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Research Article

Antiobesity Effect of *Lactiplantibacillus plantarum* Fermented Barley Extracts via the Interactions with Gut Microbiota of the Obese Adult Humans

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Extracts of fermented barley with *Lactiplantibacillus plantarum*dy-1 (LFBE) have been reported to exert antiobesity activity *in vivo* and *in vitro*. However, whether it worked in clinical trials remained uncovered. In the current study, we conducted a single-blind experiment by enrolling on some obese adult humans with hyperlipemia to test the effects of products containing LFBE (No. ChiCTR1800019614 by the Chinese Clinical Trial Registry). Results indicated that LFBE intervention could ameliorate the symptoms of the obese, characterized by the decrease of body fat percentage, visceral fat area, and serum lipid levels. More interestingly, products containing the β-glucan ingredients had similar antiobesity effects. 16S rRNA sequencing revealed that LFBE modulated the community structure of the gut microbiota of the obese adults as shown by the diversity analysis. For gut microbiota composition, LFBE significantly increased the *Bacteroides* genus while decreasing *Streptococcus* and *Haemophilus* genus. At the species level, beneficial microorganisms such as *Bifidobacterium_longum*_subsp_*longum*, *Alistipes*_sp_AL-1, *Bacteroides_plebeius*, and *Bacteroides_vulgatus* were enriched by LFBE, which was different from the effects of β-glucan. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis indicated that LFBE particularly regulated the steroid biosynthesis and hypertrophic cardiomyopathy pathways. Furthermore, correlation analysis suggested that body fat percentage and visceral fat area were significantly positively correlated with *Desulfovibrio* and *Sutterella*, the basal metabolic rate was negatively correlated with *Haemophilus*, and fasting blood glucose was positively correlated with *Dialister*. In all, this clinical study demonstrated the antiobesity function of LFBE in humans, probably via its interactions with the gut microbiota, and LFBE could be developed as a prebiotic ingredient with antiobesity effects for a healthy diet. This trial is registered with ChiCTR1800019614.

1. Introduction

Hyperlipidemia (HLP) mainly refers to the elevated serum total cholesterol (TC) and total triglycerides (TG) levels caused by abnormal lipid metabolism in humans [1]. HLP may induce multiple serious diseases, such as atherosclerosis. Normally, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) highly associated with atherosclerosis are added to the monitoring indicators in clinical diagnosis. In addition, HLP is often referred to as "dyslipidemia." Most obese patients are complicated by hyperlipidemia [2].

As one of the staple grains in the world, barley is rich in dietary fiber, polyphenols, gamma-aminobutyric acid, and other bioactive components, which can regulate human sugar and lipid metabolism [3]. Especially the fermentation

product of the barley by lactic acid bacteria with a series of changes in its components, such as the increased contents of bioactive polyphenols and proteins, can regulate the lipid metabolism. Previous studies carried out by our group have demonstrated that extracts of fermented barley with *Lactiplantibacillus plantarum*dy-1 (LFBE) could reduce the accumulation of lipid droplets in nematodes and cell lines [4] and exhibited a significant hypolipidemic effect in animal experiments [5]. However, HLP in human has complicated pathogenesis and is associated with obesity, diet, exercise habits, age, hormone levels, and even genetic inheritance, which have been manifested as metabolic abnormalities in the body. Therefore, multidirectional and multiindex analysis is also required for the study of HLP.

A lot of evidence suggests that the changes in the gut microbiota play an important role in the pathogenesis of obesity [6]. The gut microbiota in obese patients and normal people is different. Furthermore, the microbiome in obese patients is more capable of obtaining energy from the diet [7]. Consistent with the results of animal studies, the changes in the gut microbiota in obese patients are associated with the abundance of the *Firmicutes* phylum, *Bacteroidetes* phylum, and *Prevotella* genus [8]. The data from clinical studies showed that diet was one of the decisive factors for the composition and function of intestinal bacteria, and 57% of the changes of intestinal bacteria were caused by dietary changes [9]. Therefore, changing the composition and function of the gut microbiota through diet intervention is a safe and effective strategy to prevent obesity [10]. For instance, compared with infants without prebiotics, the levels of *Bifidobacterium* in the intestinal tract of the infants fed with galactose oligosaccharide (GOS) and fructose-oligosaccharide (FOS) were elevated [11].

Therefore, in this paper, the intervention of LFBE on abnormal lipid metabolism was studied by clinical trials on the basis of our previous cell and animal experiments [4, 5]. 30 obese adults with HLP were included. Experimental parameters such as detecting serum glucose and lipid levels, insulin levels, and body composition analysis were carried out by ingesting LFBE chewable tablets to analyze and verify the intervention effect of fermented barley extract on obese men and women with HLP. Besides, the regulatory effect of LFBE chewable tablets on the gut microbiota structure of men and women was analyzed in depth by 16S rRNA sequencing to predict the changes in the function of the gut microbiota and explore the mechanism of improving the lipid metabolism of fermented barley extract.

2. Materials and Methods

2.1. Materials. The fermented barley extracts by *Lactiplantibacillus plantarum*dy-1 (LFBE) were prepared the same as the previous method [12]. The tablets of fermented barley extracts contained 20.0% LFBE, 20.0% microcrystalline cellulose, 14.6% inulin, and 8.0% cranberry fruit powder. Other ingredients include 36% sorbitol, 0.8% silica, and 0.6% magnesium stearate. For the tablets of β-glucan, LFBE was replaced with β-glucan and remained the same contents of other components with LFBE tablets. In addition, the β-glucan we used was the extraction of oat, purchased from Guangzhou Sinocon Food Co., Ltd. In order to ensure the smooth implementation of the single-blind experiment on obese adults, the shape, color, and packaging of the LFBE chewable tablets and β-glucan chewable tablets prepared in this study were basically the same.

2.2. Enrolled Obese Adults. Men and women aged 45~70 years with a body mass index (BMI) of 28–35 kg/m^2, stable bodyweight (<5% reported change during the previous 3 months), and known or newly detected isolated hyperlipidemia attending a participating clinical trial center were eligible for enrollment.

Inclusion criteria included the following characteristics: the waist circumference was more than 85 cm in male and 80 cm in female. Baseline low-density lipoproteins cholesterol (LDL-C) levels were at least 3.37 mmol/L, or total cholesterol (TC) levels were at least 5.18 mmol/L, or triacylglycerols (TG) levels were more than 1.7 mmol/L, or high-density lipoproteins cholesterol (HDL-C) levels were less than 1.04 mmol/L.

Referring to the previous study [13], we excluded individuals with a history or clinical manifestation of: TG level $\geq$ 5.65 mmol/L; serious heart, liver, brain, kidney function abnormalities, or severe gastrointestinal diseases; taking insulin, weight loss, or glucocorticoids-related drugs within a short time; taking antibiotics, microecological preparations, PPI inhibitors, herbal preparations, or dietary supplements in the past 4 weeks; digestive disorders (irritable bowel syndrome, IBS), gastrointestinal disorders, inflammatory bowel diseases (such as ulcerative colitis and Crohn's disease and colorectal cancer); pregnant or lactating women; and allergic to the ingredients in the study product. Written informed consent was obtained from all individuals. The protocol was approved by the ethics committees of the hospital (No. KY2021H1230-2).

2.3. Experiment Design. 14 men and 16 women ($n = 30$) were randomly assigned, and 28 completed the trial (Figure 1). This 12-week clinical trial was a single-blind, randomized, placebo-positive controlled, parallel study performed in the affiliated hospital of Jiangsu University from October 2018 to April 2019. The trial has already been conducted at the Clinical Department of Endocrinology and Metabolism, Medical University of China. This study was registered in the World Health Organization International Clinical Trials Registry Platform (No. ChiCTR1800019614).

Participants with obesity and hyperlipidemia were randomly assigned into a trial group (LFBE, $N = 10$), a control group (low-calorie and fat diet, $N = 10$), and a positive group (β-glucan, $N = 10$) of 12 weeks intervention. For obese adults in the diet control group, a 500 kcal low-calorie diet was reduced daily on the basis of the total daily calories calculated by an individual's standard body weight, and no chewable tablet was distributed, which lasted for 12 weeks. For the fermented barley extract (LFBE) group, a 500 kcal low-calorie diet was also reduced daily on the basis of the total daily calories calculated by an individual's

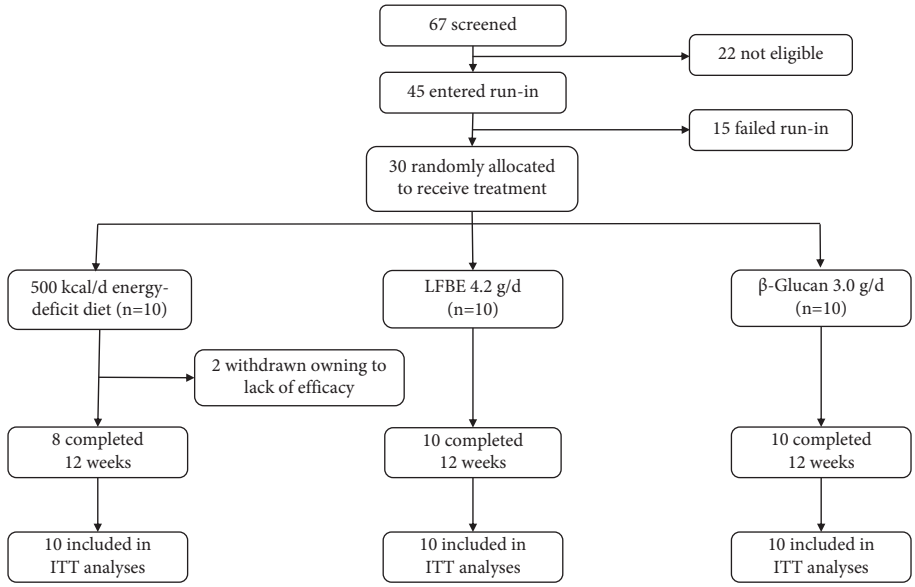

Figure 1: Study design of the human trial. 14 men and 16 women ($n = 30$) were randomly assigned and 28 completed in the control group (low-calorie and fat diet, $N = 8$), the trial group (LFBE, $N = 10$), and the positive group (β-glucan, $N = 10$). This 12-week clinical trial was a single-blind, randomized, placebo-positive controlled, parallel study.

standard body weight, while obese adults meanwhile ingested fermented barley extract at the dose of 4.2 g/d, 3 times/d, or 8 tablets/time for 12 weeks. For the BGL (β-glucan) group, a 500 kcal low-calorie diet was also reduced daily on the basis of the total daily calories calculated by an individual's standard body weight, while obese adults, meanwhile, took β-glucan at the dose of 3 g/d, 3 times/d, or 8 tablets/time for 12 weeks. Physical examinations and laboratory examinations were performed at the beginning (the first day of week 1), at the middle (the first day of week 7), and at the end (the last day of week 12) of each group by the study physician to ensure the health status of all participants. At the baseline and the end of the study, the feces of participants were collected for subsequent tests.

2.4. Anthropometric Indexes. Weight, BMI, body fat percentage (BF%), visceral fat area (VFA), and basal metabolic rate (BMR) were measured using a TANITA MC-180 bioelectrical impedance body composition analyzer (Tanita Co., Tokyo, Japan). Waist circumferences (WCs) were measured at the narrowest point between the lowest rib and the uppermost lateral border of the right iliac crest. Also, hip circumference (HIP) was also detected. After resting for at least 10 minutes in a quiet room, each participant's blood pressure (systolic blood pressure (SBP) and diastolic blood pressure (DBP)) was measured using an automatic blood pressure monitor (YE690A, Yuwell medical equipment and supply Co., Ltd., Jiangsu, China).

2.5. Biochemical Analysis. The evaluated biochemical indexes included TG, TC, HDL-C, LDL-C, fasting serum glucose (FSG), fasting serum insulin (FINS), homeostasis model assessment of insulin resistance (HOMA-IR), and homeostasis model assessment of insulin secretion (HOMA-β). FSG was measured using a glucose oxidase method, and the fasting serum concentrations of TC and TG were measured using an enzyme method with a Beckman-700 biochemical analyzer (Fullerton, CA, USA). FINS was measured using an access ultrasensitive insulin kit and a UniCelTMDxl 800 immunoassay analyzer (Beckman Coulter, Brea, CA, USA). The HOMA-IR index was used as a measure of insulin sensitivity and was calculated as follows: fasting insulin (mIU/mL) × fasting blood glucose (mmol/l)/22.5.

2.6. Fecal DNA Extraction and Sequencing. Fresh stool samples from a total of 20 participants (6 from the control group, 7 from the LFBE group, and 7 from the BGL group) before and after intervention were obtained immediately after defecation and transported in the shortest possible time to −80°C for storage until analysis of the gut microbiota. The sequencing protocol followed the previous report with slight modification [14]. The total bacterial DNA was extracted from the fecal samples using QIAamp DNA Stool Mini Kit (Qiagen, Germany). The extracted DNA (30 ng) was used as the template to amplify the V4 region of the 16S rRNA gene using primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The products from different samples were sequenced pair end on the MiSeq Illumina Sequencing Platform (San Diego, CA, USA) with the sequencing strategy PE250 (PE + 251 + 8 + 8 + 251; Miseq Reagent kit) [15]. All high-quality sequencing reads were clustered using USEARCH (v7.0.1090) with 97% similarity, and operational taxonomic units (OTUs) were obtained with a certain threshold. Each OTU was selected as the representative sequence and subjected to RDP Classifier software (v2.2) for taxonomical assignment. The number of sequences per sample was corrected for differences in sequencing depth between

samples by rarefication; that is, the same number of reads is randomly subsampled in each sample. Second, the absolute number of sequences of each OTU in each sample was converted to relative abundance to reduce the effect of differences in sequence reads. The representative sequences, together with the abundance data, were used for taxon-based analysis.

2.7. Statistical Analysis. Statistical analysis was accomplished by using SPSS statistics software version 17.0 (SPSS, Chicago, IL, USA). A *t*-test or analysis of variance (ANOVA) was used to analyze the differences between two groups or among three groups, respectively. Correlation analysis and the corresponding P value were calculated using the MATLAB function (the MathWorks, Natick, MA). The data were expressed as the arithmetic mean ± standard deviation (SD). Statistical significance was set at $P < 0.05$ in all analysis.

3. Results and Discussion

3.1. LFBE Prevented the Anthropometric Indexes of Obese Adults. Table S1 shows that there were no significant differences in anthropometric indexes among the three groups before intervention ($P > 0.05$). After intervention, the corresponding anthropometric variables of the obese adults are summarized in Table 1. In the LFBE and BGL groups, a significant decrease in body fat percentage, visceral fat area, and waistline was observed after 12 weeks of intervention ($P < 0.05$). In addition, weight gain and BMI of LFBE and BGL groups were lower than the baseline, especially for bodyweight, which decreased 2 kg in the BGL group. However, for the above parameters, which were not decreased in the control group by contrast, bodyweight and BMI have increased for the control group. Moreover, other anthropometric indexes such as systolic blood pressure and diastolic blood pressure showed no significant difference after the intervention.

Weight loss induced by a low-calorie diet also varies from person to person. In the study of Whytock et al. [16], 23 obese patients maintained a 900–1000 kcal/d diet for 28 days. It was confirmed a decrease in energy consumption lower than the body's basic metabolic requirements, which resulted in the increase of serum 3-hydroxybutyric acid, citric acid, leucine/isoleucine, acetyl carnitine, and 3-hydroxybutylcarnitine levels. The activity of fat mobilization was impaired, preventing the weight loss. Another study also revealed that no significant variations in the anthropometric values and weight loss in the obese were observed after 8 weeks of the low-calorie diet (1800 kcal/day) although there was a tendency for all the parameters to decrease after the diet [17]. Similarly, after 12 weeks of intervention in this study, there was no significance in the weight loss of obese adults by reducing 500 kcal of daily diet according to the standard body weight.

3.2. LFBE Intervention Affected the Serum Glucose and Lipids Level in the Obese. The changes in blood glucose parameters during the intervention are shown in Table 1. After 12-week intervention, compared with before intervention, FSG, FINS, HOMA-IR, and HOMA-β in the dietary control group were slightly increased without statistical significance ($P > 0.05$). Compared with before intervention, FSG in the LFBE group showed a downward trend from 6.46 mmol/L to 6.34 mmol/L, with a change rate of −1.9%, while FINS, HOMA-IR, and HOMA-β were all slightly increased without statistical significance ($P > 0.05$). FSG, FINS, and HOMA-IR in BGL group were slightly increased

Table 1: Changes and rate of biochemical and anthropometric indexes of three groups after intervention.

Parameters	Control group	LFBE group	BGL group
Weight (kg)	74.85 ± 7.35 (0.91%)	73.59 ± 8.29 (−1.06%)	72.40 ± 6.63$^{\&}$ (−2.70%)
BMI (kg/m^2)	28.23 ± 1.69 (0.91%)	27.41 ± 1.32 (−1.06%)	26.59 ± 1.22$^{\&}$ (−1.28%)
WC (cm)	98.99 ± 9.78 (−0.70%)	95.30 ± 4.67 (0.18%)	95.05 ± 8.16$^{\&}$ (−3.47%)
HIP (cm)	102.35 ± 6.11 (−1.93%)	101.40 ± 2.37$^{\#}$ (−0.54%)	97.65 ± 2.78 (−1.43%)
SBP (mmHg)	123.50 ± 10.90 (0.22%)	122.60 ± 8.03 (0.48%)	126.20 ± 8.83 (−1.50%)
DBP (mmHg)	79.80 ± 8.59 (0.23%)	75.90 ± 8.25 (2.87%)	79.10 ± 8.18 (2.65%)
FSG (mmol/L)	6.65 ± 0.61 (2.64%)	6.34 ± 0.59$^{\#}$ (−1.9%)	6.28 ± 0.75 (1.94%)
FINS (μIU/mL)	6.31 ± 3.11 (28.25%)	6.33 ± 3.11 (6.85%)	6.65 ± 3.06 (4.86%)
HOMA-IR	1.91 ± 1.03 (35.38%)	1.80 ± 0.94 (2.31%)	1.82 ± 0.73 (12.46%)
HOMA-β	39.26 ± 17.68 (15.40%)	44.95 ± 20.55 (23.49%)	53.90 ± 36.13 (−7.58%)
TG (mmol/L)	2.99 ± 0.69 (9.00%)	1.99 ± 0.83$^{\#\#}$ (−21.69%)	1.87 ± 0.80$^{\&}$ (−21.48%)
TC (mmol/L)	5.22 ± 0.44* (−4.81%)	5.03 ± 0.73$^{\#\#}$ (−18.33%)	4.48 ± 0.56$^{\&\&}$ (−20.34%)
HDL-C (mmol/L)	0.98 ± 0.31 (8.00%)	1.09 ± 0.35 (9.00%)	1.14 ± 0.35$^{\&\&}$ (14.00%)
LDL-C (mmol/L)	3.83 ± 0.50** (−4.12%)	3.59 ± 0.96$^{\#\#}$ (−24.30%)	2.76 ± 0.54$^{\&\&}$ (−13.88%)
BF (%)	30.80 ± 4.35 (−0.26%)	27.58 ± 2.98$^{\#}$ (−4.74%)	27.04 ± 3.47$^{\&}$ (−5.41%)
BMR	1342.6 ± 116.3 (−0.95%)	1345.0 ± 129.7 (4.78%)	1420.4 ± 126.2 (1.25%)
VFA (cm^2)	124.02 ± 26.83 (1.85%)	114.02 ± 18.78$^{\#}$ (−7.49%)	112.09 ± 19.44$^{\&}$ (−11.11%)

BMI: body mass index; WC: waist circumferences, HIP: hip circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, FSG: fasting serum glucose, FINS: fasting serum insulin, HOMA-IR: homeostasis model assessment of insulin resistance, HOMA-β: homeostasis model assessment of insulin secretion, TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, BF%: body fat percentage, VFA: visceral fat area, and BMR: basal metabolic rate. Data in the bracket meant the change rate = (postintervention-preintervention)/preintervention*100%. *Control group before vs. after intervention: $^{*}p < 0.05$ and $^{**}p < 0.01$; $^{\#}$LFBE group before vs. after intervention: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$; $^{\&}\beta$-glucan group before vs. after intervention: $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$.

after intervention, but the change rates were lower than those in the dietary control group without statistical significance ($P > 0.05$).

As expected, TG, TC, and LDL-C in the LFBE group were decreased by 21.69%, 18.33%, and 24.30%, respectively, while HDL-C in the LFBE group was increased by 9.00%. There were no significant changes of TG and HDL-C levels in the control group, while TC and LDL-C levels obviously decreased after intervention in the control group. LFBE significantly decreased the levels of serum TG, TC, and LDL-C level ($P < 0.05$) and slightly increased the serum HDL-C level, as well as β-glucan.

Serum lipid levels, including TG, TC, and LDL-C, which were considered to be associated with obesity, were also significantly reduced with the administration of LFBE. Our results were in accordance with the previous studies in rats [18]. However, LFBE and β-glucan did not significantly change insulin sensitivity at the selected dose, perhaps due to different models. Belobrajdic et al. showed that wholegrain barley β-glucan suppressed food intake and promoted caecal fermentation but did not improve postprandial glucose control or insulin sensitivity in rats fed a high-fat diet [19], similar to our study.

3.3. LFBE Administration Altered the Overall Composition of Gut Microbiota. In the following-up experiments, we further explored the potential pathway of LFBE alleviating the obesity from the point of gut microbiota by 16 rRNA sequencing. Results showed that after the optimization of the original sequence, a total of 3915289 optimized sequences were obtained from 40 samples, and the average length of sequences of all samples ranged from 301 to 470 bp (Tables S2–S4). In addition, a total of 30378 OTUs were obtained from 40 samples sequenced at a 97% homology cut off (Table S5). Alpha diversity refers to the diversity of bacteria in the body. Figure S1 A–F and Table S6 show that after LFBE intervention, Sobs, Chao1, and ACE indexes showed significant differences ($P = 0.01$, 0.01 and 0.02, respectively) and showed a decreasing trend of diversity. After β-glucan intervention, the diversity index of goods coverage decreased significantly ($P = 0.02$). In the diet control group, Sobs, Shannon, Simpson, Chao1, ACE, and goods-coverage indexes showed no significant differences ($P > 0.05$). In conclusion, both LFBE and β-glucan intake could affect the diversity of gut microbiota, in which the effect of LFBE was stronger due to the fact that LFBE contained polyphenols, proteins, polysaccharides, and other active substances in addition to β-glucan.

Adonis analysis based on the distance matrix (Figure 2 and Table 2) showed that the three groups were close to each other before intervention, without difference among the three groups ($P = 0.078$), while the three groups were far apart after intervention ($P = 0.001$). In addition, the comparison of each group before and after intervention showed that no significant difference was observed in the control group ($P = 0.683$), while the LFBE group and BGL group suggested significant separation ($P = 0.021$ and 0.002, respectively). These results indicated that LFBE and β-glucan

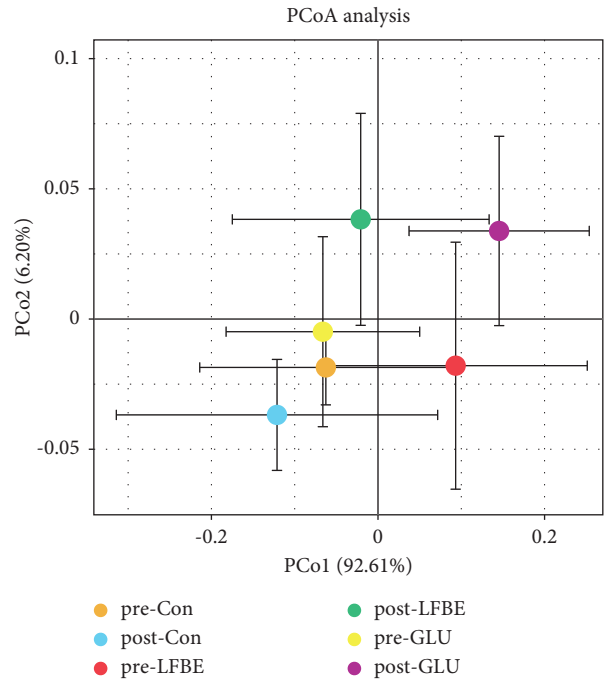

Figure 2: PCoA scores plot of gut microbiota before and after intervention. Pre-Con and post-Con: before intervention and after intervention in the control group; pre-LFBE and post-LFBE: before intervention and after intervention in the LFBE group; pre-BGL and post-BGL: before intervention and after intervention in the β-Glucan group.

exerted a certain regulatory effect on the overall structure of the human gut microbiota. However, different from our result, no differences of Shannon indices (i.e., alpha diversity) at baseline and after intervention in the elderly taken oat β-glucan were observed in a clinical trial, which was largely because the responses of oat β-glucan was disparate for individuals with different syndromes [20].

3.4. Effects of LFBE on the Gut Microbiota Composition at Phylum and Family Levels. *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* are the main components of the gut microbiota at the phylum level. As shown in Figure 3(a), changes in the gut microbiota composition of obese adults in the control group were minor, indicating that the low-calorie diet had little effect on the composition of gut microbiota at the phylum level. However, after LFBE intervention, the abundance of *Proteobacteria* which generates endotoxin significantly decreased and the proportion of *Firmicutes*/*Bacteroidetes* (F/B) also decreased. Previous studies have shown that the decrease of *Proteobacteria* was beneficial for reducing obesity-associated inflammation [21]. After β-glucan intervention, the F/B ratio was significantly elevated and the abundance of *Actinobacteria* increased. It is controversial that the F/B ratio can be regarded as a marker of obesity. Some studies suggested that the intestinal *Firmicutes* in the obese obviously increased while *Bacteroidetes* decreased; that is, the F/B ratio decreased [22, 23]. However, other studies reported that the F/B ratio in the obese did not

Table 2: Adonis analysis based on sample distance matrix.

Differences	Mean sqs	F values	R^2	P values
Pre-Con vs. post-Con	0.0242	0.3081	0.0299	0.683
Pre-LFBE vs. post-LFBE	0.377	3.2423	0.2127	0.021*
Pre-BGL vs. post-BGL	0.5838	7.0292	0.3694	0.002**
Pre-Con vs. pre-LFBE vs. pre-BGL	0.1892	2.4747	0.2255	0.078
Post-Con vs. post-BGL vs. post-LFBE	0.5342	4.8395	0.3628	0.001**

Pre-Con and post-Con: before intervention and after intervention in the control group; pre-LFBE and post-LFBE: before intervention and after intervention in the LFBE group; pre-BGL and post-BGL: before intervention and after intervention in the β-Glucan group.

change [24, 25]. To sum up, the intervention with LFBE and β-glucan could regulate the composition of the gut microbiota at the phylum level in the obese adults.

Likely, minor changes in the control group at the family level were observed. However, LFBE intervention could downregulate the abundance of *Pasteurellaceae*, *Ruminococcaceae*, and *Streptococcaceae* and upregulate the abundance of *Bacteroidaceae*, *Veillonellaceae*, and *Bifidobacteriaceae*, in which the changes in *Pasteurellaceae* and *Veillonellaceae* were the most remarked. It has been reported that the levels of *Pasteurellaceae* are elevated in the body with dyslipidemia, and high-intensity interval training could inhibit the growth of *Pasteurellaceae* [26]. *Veillonellaceae* is an important component involved in the digestive tract. Brasili et al. found that the intake of orange juice for 7 weeks could increase the abundance of *Veillonellaceae* and *Enterococcaceae* and reduce the abundance of *Ruminococcaceae*, which was similar to the LFBE intervention [27].

Different from LFBE, β-glucan intervention was relatively altered, represented by the downregulation of *Bacteroidaceae* and *Rikenellaceae* and the upregulation of *Veillonellaceae, Bifidobacteriaceae*, and *Streptococcaceae*. The changes in *Bacteroidaceae* and *Rikenellaceae* were the most significant, totally distinguished from the changes in the LFBE group. *Bacteroidaceae* belongs to *Bacteroidetes*; the variation trend of both at the family and phylum levels was the same with β-glucan intervention, while LFBE decreased, indicating that different mechanisms may exist in β-glucan and LFBE to relieve the obesity. In line with other studies, both *Schizophyllum* commune β-glucan [28] and low molecular β-glucan in barley [29] could reduce abdominal fat deposits of the subjects and increase the levels of *Bifidobacteriaceae* and *Bacteroidaceae*. *Rikenellaceae* exists in the fecal samples and digestive tracts of various animals, such as egg layers, mice, and humans. However, no direct study has shown that the members of *Rikenellaceae* are associated with the metabolic disease. *Streptococcaceae* is also one of the main contributors to distinguish the intestinal probiotic effects of LFBE and β-glucan. Studies have shown that *Streptococcaceae* was associated with the development of obesity, nonalcoholic fatty liver disease, and other metabolic diseases [30, 31]. High-fat diet (HFD) could enrich *Streptococcaceae*, while prebiotics intake decreased the abundance of *Streptococcaceae* [32]. Therefore, the probiotic effects of β-glucan partially depended on the upregulation of *Veillonellaceae* and *Bifidobacteriaceae*, not the increase of *Streptococcaceae*.

3.5. Effects of LFBE on the Gut Microbiota Composition at Genus and Species Levels. Changes in the composition of gut microbiota at the genus level were observed (Figure 3(c)). Consistent with the variation trend at family level, *Bacteroides* (*Bacteroidaceae*) and *Streptococcus* (*Streptococcaceae*) were upregulated and downregulated by the LFBE administration, respectively. What is worth mentioning is that, we found that the levels of *Haemophilus* were significantly downregulated, which was a member of *Pasteurellaceae*. The proinflammatory activity of *Haemophilusis* is reported to stimulate the Th2 inflammatory pathway, thereby causing a variety of inflammatory diseases, such as nephritis and peritonitis [33]. It was speculated that the LFBE supplement could reduce the levels of *Haemophilus*, a proinflammatory pathogen, alleviating obesity-associated inflammation. Moreover, *Dialister*, regarded as a producer of short-chain fatty acids, especially the butyric acid, can metabolize carbohydrates for providing energy and plays a beneficial role in the intestinal tract, which was enriched significantly by LFBE. However, β-glucan exhibited different impacts on the composition of gut microbiota at the genus level. There was no change in the *Haemophilus* level with β-glucan intervention, while the abundances of *Alistipes* and *Bacteroides* were decreased; the abundances of *Dialister* and *Bifidobacterium* were increased. Studies have demonstrated that HFD would like to enrich *Alistipes*, which was closely associated with obesity phenotypes [34]. Both animal and human clinical studies have reported that the consumption of oat β-glucan could significantly increase the abundance of *Bifidobacterium* [35]. In a double-blind placebo-controlled crossover study, galactose consumption in overweight adults for 12 weeks was able to increase the *Bifidobacterium* abundance and decrease the *Bacteroides* level [36]. It was speculated that the prebiotic effects of β-glucan and galactose were similar. Studies have indicated that the oat β-glucan, after *in vitro* human fecal fermentation, enhanced the growth of short-chain fatty acid producers, such as *Blautia* and *Dialister*, which were beneficial for the intestinal health [37]. In addition, oat meal rich in β-glucan also increased the relative abundance of *Dialister* in mildly hypercholesterolemic obese adults, which inferred that LFBE showed similar regulatory effects on the level of *Dialister* with oat β-glucan [38]. In conclusion, the probiotic effects of β-glucan largely depend on the reduction of the pernicious bacteria *Alistipes* and the increase of beneficial bacteria, such as *Dialister* and *Bifidobacterium*.

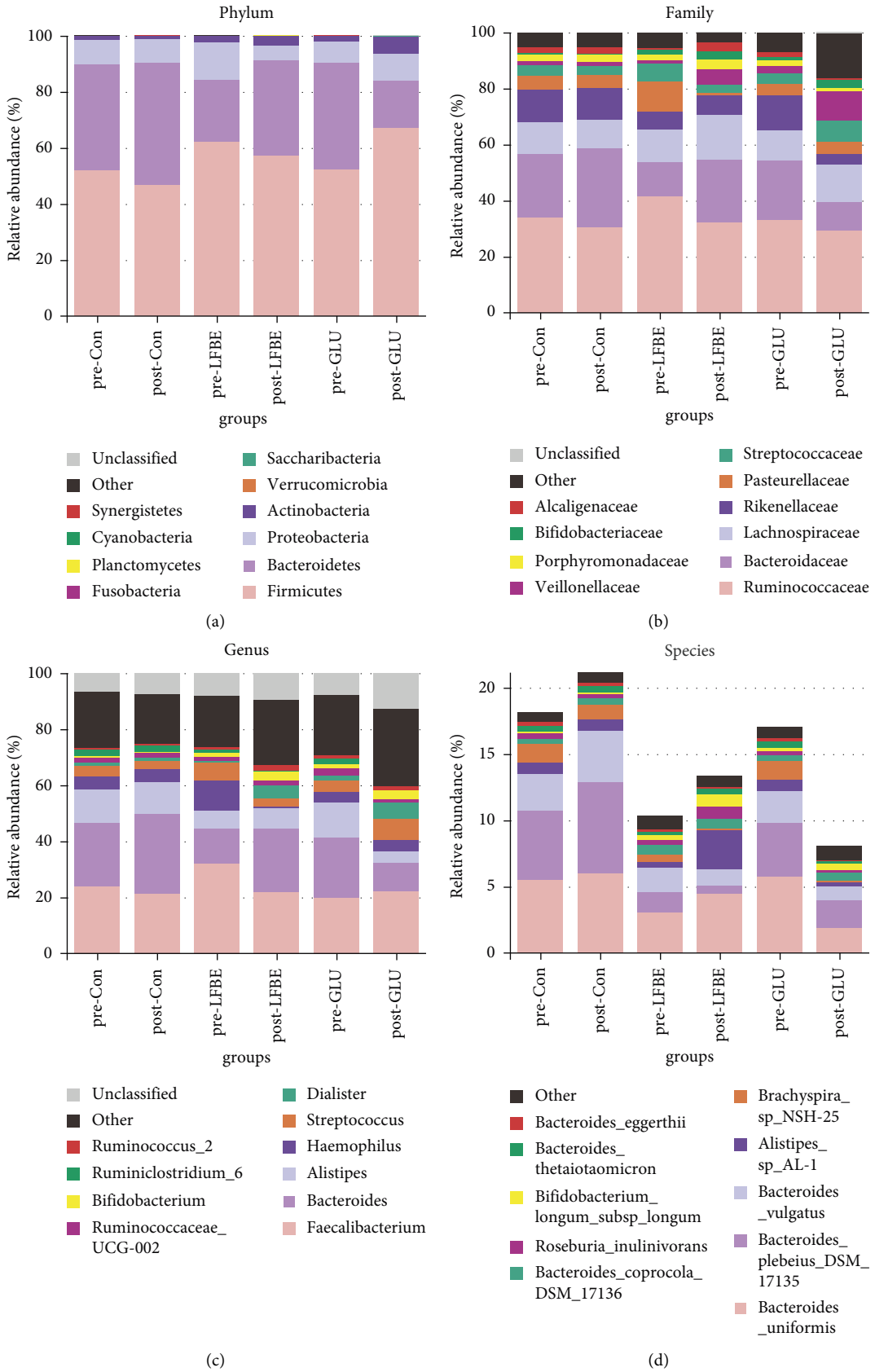

Figure 3: The taxonomic barcharts of samples at phylum (a), family (b), genus (c) and species (d) levels before and after intervention. Pre-Con and post-Con: before intervention and after intervention in the control group; pre-LFBE and post-LFBE: before intervention and after intervention in the LFBE group; pre-BGL and post-BGL: before intervention and after intervention in the β-Glucan group.

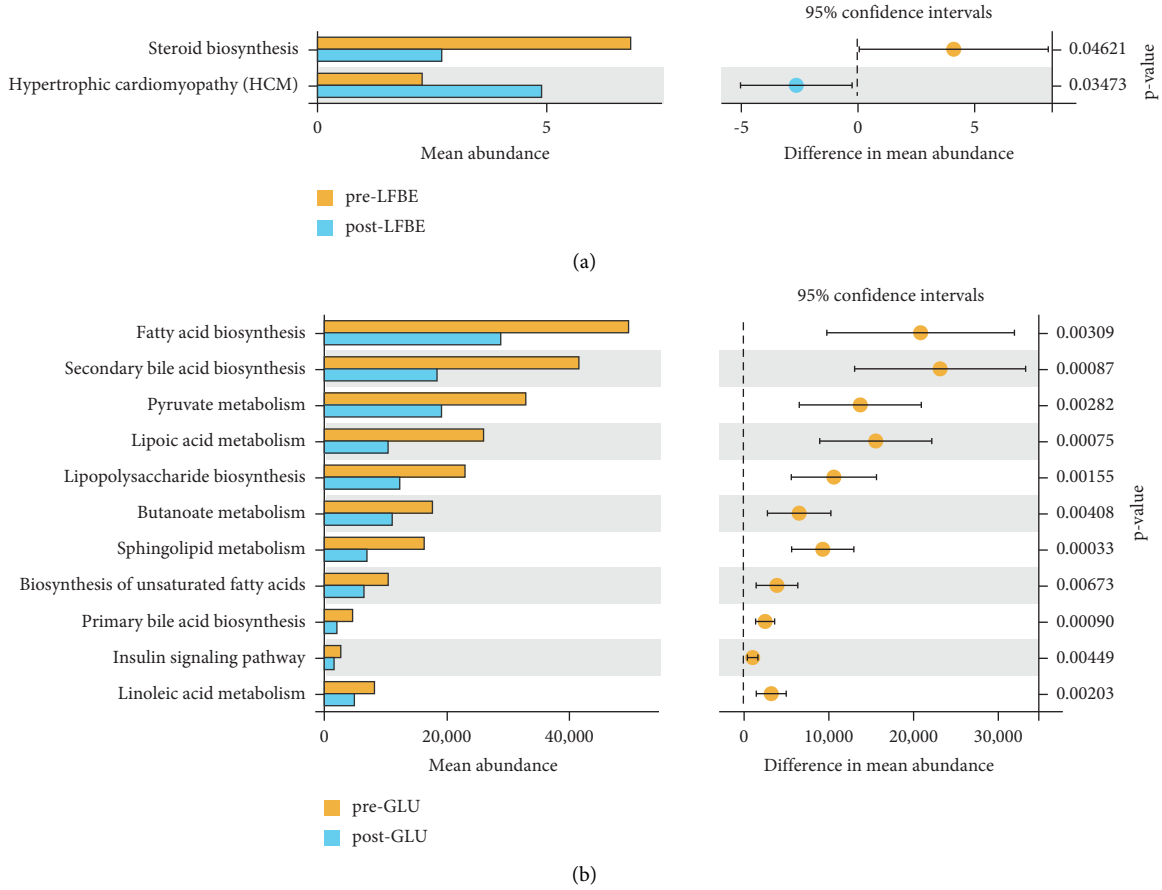

Figure 4: Effects of LFBE (a) and β-glucan (b) interventions on KEGG (L3 level) predicted by gut microbiota in volunteers. PICRUSt analysis was applied to predict the pathways according to the composition of the gut microbiota.

The regulations of gut microbiota at the species level were slight with LFBE and β-glucan, focusing on the *Bacteroides*, such as *Bacteroides_uniformis, Bacteroides_plebeius, and Bacteroides_vulgatus*. β-glucan could reduce the abundance of *Bacteroidetes*, *Bacteroidaceae*, and *Bacteroides* at the phylum, family, genus, and species levels, respectively. However, LFBE upregulated it at each level, indicating different regulatory pathways existed between LFBE and β-glucan. Meanwhile, the influences of LFBE and β-glucan on *Alistipes*_sp_*AL-1* differed to some extent: LFBE significantly upregulated it, while β-glucan downregulated it, which was consistent with the effects of β-glucan on *Alistipes*. However, the *Bifidobacterium_longum*_subsp_*longum* in probiotics *Bifidobacterium* showed an upward trend after LFBE and β-glucan interventions. Therefore, both LFBE and β-glucan could relieve the obese adults' hyperlipidemia by regulating the composition of the gut microbiota differentially.

3.6. KEGG Analysis Based on the Effects of LFBE and β-Glucan on Obese Adults' Gut Microbiota. As shown in the Figure 4(a), the intervention with LFBE mainly affected the steroid biosynthesis and hypertrophic cardiomyopathy pathways, which were closely related with the lipid-lowering effect of LFBE. As to β-glucan, the predicted pathways such as fatty acid biosynthesis, secondary bile acid biosynthesis, sphingolipid biosynthesis, and biosynthesis of unsaturated fatty acids were also involved in the lipid metabolism. In addition, lipopolysaccharide (LPS) biosynthesis was inhibited by β-glucan, hinting that some specific LPS-producers were reduced by β-glucan, such as some species belonging to *Desulfovibrionaceae* and *Enterobacteriaceae* [39]. Further analysis showed that β-glucan could significantly suppress the growth of *Desulfovibrionaceae* and *Desulfovibrio* at a relatively low abundance (family level: pre-BGL = 1.532686, post-BGL = 0.3526, and $P = 0.02$; genus level: pre-BGL = 1.474486, post-BGL = 0.262786, and $P = 0.02$). In all, β-glucan exhibited more regulations (including lipid metabolism and LPS biosynthesis) on the functions of the gut microbiota, while LFBE focused on the regulation of lipid metabolism, thereby relieving the hyperlipidemia.

3.7. Correlations between Obese Adults' Clinical Indicators and Gut Microbiota. Since gut microbiota is closely related to metabolic physiological indicators in the body, the correlation between the clinical physiological indicators and the composition of the gut microbiota was further analyzed in this study. In other words, the changed gut microbiota at the genus level and 11 clinical indicators were analyzed as shown in Figure 5. Results showed that the body fat percentage, basal

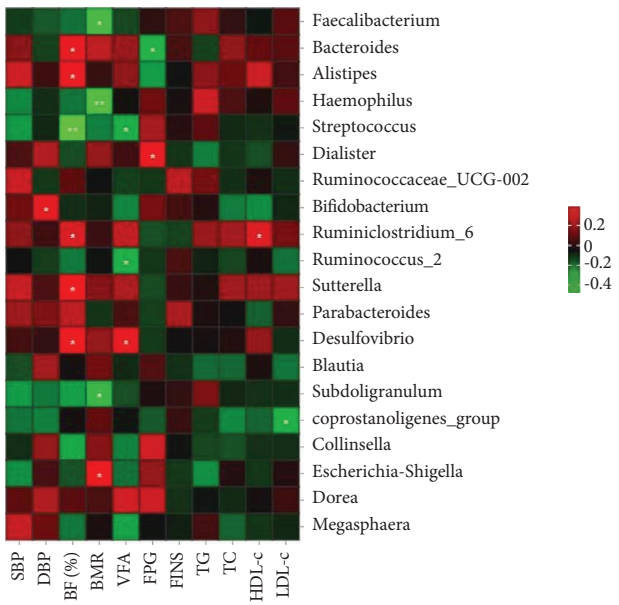

Figure 5: Correlations between clinical indexes and the gut microbiota. BF%: body fat percentage, VFA: visceral fat area, BMR: basal metabolic rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, FSG: fasting serum glucose, FINS: fasting serum insulin, TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, and HDL-C: high-density lipoprotein cholesterol.

metabolic rate, visceral fat area, and fasting plasma glucose were dramatically correlated to gut microbiota. For instance, the body fat percentage and visceral fat area were positively correlated to *Desulfovibrio* and negatively correlated to *Streptococcus*. As we all know, *Desulfovibrio*, a main genus producing LPS, is enriched in the gut microbiota of obese patients and closely correlated to some obesity-related phenotypes [40–42]. For *Streptococcus*, it was increased after Roux-en-Y gastric bypass surgery of the obese and negatively correlated to obesity-relevant indicators [43]. Moreover, body fat percentage was also notably positively correlated to *Bacteroides*, *Alistipes*, *Ruminiclostridium*_6, and *Sutterella*. Similar to *Desulfovibrio, Sutterella* was also an LPS-producer and positively correlated to chronic low-level inflammation of the obese [44–46].

Most of all, basal metabolic rate was negatively correlated to *Faecalibacterium, Haemophilus*, and *Subdoligranulum*. Also, fasting blood glucose was positively correlated to *Dialister* and negatively correlated to *Bacteroides*. We speculated that as a pathogenic bacterium in the intestinal tract, the decrease of *Haemophilus* could accelerate the body's basal metabolic rate [47]. *Dialister* was responsible for the carbohydrates hydrolysis for providing energy [48, 49] and ascribed to the reason for the positive correction with fasting blood glucose.

4. Conclusion

In all, our clinical study demonstrated that LFBE exerted excellent antiobesity activity, characterized by declining the body fat percentage, visceral fat area, and serum lipid levels. 16S rRNA sequencing data suggested that LFBE regulated the composition of the gut microbiota, different from the β-glucan, especially at the family and genus levels. Correlation analysis demonstrated that some specific gut microbiota at the genus level was significantly correlated with obesity-related indicators, either positively or negatively. These findings may provide insights into the probiotic role of fermented barley extracts on antiobesity from the clinical point of view.

Data Availability

The data supporting the findings of this study are included within the article.

Additional Points

Practical Applications. Previous studies conducted by our group showed that fermentation of barley extracts with *Lactiplantibacillus plantarum* dy-1 (LFBE) inhibited fat accumulation in nematodes, cell lines, and animal experiments. However, LFBE could exhibit the antiobesity effect and its potential mechanism in clinical trials remained uncovered. The current results suggested that chewable tablets containing LFBE could improve the symptoms of obese individuals by reducing body fat percentage, visceral fat area, and blood lipid levels. In addition, the mechanistic study demonstrated that the antiobesity function of LFBE in humans was possibly through interaction with the gut microbiota. In all, LFBE could be used as a kind of functional dietary component for treating obesity.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Ruirong Pan conceptualized the study and validated the study. Ruirong Pan, Juan Bai, and Xiang Xiao reviewed and edited the article. Jie Yuan, Juan Bai, and Jiayan Zhang proposed the methodology. Jinfu Zhang performed formal analysis. Yaoguang Gu, Song Xia, Minye Qu, and Qiang Liu investigated the study. Ying Dong performed project administration.

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Supplementary Materials

Table S1: comparison of clinical parameters of three groups before intervention. Table S2–S4: counts of tags and sequences length in the three groups before and after intervention. Table S5: counts of OTU in per sample. Table S6: comparative analysis of alpha diversity index in the three groups before and after intervention. Figure S1: alpha diversity analysis of the gut microbiota, including (A) Rarefaction curves, (B) Shannon diversity index, (C) Simpson diversity index curves, (D) Chao1 diversity index, (E) ACE diversity index, and (F) Goods Coverage diversity index. (*Supplementary Materials*)

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