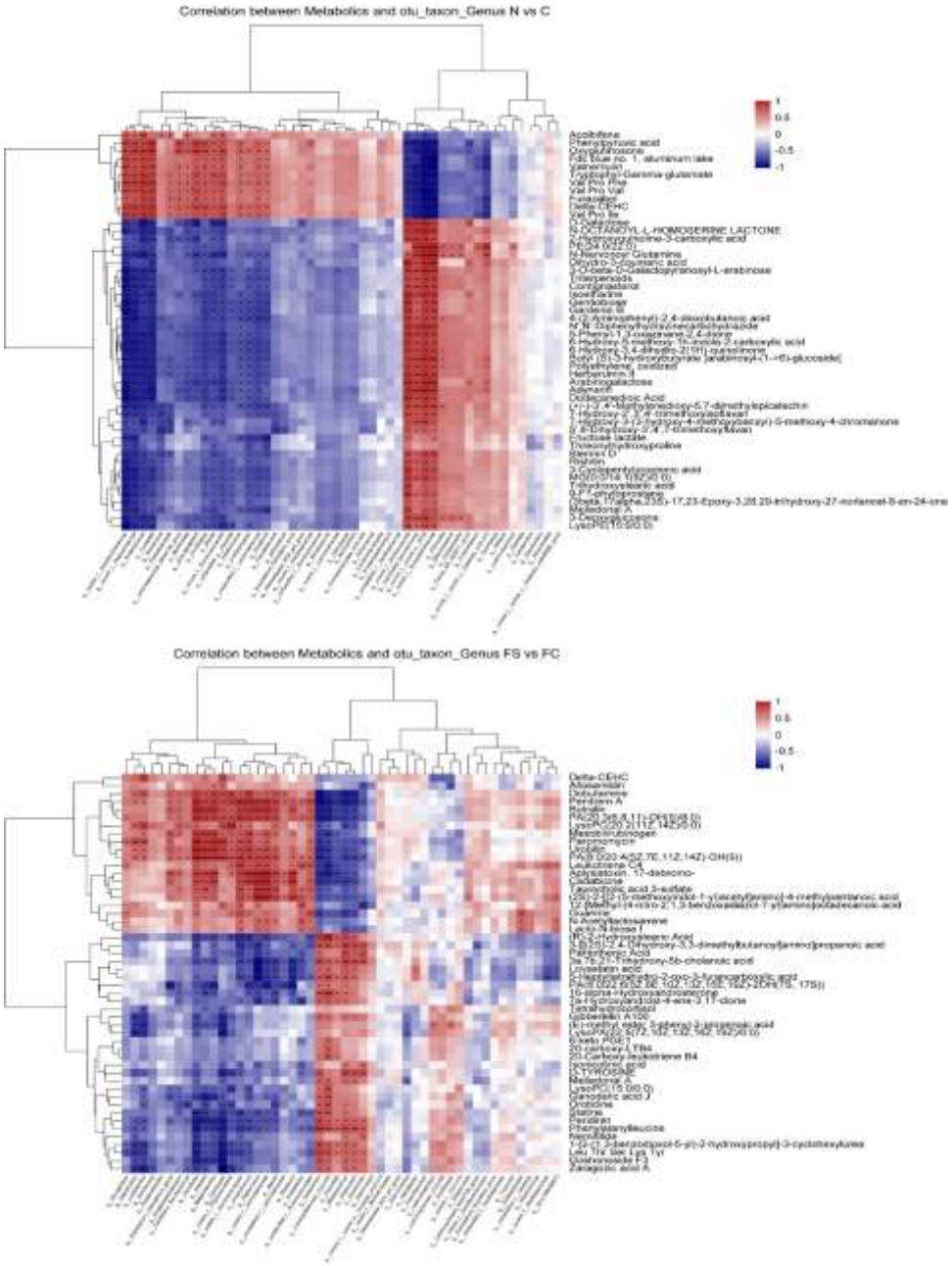

Figure 6. Joint Analysis of Gut Microbiota and Metabolomics in Obese Mice Affected by water-soluble dietary fiber Correlations between disturbed gut microbiota genera and altered fecal metabolites in each group, with red indicating high correlation and blue indicating low correlation.

4. Discussion

Obesity is a global public health issue closely linked to various chronic diseases. In recent years, the gut microbiota has been recognized as a significant factor influencing the host's energy collection and storage[18] . Additionally, water-soluble dietary fibers have shown potential in modulating the structure and function of the gut microbiota[19] . Oligosaccharides, a type of water-soluble dietary fiber, may combat obesity by altering the composition of the gut microbiota[20] .

This study investigated the impact of water-soluble dietary fiber on the gut microbiota of obese mice through feeding trials, utilizing 16S rRNA gene sequencing and metabolomics approaches. It was found that water-soluble dietary fiber significantly altered the gut microbiota composition in obese mice, increasing the abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while reducing certain harmful bacteria associated with obesity. Moreover, water-soluble dietary fiber altered the metabolic activities of the gut microbiota, affecting pathways related to energy and lipid metabolism. These changes contribute to alleviating obesity and improving metabolic health. The relationship between gut microbiota and obesity has been extensively studied. Research indicates that the gut microbial composition is closely related to the host's energy metabolism, fat storage, and inflammatory responses[21] . Particularly, the ratio of *Firmicutes* to *Bacteroidetes* has been associated with obesity[22] .

In this study, Firmicutes were the most abundant in all mouse groups, consistent with findings[23] .which reported an increased proportion of Firmicutes in obese individuals. For mice that did not gain significant weight after a high-fat diet, this might indicate individual differences in gut microbiota's role in energy intake and storage[24] . Some studies have shown that certain gut microbiota can affect the host's energy balance, thereby influencing weight[25] . In our experiment, the Non-obese (N) and Obese (F) groups showed an increase in *Helicobacter* abundance, though less so in the Non-obese group. This suggests a potential role of Helicobacter in obesity development, but its exact mechanism requires further investigation. The impact of water-soluble dietary fiber on gut microbiota is also noteworthy. Water-soluble dietary fibers are considered important factors in modulating gut microbiota composition, promoting the growth of beneficial bacteria such as *Lachnospiraceae[26]* .

In our study, feeding water-soluble dietary fiber reduced the abundance of harmful Helicobacter and increased beneficial genera, aligning with the findings that water-soluble dietary fiber can improve gut microbiota composition and promote health[27] . Regarding the similarities and differences in gut microbiota between the Non-obese group and the water-soluble dietary fiber group, our results indicate that both groups had a higher abundance of Lachnospiraceae. This might suggest a significant role of Lachnospiraceae in maintaining gut health and preventing obesity. However, the main difference between the two groups was in the abundance of Ileibacterium, which was more pronounced in the water-soluble dietary fiber group. This indicates that water-soluble dietary fibers may exert their effects through specific changes in microbiota. In summary, the composition and diversity of gut microbiota are closely related to obesity, and water-soluble dietary fibers can significantly improve the health status of the gut microbiota. These findings provide a basis for further research into the complex relationship between gut microbiota and obesity and potential strategies for developing gut microbiota-based obesity treatments. The link between differential metabolites and metabolic pathways of gut microbiota and obesity is a complex and evolving field of research. Recent studies suggest that gut microbiota, through their metabolic products, directly influence the host's energy balance and metabolic health, playing a key role in the development of obesity[28] . For instance, short-chain fatty acids (SCFAs), produced by the fermentation of water-soluble dietary fibers by gut microbiota, have been shown to regulate adipogenesis and inflammation in adipose tissue, thereby influencing obesity development[29] .

In our study, the Obese (F) group and the Non-obese (N) group displayed significant differences in fecal metabolites, potentially reflecting metabolic adjustments of gut microbiota under a high-fat diet. Particularly, supplementation with water-soluble dietary fiber (FS group) appeared to alter these metabolites, suggesting that water-soluble dietary fibers can influence metabolic health by altering the composition and function of gut microbiota[30] .

When analyzing differential metabolic pathways, the differences observed in the Obese mice in pathways such as glycerophospholipid metabolism might be related to lipid metabolism dysregulation and energy imbalance[31] . In contrast, the enrichment of differential metabolites in pathways like protein digestion and absorption in the Non-obese group (N) may reflect a more efficient energy utilization mode[21] . These findings align with recent studies suggesting that gut microbiota can influence the host's energy metabolism through various metabolic pathways[22] . Furthermore, joint analysis of gut microbiota and metabolomics data can provide a more comprehensive understanding of obesity mechanisms. For example, the strong positive correlation between *Erysipelotrichaceae* and specific metabolites may indicate a key role of this group in gut metabolism[32] . The correlation of *Ileibacterium* with specific metabolites may reveal the specific impact of water-soluble dietary fiber on gut microbiota[26] .

These findings emphasize the multifaceted role of gut microbiota in the development of obesity and potential strategies for treating or preventing obesity by modulating gut microbiota. In conclusion, the metabolic activities of gut microbiota are closely related to the development of obesity. High-fat diets and water-soluble dietary fiber significantly impact gut microbiota and metabolism, potentially regulating obesity through changes in metabolic pathways and microbiota composition. These findings provide important clues for further research into the relationship between gut microbiota and obesity and offer new perspectives for developing gut microbiota-based obesity treatment strategies.

5. Conclusions

This study, through 16S rRNA gene sequencing and metabolomics analysis, explored the impact of water-soluble dietary fiber on the gut microbiota of obese mice. The results showed that water-soluble dietary fiber significantly altered the gut microbiota composition of obese mice, increased the abundance of beneficial bacteria, improved metabolic pathways, and contributed to alleviating obesity and improving metabolic health. These findings reveal the potential value of water-soluble dietary fiber as a dietary intervention in improving obesity and metabolic disorders, providing new insights into nutritional treatment for obesity.

microbiota-based obesity treatment strategies.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Authors' contributions

Jianfeng Long: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. Linmim Deng: Software, Methodology, Funding acquisition, Conceptualization. Qijun He: Data curation, Resources, Software. Qiran Zhou: Data curation, Project administration, Resources, Software. Shijie He:Methodology,Resources, Software.Shiping Liu: Writing – review & editing, Supervision, Funding acquisition, Validation.Kang Zhou:Writing – review & editing, Supervision, Funding acquisition, Visualization, Validation.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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References

1. EJTAHED H S, ANGOORANI P, SOROUSH A R, et al. Gut microbiota-derived metabolites in obesity: a systematic review[J]. Biosci Microbiota Food Health. 2020,39(3):65-76. https://doi.org/10.12938/bmfh.2 019-026.
2. LYNCH S V, PEDERSEN O. The Human Intestinal Microbiome in Health and Disease[J]. N Engl J Med. 2016,375(24):2369-2379. https://doi.org/10.1056/NEJMra1600266.
3. GUPTA A, OSADCHIY V, MAYER E A. Brain-gut-microbiome interactions in obesity and food addiction[J]. Nat Rev Gastroenterol Hepatol. 2020,17(11):655-672. https://doi.org/10.1038/s41575-020-0341-5.
4. LEIGH S J, MORRIS M J. Diet, inflammation and the gut microbiome: Mechanisms for obesity-associated cognitive impairment[J]. Biochim Biophys Acta Mol Basis Dis. 2020,1866(6):165767. https://doi.org/10.1016/j.

bbadis.2020.165767.
5. LEE C J, SEARS C L, MARUTHUR N. Gut microbiome and its role in obesity and insulin resistance[J]. Ann N Y Acad Sci. 2020,1461 (1): 37-52. https://doi.org/10.1111/nyas.14107.
6. LONG J, JIEPINGHENNING, SUSANNE M.WOO, et al. Xylooligosaccharide supplementation decreases visceral fat accumulation and modulates cecum microbiome in mice[J]. Journal of Functional Foods. 20 19,52.
7. ZHANG Z, LIN T, MENG Y, et al. FOS/GOS attenuates high-fat diet induced bone loss via reversing microbiota dysbiosis, high intestinal permeability and systemic inflammation in mice[J]. Metabolism. 2021,119(154767. https:// doi.org/10.1016/j.metabol.2021.154767.
8. QIN Y, ROBERTS J D, GRIMM S A, et al. An obesity-associated gut microbiome reprograms the intestinal epigenome and leads to altered colonic gene expression[J]. Genome Biol. 2018,19(1):7. https://doi.org/10.1186/s13059-01 8-1389-1.
9. SINGER-ENGLAR T, BARLOW G, MATHUR R. Obesity, diabetes, and the gut microbiome: an updated review[J]. Expert Rev Gastroenterol Hepatol. 2019,13(1):3-15. https://doi.org/10.10 80/1747 4124.2019.1543023.
10. ZHI C, HUANG J, WANG J, et al. Connection between gut microbiome and the development of obesity[J]. Eur J Clin Microbiol Infect Dis. 2019,38(11):1987-1998. https://doi.org/10.1007 /s10096-019-03623-x.
11. CHEN X, DEVARAJ S. Gut Microbiome in Obesity, Metabolic Syndrome, and Diabetes[J]. Curr Diab Rep. 2018,18(12):129. https://doi. org/10.1007/s11892-018-1104-3.
12. LEONG K S W, DERRAIK J G B, HOFMAN P L, CUTFIELD W S. Antibiotics, gut microbiome and obesity[J]. Clin Endocrinol (Oxf). 2018,88(2):185-200. https://doi.org/10.1 111/cen.13495.
13. AOUN A, DARWISH F, HAMOD N. The Influence of the Gut Microbiome on Obesity in Adults and the Role of Probiotics, Prebiotics, and Synbiotics for Weight Loss[J]. Prev Nutr Food Sci. 2020,25(2):113-123. https://doi.org/ 10.3746/pnf.2020.25.2.113.
14. SERGEEV I N, ALJUTAILY T, WALTON G, HUARTE E. Effects of Synbiotic Supplement on Human Gut Microbiota, Body Composition and Weight Loss in Obesity[J]. Nutrients. 202 0,12(1). https://doi.org/10.3390/nu12010222.
15. ALPERS C E, HUDKINS K L. Mouse models of diabetic nephropathy[J]. Curr Opin Nephrol Hypertens. 2011,20(3):278-284. https://doi.org/ 10.1097/MNH.0b013e3283451901.
16. TONG L, FENG Q, LU Q, et al. Combined (1)H NMR fecal metabolomics and 16S rRNA gene sequencing to reveal the protective effects of Gushudan on kidney-yang-deficiency-syndrome rats via gut-kidney axis[J]. J Pharm Biomed Anal. 2022,217:114843. https://doi.org/ 10.1016/j.jpba.2022.114843.
17. HE F, ZHAI J, ZHANG L, et al. Variations in gut microbiota and fecal metabolic phenotype associated with Fenbendazole and Ivermectin Tablets by 16S rRNA gene sequencing and LC/MS-based metabolomics in Amur tiger[J]. Biochem Biophys Res Commun. 2018,499(3): 447-453. https://doi.org/10.1016/j.bbrc.2018.0 3.158.
18. CAI Z, YANG Y, ZHANG J. Obesity is associated with severe disease and mortality in patients with coronavirus disease 2019 (COVID-19): a meta-analysis[J]. BMC Public Health. 2021,21(1):1505. https://doi.org/10.118 6/s12889-021-11546-6.
19. FAN Y, PEDERSEN O. Gut microbiota in human metabolic health and disease[J]. Nat Rev Microbiol. 2021,19(1):55-71. https://doi.org/10. 1038/s41579-020-0433-9.
20. MYHRSTAD M C W, TUNSJø H, CHARNOCK C, TELLE-HANSEN V H. Dietary Fiber, Gut Microbiota, and Metabolic Regulation-Current Status in Human Randomized Trials[J]. Nutrients. 2020,12(3) https://doi.org/10.3390/nu12030859.
21. LEY R E, TURNBAUGH P J, KLEIN S, GORDON J I. Microbial ecology: human gut microbes associated with obesity[J]. Nature. 20 06,444(7122):1022-1023. https://doi.org/10.10 38/4441022a.
22. TURNBAUGH P J, HAMADY M, YATSUNENKO T, et al. A core gut microbiome in obese and lean twins[J]. Nature. 2009, 457 (7228):480-484. https://doi.org/10.1038/nature 07540.
23. TURNBAUGH P J, LEY R E, MAHOWALD M A, et al. An obesity-associated gut microbiome with increased capacity for energy harvest[J]. Nature. 2006,444(7122):1027-1031. https://doi.org/10.1038/ nature05414.
24. MURPHY E F, COTTER P D, HEALY S, et al.

Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models[J]. Gut. 2010, 59 (12):1635-1642. https://doi.org/10.1136/gut.2010.215665.

25. ZIĘTEK M, CELEWICZ Z, SZCZUKO M. Short-Chain Fatty Acids, Maternal Microbiota and Metabolism in Pregnancy[J]. Nutrients. 2021,13(4). https://doi.org/10.3390/nu13041244.
26. FLINT H J, SCOTT K P, DUNCAN S H, et al. Microbial degradation of complex carbohydrates in the gut[J]. Gut Microbes. 2012,3(4):289-306. https://doi.org/10.4161/gmic.19897.
27. SLAVIN J. Fiber and prebiotics: mechanisms and health benefits[J]. Nutrients. 2013,5(4):1417-1435. https://doi.org/10.3390/nu5041417.
28. SONNENBURG J L, BäCKHED F. Diet-microbiota interactions as moderators of human metabolism[J]. Nature. 2016,535(7610):56-64. https: //doi.org/10.1038/nature18846.
29. DELZENNE N M, OLIVARES M, NEYRINCK A M, et al. Nutritional interest of dietary fiber and prebiotics in obesity: Lessons from the MyNewGut consortium[J]. Clin Nutr. 2020,39(2):414-424. https: //doi.org/10.1016/j.clnu.2019.03.002.
30. DAVID L A, MAURICE C F, CARMODY R N, et al. Diet rapidly and reproducibly alters the human gut microbiome[J]. Nature. 2014,505 (7484):559-563. https://doi.org/10.1038/nature12820.
31. HERNANDEZ J, RHIMI S, KRIAA A, et al. Domestic Environment and Gut Microbiota: Lessons from Pet Dogs[J]. Microorganisms. 2022,10(5). https://doi.org/10.3390/microorganisms10050949.
32. RIDAURA V K, FAITH J J, REY F E, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice[J]. Science. 2013, 341(6150):1241214. https://doi.org/10.1126/science.1241214.