Lactobacillus acidophilus CICC 6075 Attenuates HFD-induced Obesity by Improving Gut Microbiota Composition and Histidine Biosynthesis

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Abstract

This study aimed to investigate the potential anti-obesity efficacy of *Lactobacillus acidophilus* CICC 6075. The study analyzed metagenomic data from 120 obese and 100 non-obese individuals and found that the abundance of *Lactobacillus acidophilus* was significantly higher in normal-weight individuals. High-fat diet (HFD)-induced obese mice were treated with *L. acidophilus* via daily oral gavage for 12 weeks. After the experiment, the obesity phenotype was assessed, and the fecal 16S rRNA gene sequence was analyzed. Administration of *L. acidophilus* attenuated excessive weight gain and fat accumulation and maintained the intestinal barrier in HFD-induced obese mice. Sequencing results showed that HFD hindered α and β diversity while reducing the relative abundance of *Lactobacillus* and *norank_f_Muribaculaceae* and significantly increasing the relative abundance of *Ilebacterium*. *L. acidophilus* reversed these results and reduced the Firmicutes/Bacteroidetes ratio. Supplementation of *L. acidophilus* enhanced histidine biosynthesis, inhibited the NF-κB pathway, and significantly reduced the expression levels of inflammatory factors in adipose tissue. These results indicate that *L. acidophilus* CICC 6075 can significantly improve intestinal microbiota function, promote histidine synthesis, reduce systemic inflammation, and thereby inhibit obesity progression. This suggests that *L. acidophilus* CICC 6075 may be a good candidate probiotic for preventing obesity.

Introduction

Obesity is an important health problem that has attracted widespread attention, and the latest statistics show that overweight/obese people worldwide account for approximately 30% of the global population [1, 2]. During clinical observations, obesity often presents with changes in body shape and is strongly correlated with disorders of lipid metabolism, chronic inflammation, and oxidative stress. Furthermore, obesity is a recognized risk factor for various diseases including diabetes, cancer, and cardiovascular diseases [3-5]. Drugs used clinically to combat obesity, such as orlistat, lorcaserin, bupropion, and liraglutide, show limited effectiveness and significant side effects [6-10]. Effective and safe treatment strategies have yet to be identified.

Gut microbes influence nutrient acquisition, energy regulation, and fat storage [11, 12]. Recent studies have suggested that an imbalance in natural gut bacteria, referred to as dysbiosis, could contribute to an increased risk of obesity [13-15]. Probiotics secrete beneficial substances, including short-chain fatty acids, amino acids, and vitamins, as well as products with anti-inflammatory and antioxidant capabilities that regulate host energy metabolic processes and maintain metabolism and energy balance [16-18].

*Lactobacillus acidophilus* serve as one of the most commonly used probiotics. Orally administered *Lactobacillus acidophilus* regulates lipid metabolism, improves insulin resistance, inhibits inflammation and oxidative stress, and modulates intestinal microbiota homeostasis, thereby providing health benefits [19-24]. *L. acidophilus* CICC 6075 modulates the activity of β-galactosidase, and accumulation of senescent adipocytes is crucial in initiating pathological remodeling and disrupting energy balance in
adipose tissue [25, 26]. However, the related mechanisms of *L. acidophilus* CICC 6075 against obesity have not yet been reported.

Thus, this study aimed to evaluate the effect of *L. acidophilus* CICC 6075 on lipid metabolism, the intestinal barrier, and intestinal flora in an HFD-induced obese mice. In this present study, *L. acidophilus* CICC 6075 can significantly improve intestinal microbiota function, promote histidine synthesis, reduce systemic inflammation, and thereby inhibit obesity progression.

**Materials And Methods**

**Study Inclusion and Data Acquisition**

We searched PubMed for studies that published fecal shotgun metagenomic data from normal-weight controls (n = 100) and patients with obesity (n = 120). The raw data were categorized based on the World Health Organization's (WHO) obesity classification. In brief, individuals were designated as part of the Normal group if their body mass index (BMI) was less than 25 kg/m². Conversely, individuals were classified as the Obesity group if their BMI was 30 kg/m² or higher and if they concurrently presented with at least one comorbidity, including type 2 diabetes mellitus, hypertension, or obstructive cardiovascular disease. Raw FASTQ files of the two included studies were downloaded from the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) using the following NCBI identifiers: PRJEB12123 for Liu et al [27]. and PRJEB14215 for Lesley et al [28].

**Study Design**

Male C57BL/6J mice (8-week-old; weight, 25–28 g; n = 12) were purchased from GemPharmatech LLC (Nanjing, Jiangsu, China). All mice were retained in a pathogen-free facility at Xuzhou Medical University (Xuzhou, Jiangsu, China) and maintained under a 12-h light–dark cycle at constant temperature and humidity (23 ± 1°C and 55–65%, respectively) with free access to water and the same quality of food. Mice were fed either a normal chow diet (NCD; 10% kcal fat; Jiangsu Xietong Pharmaceutical Bio-engineering Co, Ltd., Nanjing, China) or an HFD (60% kcal fat, Jiangsu Xietong Pharmaceutical Bio-engineering Co, Ltd.) *ad libitum* for 12 weeks (Fig. 1). Mice were randomly divided into four groups (n = 3): NCD-fed control group, HFD-fed phosphate-buffer saline (PBS)-treated group, HFD-fed with *L. acidophilus* treated groups (1Í10⁹ CFU/day), and HFD-fed with heat-killed *L. acidophilus* treated groups (1Í10⁹ CFU/day). At the end of the experimental period, mice were subjected to a 12-hour fasting period and then anesthetized using an isoflurane chamber (RWD, Shenzhen, China) before collecting organs and blood samples.

**Culture and Administration of *Lactobacillus acidophilus* CICC 6075**

*L. acidophilus* CICC 6075 was purchased from the China Industrial Microbial Strain Collection and Management Center (CICC; Beijing, China). It was then grown in De Man, Rogosa, and Sharpe (MRS) medium for 48 h at 37 °C under anaerobic conditions. The bacterial concentration was calculated by
measuring the absorbance at 600 nm. C57BL/6J mice with HFD were orally gavaged daily with \(1 \times 10^9\) CFU or \(1 \times 10^9\) CFU of heat-killed *L. acidophilus* in 200 μL of PBS. The control C57BL/6J mice (NCD and HFD) were orally gavaged daily with 200 μL PBS at the same time. *L. acidophilus* CICC 6075 were heat killed at 121°C under 225-kPa pressure for 15 minutes.

**Weight Monitoring**

The body masses of the mice were measured every Saturday at 10:00 a.m. until the conclusion of the experiment. By the 12th week, the HFD group exhibited a 20% increase in body mass compared with the NCD group, indicating the success of the model.

**Tissue Extraction and Hematoxylin and Eosin Staining**

After completion of the experiment, the mice were euthanized. Liver, fat, and intestinal tissues were carefully extracted and weighed while their physical appearance was observed. Liver and colon tissues were subsequently fixed in paraffin for 24 h before being sliced into 3-μm sections and stained with hematoxylin for 10 min to highlight the nuclei, then the sections washed in tap water. Next, the slides were transferred to a staining jar containing eosin solution for 3 s and washed again with tap water. The slides were then dehydrated using a process that involved 80% ethanol for 20 s, 90% ethanol for 20 s, 95% ethanol for 20 s, 100% ethanol for 5 min, and xylene for 5 min. Once removed from the xylene, the slides were left to dry in a fume hood. Hematoxylin and eosin (HE)-stained images were captured using inverted microscope equipped with a digital microscope (Olympus, Tokyo, Japan).

**Histopathology Analysis**

The severity of nonalcoholic fatty liver disease (NAFLD) activity score was assessed using the steatosis, activity, and fibrosis (SAF) scoring system [29]. Steatosis, inflammatory infiltrates, and ballooning were quantified, and the NAFLD grades were determined. Steatosis was categorized into grades 0–3 based on the percentage of intracytoplasmic lipid droplets in the liver parenchyma (grade 0, <5%; grade 1, 5–33%; grade 2, 34–66%; grade 3, >67%). Inflammatory infiltrates were categorized into grades 0–3 based on the number of inflammatory cell foci per 200× field of view (grade 0: 0 foci; grade 1, 1–2 foci; grade 2, 3–4 foci; and grade 3: >4 foci). Ballooning lesions were categorized into grades 0–3 based on the morphology and number of ballooning hepatocytes per 200× field of view (grade 0: normal hepatocytes; grade 1: the presence of round and pale cytoplasmic hepatocytes; grade 2: one enlarged ballooning hepatocyte; and grade 3: multiple enlarged ballooning hepatocytes).

**In Vivo Gut Permeability Assays**

Mice were fasted for 12 h and then orally administered fluorescein isothiocyanate (FITC)-labeled dextran (DX-4000 FITC, 600 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA). Serum samples were collected by orbital blood sampling after four hours, incubated at 25 °C for 30 min, and then centrifuged at 1,500 g for 20 min. The serum was diluted with PBS at a dilution ratio of 1:9. The concentration of DX-
4000 FITC in the serum was measured using a fluorescence spectrophotometer (Synergy H1, BioTek, Winooski, VT, USA) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

**Histidine analysis**

Mouse serum samples were spiked with acetonitrile, vortex-mixed for 1 min, centrifuged for 5 min at 16,000 g at 4 °C and the supernatant was harvested. Derivatization and gradient elution of serum amino acids were performed using standard chemicals (Waters, Milford, USA) according to the manufacturer's instructions. A Waters UPLC I-Class system was used to perform ultra-performance liquid chromatography separations using a reversed-phase liquid chromatography column. A Waters TQ-XS mass spectrometer equipped with an electrospray ionization (ESI) probe was used. The positive ESI source temperature was 150 °C, the capillary voltage was 1.5 kV, and the cone voltage was 20 V. The liquid chromatography-mass spectrometry system was controlled using the Masslynx software (Waters), and data were collected and processed using the same software.

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**Enzyme-Linked Immunosorbent Assay (ELISA)**

Serum from each group was preserved and stored at -80 °C until analysis. Serum levels of tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), and interleukin-6 (IL-6) were quantified by ELISA following the manufacturer's instructions (Nanjing Yifeixue Biotech Co., Ltd., Nanjing, China).

**Real-Time Quantitative PCR (qPCR)**

RNA was extracted from the adipose tissue samples using the RNA-Quick Purification Kit (esunbio, Shanghai, China) following the manufacturer's protocol. The extracted RNA was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific™, Massachusetts, USA). Real-time PCR (RT-PCR) analyses were conducted using the ABI 7900HT real-time PCR system (Applied Biosystems, San Francisco, USA) with Super SYBR Green qPCR Master Mix (esunbio, Shanghai, China). β-actin expression was used as the reference for normalization, and results were calculated based on the comparative cycle threshold method ($2^{-\Delta\Delta C_T}$). The primer sequences used in this study were as follows: (AGCGGGAACTGAGTGAGATGA and GCACCCAGTGTATCGG).
DNA Extraction and 16S rRNA Gene Sequencing

Fecal samples were collected at the end of the experiment. Each fecal sample was snap-frozen in liquid nitrogen within minutes of donation and then kept at −80 °C. Bacterial genomic DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The 16S rRNA V3–V4 region was amplified by PCR and sequenced using the MiSeq platform (Illumina, San Diego, CA, USA). The sequences of the primers were as follows: 338F (5’-ACTCCTACGGGAGGCAGCA G-3’), 806R(5’-GGACTACHVGGGTWTCTAAT-3’).

Metagenomics Analysis

Adaptor contamination and low-quality reads were discarded from the raw sequencing reads, and the remaining reads were filtered to eliminate the human host DNA based on the human genome reference (hg37). We acquired 1,430 Gb of high-quality paired-end reads for 220 samples, with an average of 6.5 Gb per sample, after removing human DNA reads. Kraken2 software was used to classify the reads from the metagenome samples. All classifications were performed using default settings in Kraken2. Bracken software was designed to re-estimate the abundances at a fixed rank by distributing reads from higher ranks into lower ranks, based on conditional probabilities estimated from the database content. A Bracken database (100-mers) was created for the HumGut_97.5 database and used to re-estimate all abundances at the species rank.

Bioinformatic Analysis

The 16S rRNA sequencing data were processed using Quantitative Insights into Microbial Ecology (QIIME2 V.2021.11) [30]. DADA2 software integrated into QIIME2 was used to filter the sequencing reads and generate the feature table. Operational taxonomic units (OTUs) were clustered at 97% identity using the search plugin, and taxonomic classification was performed using the Silva (SSU138) 16S rRNA database (V.13.8). To ensure data reliability, OTUs comprising <0.005% of the total number of sequences were excluded. The resulting data yielded an average of 32,708 reads per sample (minimum: 47,213; maximum: 56,203). The sequences were aligned using MAFFT and a phylogenetic tree was constructed using the FastTree plugin. The diversity of the gut microbiome was analyzed using q2-diversity with a rarefied sampling depth of 47,213. Finally, we employed Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to estimate the metagenomes of the gut microbiome based on 16S rRNA sequences, as previously described [31].

Alpha-diversity indices were compared using the Kruskal–Wallis test, followed by pairwise Mann–Whitney U comparisons. To analyze the structural variation in microbial communities across samples, beta-diversity analysis was conducted using unweighted unifrac distance and visualized via principal coordinate analysis (PCoA). The Bonferroni method was used to correct the resulting P values. Furthermore, differences in unweighted unifrac distance among groups were determined using analysis of similarities. To compare the relative levels of abundant taxa at the phylum, family, and genus levels...
among the groups, one-way analysis of variance (ANOVA) and post-hoc least significance tests were performed using GraphPad Prism 6. Additionally, the linear discriminant analysis (LDA) effect size (LEfSe) was used to identify biomarkers for both abundant taxa and functional pathways by calculating the LDA score ($\geq 3.5$) across groups. A heatmap was plotted using the R package 4.2.1. PICRUSt based on OTUs was employed to predict the abundance of functional categories using Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KO). Data are expressed as mean ± standard error of the mean (SEM), and significance was set at $P < 0.05$.

Statistical Analysis

All data, except those obtained by 16S rRNA gene sequencing, were analyzed using GraphPad Prism 8 software, and the mean (± SEM) was utilized. To determine statistical significance, unpaired two-tailed Student’s $t$-test was used for comparisons between two groups, whereas one-way ANOVA was used for comparisons between more than two groups. Statistical significance was set at $P < 0.05$.

Ethics approval and consent to participate

The procedures of animal study performed under the ethical guidelines of Xuzhou Medical University Laboratory Animal Ethics Management Committee, approval number [4120185]. The contents of this study are under full compliance with government policy and ARRIVE guidelines.

Results

Signatures of Intestinal Microbiota in Patients with Obesity

To investigate the role of gut microbiota in obesity, a cohort of 120 individuals with obesity and 100 normal individuals underwent metagenomic analysis of their gut microbiota. Our findings demonstrated a significant reduction in alpha-diversity, as indicated by the Shannon diversity index, in the obese group compared to that in the normal group (Fig. 2A and B). Moreover, the gut bacterial composition differed considerably between the two groups, as illustrated by the PCoA results (Fig. 2C). At the genus level, we observed a distinct enrichment of *Odoribacter, Lactobacillus*, and *Parabacteroides* among healthy individuals, whereas these genera were less abundant in the obese group (Fig. 2D). The abundance of the top 20 species of *Lactobacillus* in each individual was presented as a heatmap (Fig. 2E). Notably, *L. acidophilus* exhibited a significantly lower relative abundance in the obese group than in the normal group (Fig. 2F). *Lactobacillus paracollinoides* and *Lactobacillus helveticus*, which were less abundant than *L. acidophilus*, did not differ between the obese and normal groups (Fig. 2G and I). However, the abundance of *Lactobacillus heilongjiangensis* had significantly higher relative abundance in the obese group than that in the normal group (Fig. 2H).

*L. acidophilus* Administration Ameliorated HFD-induced Adiposity and Fatty Liver in Mice

Eight-week-old male mice were fed either NCD or HFD along with oral supplementation of *L. acidophilus* CICC 6075 at varying concentrations for 12 weeks. Administration of *L. acidophilus* CICC 6075 to mice
fed an HFD (HFD-L) resulted in a significant reduction in body weight gain (by ~40%), liver weight (by 20%), total white adipose tissue (by 45%), and total white fat weight/body weight and liver weight/body weight ratios compared to those in HFD-fed mice (Fig. 3A–E). Although HFD-fed mice administered heat-killed *L. acidophilus* (HFD-H) exhibited similar trends to HFD-L, the effect on total white adipose tissue weight and liver weight was not statistically significant (Fig. 4A–C).

To investigate the effect of *L. acidophilus* on tissue lipid accumulation, tissue sections were examined histologically. HFD-L mice exhibited a significant improvement in liver appearance and a 39% reduction in liver lipid accumulation compared with HFD-fed obese mice (Fig. 4D). Another key finding was that the mean area of adipocytes in the adipose tissue of the epididymis was significantly higher in the HFD group than in the NCD group. In addition, the mean adipocyte area was significantly lower in HFD-L mice than in HFD mice, but there was no difference in the mean adipocyte area between HFD-H and HFD mice (Fig. 4E).

Given that intestinal dysbiosis in HFD-fed animals may affect gut permeability, thereby causing the release of bacterial LPS into circulation [32], we investigated whether *L. acidophilus* modulates gut integrity. Our results showed that in vivo intestinal permeability, as determined by oral administration of DX-4000-FITC, followed by measurement of its circulating concentration, was significantly higher in the HFD group than in the NCD group, and HFD-induced increases in intestinal permeability and intestinal mucosal disruption were blocked by *L. acidophilus* treatment (Fig. 4F). Compared to the NCD group, there was no significant difference between the HFD-L and HFD-H groups. However, these effects were completely restored by *L. acidophilus* treatment. These results suggest that *L. acidophilus* can effectively reduce weight gain and fat accumulation and restore the intestinal barrier in HFD-fed mice, with the HFD-L group showing the most notable improvements.

**Effects of *L. acidophilus* on Gut Microbial Diversity and Richness**

The gut microbiota of obese humans and HFD-fed mice are characterized by reduced alpha-diversity, altered beta-diversity, increased ratios of Firmicutes to Bacteroides, elevated endotoxin-producing bacteria, and reduced numbers of immunostable bacterial species [33-35]. The effect of *L. acidophilus* on the composition of the intestinal microbiota was examined using MiSeq sequencing-based analysis of bacterial 16S rRNA gene sequences (V3–V4 region) in feces.

The bacterial libraries derived from our samples effectively captured the diversity of the microbial communities, as evidenced by the near-saturation of the rarefaction curves (Fig. 5A). To assess differences in microbial composition, we used PCoA to calculate beta-diversity (Fig. 5B). The overall composition of the gut flora differed significantly among the groups according to ANOVA. Additionally, alpha-diversity indicators, such as richness and diversity (measured using the Sobs and Shannon indices, respectively), were computed. HFD feeding had a profound impact on gut microbial alpha-diversity, resulting in a marked decrease (Fig. 5C and D), whereas supplementation with *L. acidophilus* effectively restored these levels.
To gain a better understanding of the shared richness among each group and to assess the extent of variation in the samples, we performed hierarchical clustering and generated a Venn diagram to illustrate the overlap between groups. At the genus level, there was less variation in microbial composition within the groups (Fig. 6A). Of the 995 OTUs, only 152 were shared among all groups, whereas the remaining OTUs were distributed among the three groups, two groups, and each group (Fig. 6B). Furthermore, the analysis revealed that *L. acidophilus* treatment significantly increased the number of OTUs in the HFD-fed mice, indicating that it had a positive impact on the richness and diversity of the intestinal microbiota. These results highlight the significance of *L. acidophilus* treatment on the richness and diversity of the intestinal microbiota.

**Effects of *L. acidophilus* on the Gut Microbiota Composition**

The ratio of saprophytes to bacteria is commonly used to indicate dysbiosis of the gut flora, which is linked to obesity and related diseases [36]. Feeding on an HFD had a significant impact on the relative abundance of Firmicutes and the Firmicutes/Bacteroidetes ratio when compared with that in the NCD group (Fig. 7A and B). Notably, treatment with *L. acidophilus* under HFD, particularly in the HFD-L group, reduced the relative abundance of Firmicutes and the Firmicutes/Bacteroidetes ratio, and substantially increased the abundance of Actinobacteria (Fig. 8A and B).

Among the top 15 families, *L. acidophilus* treatment markedly increased the abundance of *Erysipelotrichaceae* and *Atopobiaceae*, whereas the relative abundances of *Oscillospiraceae*, *Lachnospiraceae*, and *Marinilaceae* decreased. Interestingly, *L. acidophilus* supplementation increased the abundance of *Lactobacillaceae* (Fig. 8C and D). At the genus level, *L. acidophilus* treatment, particularly in the HFD-L group, caused an increase in *Ileibacterium* (*P* < 0.05), *Lactobacillus*, *norank_f_Muribaculaceae*, and *Helicobacter*, and a reduction in *unclassified_f_Lachnospiraceae* (*P* < 0.05), *Lachnospiraceae_NK4A136*, *Lachnospiraceae_UCG-006* (*P* < 0.05), *unclassified_f_Oscillospiraceae*, and *Odoribacter* compared to HFD mice (*P* < 0.05) (Fig. 8E and F).

In addition, the bacterial community structure with notable differences among the NCD, HFD, HFD-L, and HFD-H groups was further analyzed using the LEfSe method (Fig. 9A and B). It was determined that taxa at different levels were differentially abundant among the four groups. In addition, *unclassified_f_Prevotellaceae*, *Muribaculum*, *Eubacterium_siraeum_group*, Gammaproteobacteria, and Proteobacteria played critical roles and could be used as biomarkers in the NCD group. However, *unclassified_f_Lachnospiraceae* and *Lachnospiraceae_UCG-006* functioned markedly and could be used as biomarkers in the HFD group. Actinobacteriota, *Coriobacteriales*, *Atopobiaceae*, *Faecalibaculum*, *Bifidobacteriaceae*, *Lachnocalistrodium*, *Coriobacteriia*, and *Dubosiella* played crucial roles and could be employed as biomarkers in the HFD-L group, whereas *Erysipelotrichales*, *Ileibacterium*, *Bacilli*, *Romboutsia*, *Peptostreptococcaceae*, *Turicibacter*, *Blautia*, and *Clostridiaceae* were vital biomarkers in the HFD-H group. Furthermore, the number of taxa with differential abundance in the HFD group was lower than that in the HFD-L and HFD-H groups, indicating that *L. acidophilus* has a recovery effect on the increase in specific microbiota in the HFD group.
Effects of \textit{L. acidophilus} on the Gut Microbiota Function

The gut microbiota plays a critical role in the physiology of the host and has a significant impact on systemic metabolism and the pathology of obesity. To explore the functional potential of bacteria in the HFD group, we used PICRUSt and analyzed the data within the KEGG database. Based on these results, the enzymes, KO, modules, and pathways associated with the KEGG functional categories were identified. Compared with the NCD group, HFD feeding had a dramatic impact on the four functional categories. However, treatment with \textit{L. acidophilus} completely reversed these effects (Fig. 10A–F). Overall, this study suggests that \textit{L. acidophilus} treatment enhances the gut microbiota functions related to metabolism, immune responses, and pathogenesis.

Histidine production by \textit{L. acidophilus} reduced systemic inflammation via the NF-κB pathway and Protected against Obesity

Given to significant differences in histidine biosynthesis function among the groups, serum histidine levels were assessed. As anticipated, histidine levels were significantly lower in the HFD group than in the NCD group. Histidine levels exhibited an increase in the HFD-L group, but no significant difference was observed in the HFD-H group (Fig. 11A). To further investigate the mitigating effects of histidine on obesity, serum levels of the inflammatory cytokines TNF-α, IL-6, and IL-8 were assessed. The results showed that supplementation with low-dose \textit{L. acidophilus} significantly reduced the elevated levels of TNF-α, IL-6, and IL-8 in the HFD group, whereas no significant changes were observed in the HFD-H group (Fig. 11B–D). RT-qPCR results showed that the expression of NF-κB in adipose tissue of the HFD group was significantly higher than that of the NCD group, whereas compared to the HFD group, the expression level in the HFD-L group was significantly lower. Likewise, there were no significant difference observed between the HFD-H group and the HFD group (Fig. 11E). In summary, these results suggested that supplementation of \textit{L. acidophilus} mitigated the inflammatory response in mice with HFD-induced obesity.

Discussion

In this study, metagenomic shotgun sequencing data from cohorts of healthy and obese individuals were used to comprehensively compare the differences in gut microbes. \textit{L. acidophilus} was found in individuals with a normal body-mass index compared to that in obese individuals, \textit{L. acidophilus} abundance was significantly different. By combining human cohort metagenomic data with animal experiments, we demonstrated that supplementation with \textit{L. acidophilus} CICC 6075 ameliorated HFD-induced obesity in mice. \textit{L. acidophilus} CICC 6075 exerts critical effects by reducing weight gain, maintaining the integrity of the gut barrier, and modulating the gut microbiota. Kang et al. \cite{24} also found similar results by evaluating the effect of \textit{L. acidophilus} isolated from porcine intestines on obesity. However, in the present study, \textit{L. acidophilus} was obtained by analyzing the metagenomic results from patients with obesity and provided the first insight into the effect of \textit{L. acidophilus} CICC 6075 HFD-induced obesity.
Metagenomic data showed that obese individuals have significant differences in the gut microbiome and decreased alpha-diversity compared to healthy individuals, which is consistent with the findings of Kim et al. [37], who found that gut microbial diversity in patients with obesity with metabolic disorders decreased. Depletion of beneficial bacteria is accompanied by the progression of obesity. We compared the abundance at the species level between healthy individuals and patients with obesity from *Lactobacillus* spp., and found *L. acidophilus* decreased significantly in patients with obesity. In addition, our results show that live *L. acidophilus* CICC 6075 supplementation could effectively alleviate obesity, which has not been reported in previous obesity studies. In HFD-induced animal models, changes in intestinal microecology lead to intestinal barrier destruction, and LPS produced by gram-negative bacteria are more likely to enter the liver and intestinal circulation through the leaky gut tract to cause inflammation. These results showed that *L. acidophilus* supplementation effectively maintained the damaged intestinal barrier. Notably, *L. heilongjiangensis*, which had a significantly high relative abundance in the obese group, has not yet been studied.

Previous studies have shown that probiotic supplementation is associated with changes in intestinal microbiota, and the results of this study support this conclusion. Probiotics can promote the proliferation of beneficial bacteria and inhibit the reproduction of harmful bacteria to balance the intestinal microbiota. Therefore, we investigated significant changes in the intestinal microbiota. Although previous studies have shown that *L. acidophilus* CICC 6075 significantly alleviates HFD-induced chronic inflammatory diseases, intestinal microbes were not detected herein, and we remedied this deficiency. The ratio of Firmicutes to Bacteroides increases in the gut of obese individuals and HFD-induced animal models, and these critical phyla play a role in obesity-related inflammation [38, 39]. The results herein showed that *L. acidophilus* supplementation significantly reversed intestinal flora disorders, and the high ratio of Firmicutes to Bacteroides was restored to levels observed in mice fed a normal diet.

Additionally, HFD-induced obesity reduces *Ileibacterium* spp., and the abundance of these bacteria is negatively correlated with chronic inflammatory diseases such as obesity, atherosclerosis, and aging [40-42]. It has been reported that *Helicobacter* negatively correlates with intestinal tight junction protein expression, which increases after HFD feeding [43]. Furthermore, we described the association between HFD-induced obesity and controversial *Helicobacter*, whose abundance did not increase significantly after HFD feeding [44, 45]. *Lachnospiraceae_UCG-006* is associated with HFD-induced obesity, which is consistent with our results. Supplementation with *L. acidophilus* reduces the abundance of *Lachnospiraceae_UCG-006* [46]. These results suggest that increasing the abundance of some beneficial and reducing bacteria associated with inflammation or obesity is partly responsible for the relief of obesity with *L. acidophilus* supplementation. Thus, supplementation with *L. acidophilus* can alleviate HFD-induced obesity by regulating the intestinal microbial composition.

Moreover, predictions of the bacterial functional potential of the gut microbiome varied significantly across groups, and *L. acidophilus* supplementation reduced HFD-mediated inflammatory responses, energy metabolism, immune responses, and pathogenic gut microbiota dysfunction to levels observed in mice fed a normal diet. Furthermore, it was revealed that a possible reason for *L. acidophilus*
supplementation to maintain gut homeostasis is the induction of functional changes via altered gut microbial composition, thereby enabling new interactions between microbes, metabolites, and the immunity of the host.

It has been reported in animal studies that probiotics such as *Lachnospiraceae_NK4A136_group* and *unclassified_f_Lachnospiraceae*, are involved in the production short-chain fatty acids, maintenance of gut barrier integrity, and inhibition of inflammatory responses, indicating their potential in combating obesity [47-51]. Our results revealed that compared to the HFD-L group, the abundance of *Lachnospiraceae_NK4A136_group* and *unclassified_f_Lachnospiraceae* were reduced in the HFD-H group. This suggests that supplementation of heat-killed probiotics impede the ability of the microbiota to restore its functionality, thereby reversing the anti-obesity effects of probiotics.

Furthermore, Histidine, a key molecular regulator of lipid metabolism, is known to be reduced in individuals with obesity and type 2 diabetes [52-54]. It plays a role in inhibiting inflammation and oxidative stress via the NF-κB pathway, thereby improving insulin resistance [55-58]. In this study, supplementation of *L. acidophilus* enhanced histidine biosynthesis and suppressed the NF-κB inflammatory signaling pathway. However, supplementation of heat-killed *L. acidophilus* failed to exert the same effects. This suggests that the inability of heat-killed *L. acidophilus* supplementation to effectively alleviate obesity may be attributed to impaired regulation of metabolism, resulting in NF-κB activation and systemic inflammation that counteracts the anti-obesity effects of probiotics.

In conclusion, supplementing with *L. acidophilus* CICC 6075 alleviates systemic inflammation by inhibiting the NF-κB pathway, restores gut microbiota dysbiosis, and enhances gut microbiota function, thereby ameliorating obesity in HFD-induced mice. This study provides novel insights for the future treatment of obesity.

**Declarations**

**Ethics approval and consent to participate**

This study protocol was reviewed and approved by the Ethical Guidelines of Xuzhou Medical University Laboratory Animal Ethics Management Committee, approval number [4120185].

**Availability of data and materials**

The datasets generated during the current study are available in the figshare repository, [https://figshare.com/s/8a81a606967d18e4aeb9](https://figshare.com/s/8a81a606967d18e4aeb9).

**Author Contributions**

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Not applicable.

**Competing interests**

The author(s) declare no competing interests.

**References**


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Experimental design timeline.
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*L. acidophilus* produced histidine to reduce systemic inflammation via the NF-κB pathway and prevented obesity.

**Supplementary Files**

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