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Multi-omics analysis of the effects of dietary changes and probiotics on diet-induced obesity

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ABSTRACT

The consumption of a healthy diet is critical for maintaining and promoting human health. In the context of the rapid transformation from a high-fat diet (HFD) to a Mediterranean diet (MD) leading to major systemic changes, we explored the necessity of a transitional standard diet (TSD) between these two varied diets and the adjuvant effect of probiotics. HFD-fed mice were used for studying the changes and benefits of a dietary intervention and probiotic treatment. By measuring multiple systemic alterations such as weight (group B vs. group E, $P < 0.05$), liver function (AST, group C vs. group E, $P < 0.001$), and histopathology, we found that an MD, TSD and *Bifidobacterium longum* all contribute to alleviating lipid deposition and liver injury. The downregulation of IL-17 (group B vs. group E, $P < 0.01$) and MIP-1α (group B vs. group E, $P < 0.001$) also demonstrated the anti-inflammatory effects of the TSD. Moreover, we performed multi-omics analysis combined with the 16S sequencing, transcriptome and metabolome results and found that the TSD increased the abundance of the *Lactobacillus* genus (group C vs. group E, $P < 0.01$) and effectively lowered lipid accumulation and systemic inflammation. Furthermore, *B. longum* played an important role in the synergistic effect. The results showed that a TSD might be useful for HFD-induced obesity before drastic dietary changes, and probiotics were also beneficial.

1. Introduction

High-fat diet pattern is an extremely common dietary pattern in economically developed areas nowadays. Within the context of the harm of an HFD, the morbidity rates of nonalcoholic fatty liver disease (NAFLD), which was defined as more than 5% of hepatocytes accumulating lipids, have been substantially increasing (Aron-Wisnewsky et al., 2020). A high-fat dietary habit gradually leads to the development of various liver diseases through the accumulation of triacylglycerols in the liver (Hodson and Gunn, 2019), such as fatty liver, liver cancer (Broadfield et al., 2021) et al. In addition, a continuous HFD can lead to a range of metabolism-related diseases and intestinal disorders, such as obesity, diabetes (Serino et al., 2012), altered intestinal permeability (Bowser et al., 2020), altered intestinal flora distribution (Tomas et al., 2016; Lin et al., 2022) and constipation (Tan et al., 2021). A trial of 217 nonobese young adults consuming varying proportions of a HFD was conducted by scientists over 6 months. Changes such as significantly lowered short-chain fatty acids, increased plasma proinflammatory factors, and increased *Alistipes* spp. and *Mycobacterium* spp. suggested that a HFD may lead to long-term health risks (Wan et al., 2019). Due to the harsh effects of HFD-induced fat accumulation, the issue of dietary pattern shift should be further investigated.

Known as the anti-inflammatory diet, the Mediterranean diet represents a healthy weight loss diet (Romero-Gomez et al., 2017; Muralidharan et al., 2021). The MD can improve fat accumulation and has anti-inflammatory effects due to its diverse and rational composition.

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Table 1
Composition of standard diet (STD), mediterranean diet (MD), and high fat diet (HFD).

Ingredient	STD	MD	HFD
	gm	gm	gm
Casein	200	44	200
Fish Protein Isolate	0	27	0
Maltodextrin 10	0	0	125
Egg White	0	9	0
Beef, Cooked	0	61.9	0
Lard	0	0	245
L-Cystine	3	3	3
Corn Starch	641	0	0
Wheat tarch	0	365	0
Sucrose	0	61	68.8
Fructose	0	19	0
Cellulose	75	40.7	50
Inulin	25	13.5	0
Soybean Oil	70	0	25
Menhaden Oil (200 ppm tBHQ)	0	13.2	0
Palm Kernal Oil	0	9.9	0
Butter, Anhydrous	0	7.4	0
Flaxseed Oil	0	6.1	0
Olive Oil	0	117.7	0
t-BHQ	0.0049	0.0023	0
Mineral Mix S10026	10	10	10
Dicalcium Phosphate	13	13	13
Calcium Carbonate	5.5	5.5	5.5
Potassium Citrate, 1 H20	16.5	16.5	16.5
Vitamin Mix V10001	10	10	10
Biotin (1%)	0	0.014	0
Choline Bitartrate	2	2	2
Red Wine Extract	0	0.045	0
FD&C Red Dye #5	0	0.05	0.05
FD&C Yellow Dye #40	0.05	0	0
Total	1071.0549	855.5113	773.85

Moreover, the MD can protect against the formation of chronic diseases such as NAFLD (Hydes et al., 2020), colon cancer (Piazzi et al., 2019), and breast cancer (Augimeri et al., 2021). Cancello et al. (2019) performed a clinical study based on a short-term MD and probiotic intervention in older obese women, whereas the optimal duration and intensity of dietary interventions for obese groups is not yet further studied(Hoare et al., 2021). In our study, we assumed that a regular diet should be consumed before switching to a healthy diet for people with unhealthy diet habits drastically, and we innovatively proposed a novel concept-TSD. Additionally, as a potential lipid-lowering probiotic, the anti-obesity function of *B. longum* was widely verified(Rahman et al., 2021; Schellekens et al., 2021) by several studies. However, the synergistic effects of *B. longum* combined with dietary patterns have never been further explored yet.

The study was designed to further explore not only the potential effects of Mediterranean diet and TSD intervention but also the synergic effect of lipid-lowering probiotic on HFD-induced obese mice. Based on a 4-week HFD-induced obesity model (Chai et al., 2020), we subsequently examined various physiological alterations of obese mice by dietary interventions and probiotic administration and multi-omics studies including 16S rRNA sequencing, transcriptome and metabolome were applied for tracking specific changes and potential mechanisms. Additionally, we performed a comprehensive correlation analysis between different omics, providing new theoretical basis and research perspective for further understanding of the occurrence and development of HFD-induced obesity.

2. Materials and methods

2.1. Probiotic culture

In an anaerobic bag at 37 °C, *Bifidobacterium longum* subsp. *Longum* (ATCC 15707) was cultured in trypticase-phytone-yeast broth medium (RiShui, Ltd., Qingdao, China) for 24 h. Subsequently, ice-cold saline was used to wash and configure the probiotic suspension at a concentration of 1×10^8 CFU/ml(Celiberto et al., 2017). Subsequently, it was administered to each mouse in groups D and F at 0.2 ml per day for 7 days.

2.2. Animal experiments

Eighty C57BL/6J mice (male, 5–6 weeks old, 20 ± 3 g, Zhejiang Experimental Animal Center, Zhejiang, China) were randomly divided into 8 groups, labeled A to H. After acclimatizing for 1 week, the mice in groups A to G were fed an HFD diet (PD6001, Changzhou SYSE Bio-Tec. Co., Ltd.) for a total of 4 weeks, and those in group H were fed standard diet (Table 1). 4 weeks later, groups G and H were sacrificed for model verification.

After the establishment of the obese model, the diet of the groups A, B, C were switched to chow, HFD and MD (PD12052702, Changzhou SYSE Bio-Tec. Co., Ltd.) respectively. In order to study the TSD, group E was fed chow for 1 week and then changed to MD for next week. For the purpose of studying probiotic, groups D and F was given *B. longum* gavage at week 5.

The mice were group-housed in a constant housed environment with 12 h of light/darkness and free access to food and water. The mice were weighed every week and fed every two days, and their diets were recorded (measured/cage, n = 5/cage). At the end of the experiment, all mice were anesthetized and sacrificed. This experiment was approved by the Animal Experimental Ethics Committee of the First Affiliated Hospital of Zhejiang University (no. 20221262).

2.3. Liver function test and cytokine assay

The collected blood samples were centrifuged, and the supernatant was isolated and frozen at −80 °C for liver function and cytokine testing. Serum alanine transaminase (ALT) and aspartate aminotransferase (AST) levels were measured using a Hitachi 7600-210 automated analyzer (Hitachi, Tokyo, Japan).

The serum cytokines were measured by a Bio-PlexTM Pro Mouse Cytokine Grp 1 Panel 23-Plex Assay Kit (Bio-Rad Laboratories, Inc.), including IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17α, IL-18, MCP-1, MIP-1α, MIP-3α, RANTES, TNF-α, IFN-γ, G-CSF, GM-CSF, GRO/KC, M-CSF, and VEGF. Then, the test was performed as recommended in the kit's operating manual(Yamawaki et al., 2016).

2.4. Histological examination

The liver and ileal tissues were fixed in 10% formalin overnight for histologic evaluation, followed by subsequent embedding and slicing. Afterward, hematoxylin-eosin (HE) staining was conducted according to routine protocols(Cardiff et al., 2014). For assessment of hepatic fat accumulation, Oil Red O staining was used in subsequent cryosections. Periodic acid-Schiff (PAS) staining was applied to measure liver sugars, while Masson trichrome staining was performed to highlight the fibers and inflammatory factors. After fixation in Carnoy's fluid, the mucus produced by the ileum was stained according to the manufacturer's protocol of the Alcian Blue Periodic Acid Schiff/AB-PAS Stain Kit (Solaibao, Beijing, China)(Genta et al., 2018). Panoramic Viewer Software (3DHISTECH, Budapest, Hungary) was used for image acquisition.

2.5. Transcriptome analysis

Total RNA was extracted from liver tissue cells, and then, the library was constructed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) for quality control. According to the principle of sequencing by synthesis, Illumina sequencing is performed after pooling different libraries given the requirements of effective concentration and target offline data amount. Through original

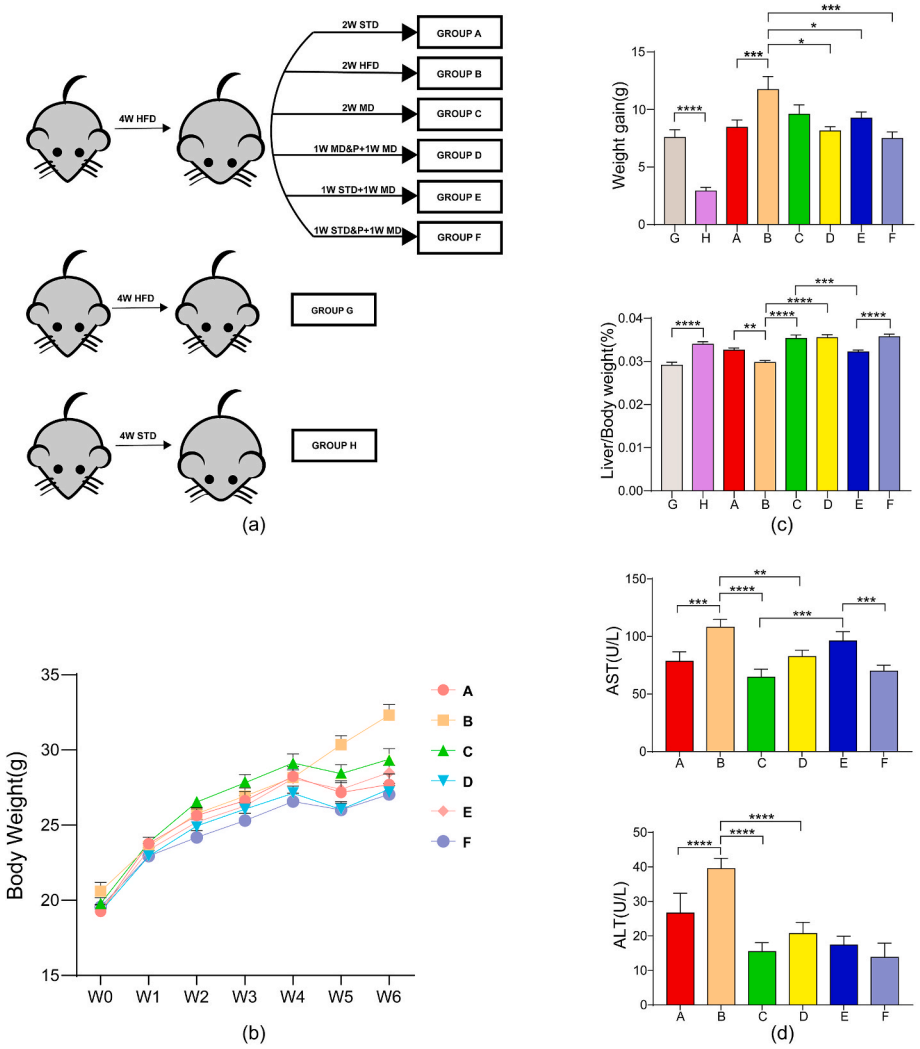

Fig. 1. Beneficial effects of dietary transition and *B. longum* administration on HFD-induced weight gain and liver damage. (a) Schematic design of the animal experiment. (b) Longitudinal body weight changes in the different groups. (c) Weight gain and liver-to-body weight ratios. (d) Serum levels of AST and ALT. Data are presented as the mean ± SE. n = 9, *P < 0,05, **P < 0.01, ***P < 0.001, ****P < 0.0001. HFD, high-fat diet; STD, standard diet; MD, Mediterranean diet; P, *Bifidobacterium longum*.

data filtering, sequencing error rate checking and GC-content distribution checking, clean reads were obtained. Then, clean reads were compared with the reference genome using HISAT2 software (v2.0.5) quickly and accurately(Jing et al., 2021). Differentially expressed genes (DEGs) were identified in the intergroup comparison. P value < 0.05 and |log2 Fold Change |≥0.0 were applied for the criteria of differentially expressed gene screening. Next, principal component analysis (PCA) was performed on the expected number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM) of all samples. Cluster analysis heatmaps were generated by using the R package ggplot2 (version 3.0.3). In the final step, the ClusterProfiler R package (3.8.1)(Yu et al., 2012) from Bioconductor was adopted for Gene Ontology (GO) functional enrichment analysis and Reactome pathway enrichment analysis. In addition, on the basis of the Padj <0.05 threshold, the top 10 differentially expressed gene regulation terms of biological process (BP) and molecular function (MF) were identified.

2.6. Fecal metabolomics

Untargeted gas chromatography-mass spectrometry (GC-MS)-based metabolomics was applied to further explore the alterations of fecal metabolites. As previously described (Ye et al., 2018; Jiang et al., 2021), 20 mg of feces was homogenized and filtered, and then, 1 mg/ml heptadecanoic acid (Sigma-Aldrich, St. Louis, MO, United States) was added as an internal reference to complete machine detection. Pyridine (Sigma-Aldrich, St. Louis, MO, United States) and methoxylamine hydrochloride(Sigma-Aldrich, St. Louis, MO, United States) were mixed up at the concentration of 15 mg/ml. Using a nitrogen stream (Aosheng, Hangzhou, China), samples were died and then reconstituted with 50 μl methoxylamine pyridine. After incubating at 37 °C for 24 h, next step is to add 50 μl N,O-bistrifluoroacetamide [with 1% trimethylsilyl chloride] (Sigma-Aldrich, St. Louis, MO, United States). Finally, the mixture was incubated in the dark at 70 °C for 2 h. The derivatived samples were analyzed on an Agilent 7890A gas chromatography system coupled to an Agilent 5977A MSD system (Agilent Technologies Inc., CA, USA). The raw GC/MS data (.D format) were converted into the analysis basic file. abf format by Analysis Base File Converter software for fast data retrieval (Kind et al., 2009). Qualitative and relative quantitative analysis and standardized preprocessing of raw data were performed by MS-DIAL software and the LUG database (Tsugawa et al., 2015), which involved a series of processing steps performed on the imported data and finally exported the original data matrix. For headspace sampling GC–MS experiments of volatiles, we used the NIST database (https://webbook.nist.gov/chemistry/) for qualitative analysis of the substances.

For multivariate statistical analysis, we first used unsupervised PCA to observe the overall distribution among the samples and the stability of the entire analysis process. Then, supervised partial least squares analysis (PLS-DA) and orthogonal partial least squares analysis (OPLS-DA) were used to identify overall differences in metabolic profiles. Metabolic pathway enrichment analysis of differentially abundant metabolites was based on the Kyoto Encyclopedia of Genes and Genomes

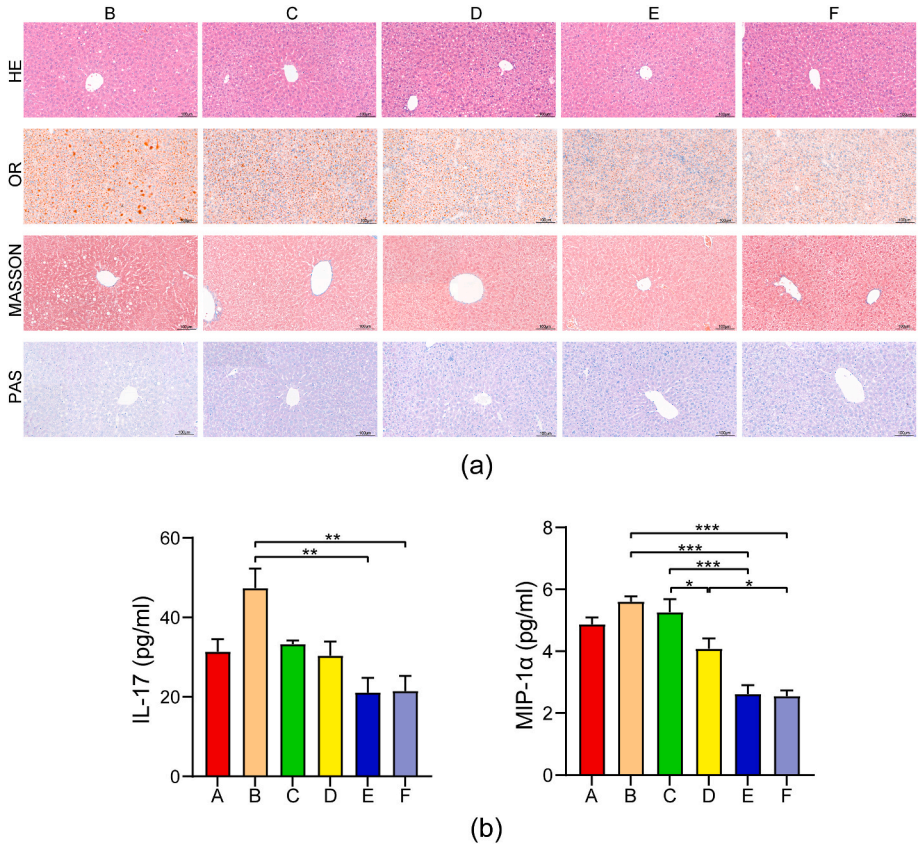

Fig. 2. Dietary changes and *B. longum* administration ameliorate lipid accumulation and inflammation. (a) H&E staining, Oil Red O staining, Masson trichrome staining and PAS staining of liver tissues from different groups. Scale bars: 100 μm. (b) Altered serum levels of inflammatory cytokines. Data are presented as the mean ± SE. n = 8, *P < 0,05, **P < 0.01, ***P < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(KEGG) database (https://www.kegg.jp/), and bubble plot drawing was used for selected significant enrichment pathways.

2.7. Sequencing of 16S V3-V4

The genomic DNA of the fecal samples was extracted by the sodium dodecyl sulfate (SDS) method, and then, the purity and concentration of the extracted DNA were detected using agarose gel electrophoresis. The diluted genomic 16S V3-V4 DNA was used as a template for PCR amplification(Sonnenburg et al., 2016). According to the manufacturer's instructions, library construction was performed using the NEBNext® Ultra™ IIDNA Library Prep Kit (Cat No. E7645), and the library was sequenced using the Illumina NovaSeq6000 platform(Zhang et al., 2020). Collectively, the amplicon sequence variants (ASVs) were obtained by splicing, filtering and noise reduction of the sequenced data, and for further analysis of the differences in community structure among the grouped samples, the species composition and community structure of the grouped samples were tested for significance of differences by linear discriminant analysis effect size (LEfSe) software (Version 1.0).

2.8. Statistical analysis

Data are presented as the means ± standard errors (SE). Independent-samples t tests were applied to the comparison of two groups, while one-way ANOVA was used for comparisons of more than two groups (GraphPad Prism 9, La Jolla, CA, USA). The LSD post hoc test was applied when the chi-squared test indicated a normal distribution, and the Bonferroni post hoc test indicated that the condition was not met. By definition, P values < 0.05 were considered statistically significant.

3. Results

3.1. Dietary transition ameliorates lipid accumulation, inflammation, liver function and pathology in HFD-fed mice

As outlined in Fig. 1a, mice were divided into groups A-H with different food arrangements and probiotic combinations. After dietary transitions, the body weights of the mice in the intervention groups were lower than those in the group B at 5 weeks (Fig. 1b). After 4 weeks of dietary modeling, weight gain (group G 7.57 ± 0.67 vs. group H 2.92 ± 0.31, P < 0.0001) and the ratio of liver weight/body weight (group G 0.0291 ± 0.0007 vs. group H 0.0340 ± 0.0006, P < 0.0001) were statistically significant in mice treated with HFD. A decrease in body weight in the mice after dietary intervention was found. Compared to group B, the weight gain of group A (11.54 ± 1.19 vs 7.97 ± 0.70, P < 0.001), D (11.54 ± 1.19 vs 8.77 ± 0.55, P < 0.05), E (11.54 ± 1.19 vs 8.77 ± 0.57, P < 0.05), F (11.54 ± 1.19 vs 7.85 ± 0.54, P < 0.001) were marked decreased. The ratio of liver weight/body weight indicated a decrease in liver fat content through TSD (group C 0.0353 ± 0.0008 vs. group E 0.0322 ± 0.0004, P < 0.001) or probiotic supplementation (group E 0.0322 ± 0.0004 vs. group F 0.0357 ± 0.0004, P < 0.001) (Fig. 1c). Moreover, the levels of ALT and AST were ameliorated by dietary intervention (Fig. 1d).

As shown in Fig. 2a, H&E staining and Oil Red O staining of liver sections demonstrated that anti-inflammatory dietary intervention and probiotic adjuvant therapy have critical regulatory effects. At the experimental endpoints, the liver pathology of continuous HFD-feeding mice was the most impaired among all groups. Large red lipid droplets were found scattered in the hepatocytes, indicating exacerbated hepatic steatosis. Moreover, the mice administered *B. longum* or a TSD had a notably lower volume of lipid droplets than group B. Masson trichrome staining of murine livers depicted fibrosis in all groups. Additionally, hepatic glycogen detected by PAS staining was undifferentiated. All

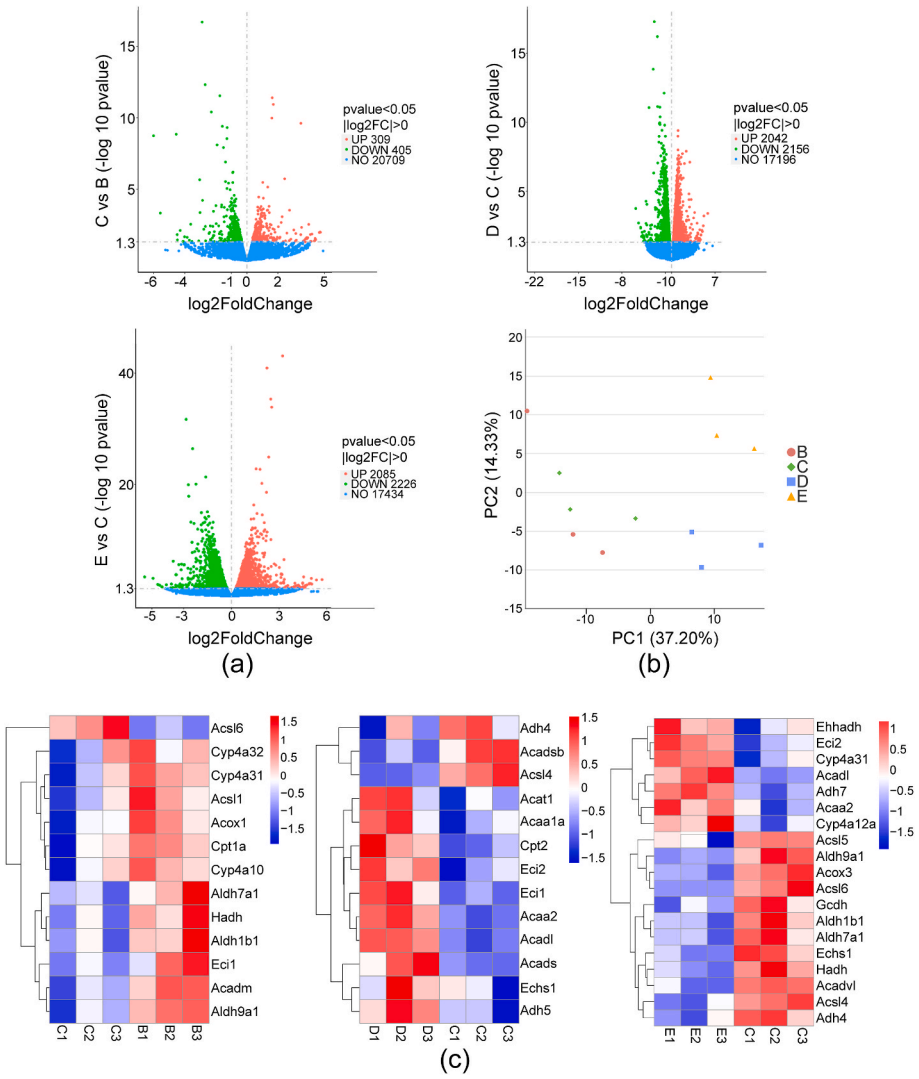

Fig. 3. Mediterranean diet, transitional standard diet and *B. longum* supplementation modulate the liver transcriptome. (a) Volcano plot of DEGs (group C vs. group B, group D vs. group C, group E vs. group C). (b) Principal component analysis (PCA) plot. (c) Heatmaps of differentially expressed lipid metabolism-associated genes (group C vs. group B, group D vs. group C, group E vs. group C). Red denotes upregulation and blue downregulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

these pathological findings contributed to the fact that both a TSD and *B. longum* administration play important roles in ameliorating lipid deposition.

To further explore the effects of dietary factors and probiotic intervention on inflammatory modulation in the HFD-induced obese mice, we measured the levels of multiple serum cytokines. As the figure shows, we found that the serum levels of IL-17 and MIP-1α in the HFD group were markedly increased. Specifically, the levels of these two cytokines obviously decreased under TSD conditions (IL-17: group B 47.40 ± 4.87 vs. group E 21.08 ± 3.70, $P < 0.01$; MIP-1α: group B 5.60 ± 0.17 vs. group E 2.61 ± 0.29, $P < 0.001$) (Fig. 2b). Additionally, the serum level of MIP-1α was significantly reduced after *B. longum* administration (group C 5.17 ± 1.02 vs. group D 4.08 ± 0.95, $P < 0.05$) (Fig. 2b).

3.2. Genes associated with lipid metabolism by dietary intervention changed significantly according to liver transcriptome analysis

Illumina sequencing was applied to liver samples, and corresponding pairwise comparisons were subsequently performed. DEG counts are shown in Fig. 3a. Compared to HFD-treated group, 309 DEGs were upregulated and 405 DEGs were downregulated in MD-treated group. When comparisons were made between the MD groups with and without *B. longum*, we found that 2042 DEGs were increased and 2156 DEGs were decreased. To study the effects of TSD on the transcriptome, we identified 2085 upregulated genes and 2226 downregulated genes. DEGs were then further researched by cluster grouping analyses (Fig. 3b). Heatmaps based on the lipid metabolism-associated genes of group C versus B, group D versus C and group E versus C are displayed in Fig. 3c.

After screening by the Illumina HiSeq high-throughput sequencing platform, we conducted the functional enrichment analysis. Biological processes, molecular functions, and cellular components, three central domains of GO analysis, were shown in Fig. 4a. The top 10 over-represented biological processes of DEGs were mainly concentrated in fatty acid, steroid and long-chain fatty acid metabolic processes; organic acid, carboxylic acid, monocarboxylic acid and lipid catabolic processes; and lipid biosynthetic processes. In addition, in regard to molecular function, oxidation-reduction reactions, biological binding, etc., were substantially transformed (Fig. 4a). Compared to that of group C, the expression of genes associated with ribosome formation obviously increased while GTPase activities were decreased in group D. Then, with the addition of the transition diet, hepatic glycogen metabolism was weakened in group E.

Our further pathway enrichment analysis focused on changes in molecular pathways. The Reactome pathway database was applied to up- and downregulated DEGs (Fig. 4b). Notably, the top upregulated pathways in the MD group were biosynthesis of maresins compared to those of the HFD group. With a healthy eating pattern, the gene expression of fatty acid metabolism was decreased correspondingly. In addition, a transitional diet could lower the metabolism of water-soluble

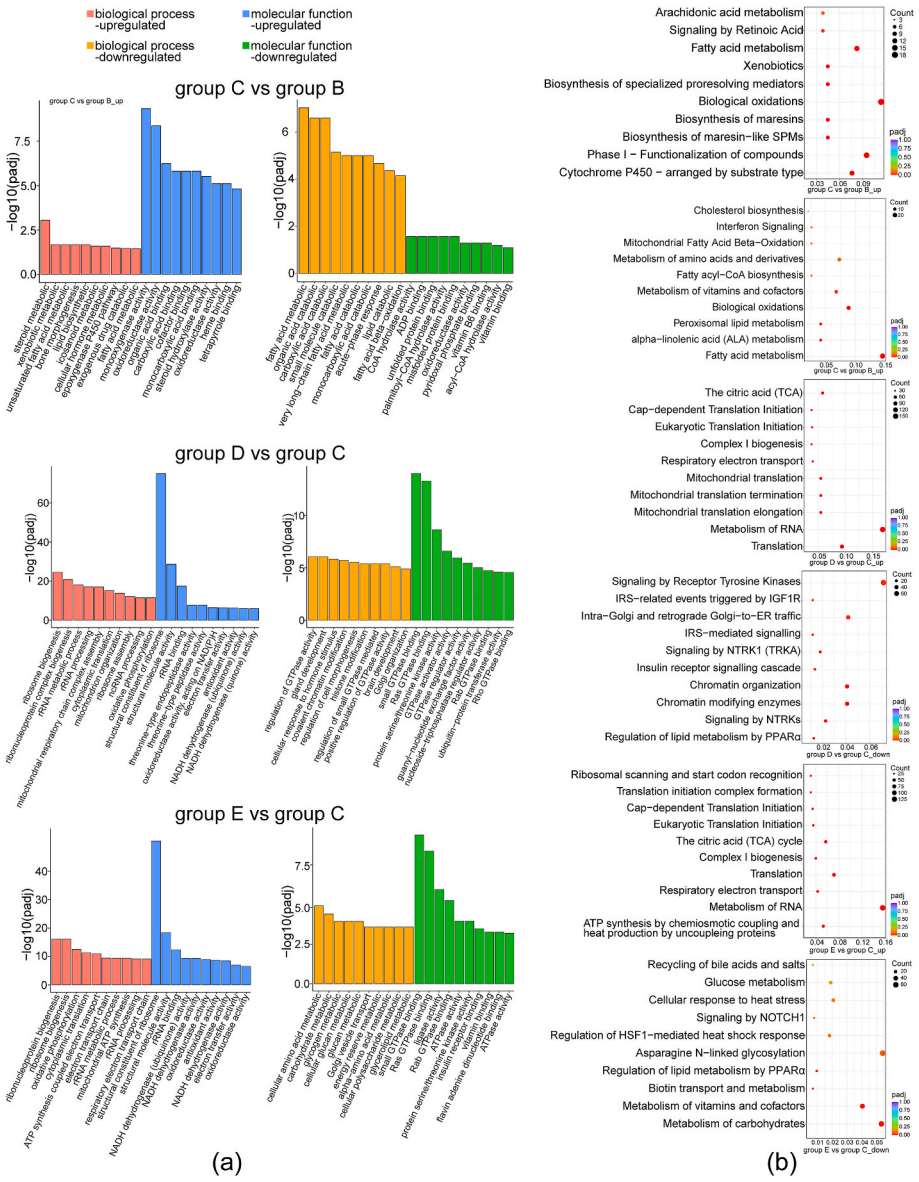

Fig. 4. Improved material and energy metabolism by dietary changes and *B. longum* administration. (a) The top 10 up- and downregulated terms in the biological processes and molecular functions categories by GO enrichment analysis. Red and blue indicate upregulation, while yellow and green denote downregulation. (b) Up- and downregulated bubble diagram by Reactome functional enrichment analysis (group C vs. group B, group D vs. group C, group E vs. group C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

vitamins and cofactors, carbohydrates, vitamins and cofactors.

3.3. The TSD ameliorated intestinal damage via an increase in the *Lactobacillus* genus

According to Shannon index analysis, which was used to reflect the diversity of the intestinal microbiota population, a TSD improves the diversity of the flora. Instead, this factor was markedly lowered after treatment with *B. longum* (Fig. 5a). Moreover, the results of principal coordinates analysis (PCoA) were depicted in Fig. 5b, displaying 37.77% variance on PC1 and 25.79% on PC2. By using the LEfSe method, we further explored the discrepancies between groups (Fig. 5c). Through intragastric administration with *B. longum*, both group D vs. F and group C vs. E had no significant differences in the intestinal microbiota structure. For group C, the abundance of *Lactobacillus* and *Clostridia* was improved at the order, family, and genus taxonomic levels in group E. Similarly, the distribution of gut microbiota of group F showed an increase in *Lactobacillus* content, which made a difference in microbial taxa compared to that in group D. Moreover, the changes in microflora between group C and E, and group D and F, suggesting that *Lactobacillus* may play a direct role in the advantages of a TSD.

Accordingly, for mice colonic tissues, more orderly and tight ileal villi were observed in the other groups than in group B (Fig. 5d). Through AB-PAS staining, goblet cells were estimated more accurately. Due to the MD, TSD and *B. longum* intervention, the number of goblet cells in the ileum increased while accumulated glycogen correspondingly declined.

3.4. A TSD and B. longum supplementation ameliorated lipid accumulation by enhancing the processes of carbohydrate digestion and absorption

The benefits of TSD with *B. longum* supplementation were further studied. Through KEGG functional enrichment analysis, group D vs. group C and group E vs. group C were grouped to screen signal pathways related to lipid metabolism, and their DEGs were screened out for correlation analysis. We next adopted Spearman's correlation analysis to determine the association between the DEGs and the top 8 genera (Fig. 6).The characteristic microbial environment suggested that the relevant metabolites may also be influenced to change. To reveal the metabolic phenotypes, we performed metabolic profiling of the mice feces via untargeted GC-MS and we found that there existed significant

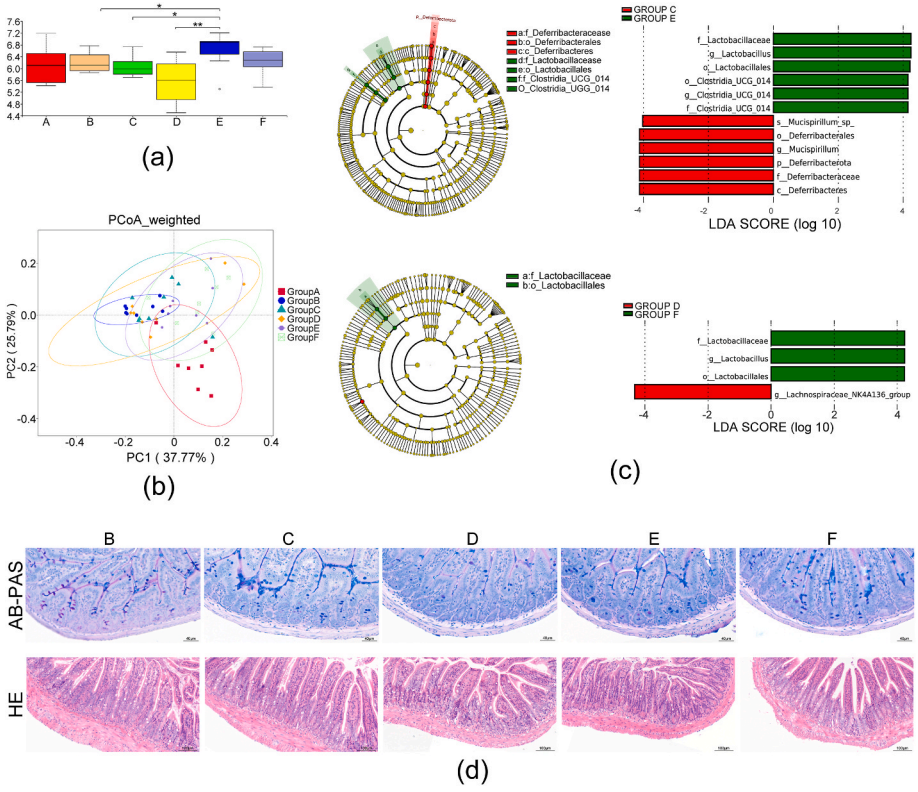

Fig. 5. Dietary changes and *B. longum* administration altered the composition of intestinal flora and histopathological features of the ileum. (a) Shannon index. (b) Principal coordinate analysis (PCoA) plot using the weighted UniFrac distance. (c) Cladograms and LDA scores are used for presenting discrepant gut microbiota profiles (group E vs. group C, group F vs. group D). (d) AB-PAS staining (Scale bars: 40 μm) and H&E staining (Scale bars: 100 μm) of ileum tissues from different groups. *P < 0,05, **P < 0.01.

differences in the metabolic profiles between different treatment groups. Next we constructed PCA and PLS-DA models based on the identified metabolites (Fig. 7a), which intuitively reflected the similarity or difference between samples. Consistently, the scores differed markedly in metabolic features between various groups. The quantity of differentially abundant metabolites between compared groups was displayed in a bar chart(Fig. 7b), which were further shown in the heatmaps (Fig. 7c). Furthermore, enrichment analysis results on signal pathways based on differential enrichment metabolites were shown in bubble diagrams (Fig. 7d).

Compared to those of group B, some essential metabolites participating in fatty acid biosynthesis pathways was significantly enriched in group C such as myristic acid, oleic acid, palmitic acid and dodecanoic acid, which could ameliorate hyperlipidemia and modulate glucose metabolism and inflammation (Zhao et al., 2022). Moreover, after *B. longum* administration, the biosynthesis of some intermediates was enhanced, including glucose 6-phosphate glucose, maltotriose and butyric acid. And these metabolites were critically enriched in the metabolic pathways of carbohydrate digestion and absorption which was evidenced to be the essential cellular processes providing energy and supporting to build blocks for cell growth., Furthermore, KEGG analysis showed that in the comparison between group F and D, the pathways of central carbon metabolism in cancer, the fundamental process of cellular transformation and tumor progression (Folmes et al., 2011; Wong et al., 2017), were significantly enriched. Overall, significantly altered metabolic profiles were observed after dietary Changes and probiotics administration.

4. Discussion

In the past half century, people with overweight have become increasingly common, and uncontrolled weight gain has become a global pandemic. The main driving forces are attributed to the global food system transition and sedentary behavior (Blüher, 2019). Apart from decreasing lipid droplet formation and ameliorating liver function, we further explored the strengths of Mediterranean diet. The MD has long been considered the most healthy dietary pattern worldwide due to its food structure, which is rich primarily in antioxidants and anti-inflammatory nutrients (Slomski, 2022). Given the expression profiles of the liver transcriptomes, the biosynthesis of maresins was increased with the MD. Maresins, a family of proresolving lipid mediators, not only inhibit inflammation and modulate the immune response but also enhance lipid breakdown (Serhan, 2014). Moreover, in the MD-treated group, the biosynthesis of fatty acids was significantly decreased, indicating the beneficial effects of MD on disordered lipid metabolism. In our study, we investigated the impact of TSD on the systemic alterations of HFD-induced obesity, especially with lipid-lowering probiotic supplementation, and further studies investigating potential therapeutic targets are discussed.

Innovatively, we put forward a novel concept of TSD. After intervention with the TSD, an critically increased abundance of *Lactobacillus* was found in the intestinal tract of the mice with or without interference by probiotic supplementation. *Lactobacillus*, a major class of probiotics, has been widely evidenced to play an beneficial roles in the regulation of immune system and anti-inflammatory effects. Researches Rodrigues et al. (2021) have revealed that *Lactobacillus* acted on mitochondria in the liver, resulting in an improvement in lipid metabolism in Western-style diet-treated mice. Moreover, strains belonging to the *Lactobacillus* genus were studied for their antimicrobial activities, immunoregulatory effects, gut microbiome regulation, metabolites, antitumor activities, etc. (Jeong et al., 2022). Here, we reported that the livers and intestines of mice get improved to some extent after administration of a TSD, which is consistent with the increased abundance of *Lactobacillus* by LefSe analysis. *Lactobacillus* augments intestinal barrier function by overproducing mucus, secretory immunoglobulin A (sIgA) and antimicrobial peptides, as well as strengthening tight junctions (Dempsey and Corr, 2022). Combined with the outcomes from liver transcriptome analyses, energy expenditure-related pathways were relatively highly expressed in the TSD groups. Consistent with the decrease in the transcriptional expression of lipids and glucan, the total

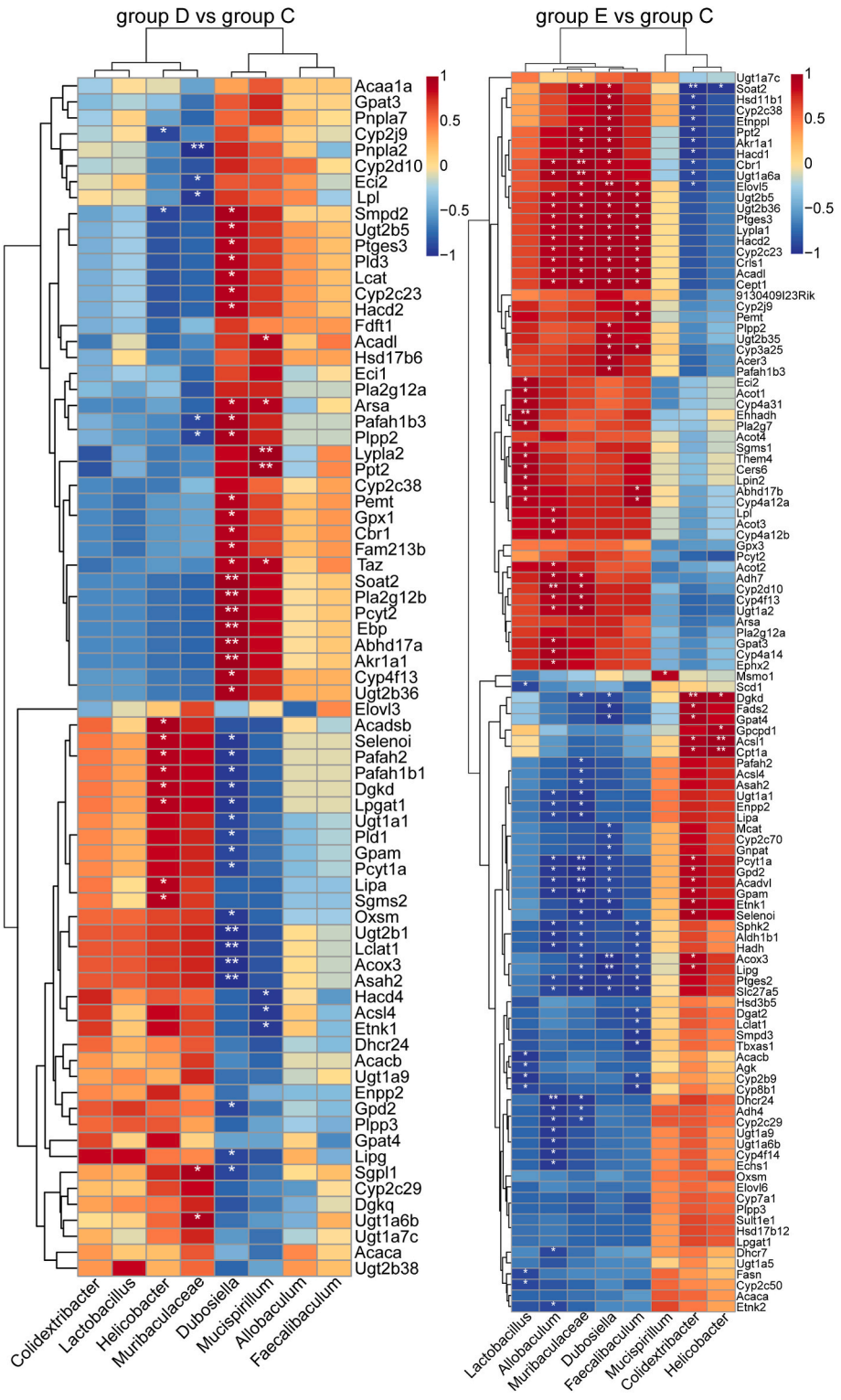

Fig. 6. Altered microbes are associated with lipid synthesis- and metabolism-related genes. Heatmaps of Spearman correlation analysis among the top 8 most abundant bacterial genera and gut and lipid synthesis- and metabolism-associated transcripts (group D vs. group C, group E vs. group C). The color depth represents the strength of the correlation. Red indicates a positive correlation, and blue indicates a negative correlation. *P < 0.05, **P < 0.01. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

liver lipid content of group E apparently decreased after dietary modifications. Our metabolomic profiling results suggest that inflammatory mediator regulation of TRP channels and butanoate metabolism was markedly changed with the above transitional dietary interventions. In previous literature, *Lactobacillus* was applied to modulate oxidative stress in the gastrointestinal tract (Kong et al., 2020). Likewise, similar changes were observed in the liver transcriptomes, as shown by the findings on oxidative phosphorylation, respiratory electron transport chain, etc.

Furthermore, we found that the expression levels of several cytokines, including IL-17 and MIP-1α, were strongly regulated by a transitional diet with or without probiotic intervention. Previous studies indicated that IL-17 was a pivotal cytokine involved in activating NLR Family Pyrin Domain Containing 3 (NLRP3), a caspase-1 activation platform exerting critical effects on magnifying and perpetuating liver inflammation and fibrosis (Wree et al., 2018; de Morales et al., 2020). MIP-1α is a macrophage inflammatory protein that has been a research hotspot in recent years for its critical roles in diverse diseases (Teufel

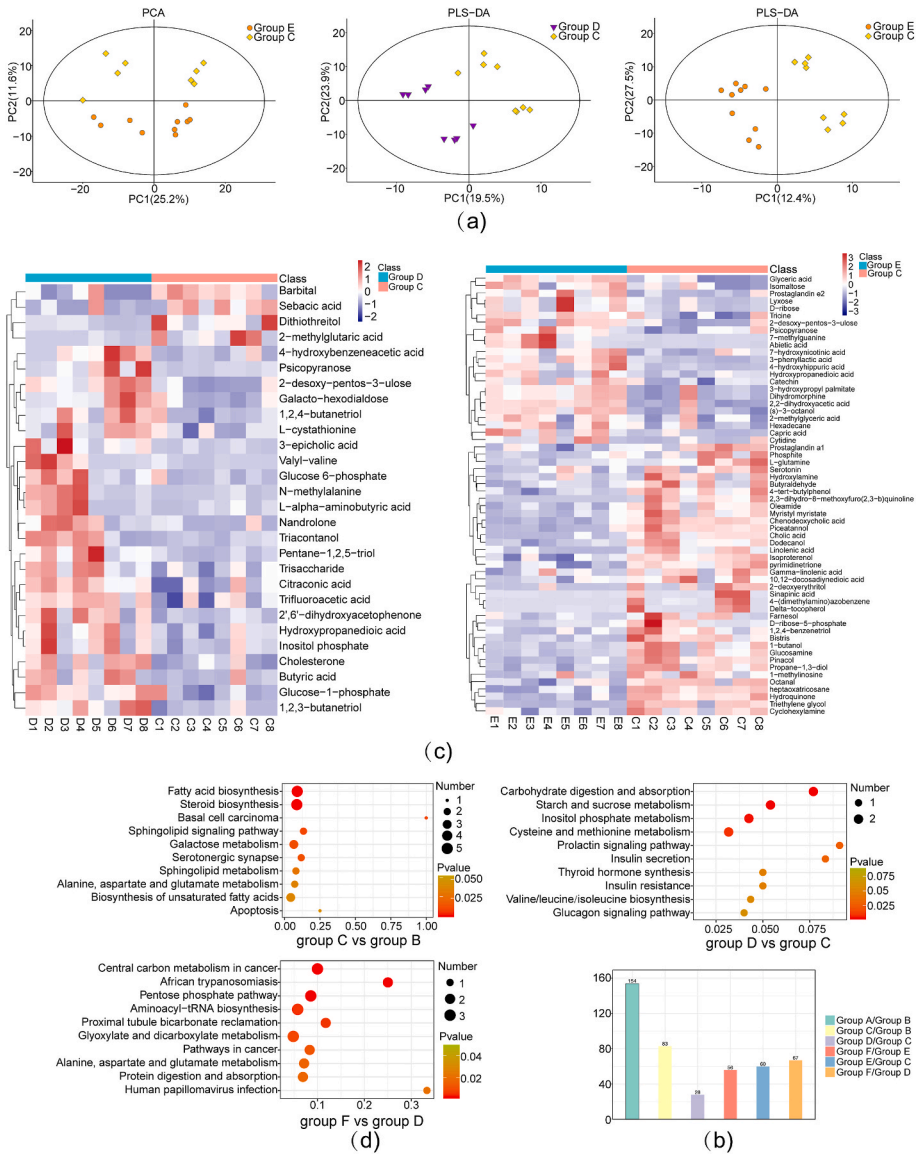

Fig. 7. Altered metabolic profiles associated with the Mediterranean diet, transitional standard diet and *B. longum* supplementation. (a) PCA and partial least squares-discriminant analysis (PLS-DA) plots (group D vs. group C, group E vs. group C). (b) Amounts of differential metabolites among the different groups. (c) Heatmaps of differential metabolites (group D vs. group C, group E vs. group C). (d) Top 10 metabolic pathways by KEGG enrichment analysis (group C vs. group B, group D vs. group C, group F vs. group D).

et al., 2019). Accumulating studies have shown that MIP-1α could accelerate the progression of steatohepatitis by facilitating macrophage infiltration into the liver and M1 polarization (Xu et al., 2021), and the combination of a transitional diet and probiotics may protect the liver by blocking this process. An important implication can be drawn from the results in our study: the TSD performed reasonably well in ameliorating obesity in mice via the increase in the *Lactobacillus* genus.

B. longum, a common lipid-lowering probiotic used in nutraceuticals and drugs, was chosen as our probiotic intervention. In addition to ameliorating antioxidative stress effects, *B. longum* also limits weight gain, inflammation and metabolic function (Alard et al., 2021). The gene expression of citric acid was obviously upregulated in group D compared with group C. As an antioxidant (Zhang et al., 2022), citric acid shows strong health benefits combined with *B. longum* (Bianchi et al., 2019). Changes in metabolites reveal alterations at the endogenous substance or gene expression level, which makes the research objectives more intuitive. Not surprisingly, the synergistic effect of a TSD and *B. longum* was the most effective fat mediator. At the final end point, the mice of group F were the lightest among all groups. Most importantly, the synergistic effect reduced the levels of L-alanine and L-glutamine, which are related metabolites in the central carbon metabolic pathway in cancer (Sammad et al., 2022). Accumulating experimental evidence shows that *B. longum* has beneficial regulatory effects on host metabolism, consistent with the results of GC-MS-based metabolomic analysis in this study (Strazar et al., 2021). Dietary factors may act synergistically with probiotic application in this process, but further studies are needed to elucidate the underlying mechanisms (Mentella et al., 2019). Overall, the markedly changed metabolic signature indicated that the combination exerted significant effects on the metabolic patterns, which may be achieved by modulating the gut microbiota.

However, the limitations of our research are listed as follows. First, the duration of a transitional diet needs to be further tested for more precise conclusions. Additionally, more probiotics with the ability to reduce body fat could be combined for adjuvant therapy of fat loss. Finally, the relevant mechanisms could be further investigated.

5. Conclusion

In summary, we investigated the impact of dietary conversion and lipid-lowering probiotic administration on liver fat accumulation by histomorphological studies and multi-omics analyses. In general, our findings provide evidence that a TSD may ameliorate fat accumulation by increasing the proportion of the *Lactobacillus* genus. Correspondingly, a TSD improved the bioprocess of lipid metabolism and exerted anti-

inflammatory effects. Thus, not only were metabolic pathways of water-soluble vitamins and cofactors, carbohydrates, vitamins and cofactors modulated, but also the levels of some inflammatory cytokines including IL-17 and MIP-1α were markedly decreased. Through evaluating histomorphology and cytokines levels, we demonstrated that a TSD, combined with the synergistic effect of *B. longum* might be more beneficial than drastic dietary change by enhancing the relative abundance of *Lactobacillus*, providing theoretical basis and novel research perspective for further understanding the initiation and progression of HFD-induced obesity.

CRediT authorship contribution statement

Shiman Jiang: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Yuanshuai Su:** Formal analysis, Drawing figures, Writing – original draft, Writing – review & editing. **Qiangqiang Wang:** Methodology, Writing – review & editing. **Longxian Lv:** Writing – review & editing. **Chen Xue:** Writing – review & editing. **Lvwan Xu:** Writing – review & editing. **Lanjuan Li:** Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100435.

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