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Bifidobacterium longum CCFM1077 Attenuates Hyperlipidemia by Modulating the Gut Microbiota Composition and Fecal Metabolites: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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ABSTRACT

An increasing number of studies have indicated that gut microbiota and its metabolites are crucial in the development of hyperlipidemia. *Bifidobacterium longum* (*B. longum*) CCFM1077 has been shown to have lipid-lowering effects in animals. This study aimed to evaluate the potential of *B. longum* CCFM1077 in lowering the lipid levels in patients with hyperlipidemia and investigate the effect of this bacterium on serum lipid abnormalities, gut microbiota, and fecal metabolites in these patients. This study was a six-week, randomized, double-blind, and placebo-controlled pilot clinical trial. Subjects with hyperlipidemia (N = 62) were randomly assigned to receive placebo (N = 31) or *B. longum* CCFM1077 (1×10^{10} colony-forming units (CFUs) per day; N = 31). Serum lipid levels including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), total triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were examined at the baseline and interventional endpoints. Changes in the gut microbiota composition and diversity were measured based on 16S ribosomal RNA (rRNA) sequencing of the V3–V4 region at the end of the intervention period. Non-targeted metabolomics of the feces was performed using ultra-performance liquid chromatography (UPLC)-Q-Exactive Orbitrap/mass spectrometer. Oral administration of *B. longum* CCFM1077 for six weeks significantly decreased the serum levels of TC ($p < 0.01$) and LDL-C ($p < 0.01$) in patients with hyperlipidemia. *B. longum* CCFM1077 treatment markedly increased gut microbiota diversity and the relative abundance of anti-obesity-related genera, including *Lactobacillus*, *Butyricicoccus*, *Bifidobacterium*, and *Blautia*, whereas it decreased the relative abundance of obesity-related genera, including *Alistipes*, *Megamonas*, and *Catenibacterium*. Additionally, some key metabolites (bile acids (BAs), biotin, and caffeine) and their corresponding metabolic pathways (primary BA biosynthesis, and taurine and hypotaurine, biotin, purine, and caffeine metabolisms) were enriched by *B. longum* CCFM1077, and thus it may lower lipid levels. *B. longum* CCFM1077 is a probiotic strain with the potential to lower serum TC and LDL-C levels patients with hyperlipidemia. The underlying mechanism may be related to the increased abundance of anti-obesity-related genera and fecal metabolites. These findings provide a foundation for future clinical applications of lipid-lowering probiotics in managing individuals with hyperlipidemia.

1. Introduction

In recent years, dietary patterns characterized by high-calorie foods occupy a large proportion of the human daily diet, leading to a dramatic rise in metabolic syndromes such as hyperlipidemia,

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fatty liver, and type II diabetes [1]. Hyperlipidemia is a major risk factor for the occurrence and progression of atherosclerosis and other cardiovascular diseases and is a global public health concern [1]. By 2022, it was estimated that approximately 78 million individuals globally would develop from hyperlipidemia [2], which is characterized by the abnormal accumulation of lipids in the blood and liver [3]. Several lipid-lowering medications have been developed for decades to treat hyperlipidemia; however, clinical data show that most of these medications have undesirable side effects [4]. Therefore, it is imperative to develop safe and effective alternatives, such as functional foods, to control the levels of serum lipid profiles with fewer adverse effects and avoid or minimize the onset and progression of hyperlipidemia.

Probiotics are live microbial supplements administered in sufficient quantities while providing physiological benefits to the host [5]. They have drawn increasing attention in the past few years to control abnormal lipid metabolism. In recent years, probiotics have been extensively utilized to prevent and alleviate hyperlipidemia [6,7]. Bacteria of the genera *Lactobacillus* and *Bifidobacterium* are the most well-known and frequently administered probiotics for improving lipid metabolism abnormalities [8]. The lipid-lowering effects of probiotics have been demonstrated in animal models [9]. Mechanistically, there is growing evidence from preclinical studies that probiotics can provide considerable protection against hyperlipidemia by activating the adenosine monophosphate-activated protein kinase (AMPK) and antioxidant signaling pathways [10], normalizing gene expression related to cholesterol metabolism and decreasing lipid-related microRNA (miRNA) levels [11]; upregulating gene expression related to the liver X receptor (LXR), and downregulating the expression of the farnesoid X receptor gene (*FXR*) [12,13] and improving gastrointestinal function by decreasing the expression of intestinal Niemann-Pick C1-like 1 and microsomal total cholesterol (TC) transfer protein [14]. In addition, *Lactobacillus reuteri* (*L. reuteri*) A9 and *L. mucosae* A13 isolated from the oldest living Chinese people, were observed to exert cholesterol-lowering effects via bile salt hydrolase (BSH), an enzyme from probiotics that hydrolyzes bile salts into free amino acids and bile salts [15]. *Bifidobacterium animalis* subsp. *Lactis F1-7* and *L. vaginalis* FN3 reduced triglyceride (TG) and TC levels in the liver of a mouse model of hyperlipidemia, while these two probiotic strains increased the total bile acid (BA) concentration in the feces [13].

A recent study including 38 meta-analyses systematically examined the effect of probiotics on individuals with hyperlipidemia. It reported that probiotic supplementation substantially lowered the levels of TC (95% confidence interval (95% CI): −0.61, −0.30, $p < 0.001$), TG (95% CI: −0.23, −0.04, $p = 0.006$), and low-density lipoprotein cholesterol (LDL-C; 95% CI: −0.40, −0.19, $p < 0.001$) in patients with hyperlipidemia. However, probiotics did not significantly increase high-density lipoprotein cholesterol (HDL-C; 95% CI: −0.04, 0.08, $p = 0.519$) levels [16]. Similarly, another meta-analysis comprising 13 randomized, controlled trials (RCTs) showed that probiotic consumption effectively reduced TC and LDL levels in individuals with hyperlipidemia [17]. In addition, probiotic intervention groups with a single probiotic strain, such as *Bifidobacterium longum* (*B. longum*) BB536 [18] or *L. plantarum* CECT 7527 [19], have been shown to improve dyslipidemia, which significantly reduced the levels of TC and LDL-C compared with that in the placebo group. However, most RCTs of probiotics for hyperlipidemia treatment that have been published so far tested only the efficacy of probiotics [19–21], the mechanisms underlying their lipid-lowering effect have rarely been investigated.

A growing number of studies have shown that patients with hyperlipidemia present with marked gut microbiome dysbiosis compared to healthy controls, with a strong correlation between gut microbiota dysbiosis and serum lipid abnormalities [22–24]. This suggests that the intestinal microbiome may be a therapeutic target for hyperlipidemia. To the best of our knowledge, almost no studies have investigated whether probiotics can alleviate hyperlipidemia by regulating the intestinal microbiota and its metabolites. *B. longum* CCFM1077 is a probiotic strain isolated from one of the oldest living Chinese people and has excellent physiological characteristics. Previous studies have shown that *B. longum* CCFM1077 can exert cholesterol-lowering effects by increasing BSH activity in a cholesterol-induced hyperlipidemia model in Sprague-Dawley rats [25]. We hypothesized that *B. longum* CCFM1077 has the potential to improve lipid abnormalities in individuals with hyperlipidemia. To test this hypothesis, we designed a randomized, double-blind, placebo-controlled clinical study to investigate the efficacy of *B. longum* CCFM1077 against hyperlipidemia. Specifically, this study aimed to investigate the protective effect of *B. longum* CCFM1077 against hyperlipidemia and its underlying mechanism using 16S ribosomal RNA (rRNA) sequencing of the gut microbiome and liquid chromatography–mass spectrometry (LC–MS)-based untargeted metabolomics of the feces. Moreover, this study determined whether the consumption of *B. longum* CCFM1077 changed the gut microbiota composition and investigated the relationship between the lipid metabolic phenotype and gut microbes and their metabolites. Hence, this study provides a foundation for the development of functional probiotics that can help treat hyperlipidemia.

2. Materials and methods

2.1. Patient recruitment and ethics approval

Subjects with hyperlipidemia were recruited in Yancheng, China, from May to June 30, 2020. The inclusion criteria for patients with hyperlipidemia were based on the *Guidelines for the prevention and treatment of dyslipidemia in Chinese adults* (revised edition in 2016). Subjects were eligible if they were: healthy adults aged 18–75 years, with fasting TC level $\geq$ 5.7 mmol·L^{-1}, or fasting TG level $\geq$ 1.7 mmol·L^{-1}, or fasting LDL-C level $\geq$ 3.1 mmol·L^{-1}, or HDL-C level $\leq$ 1.1 mmol·L^{-1}. Eighty-seven patients were recruited from Yancheng Tinghu District People's Hospital, China; however, 25 individuals were not permitted to participate in the clinical trial because of the inclusion and exclusion criteria. The 62 participants who were eligible for the study were divided into two groups of 31 people each, with one group receiving a placebo and the other receiving *B. longum* CCFM1077. Patient randomization was performed using software that generated a random number sequence by the staff of the Yancheng Tinghu District People's Hospital, and the participants and associated experimental employees were unaware of the groupings.

Both the clinical protocol and ethics statements were submitted to and approved by the Ethics Committee of Yancheng Tinghu District People's Hospital (approval No. ET2020004). This study was registered at www.chictr.org.cn with the code ChiCTR2000033845. The study was conducted in accordance with the *Helsinki Declaration*, and informed consent was obtained from each participant.

2.2. Study design and intervention

This investigation was a six-week-long, double-blind, randomized, placebo-controlled trial with two parallel groups. After the initial screening and two-week run-up phase, 62 eligible subjects were randomized to receive either the probiotic *B. longum* CCFM1077 ($N = 31$) or placebo ($N = 31$). Patients with hyperlipidemia who met the inclusion criteria were randomly assigned to the probiotic group or placebo group. A staff member who was excluded from participating data management or statistical analy-

sis prepared the allocation sequence using random, computer-generated numbers. A sealed envelope for each subject was offered to the principal investigator and could be opened only after storing the relevant data on a computer. The patients participating in the study and all health care professionals were all blinded to treatment allocation until statistical analyses was completed. In addition, the preparations used in this trial (including *B. longum* CCFM1077 and placebo) were all powders with the same appearance and packaging to ensure double-blinding during the trial. The experimental staff collected the samples for the measurement of each index. Blinding was uncovered after the completion of the experimental index testing and analysis. The participants were asked to maintain their regular lifestyle, exercise intensity, and eating habits. However, during the intervention period, they were prohibited from consuming fermented food that may have contained large quantities of probiotic strains, such as yogurt and pickled vegetables. According to the instructions, the subjects were required to ingest one pouch of the treatment or placebo after a meal for six weeks.

2.3. Study products

In the probiotic group, patients with hyperlipidemia were assigned to consume a probiotic preparation containing 5×10^9 colony-forming units (CFUs) of *B. longum* CCFM1077 daily. The *B. longum* CCFM1077 preparation and placebo products were provided by Wecare-Bio Science and Technology Company (China). The products were in powder form and had the same packaging (2 g·pouch^{-1}). The placebo product used was maltodextrin. The *B. longum* CCFM1077 preparation contained *B. longum* CCFM1077 (> 5×10^9 CFU·g^{-1}) and maltodextrin. All of the products had to be stored at 4 °C. The viable bacterial amount of *B. longum* CCFM1077 in the product remained over 5×10^9 CFU·g^{-1} over three months.

2.4. Clinical measures

Patients with hyperlipidemia who were included in the study underwent a comprehensive health examination at the Physical Examination Center of the Yancheng Tinghu District People's Hospital (China), before the intervention period. This examination, which was performed in Yancheng, China, included general parameters (height, weight, blood pressure, waist circumference (WC), hip circumference (HC), thigh circumference, and body mass index (BMI)), blood biochemistry, liver function, routine urinalysis, routine fecalysis, and body composition analyses. Blood samples collected in plasma separator tubes were used to measure the TC, TG, LDL-C, and HDL-C levels. Parameters included in the Framingham risk algorithm were also recorded, such as age, sex, medical history, dietary habits, frequency of exercise, defecation, yogurt or probiotic product consumption, antibiotic use, diabetes, hypertension, and smoking status.

2.5. Preparation of fecal extracts for untargeted metabolomic analysis

Fecal extraction was performed as previously described [26], with slight modifications. Briefly, the test samples were thawed at 4 °C, then a 100 mg aliquot of feces was transferred to 1.5 mL centrifuge tubes. Thereafter, 400 μL of precooled methanol was added to each tube and vortexed thoroughly for 30 s. The samples were centrifuged at 12 000 revolutions per minute (rpm) for 15 min at 4 °C. Then, 400 μL of the supernatant in each tube was transferred into a new centrifuge tube, which was concentrated using a CentriVap Concentrator (Thermo Fisher Scientific, USA). Subsequently, 200 μL of methanol solution (methanol:water, 4:1) was added, and the mixture was vortexed thoroughly for 30 s. After centrifugation at 12 000 rpm for 5 min at 4 °C, the supernatant was collected as the sample extract for high performance liquid chromatography (HPLC)–MS. Twenty microliters of each prepared sample was extracted and mixed as the quality control sample.

2.6. Ultra-performance liquid chromatography (UPLC)-Q-Exactive Orbitrap/mass spectrometer-based metabolomic analysis

Samples were analyzed for non-targeted metabolites using an UltiMate-3000 UPLC system coupled with a high-resolution Q-Exactive mass spectrometer (Thermo Fisher Scientific). An Acquity UPLC T3 column (2.1 mm × 100.0 mm, 1.8 μm; Waters Corporation, USA) was used, with an operating temperature of 35 °C. In the positive mode, mobile phase A was 0.1% formic acid and mobile phase B was 100% methanol; in the negative mode, mobile phase A was 5 mmol·L^{-1} ammonium acetate and mobile phase B was 100% methanol. The gradient elution conditions were: 0–1 min, 2% B; 1–10 min, 2%–98% B; 10–12 min, 98% B; 12–15 min, 2% B. The flow rate was maintained at 0.3 mL·min^{-1} and the injection volume of both phases was 2 μL. The heated electrospray interface (HESI) source was operated under the following conditions: nitrogen flow of 35 and 15 arbitrary units for the sheath and auxiliary gas, respectively; probe and capillary temperature of 450 and 320 °C, respectively; and spray voltage of 3.8 and 2.8 kV in the positive and negative modes, respectively. For MS/MS spectra acquisition at later unknown structural annotation stages, the mass spectrometer scanned for 50–1070 Dalton in full-scan MS1 mode (full width at half height, 70 000; automatic gain control target, 1e6; maximum injection time, 100 ms) alternating with a parallel reaction monitoring MS/MS mode with normalized collision energy averaging 20, 40, and 60 eV (full width at half height, 17 500; automatic gain control target, 5e4; maximum injection time, 50 ms; dynamic exclusion, 10 s).

2.7. Collection of fecal samples and analysis of gut microbiota

On the last day of the intervention period (day 42), fecal samples were collected from each subject at the Yancheng Tinghu District People's Hospital and immediately stored at –80 °C until further analysis. Total genomic DNA was extracted from each fecal sample (50 mg·sample^{-1}) using a FastDNA® Spin Kit for Feces (MP Biomedicals, USA) according to the manufacturer's instructions. The quantity and quality of DNA isolated from each sample were analyzed using a Nanodrop 2000 spectrophotometer (USA). Polymerase chain reaction (PCR) was used to amplify the V3–V4 region of the 16S rRNA gene in each sample. The primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) were used with a Premix TaqTM reagent (Takara, China) according to the manufacturer's instructions. Amplification comprised an initial denaturation phase at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. After amplification, the products were maintained at 4 °C. The PCR products were purified using a DNA gel/PCR Purification MiniPrep Assay Kit (BM-DC3511; Biomiga, China). Library preparation, sequencing, and bioinformatic analyses were performed as previously described [27]. The analytical methods for subsequent 16S rRNA sequencing are detailed in Appendix A.

2.8. Statistical analysis

The results were expressed as the mean ± standard deviation (SD). One-way analysis of variance was analyzed using the non-parametric Kruskal–Wallis test, whereas multiple tests between multiple groups were analyzed using Dunnett's multiple comparison test. Differences were considered significantly different at p values < 0.05. Statistical analyses were performed using GraphPad

Prism 8.0 software (GraphPad Software, USA). The composition and diversity of gut microorganisms were analyzed using the QIIME II platform[†] and MicrobiomeAnalyst platform[‡]. Non-targeted metabolomics was analyzed using the MetaboAnalyst 5.0 platform[††]. Differential intestinal microbial genera between groups were analyzed using the linear discriminant analysis (LDA) effect size (LEfSe) module of the Galaxy/Hutlab platform[‡‡].

3. Results

3.1. Subjects and baseline characteristics

The participants were screened from May to June 2020, while the intervention period was from July to August 2020. After assessing eligibility, 62 participants who met the inclusion criteria were randomly assigned to two groups, the placebo group (N = 31) or the *B. longum* CCFM1077 group (N = 31) (Fig. 1). The same approximate sex ratios and ages were maintained in each group. After six weeks of treatment, seven participants withdrew because of the following exclusion criteria: antibiotic consumption (N = 2), drop out (N = 2), and low-compliance with the intervention protocol (N = 3). Finally, 55 subjects successfully completed the trial according to the trial procedures, and their baseline characteristics are shown in Table 1. No differences in adherence and compliance between the placebo and *B. longum* CCFM1077 groups were observed. Similarly, no adverse effects were reported in either group.

3.2. Effects of B. longum CCFM1077 on serum lipids in subjects with hyperlipidemia

The serum lipid levels of the subjects before and after the intervention are shown in Table 2. At the baseline, there were no remarkable variations in serum lipid levels, including TC, TG, LDL-C, and HDL-C, between the placebo and *B. longum* CCFM1077 groups. After the intervention period, participants who were assigned the placebo showed no obvious changes in their serum lipid levels compared with the baseline ($p > 0.05$). In contrast, the levels of TC and LDL-C in the *B. longum* CCFM1077 group showed a significant decrease ($p < 0.05$). Additionally, when compared to the control group, daily supplementation with *B. longum* CCFM1077 for six weeks significantly decreased TC ((-0.95 ± 1.30) mmol·L^{-1}) and LDL-C ((-0.59 ± 0.80) mmol·L^{-1}) levels ($p < 0.05$). In addition, there was no significant shift in TG and HDL-C levels in the *B. longum* CCFM1077 group ($p > 0.05$) throughout the intervention.

3.3. Effects of B. longum CCFM1077 treatment on gut microbiota richness, diversity, and composition in subjects with hyperlipidemia

We performed 16S rRNA amplicon sequencing to investigate whether *B. longum* CCFM1077 influenced the composition of intestinal bacteria in individuals with hyperlipidemia. After trimming and filtering, 21 428 115 excellent quality reads were obtained for all samples. These reads were grouped into 1793 operational taxonomic units, with a sequence similarity of 97%. We conducted a β-diversity analysis to measure the degree of similarity between the microbial structures of the two groups. The Bray–Curtis index-based principal component (PCoA) was used to measure β-diversity and visualize the differences in bacterial composition between the two groups. As shown in Fig. 2(a), there was a statistically significant variation in clustering between the two groups (permutational multivariate analysis of variance (PERMANOVA), $p < 0.001$). The contributions of the first principal component (PC1) and PC2 to the total variation were 24% and 15%, respectively. Similarly, β-diversity measured using non-metric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarity index also showed a significant difference (PERMANOVA), $p < 0.001$; stress < 0.2) (Fig. 2(b)). The α-diversity can be used to evaluate the richness and diversity of microbial communities within a sample. As shown in Figs. 2(c)–(e) and Figs. S1(a) and S1(b) in Appendix A, subjects with hyperlipidemia administered with *B. longum* CCFM1077 had a significantly increased gut microbial community diversity, evident by their ACE ($p < 0.001$), Chao1 ($p < 0.001$), Fisher ($p < 0.001$), observed ($p < 0.001$), and Shannon ($p < 0.001$) indices. Differences in the relative abundance of the gut microbiome between the two groups at the phylum level were represented as stacked histograms. Firmicutes, Bacteroidetes, and Proteobacteria were the predominant phyla, accounting for more than 90% of the microbial community (Fig. 2(f)). Classical univariate statistical comparisons measured using the Mann–Whitney/Kruskal–Wallis tests/zero-inflated Gaussian fit model showed that the proportions of Chloroflexi ($p < 0.001$), Acidobacteria ($p < 0.001$), Cyanobacteria ($p < 0.01$), Euryarchaeota ($p < 0.001$), Gemmatimonadetes ($p < 0.001$), and Nitrospirae ($p < 0.001$) were significantly decreased in the *B. longum* CCFM1077 intervention group compared to that in the control group (Figs. 2(g)–(l)).

3.4. The gut microbiota in the B. longum CCFM1077 group differed from that in the placebo group

The results of the β-diversity test suggest substantial variations in the microbial communities between the two groups. We used LEfSe analysis to identify the specific bacterial taxa driving the differential abundances in the two groups. The LEfSe analysis showed that *B. longum* CCFM1077 significantly affected the abundance of 50 bacterial genera, of which the abundance of the 40 genera decreased, and the relative abundance of 10 genera increased compared to those in the placebo group (Fig. 3(a)). A six-week *B. longum* CCFM1077 intervention significantly reduced the increase in the proportion of *Alistipes* ($p < 0.0001$, LDA = 4.82) and *Megamonas* ($p < 0.0001$, LDA = 4.74). In contrast, the relative abundances of *Lactobacillus* ($p < 0.002$, LDA = −3.74), *Butyricicoccus* ($p < 0.0001$, LDA = −4.44), and *Lachnospira* ($p < 0.05$, LDA = −4.01) were markedly increased. The changes in the gut microbiota at the genus level, as analyzed using the DESeq2 method, identified 131 differentially abundant taxa ($p < 0.05$) (Table S1 in Appendix A). Specifically, the abundances of *Lactobacillus*, *Bifidobacterium*, *Butyricicoccus*, *Blautia*, *Lachnospiraceae_NK4A136_group*, and *Ruminococcaceae_UCG_013* were higher, whereas the abundances of *Bosea*, *Alistipes*, *Megamonas*, *Catenibacterium*, and *Ralstonia* were lower in the *B. longum* CCFM1077 group than in the placebo group (Fig. 3(b)).

Pattern search analysis was also performed to determine the correlation between the core bacteria in the gut microbial community and the abundance between groups (*B. longum* CCFM1077 group vs placebo group pattern). *Butyricicoccus* (correlation coefficient (R) = 0.7930, false discovery rate (FDR)-adjusted $p < 0.05$), *Blautia* (R = 0.7751, FDR-adjusted $p < 0.05$), and *Bifidobacterium* (R = 0.6337, FDR-adjusted $p < 0.05$) were positively correlated with *B. longum* CCFM1077 intake in subjects with hyperlipidemia. Conversely, *Bosea* (R = −0.3299, FDR-adjusted $p < 0.05$) was negatively correlated with *B. longum* CCFM1077 supplementation (Fig. 3(c)). We extended our understanding of the correlations between *Bifidobacterium* and 25 genera by applying the same algorithm. *Bifidobacterium* was positively correlated with *Lactobacillus* (R = 0.6822, FDR-adjusted $p < 0.05$), *Blautia* (R = 0.8257, FDR-adjusted $p < 0.05$), *Butyricicoccus* (R = 0.7761, FDR-adjusted $p < 0.05$),

[†] https://view.qiime2.org/.
[‡] www.microbiomeanalyst.ca.
[††] https://www.metaboanalyst.ca/.
[‡‡] http://huttenhower.sph.harvard.edu/galaxy/.

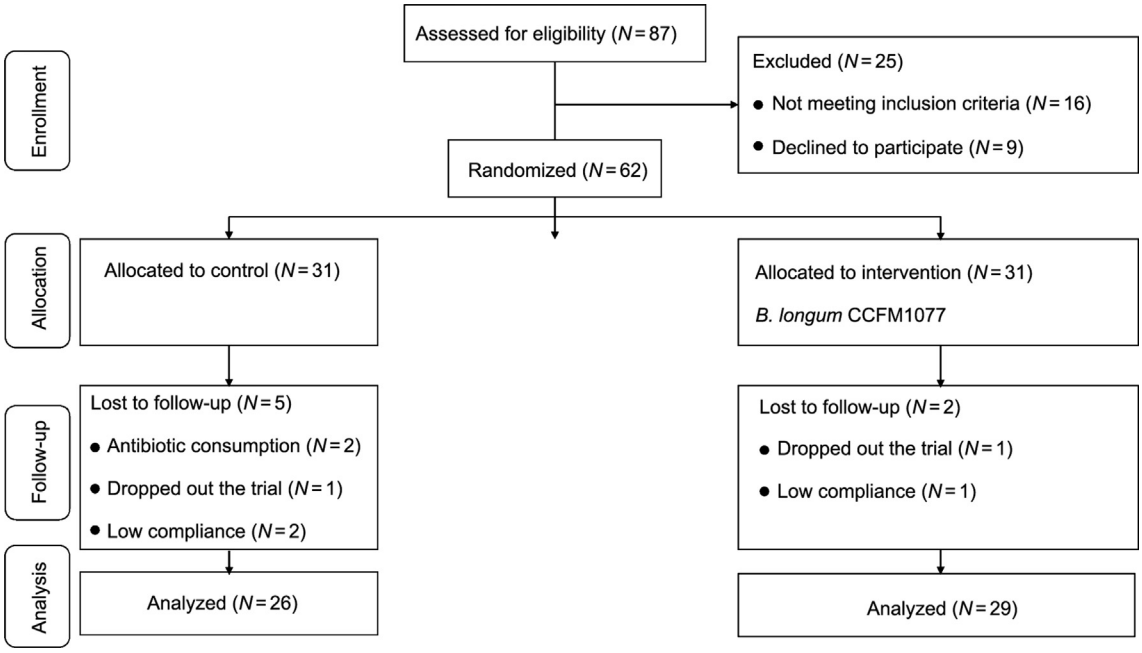

Fig. 1. Flowchart of the status of the participants in the *B. longum* CCFM1077 and placebo groups.

Table 1
Baseline demographic and clinical characteristics of the recruited patients.

Characteristics	Placebo	*B. longum* CCFM1077
Demographic parameter		
N	26	29
Age (year)	58.27 ± 8.39	55.76 ± 8.87
Male	10 (38.46%)	12 (41.38%)
Lifestyle factors		
Current smokers	4 (15.38%)	5 (17.24%)
Current drinkers	6 (23.08%)	8 (27.59%)
Current exerciser ($\geq$ 3 times·week^{-1})	5 (19.23%)	7 (24.14%)
Meat consumption ($\geq$ 1 meal·day^{-1})	20 (76.92%)	23 (79.31%)
Anthropometry parameters		
Height (m)	162.00 ± 7.94	165.28 ± 7.10
Weight (kg)	64.92 ± 10.83	71.28 ± 11.44
BMI (kg·m^{-2})	24.35 ± 2.67	26.02 ± 3.09
WC (cm)	80.83 ± 14.31	84.07 ± 8.76
HC (cm)	91.96 ± 13.06	96.44 ± 8.26
WHR	0.88 ± 0.08	0.87 ± 0.08
Thigh circumference (cm)	48.61 ± 3.93	52.04 ± 5.23
Family history of hyperlipidemia	12 (46.15%)	15 (51.72%)
Stroke	0 (0)	1 (3.45%)
Coronary heart disease	1 (3.85%)	1 (3.45%)
Clinical measures		
WBC (10^9 L^{-1})	6.11 ± 1.51	6.06 ± 0.87
RBC (10^9 L^{-1})	4.46 ± 0.33	4.67 ± 0.44
HGB (g·L^{-1})	136.10 ± 11.15	140.61 ± 16.55
PLT (10^9 L^{-1})	194.30 ± 44.00	223.54 ± 43.24
hs-CRP (mg·L^{-1})	1.86 ± 1.29	1.70 ± 0.99
ALP (U·L^{-1})	72.38 ± 15.12	79.38 ± 18.91
ALT (U·L^{-1})	23.31 ± 7.25	26.43 ± 12.82
AST (U·L^{-1})	23.58 ± 3.84	22.96 ± 5.75
TBIL (μmol·L^{-1})	18.21 ± 6.60	15.61 ± 5.91
BUN (mmol·L^{-1})	5.52 ± 0.96	5.22 ± 1.11
Cr (μmol·L^{-1})	69.23 ± 17.44	63.57 ± 12.98
Ua (μmol·L^{-1})	331.35 ± 78.63	362.04 ± 81.44
Glu (mmol·L^{-1})	5.74 ± 0.82	6.38 ± 2.45
SBP (mmHg)	127.88 ±15.78	128.59 ± 14.24
DBP (mmHg)	78.54 ± 11.25	82.74 ± 9.31

Continuous values are presented as means ± SD, and categorical measures are presented as number (proportion).
WHR: waist to hip ratio; WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; PLT: blood platelet; hs-CRP: high-sensitivity C-reactive protein; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; BUN: blood urea nitrogen; Cr: creatinine; Ua: uric acid; Glu: glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure.

and *Alistipes* (R = 0.5951, FDR-adjusted $p < 0.05$ (Fig. 3(d) and Fig. S1(c) in Appendix A). The data revealed that *B. longum* CCFM1077 treatment neutralized the disorder in gut microbiota composition, specifically of *Lactobacillus*, *Bifidobacterium*, *Butyricicoccus*, *Blautia*, *Lachnospiraceae_NK4A136_group*, *Ruminococcaceae_UCG_013*, *Bosea*, *Alistipes*, *Megamonas*, *Catenibacterium*, and *Ralstonia*.

3.5. The predicted functional profile of the gut microbiome differed between the B. longum CCFM1077 and the placebo groups

Based on the above analysis, the consumption of *B. longum* CCFM1077 altered the composition of the intestinal bacteria in

Table 2
Alternations in serum lipid levels (△) in the *B. longum* CCFM1077 and placebo group from the baseline to post-intervention.

Lipid profile or lipid level	Placebo (N = 26)	*B. longum* CCFM1077 (N = 29)	p
TC (mmol·L^{-1})			
Baseline	6.58 ± 0.85	6.85 ± 1.22	—
After intervention	6.54 ± 0.89	5.90 ± 1.18*	—
△	−0.04 ± 0.46	−0.95 ± 1.30	< 0.01
LDL-C (mmol·L^{-1})			
Baseline	4.24 ± 0.96	4.25 ± 0.82	—
After intervention	4.18 ± 0.95	3.66 ± 0.76*	—
△	0.06 ± 0.48	−0.59 ± 0.80	< 0.01
TG (mmol·L^{-1})			
Baseline	2.51 ± 1.29	2.39 ± 1.41	—
After intervention	2.34 ± 1.10	2.24 ± 1.00	—
△	−0.17 ± 0.96	−0.15 ± 1.10	0.9149
HDL-C (mmol·L^{-1})			
Baseline	1.39 ± 0.38	1.34 ± 0.30	—
After intervention	1.34 ± 0.31	1.31 ± 0.55	—
△	−0.05 ± 0.14	−0.03 ± 0.17	0.9265

All lipid profiles data is represented as means ± SD. The unit of lipid profiles at baseline and after intervention are presented by mmol·L^{-1}. Significant changes between baseline and post-intervention are shown as follows: *$p < 0.01$.
Independent t-test was used to compare changes in outcome measurements between *B. longum* CCFM1077 and placebo group. Significant differences between groups are presented by $p < 0.01$.

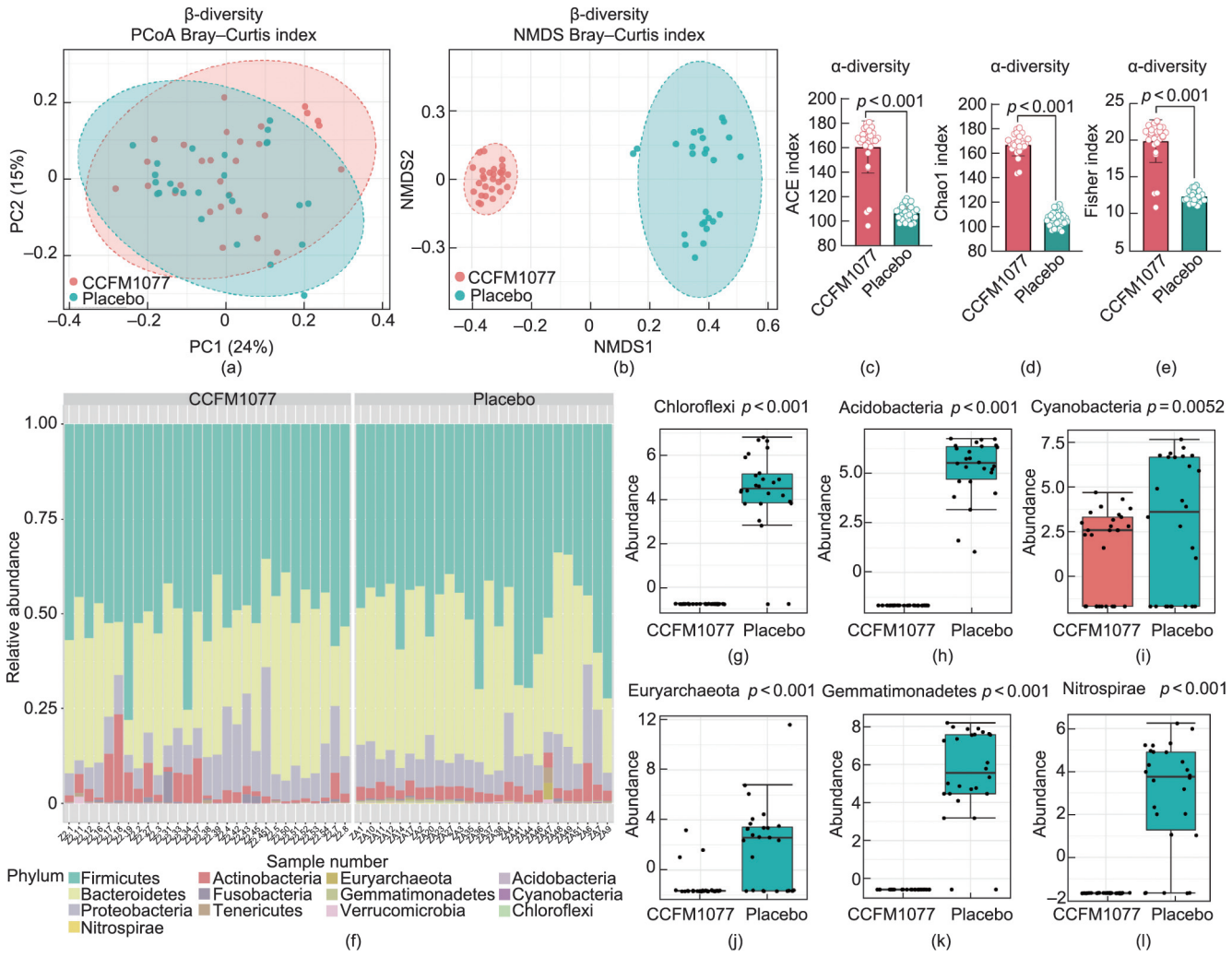

Fig. 2. The effect of *B. longum* CCFM1077 on the diversity of gut microbiota and compositions at the phylum. (a) PCoA score plot based on the Bray–Curtis dissimilarity for all participants. PERMANOVA of the Bray–Curtis dissimilarity index: *F*-value = 3.346, K^2 = 0.059348, $p < 0.001$. K^2 indicates the degree of explanation of the differences between samples by grouping factors, with larger K^2 indicating a higher degree of explanation of the differences by grouping factors. (b) β-diversity measured via NMDS. PERMANOVA of the Bray–Curtis dissimilarity index: *F*-value = 3.46, K^2 = 0.059348, $p < 0.001$, NMDS stress = 0.065147. Stress < 0.2 indicate a marked difference between the two samples. (c–e) α-diversity indices as reflected by the ACE, Chao1, and Fisher indices. *p* were calculated using Mann–Whitney tests. (f) Stacked column bar graphs depict the relative abundance of bacteria at the phylum level for individual subjects. (g–l) Classical univariate statistical comparisons between selected phyla as measured using Mann–Whitney/Kruskal–Wallis tests/zero-inflated Gaussian fit models. Significance was considered for false discovery rate (FDR)-adjusted $p < 0.05$. The *y*-axis represents the log-transformed count.

patients with hyperlipidemia. Consequently, *B. longum* CCFM1077 may result in alterations not only in the gene function of gut bacteria but also in their associated metabolic pathways. The phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) [28] method was used to predict the gene functions of gut bacteria in patients based on 16S rRNA sequencing information. As shown in Fig. 4(a), there was apparent variance in the functional predictions of the intestinal microbiota between the two treatment groups, with PC1 and PC2 contributing to 69.3% and 14.1%, respectively, of the total differences. A convergence overview of the predicted metabolic pathways revealed multiple differentially enriched pathways in these two categories (Fig. S1(d) in Appendix A). At Kyoto Encyclopedia of Genes and Genomes (KEGG) level II, the main differential pathways between *B. longum* CCFM1077 group and the placebo group included drug resistance: antimicrobial ($p < 0.001$), energy metabolism ($p < 0.001$), carbohydrate metabolism ($p < 0.001$), and nucleotide metabolism ($p = 0.022$) (Fig. 4(b)).

Following *B. longum* CCFM1077 intervention, 46 enriched metabolic pathways showed significant differences at KEGG level III (Fig. 4(c)). The altered gene functions of the gut microbiome after *B. longum* CCFM1077 treatment were mostly involved in amino sugar and nucleotide sugar metabolism ($p < 0.001$), fructose and mannose metabolism ($p = 0.015$), carbon fixation in photosynthetic organisms ($p = 0.014$), *D*-glutamine and *D*-glutamate metabolism ($p < 0.001$), methane metabolism ($p = 0.022$), the pentose phosphate pathway ($p = 0.022$), and glycolysis/gluconeogenesis ($p = 0.024$). As a result, these metabolic pathways may be associated with alleviating lipid accumulation in the serum of patients with hyperlipidemia.

3.6. B. longum *CCFM1077 affected the fecal metabolites of patients with hyperlipidemia*

Alterations in the composition of the gut microbiome and bacterial metabolites affect human metabolism and are the root cause of many metabolic illnesses. Feces derived from two groups were analyzed using non-targeted metabolomics to analyze the changes in the gut bacterial metabolites of patients with hyperlipidemia after *B. longum* CCFM1077 treatment. After combining the chromatographic data of positive and negative ions, 112 endogenous metabolites were obtained. The differences in metabolites between

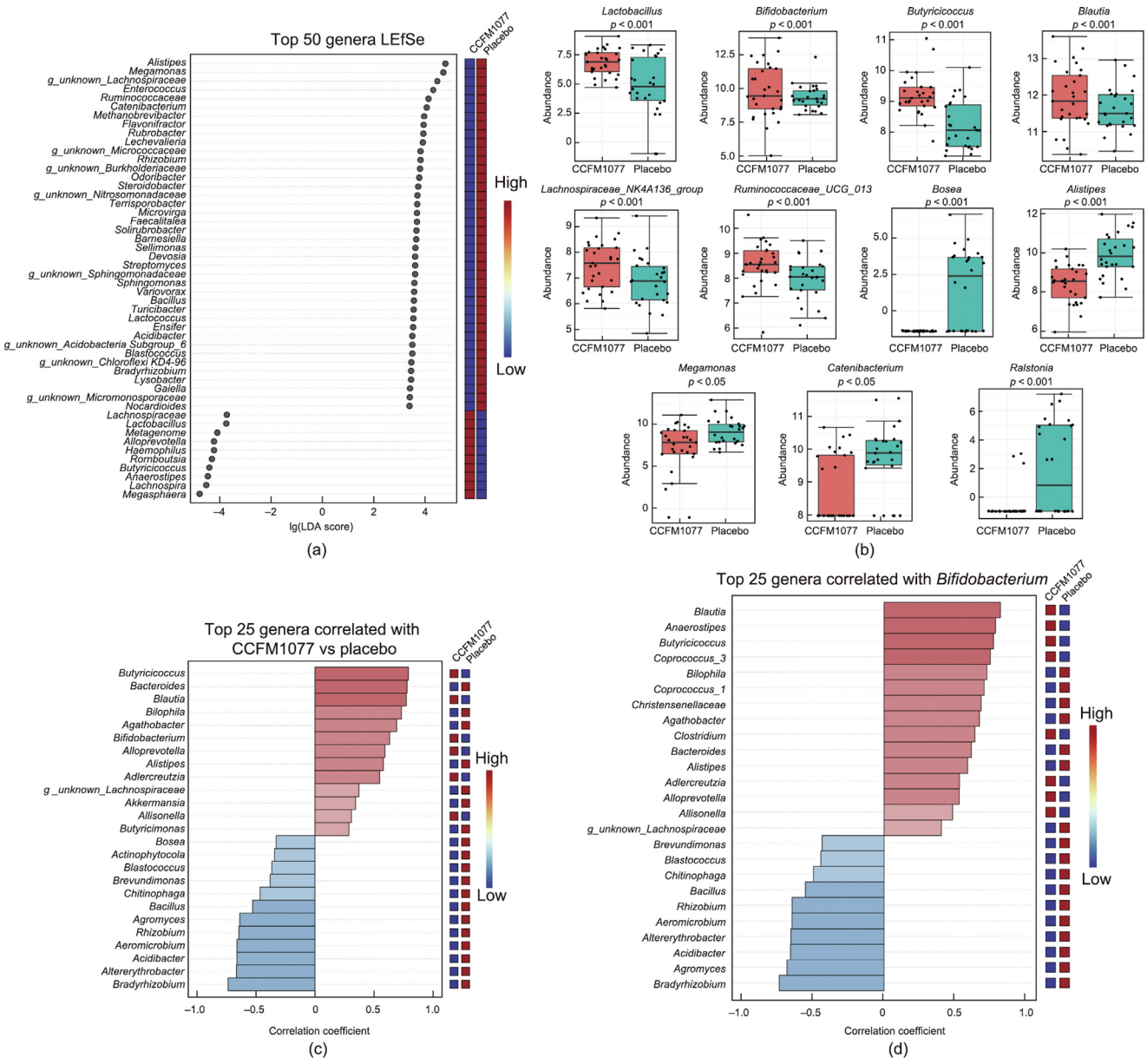

Fig. 3. Key genera identified via LEfSe and correlation analysis of core microbes between the *B. longum* CCFM1077 and placebo groups. (a) The top 50 genera from the LDA results of the gut microbiota phylotypes (lg(LDA score) > 3, FDR-adjusted $p < 0.05$). (b) Significant genera obtained from the differential abundance analysis DESeq2 (FDR-adjusted $p < 0.05$). (c) The top 25 genera from pattern search analysis based on the SparCC arithmetic (*B. longum* CCFM1077 group vs placebo group pattern). (d) Pattern search analysis via SparCC showing positive and negative correlations of *Bifidobacterium*. The bar chart (left) and the heat map (right) show the correlation between the top 25 genera.

the two groups were compared using orthogonal partial least squares-discriminant analysis (OPLS-DA) (Fig. 5(a)). The OPLS-DA plot showed that the fecal metabolites of the two hyperlipidemic populations were significantly different, indicating that the metabolites of the gut microbiome in subjects with hyperlipidemia supplemented with *B. longum* CCFM1077 were considerably different (Fig. 5(a)). The quality of the model was evaluated, and the results showed a valid predictive capacity (Q2 = 0.774) and significant explanatory power (R2Y = 0.861) as well as an acceptable OPLS-DA model fit, as ascertained by a 1000-fold permutation test ($p < 0.001$) (Fig. 5(b)). The synchronized three dimensional (3D) partial least squares-discriminant analysis (PLS-DA) plots further described 39.8% of the total variance, including Component 1 (13.1%), Component 2 (14.4%), and Component 3 (12.3%). Furthermore, the two groups were well separated (Fig. 5(c)). We also identified key metabolite signatures differentiating the two groups using a volcano plot. A total of 33 metabolites that met the criteria of the adjusted $p < 0.05$ and $|\log_2(FC)| > 1$ (FC: fold change) between the *B. longum* CCFM1077 and placebo groups were eventually identified as potential biomarkers (Table S2 in Appendix A). *B. longum* CCFM1077 upregulated 18 and downregulated 16 metabolites compared to the placebo group (Fig. 5(d)). To visualize and effectively characterize the differential metabolites between these two groups, we extracted the top 30 compounds to construct a heat map (variable important in projection (VIP) > 1, $p < 0.05$) (Fig. 5(e)).

3.7. Metabolic pathway enrichment analysis of key differential fecal metabolites

The differential metabolites were classified according to functional metabolic pathways based on the KEGG database, followed

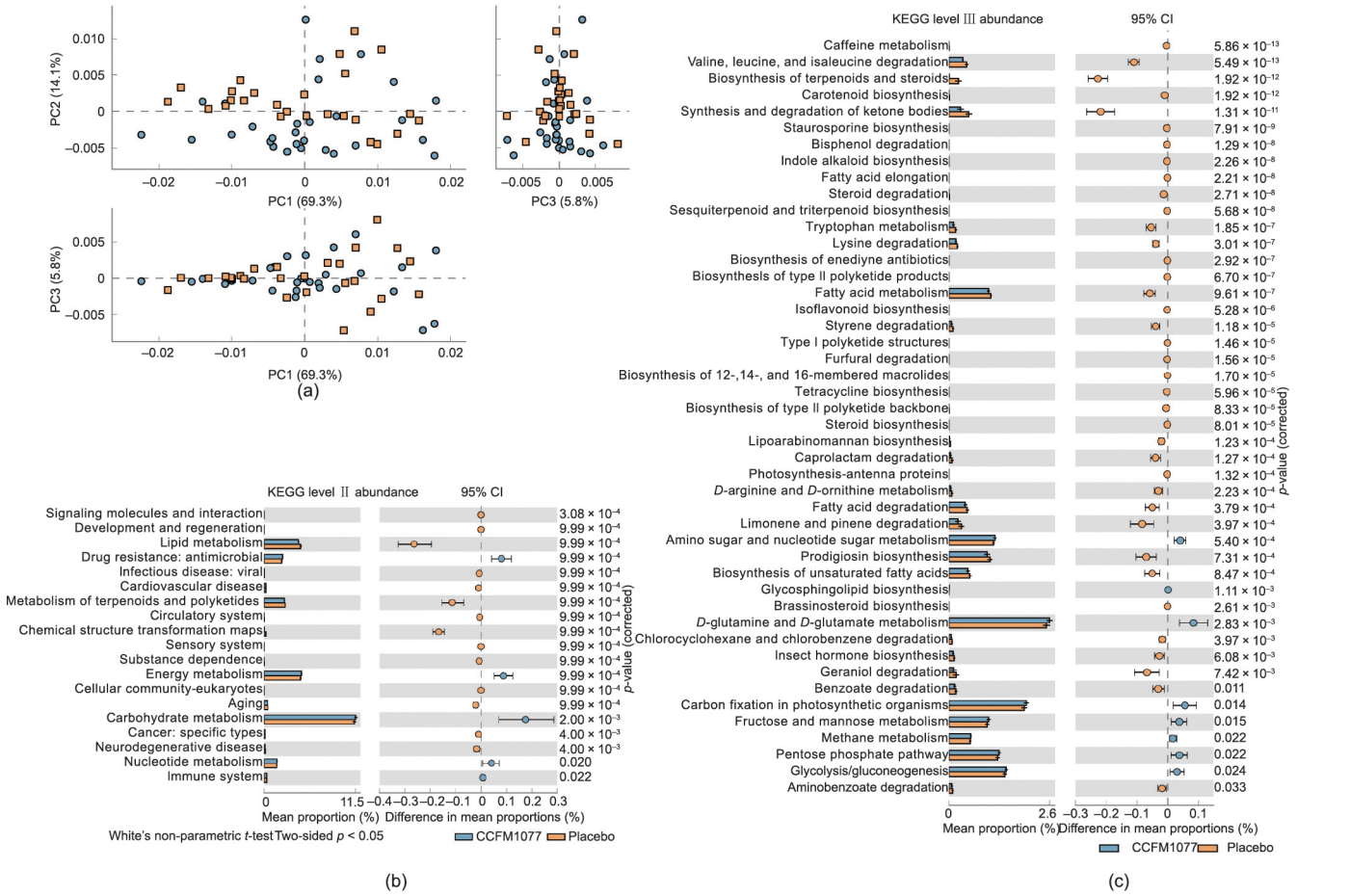

Fig. 4. PICRUSt-predicted functional metabolic pathways via 16S rRNA sequencing between the *B. longum* CCFM1077 and placebo groups. (a) PCoA analysis. Significantly altered predicted functional metabolic pathways based on KEGG analysis at (b) level II and (c) level III between the *B. longum* CCFM1077 and placebo groups. Significant metabolic pathways are displayed with their corresponding 95% CIs (Welch's *t*-test, $p < 0.05$).

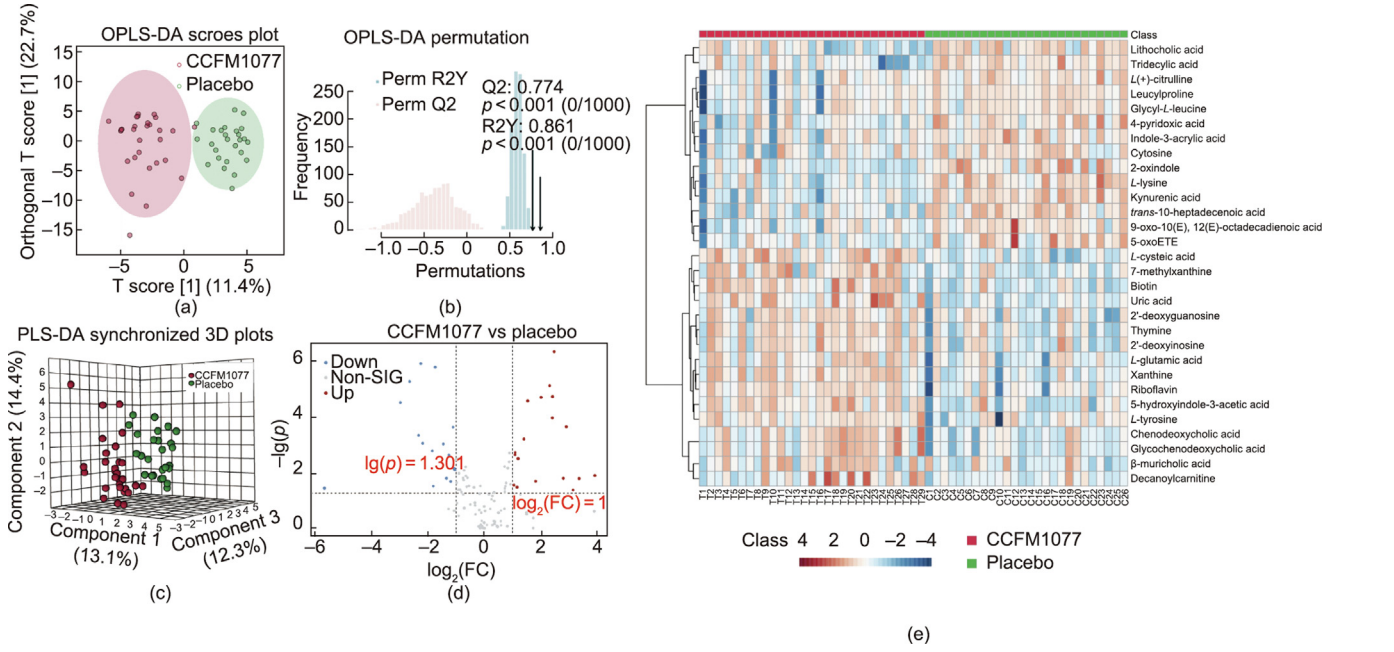

Fig. 5. *B. longum* CCFM1077 regulates the fecal metabolites during hyperlipidemia. (a) Score plot of the OPLS-DA model constructed using combined 112 different metabolites in the positive and negative ion modes. (b) Permutation testing of the OPLS-DA model using 1000 iterations (Q2, $p < 0.001$; R2Y, $p < 0.001$). (c) PLS-DA synchronized 3D plot of metabolite composition observed in the two groups. (d) Altered metabolites based on volcano plot analysis with $p < 0.05$ and $|\log_2(FC)| > 1$ between the *B. longum* CCFM1077 and placebo groups. (e) Heat map showing the abundance of the top 30 differential metabolites for each sample in each group (VIP > 1, $p < 0.05$).

by metabolic pathway enrichment analysis using Fisher's exact test and the Benjamini and Hochberg multiple correction method. The main pathways affected by *B. longum* CCFM1077 treatment were biotin metabolism ($p < 0.0001$), riboflavin metabolism ($p = 0.0053099$), tryptophan metabolism ($p = 0.0010933$), caffeine metabolism ($p = 0.0038485$), purine metabolism ($p = 0.0021886$), pyrimidine metabolism ($p = 0.0053099$), primary BA biosynthesis ($p = 0.0011178$), taurine and hypotaurine metabolism ($p < 0.0001$), glutathione metabolism ($p = 0.0060643$), aminoacyl-transfer RNA (tRNA) biosynthesis ($p = 0.00993$), and pantothenate and coenzyme A (CoA) biosynthesis ($p = 0.018151$) (Fig. 6(a)). The level of biotin was higher in the *B. longum* CCFM1077 group than in the placebo group ($p < 0.001$) (Fig. 6(b)). To obtain further insights into the effect of *B. longum* CCFM1077 on BA biosynthesis, we quantitatively analyzed the fecal metabolites related to the BA biosynthesis pathway. The levels of cholic acid (CA) ($p = 0.0559$), chenodeoxycholic acid (CDCA) ($p = 0.000212$), taurocholic acid ($p = 0.0147$), taurochenodeoxycholic acid ($p = 0.0388$), and glycochenodeoxycholic acid ($p = 0.000212$) were higher in the *B. longum* CCFM1077 group than in the placebo group (Figs. 6(c) and (d)), indicating that *B. longum* CCFM1077 can relieve hypercholesterolemia by promoting the excretion of fecal BAs, thus

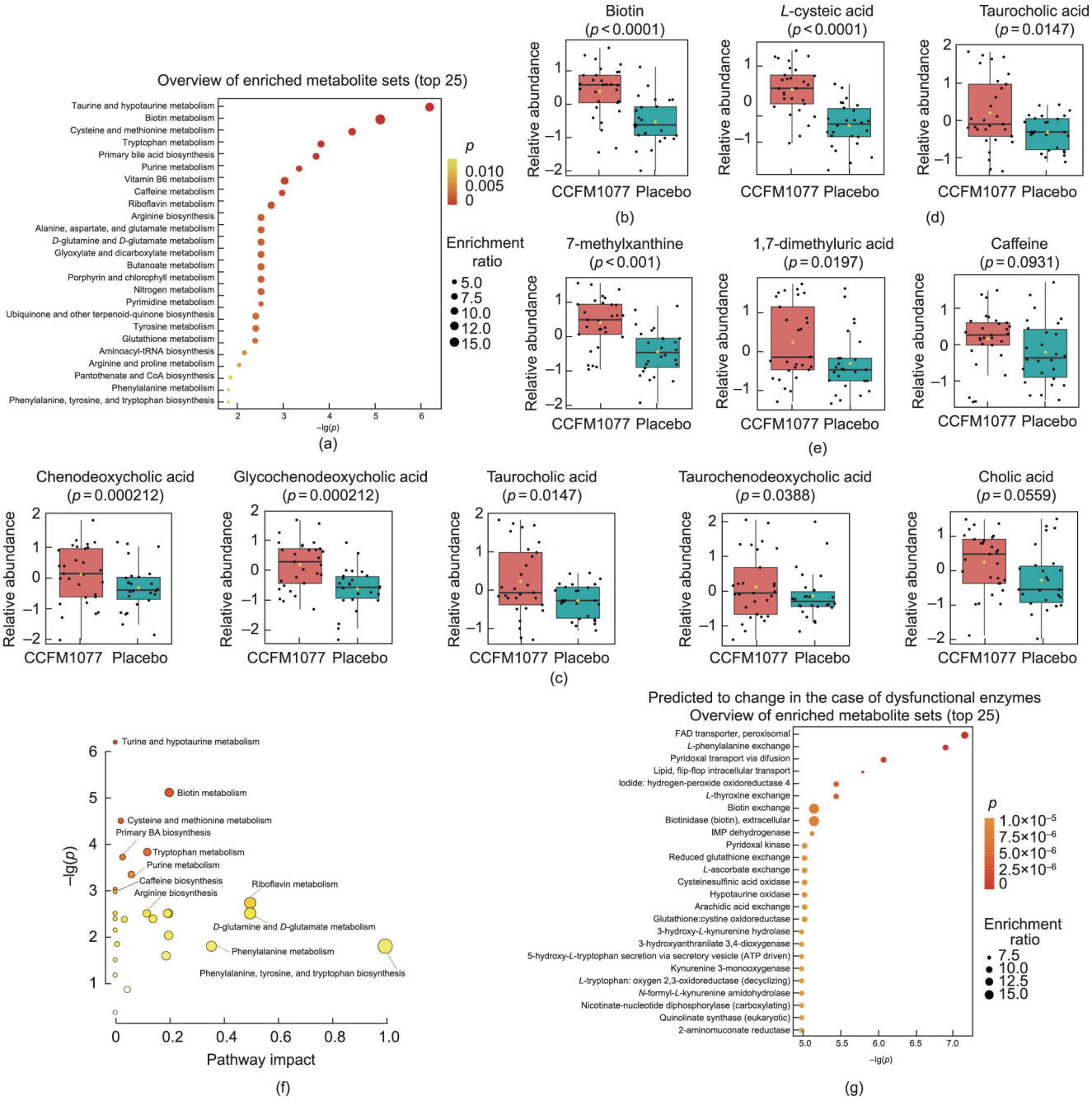

Fig. 6. Metabolic pathway enrichment analysis of key differential fecal metabolites. (a) Bubble plot showing the enriched metabolic pathways based on KEGG in MetaboAnalyst 5.0 ($p < 0.05$). (b) Biotin levels in the two groups. (c) Levels of primary BA biosynthesis-related metabolites. (d) Changes in the abundance of taurine and hypotaurine metabolism-related metabolites. (e) Changes in the abundance of caffeine metabolism-related metabolites. (f) KEGG pathway enrichment pathway analysis of differential metabolites between the *B. longum* CCFM1077 and placebo groups. (g) Enzyme enrichment analysis of differential fecal metabolites based on 912 metabolic sets predicted to change in the case of dysfunctional enzymes.

consuming more cholesterol and resulting in lower blood cholesterol. Hyperlipidemia is usually accompanied by abnormal purine metabolism [29]. We quantitatively analyzed fecal metabolites related to the purine metabolism pathway. The levels of xanthine ($p < 0.0001$), uric acid ($p < 0.0001$), 2′-deoxyguanosine ($p = 0.00209$), 2′-deoxyinosine ($p = 0.00193$), adenine ($p = 0.0442$), and hypoxanthine ($p = 0.0282$) were higher in the *B. longum* CCFM1077 group than in the placebo group (Fig. S2(a)), indicating that *B. longum* CCFM1077 can relieve hypercholesterolemia in part by modifying purine metabolism. Caffeine metabolism is a key intervention in preventing fat deposition and metabolic disorders, and we found that caffeine metabolism-related metabolites, including 7-methylxanthine ($p < 0.001$), 1,7-dimethyluric acid ($p = 0.0197$), and caffeine ($p = 0.0931$), were selectively enriched in the *B. longum* CCFM1077 group (Fig. 6(e)). Moreover, we quantitatively analyzed fecal metabolites related to hypercholesterolemia metabolic pathways, including pyrimidine, tryptophan, aminoacyl-tRNA, glutathione, pantothenate and CoA, and riboflavin metabolism. More detailed quantitative information is shown in Figs. S2 and S3 in Appendix A. Pathway enrichment analysis was then performed based on the differential metabolites. The seven pathways differentially enriched between the *B. longum* CCFM1077 and placebo groups were taurine and hypotaurine metabolism ($p < 0.0001$), biotin metabolism ($p < 0.001$), cysteine and methionine metabolism ($p < 0.001$), tryptophan metabolism ($p = 0.0010933$), primary BA biosynthesis ($p = 0.0011178$), purine metabolism ($p = 0.0021886$), and caffeine biosynthesis ($p = 0.0038485$) (Fig. 6(f)). Enzyme enrichment analysis using least absolute shrinkage and selection operator (LASSO) regression was also performed on the 912 metabolic sets expected to be altered in the case of dysfunctional enzymes (Fig. 6(g)). Two extracellular enzyme-related pathways, biotin exchange ($p < 0.0001$) and biotinidase (biotin), extracellular ($p < 0.0001$) were expected to be significantly increased in the *B. longum* CCFM1077 group. Five other enzyme-related pathways, including (flavin adenine dinucleotide) FAD transporter, peroxisomal ($p < 0.0001$), *L*-phenylalanine exchange ($p < 0.0001$), lipid, flip-flop intracellular transport ($p < 0.0001$), pyridoxal transport via diffusion ($p < 0.0001$) and Iodide:hydrogen-peroxide oxidoreductase 4 ($p < 0.0001$) were also predicted to be significantly increased in the *B. longum* CCFM1077 group. The results of enzyme enrichment analysis are shown in Table S3 in Appendix A. These results suggest that altered fecal metabolites following *B. longum* CCFM1077 treatment are beneficial in alleviating hyperlipidemia.

4. Discussion

To the best of our knowledge, this study is the first to report the alleviating effect of the probiotic *B. longum* CCFM1077 on hyperlipidemia by modulating BA biosynthesis, thereby both increasing microbial diversity and inducing favorable gut microbiota alterations. The data from this study suggest that intervention with *B. longum* CCFM1077 for six weeks regulated the serum lipid profiles of patients with hyperlipidemia. Compared with subjects in the placebo group, *B. longum* CCFM1077 significantly lowered TC levels by 5.7% and LDL-C levels by 4.7% without affecting TG and HDL-C levels. These findings may indicate a potential mechanism underlying the lipid regulatory effects in Chinese patients with hyperlipidemia following probiotic treatment.

Previous RCTs on the efficacy of probiotics in patients with hyperlipidemia were mainly conducted in Europe and the United States [17]. Different strains have been reported to alleviate hyperlipidemia by decreasing TC and LDL-C levels in several countries [6]. In a British urban-based RCT, *L. plantarum* ECGC effectively lowered TC and LDL-C levels in patients [20]. Similarly, *L. acidophilus* DDS-1, *L. reuteri* NCIMB 30, and *L. fermentum* have shown positive mitigating effects against hyperlipidemia in the United States [30], the Czech Republic [21], and Australia [31], respectively. In contrast, only two randomized RCTs in Asian countries have demonstrated the efficacy of using probiotics to regulate dyslipidemia in the past few years [32,33]. Thus, our results seem to fill the gap in knowledge regarding the efficacy of probiotics in treating hyperlipidemia in the Chinese population. In addition, most of the RCTs on the use of probiotics for alleviating hyperlipidemia have focused on *Lactobacillus,* suggesting that *B. longum* CCFM1077 isolated from one of the oldest living Chinese people (aged 93 years) may have some unique genetic and physiological properties. Future studies will need to utilize multi-omics techniques, such as comparative genomics and metabolomics, to study the functional genes and molecular pathways underlying the ability of this stain to exert lipid-lowering effects.

Recent population-based association studies have shown that the composition of individual gut microbiota can explain the differences in blood TG and HDL-C levels between individuals and can be a good predictor of metabolic responses to diet and drugs [34]. We have previously observed that *L. reuteri* A9 and *L. mucosae* A13 could alleviate hyperlipidemia by increasing gut microbial diversity and the abundance of BSH-producing bacteria in a rat model of hypercholesterolemia [15]. Likewise, the administration of *L. plantarum* HNU082 specifically resulted in a significant increase in the abundance of the genera *Clostridium*, *Natranaerovirga*, and *Odoribacter*. In addition, the pathways of secondary BA were enriched after supplementation with probiotics [35]. As expected, patients with hyperlipidemia who adhered to *B. longum* CCFM1077 treatment showed differential gut microbial composition measured by β-diversity (Figs. 2(a) and (b)). The α-diversity of the gut microbiome showed a significant increase in the Chao1, ACE, and Fisher indices after *B. longum* CCFM1077 treatment compared with the placebo group, indicating that supplementation with *B. longum* CCFM1077 increased the diversity of the fecal microbiota (Figs. 2(c)–(e)) The composition of the fecal microbiota at the phylum level is shown in Fig. 2(f). In individuals with obesity and type 2 diabetes mellitus (T2DM), the phyla Chloroflexi, Acidobacteria, Gemmatimonadetes, and Euryarchaeota are overrepresented [36–38]. Intriguingly, the populations of the four genera mentioned were remarkably decreased in the *B. longum* CCFM1077 group, indicating that dysbiosis of the gut microbiome in hyperlipidemia was amended by *B. longum* CCFM1077.

As shown in Fig. 3(a), compared to the placebo group, the relative abundance of *Lactobacillus*, *Butyricicoccus*, *Bifidobacterium*, *Blautia, Lachnospiraceae_NK4A136_group*, and *Ruminococcaceae_UCG_013* remarkably increased after intervention with *B. longum* CCFM1077 ($p < 0.05$). In contrast, the relative abundances of the genera *Alistipes*, *Megamonas*, *Catenibacterium*, and *Ralstonia* were lower in the *B. longum* CCFM1077 intervention group. The four genera mentioned above have been corroborated by various reports that investigated their obesity-promoting effects [39–41]. An increased abundance of *Bosea* in the blood and feces has been observed in patients with obesity and liver fibrosis [42]. *Ralstonia* exacerbates glucose tolerance in diet-induced obese mice and has been reported to have a considerably higher abundance in obese patients with T2DM. The three *Ralstonia* lipopolysaccharides explain the low-grade inflammation observed in patients with obesity [43,44]. *Butyricicoccus*, a butyrate-producing bacterium, acts as a biomarker for predicting obesity-related metabolic abnormalities [45]. *Butyricicoccus pullicaecorum* 25-3 was safe, well-tolerated, and possessed the potential to become a second-generation probiotic in a safety evaluation trial of 30 healthy volunteers [46]. *Blautia*, a new functional genus with potential probiotic properties [47], was significantly and negatively associated with the visceral fat area, independent of sex factors in a cross-sectional

study that included 1001 Japanese individuals aged 20–76 years [48]. Similarly, another recent report found that the abundance of *Blautia wexlerae* was negatively associated with the risk of obesity and T2DM, and that oral administration of *Blautia wexlerae* to mice alleviated the weight and inflammatory phenotypes associated with obesity and diabetes while altering the composition of the intestinal microbiota [49]. In addition, certain *Bifidobacterium* or *Lactobacillus* strains have shown anti-obesity effects [50]. Taken together, the serum lipid-lowering effects of *B. longum* CCFM1077 may be achieved by increasing the proportion of anti-obesity-related bacteria and reducing that of obesity-promoting-related bacteria.

Gut microbes affect human physiological health via their metabolites, and some species/strains and their metabolites have shown strong associations with metabolic diseases [51]. In this study, we found that, after supplementation with *B. longum* CCFM1077, primary BAs, including CA and CDCA, were significantly decreased, whereas secondary BAs, including lithocholic acid (LCA) and deoxycholic acid (DCA), were remarkably increased compared to that in the placebo-treated group (Figs. 6(c) and (d)). This result suggests that *B. longum* CCFM1077 may enhance the transformation from primary BAs to secondary BAs by remodeling the gut microbiota composition.

BAs, the primary constituents of bile, are amphiphilic steroid molecules that can reach and influence metabolic activities in multiple organs. BAs play an essential role in cholesterol metabolism, including assisting the digestion and absorption of lipids, serving as signaling molecules to activate the BA receptors FXR and TGR5, stimulating bile secretion from hepatocytes, and eliminating bile stasis, among others [52]. There is evidence that increased total BA levels exert antihyperlipidemic benefits [53]. The human BA pool comprises of two types: primary and secondary BAs. In the liver, BAs synthesized by hepatocytes directly from cholesterol are called primary BAs and include CA, CDCA, and their combination products with glycine or taurine. Secondary BAs, comprises mainly DCA, LCA, and their binding products on combining with glycine or taurine [54], and are produced after the primary BAs are deoxygenated via 7α-hydroxylation via the action of intestinal bacteria. The levels of CA, CDCA, taurocholic acid, taurochenodeoxycholic acid, and glycochenodeoxycholic acid were higher in the *B. longum* CCFM1077 group (Figs. 6(c) and (d)), suggesting one of the mechanisms of the cholesterol lowering efficacy of *B. longum* CCFM1077 is to convert cholesterol into BAs faster via a feedback mechanism, thereby reducing intracellular cholesterol content, promoting increased LDL receptor synthesis, accelerating LDL metabolism, and reducing the concentration of TC in the blood.

The fecal excretion of BAs and their metabolites is thought to be a crucial factor in lipid metabolism because it is one of the modes of lipid elimination in humans. Intestinal bacteria metabolize BAs via decoupling and dehydroxylation, increasing the diversity of their BAs [55]. The ability of gut bacteria to dissociate and convert primary BAs differs between patients with hyperlipidemia and healthy individuals is due to differences in the composition of their gut microbiome. Some intestinal bacteria have been reported to interact with BAs and modify BA metabolism via multiple modes of action: ① deconjugation of BAs by producing BSHs, such as in *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*; ② dehydrogenation of BAs by producing BA hydroxysteroid dehydrogenases, such as in *Ruminococcus*, *Clostridium*, and *Peptostreptococcus*; and ③ esterification and desulfation of BAs, such as in *Clostridium*, *Bacteroides*, *Peptococcus*, and *Lactobacillus* [55]. Similarly, data from 16S rDNA gut microbiota sequencing showed that *B. longum* CCFM1077 treatment remarkably increased the relative abundance of *Ruminococcaceae_UCG-013*, *Bifidobacterium*, and *Lactobacillus* compared to the placebo group (Figs. 3(a) and (b)). Therefore, *B. longum* CCFM1077 improves the composition of the intestinal microbiota to increase the action of enzymes in the colonic environment of patients with hyperlipidemia, synthesizes more BAs to reach the liver, and reduces TC accumulation in the serum. However, previous studies have indicated that *B. longum* CCFM1077 can exert cholesterol-lowering effects by increasing BSH activity in a cholesterol-induced hyperlipidemia model in Sprague-Dawley rats [25], suggesting that it also has a cholesterol-lowering effect by itself.

Biotin supplementation has been shown to markedly reduce plasma triacylglycerol and very low-density lipoprotein (VLDL) levels in patients with non-diabetic hypertriglyceridemia [56]. Hence, the TG-lowering effect of biotin suggests that it can be used to treat hypertriglyceridemia. Surprisingly, biotin metabolism was found to be the top metabolic pathway in this study. The relative abundance of biotin increased after supplementation with *B. longum* CCFM1077 (Fig. 6(b)). Moreover, metabolic pathways, such as purine metabolism [9,57], caffeine metabolism [58], pyrimidine metabolism [59], tryptophan metabolism [60], aminoacyl-tRNA biosynthesis [61], glutathione metabolism [62], pantothenate and CoA biosynthesis [63], and riboflavin metabolism [64] have been reported to show lipid-lowering effects. Coincidentally, the pathways mentioned above were enriched in the *B. longum* CCFM1077 group (Fig. 6 and Figs. S2 and S3).

5. Conclusions

Our findings suggest that *B. longum* CCFM1077 significantly decreased TC and LDL-C levels in the serum of patients with hyperlipidemia. *B. longum* CCFM1077 reshaped the gut microbiota composition and increased intestinal bacterial diversity. The lipid-lowering efficacy of *B. longum* CCFM1077 may be attributed to the increase in the relative abundance of anti-obesity-related bacteria and a reduction in obesity-associated bacterial genera. In addition, the enrichment of key metabolites (BA, biotin, and caffeine) and their corresponding metabolic pathways (primary BA biosynthesis, taurine and hypotaurine metabolism, biotin metabolism, purine metabolism, and caffeine metabolism) after supplementation with *B. longum* CCFM1077 may lower lipid levels in patients with hyperlipidemia. Our findings validate the lipid-lowering effect of probiotics and provide a foundation for investigating the mechanism of lipid reduction in individuals with hyperlipidemia.

Acknowledgments

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Compliance with ethics guidelines

Chuanqi Chu, Jinchi Jiang, Leilei Yu, Yiwen Li, Songli Zhang, Wei Zhou, Qun Wang, Jianxin Zhao, Qixiao Zhai, Fengwei Tian, and Wei Chen declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2023.04.010.

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