Article

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Impacts of Fecal Microbiota Transplantation on the Gut Microbiota-Short-Chain Fatty Acids-GPR43-Interleukin-18 Pathway in Rats Subjected to a High-Calorie Diet

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Background: A high-calorie diet (HCD) is a significant pathogenic factor contributing to obesity and can induce dysbiosis in the intestinal flora. Fecal microbiota transplantation (FMT) has been recognized for potentially restoring intestinal microecology. However, precise mechanisms underlying its therapeutic effects remain largely elusive. This study aimed to investigate the impact of FMT on the gut microbiota-short-chain fatty acids (SCFAs)-G protein-coupled receptor 43 (GPR43)-interleukin-18 (IL-18) pathway in HCD-induced rats. The findings provide insights and evidence for preventing and treating pediatric diseases caused by HCD.

Methods: Forty specific pathogen-free (SPF)-grade Sprague-Dawley (SD) rats were randomly allocated into six groups: normal control 1 (NC1), normal control 2 (NC2), normal control 3 (NC3), high-calorie diets model (M), fecal microbe transplantation treatment (FMTT), and Medilac-Vita (MV) groups. Antibiotic intervention simulated the state of antibiotic-treated rats, and a specialized diet was used to replicate the HCD model. Based on group assignments, rats received a normal diet bacterial solution, normal saline enema, or MV. Clinical characteristics and colonic morphology were observed, while changes in gut microbiota, SCFAs, GPR43, and IL-18 were assessed using 16SrDNA, gas chromatography-mass spectrometry (GC-MS), hematoxylin-eosin (HE), immunohistochemistry (IHC), and enzyme-linked immunosorbent assay (ELISA), respectively.

Results: FMT effectively restored the gut microbiota of antibiotic-induced rats. In the HCD-induced rats, FMTT significantly alleviated the pathological state and increased alpha indices and beta distances ($p < 0.05$). Furthermore, significant alterations in the relative abundances of gut bacterial genera associated with SCFAs production were observed. FMTT increased SCFA content in feces, especially acetic acid ($p < 0.05$). Notably, downstream pathways related to SCFAs, such as GPR43-IL-18, were modulated by FMT in HCD-induced rats ($p < 0.05$). Recognizing the crucial role of gut microbiota in SCFAs metabolism, a co-occurrence network among the Lactobacillaceae and SCFAs-GPR43-IL-18 was constructed.

Conclusion: The Lactobacillaceae-acetic acid-GPR43-IL-18 pathway emerges as a potential biological basis for the pathological state of HCD-induced rats. FMT exhibits corrective properties by influencing this pathway.

Keywords: high-calorie diet 1; fecal microbiota transplantation 2; gut microbiota-SCFAs-GPR43-IL-18 3; pathway 4; microbiota 5

Introduction

With the continuous improvement of living standards and the significant enrichment of material resources in contemporary society, excessive intake of fat and calories has emerged as a prominent alteration in the dietary structure of the Chinese population [1,2]. Parental indulgence and lack of control have resulted in the prevalent adoption of high-calorie diet (HCD) among children. Given the weaker gastrointestinal function in children, they are more susceptible to physical ailments such as diabetes and obesity following HCD [3,4]. Considering the unique growth and development phase of childhood, characterized by heightened demand for nutrition, correcting dietary structures and habits proves challenging, and effective preventive measures in clinical practice are currently lacking.

A nutrient-rich HCD can impact metabolic mechanisms by enhancing basic metabolism and accelerating cell reproduction [5], ultimately compromising health. Previous investigations have reported that HCD influences body physiology by contributing to pathological alterations in energy and lipid metabolism, as well as the activation of the immune system [6]. Our preliminary experimental research

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has indicated that short-term HCD may potentially reset the structure and function of the gut microbiota, leading to immune dysfunction [7,8]. However, the precise mechanisms underlying these phenomena remain unclear.

The gut microbiota plays pivotal roles in human food digestion, nutrition absorption, immune regulation, and resistance to pathogen infections, among other crucial physiological functions [9]. It constitutes a significant component of the intestinal mucosal barrier, contributing to a dynamic balance between antagonistic and synergistic bacterial species [10,11]. The systemic effects of the gut microbiota are primarily attributed to metabolites derived from the microbiota, such as short-chain fatty acids (SCFAs), bile acids (BAs), and amino acids (AAs) [12,13]. Among these, SCFAs play a crucial role in maintaining the integrity of intestinal epithelial cells. They activate G protein-coupled receptor 43 (GPR43) in epithelial or immune cells, triggering the massive production of Nucleotide-binding and oligomerization domain (NOD)-like receptor thermal protein domain-associated protein 3 (NLRP3) and promoting interleukin-18 (IL-18) production [14,15].

In recent years, fecal microbiota transplantation (FMT), a therapy with a longstanding history capable of reconstructing the gut microbiota, has garnered increasing attention [16]. Essentially, this involves the transplantation of functional flora from the feces of healthy individuals into the intestines of patients, thereby reconstructing the normal intestinal microecology system [17]. Given its broad applicability, significant efficacy, and minimal adverse reactions, FMT has found extensive use in treating gastrointestinal function diseases [18,19], endocrine and metabolic diseases [20], nervous system diseases [21], and other disorders related to gut microbiota. It is widely accepted that the specific mechanism of FMT involves establishing new flora-host interactions through the transplantation of functional flora as a therapeutic approach to various diseases. However, a recent perspective suggests that FMT might act as an implosive therapy, rectifying the gut microbiota disorders of patients through numerous implantations of exogenous healthy flora, thereby guiding the gut microbiota of the recipient towards a normal state under the influence of the healthy flora of the donor [22].

Based on previous research findings, we hypothesized that HCD could induce immune disorders by modulating the gut microbiota-SCFAs-GPR43-IL-18 pathway. Additionally, we postulated that FMT may have a protective effect on HCD-induced rats by correcting this pathway. Therefore, this study elucidated the immune disorders induced by HCD through gut microbiota and evaluated the impact of FMT on the "gut microbiota-SCFAs-GPR43-IL-18" pathway in rats subjected to HCD.

Materials and Methods

Animals and Diets

All animals were procured from Beijing Si Pei Fu Biotechnology Co., Ltd. (Animal license number: SYXK (Beijing, China) 2014–0006). A total of 40 female Sprague-Dawley (SD) rats, specific pathogen-free (SPF) grade, weighing 100–110 g, and aged 3 weeks, were housed in the SPF animal center of Beijing Hospital of Traditional Chinese Medicine under controlled conditions (temperature 20–25 °C, humidity 50%–70%). The standard rat maintenance feed served as the diet, while the high-calorie feed, comprising rice crackers, chocolate wafers, beef grains, and flour in a ratio of 1:2:2:1, was produced by Beijing Si Pei Fu Biotechnology Co., Ltd. The high-calorie feed provided 1828.12 kJ, with 16.10% fat content, surpassing the common feed and demonstrating good stability and repeatability (**Supplementary Table 1**) [7,23].

Experimental Models and Drug Administration

Bacterial solutions were prepared from the faeces of six randomly selected normal female SD rats [24,25]. The 40 female SD rats were then divided randomly divided into six groups: normal control 1 (NC1, n = 6), normal control 2 (NC2, n = 6), normal control 3 (NC3, n = 6), high-calorie diets model (M, n = 6), fecal microbiota transplantation treatment (FMTT, n = 8) [26], Medilac-Vita (MV, S20020037, Hanmi, Beijing, China) treatment group (n = 8). The rats underwent three days of adaptive feeding.

From days 1 to 6 of the experiment, all groups were provided free access to drinking water and diet. The NC2, NC3, M, FMTT, and MV groups received antibiotic treatment involving intragastric infusions of neomycin sulfate (1405-10-3, Sigma, Saint Louis, MO, USA) and streptomycin sulfate (3810-74-0, Sigma, Saint Louis, MO, USA) at a ratio of 1:1200 mg/kg twice daily. NC1 received equivalent volumes of normal saline [27]. On days 7, 9, and 11, the NC1, NC2, and NC3 groups were fed with a common diet, while the M, FMTT, and MV groups were fed with a special HCD, and 2 mL/100 g milk solution was administered intragastrically twice daily; and the MV group received MV solution at a concentration of 100 mg/mL twice daily. On days 8, 10, and 12, the NC1, NC2, M, and MV groups received normal saline enemas after a 2-hour fast, while the NC3 and FMTT groups received normal fecal filtrate enemas. After an 8-hour fast on day 13, rats were euthanized by intraperitoneal injection of 2% sodium pentobarbital (0.1 mL/10 g). Blood was collected from the abdominal aorta, centrifuged at 4 °C and 3000 rpm for 15 minutes after 4 hours, and the upper serum was collected and stored at –20 °C. The colon tissue was aseptically excised, rinsed with 0.9% normal saline, and stored in polyformaldehyde fixative. Colonic contents were collected and preserved at –80 °C. Euthanasia was performed with 150 mg/kg sodium pentobarbital.

16S rDNA Full-Length Sequencing and the Data Processing

Individual total genomic DNA was extracted from colon digesta using a Tiangen DNA Kit (DP304, Tiangen, Beijing, China). Bacterial 16S rDNA was amplified with unique molecular identifier (UMI) primers and KAPA HiFi HotStart ReadyMix (KK3605, Roche, Shanghai, China). The universal primers were 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′). Amplicons were purified with Beckman Agencourt AMPure XP (A63881, Beckman coulter, Brea, CA, USA) and quantified using a ThermoFisher Qubit dsDNA assay kit (Q32854, ThermoFisher, Waltham, MA, USA). The sequencing library was constructed by pooling equal amounts of purified amplicons and subjected to whole 16S rDNA sequencing on the Illumina platform.

Targeted Metabolomics for SCFAs

Acetic acid (64-19-7, Sigma, Saint Louis, MO, USA), propionic acid (79-09-4, Sigma, Saint Louis, MO, USA), isobutyric acid (79-31-2, Sigma, Saint Louis, MO, USA), butyric acid (107-92-6, Sigma, Saint Louis, MO, USA), isovaleric acid (503-74-2, Sigma, Saint Louis, MO, USA), valeric acid (109-52-4, Sigma, Saint Louis, MO, USA), and hexanic acid (142-62-1, Sigma, Saint Louis, MO, USA) were dissolved in ether to prepare a solution of standards and stored at 4 °C. For each, 100 mg of rat feces from each group were homogenized in 100 µL of phosphoric acid (15%, 7664-38-2, Sigma, Saint Louis, MO, USA) with 100 µL isocaproic acid (50 µg/mL, 646-07-1, Sigma, Saint Louis, MO, USA) as an internal standard solution (isocaproic acid, 646-07-1, Sigma, Saint Louis, MO, USA). An additional 400 µL of ether was used to homogenize for 1 minute. Subsequently, the samples were centrifuged at 4 °C at 12,000 rpm for 10 minutes, and then the supernatant was transferred into the vial prior to gas chromatography-mass spectrometry (GC-MS) analysis. The GC was performed on a capillary column Agilent HP-INNOWAX (30 m × 0.25 mm i.d. ×0.25 µm, 6890N GC, Agilent Technologies, Santa Clara, CA, USA) with the sample injected at a rate of 10:1, volume of 1 µL, and at a temperature of 250 °C.

Hematoxylin-Eosin (HE) Staining

Rat colon tissue, fixed and dehydrated in gradient ethanol, was embedded in paraffin and subsequently sectioned into 5 µm thick slices. Subsequently, paraffin sections were dewaxed, stained with hematoxylin for 2 minutes, differentiated for 10 seconds, stained with eosin for 3 minutes, and dehydrated and sealed after rinsing in tap water. An optical microscope (95.13202 Rev F, Lecia, Wetzlar, Germany) was used to capture the images.

Immunohistochemistry (IHC) Staining

Antigen recovery was conducted in an autoclave using citrate buffer following paraffin sectioning and deparaffinization in water. Sections were treated with a 3% hydrogen peroxide solution for 10 minutes, followed by incubation with a diluted primary antibody (GPR43, 1:200, orb159222, Biorbyt, Cambridge, UK) at 4 °C overnight. The secondary antibody (1:50, PV-6001, ZSGB-BIO, Beijing, China) was incubated for 40 minutes at 37 °C, and subsequently stained with diaminobenzidine (DAB, ZLI-9017, ZSGB-BIO, Beijing, China). Hematoxylin staining was performed, and the target protein was observed under an inverted microscope (95.13202 Rev F, Lecia, Wetzlar, Germany). ImageJ software (version 1.43, National Institutes of Health, Bethesda, MD, USA) was used for quantitative analysis of the images.

Enzyme-Linked Immunosorbent Assay (ELISA)

A 96 T rat IL-18 ELISA kit (E02I0310, BlueGene, Shanghai, China) was obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Serum IL-18 content was measured following the ELISA instructions, and the optical density (OD) value was read on a microplate reader (450 nm, PR4100, Bio-Rad, Hercules, CA, USA).

Statistical Methods

Correlation analysis of intestinal microflora was performed using the Illumina MiSeq platform. Statistical analysis of the data was conducted using SPSS (version 23.0, IBM SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Descriptive statistics and exploratory analysis were employed to assess the normal distribution of data. In cases where normal distribution criteria were not satisfied, the Kruskal-Wallis test was applied. When normal distribution criteria were met, one-way ANOVA was used, and the LSD method for group comparisons based on variance homogeneity. Statistical significance was determined at $p < 0.05$.

Results

Quality Evaluation of FMT and Modeling of Antibiotic-Induced Rats

FMT Restores the Clinical Characteristics of Antibiotic-Induced Rats

Fig. 1A illustrates that, during the antibiotic intervention, there were no significant differences in appearance among groups compared to the NC1 group. However, noticeable changes included yellowing and alterations in fecal shape, accompanied by varying degrees of body weight and abdominal circumference reduction. The intervention of FMT led to a gradual increase in body weight and abdominal circumference in the NC3 group over time (Fig. 1B,C).

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FMT Corrects Fecal-Associated Microbiome (FAM) Disorder Induced by Antibiotics

Various alpha indices (Sobs, Shannon, Shannoneven, Chao) were used to analyze bacterial community richness. As shown in Fig. 1D,E, bacterial community richness (Sobs and Chao) in the NC2 group significantly differed from that in the NC3 group ($p < 0.05$). Although not statistically significant, similar trends were observed with other indices (Shannon, Shannoneven) (**Supplementary Fig. 1A,B**). To evaluate the degree of bacterial community similarity, principal coordinates analysis (PCoA) based on the Bray-Curtis distance of the operational taxonomic units (OTUs) was used, demonstrating distinct distribution structures for NC1 and NC3 compared to NC2 (Fig. 1F) (analysis of similarities (ANOSIM), R = 0.3761, $p = 0.003$). Partial least squares-discriminant analysis (PLS-DA) further supported clear separation among NC1, NC2, and NC3, with NC2 showing a more centralized distribution (Fig. 1G).

The relative abundances of the top 20 families in each subject revealed significant structural differences in the three groups (Fig. 1H). These results indicate that the normal bacterial solution effectively ameliorated the antibiotic-induced flora disorder. Notably, antibiotic intervention led to changes in abundance, such as the increase of the Bacteroides S24-7 group, Verrucomicrobiaceae, and a decrease in Peptostreptococcaceae (**Supplementary Fig. 1C,D**). This intervention also impacted the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, including primary immunodeficiency and those associated with cancer and infectious diseases like prostate cancer and tuberculosis (**Supplementary Fig. 1E**).

FMT Effectively Reverses HCD-Induced Gut Microbiota Dysfunction and Downstream Metabolites Disorder

FMT Ameliorates HCD-Induced Pathological States in Rats

Previous investigations identified an imbalance in gut microbiota following FMT in rats subjected to HCD. However, the precise mechanisms require further exploration. Despite the proven efficacy of intestinal microbiota transplantation in regulating disturbances in intestinal microecology for disease treatment, its impacts on rats subjected to HCD remain unknown. Consequently, we evaluated the therapeutic potential of FMT in an antibiotic rat model induced with HCD, comprising a 52% full-fat milk powder solution and a specialized HCD (Fig. 2A).

Notably, the increase in body weight and abdominal circumference in the high-calorie diets model (M) group was slow (Fig. 2B,C). Although the structural changes of mucosal epithelial layer were not apparent, disordered cell arrangement and goblet cell aggregation were evident (Fig. 2D). Post-FMT, while short-term improvements in weight and abdominal circumference were not significant, the HE staining of histological sections of the distal colon showed a significantly lower degree of lesions in the FMTT group compared to the M group (Fig. 2B–D).

FMT Induces Alterations in Microbial Communities in Rats with HCD

Overall Structure of FAM Communities. 16SrDNA sequencing of the gut microbiota revealed that HCD induced gut microbiota disorder in normal rats characterized by decreased flora diversity and abundance and alterations in FAM structure. The Shannon index, assessing the alpha diversity of the community, revealed a significant reduction in bacterial community diversity in fecal samples of HCD-fed rats compared to NC2 (Fig. 3A). Following FMT or MV intervention, bacterial community diversity in rats increased to some extent, with a significant difference between the FMTT and M groups. Similarly, beta distance (Bray-Curtis) between samples in the four groups exhibited substantial differences, resulting in the distinct distribution in PCoA (Fig. 3B, ANOSIM, R = 0.6205, $p = 0.001$) and PLS-DA (Fig. 3C). The results demonstrated significant differences between the NC2 and M groups. Following FMT and MV intervention, the bacterial structure tended towards normalcy, and the bacterial structure of the FMTT group closely resembled that of the NC2 group and was significantly different from the M group (Fig. 3B).

Common and Distinct Flora in the Analyzed Groups. The top five most abundant families, namely Bacteroidales S24-7 group, Verrucomicrobiaceae, Bacteroidaceae, Lachnospiraceae, and Lactobacillaceae, collectively constituted 74.13% of all sequences (Fig. 3D). Bacteroidales S24-7 group was the most abundant family, representing 23.27% of the sequences. Linear Discriminant Analysis Effect Size (LEfSe) and Kruskal-Wallis methods (Fig. 3E,F) were used to identify specific bacteria that were enriched in each group. At the family level, compared to the NC2 group, Verrucomicrobiaceae and Bacteroidaceae increased, while Lactobacillaceae, Bacteroidales S24-7 group, and Ruminococcaceae decreased in the M group ($p < 0.05$). In comparison to the M group, the FMTT group exhibited increased Lactobacillaceae and Christensenellaceae, and decreased Verrucomicrobiaceae and Bacteroidaceae ($p < 0.05$). Additionally, Verrucomicrobiaceae in the MV group decreased compared to the M group, and the Bacteroidales S24-7 group showed an increasing trend ($p < 0.05$).

Functional Predictions

The function of 16SrDNA was predicted using the Tax4Fun algorithm. Notably, metabolic pathways associated with fatty acids, such as those related to fatty acid degradation, exhibited a marked enrichment in the M group (Fig. 4A). Subsequent analysis of the fatty acid degradation pathway, utilizing Statistical Analysis of Metagenomic Profiles (STAMP), revealed an enrichment of the fatty acid degradation in samples from the M and MV groups com-

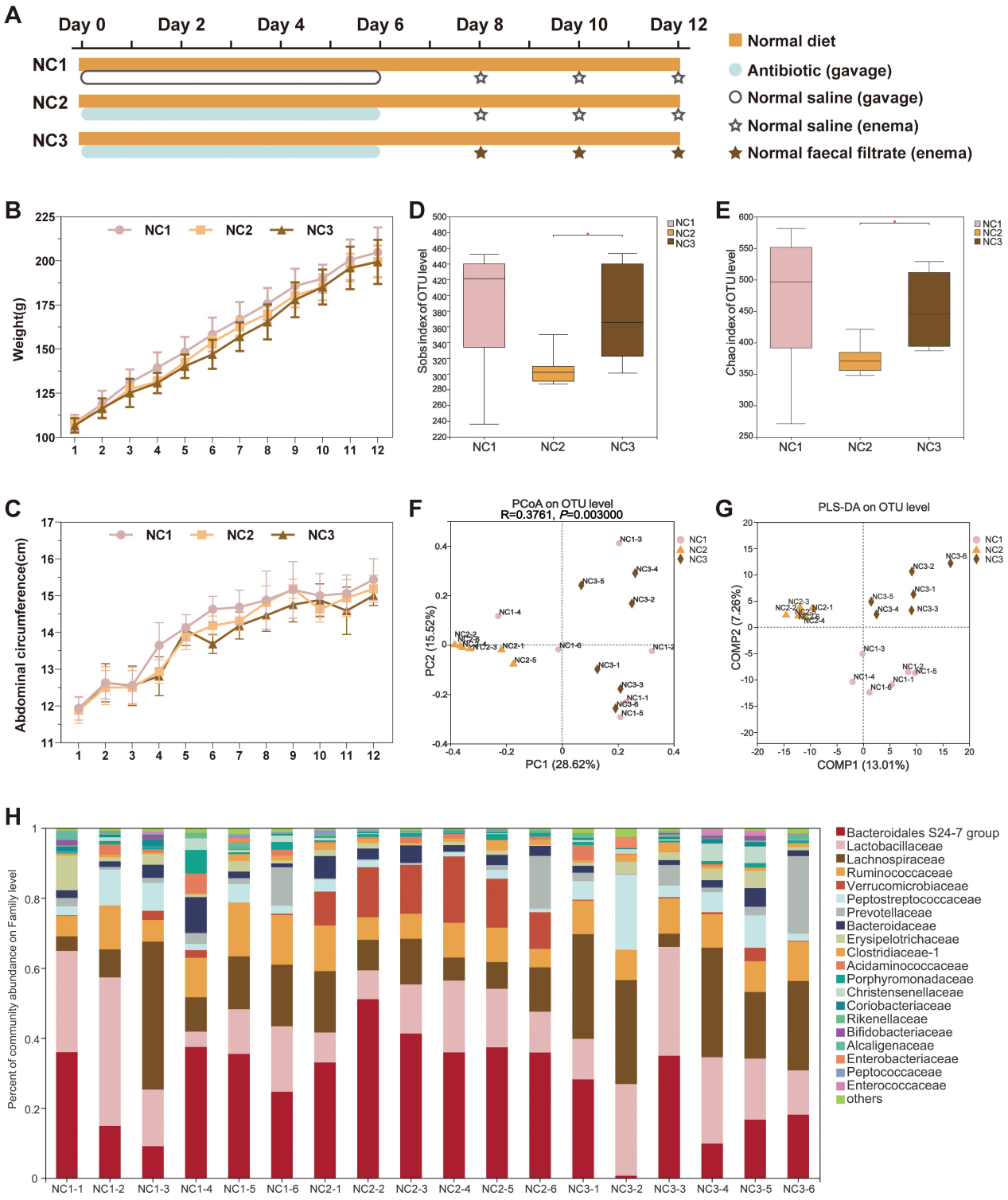

Fig. 1. Evaluation of FMT intervention. (A) Schematic representation of the experimental model for FMT intervention on neomycin sulfate and streptomycin sulfate (1:1) induced antibiotic rats. (B) Changes in body weight observed in NC1, NC2, and NC3 groups. (C) Alterations in abdominal circumference recorded in NC1, NC2, and NC3 groups. (D) Sobs index and (E) Chao index illustrating microbial diversity. (F) PCoA of FAM at the OTU level, with ANOSIM results (R = 0.3761, $p = 0.003$). (G) PLS-DA of FAM at the OTU level. (H) Composition of bacterial communities at the family level across samples, with different colors representing distinct groups (n = 6). NC1, normal control 1; NC2, normal control 2; NC3, normal control 3; FMT, fecal microbiota transplantation; FAM, fecal-associated microbiome; PLS-DA, partial least squares-discriminant analysis; PCoA, principal coordinates analysis; OTU, operational taxonomic unit; PC, principal coordinates; COMP, components to include in the model; ANOSIM, analysis of similarities. *$p < 0.05$.

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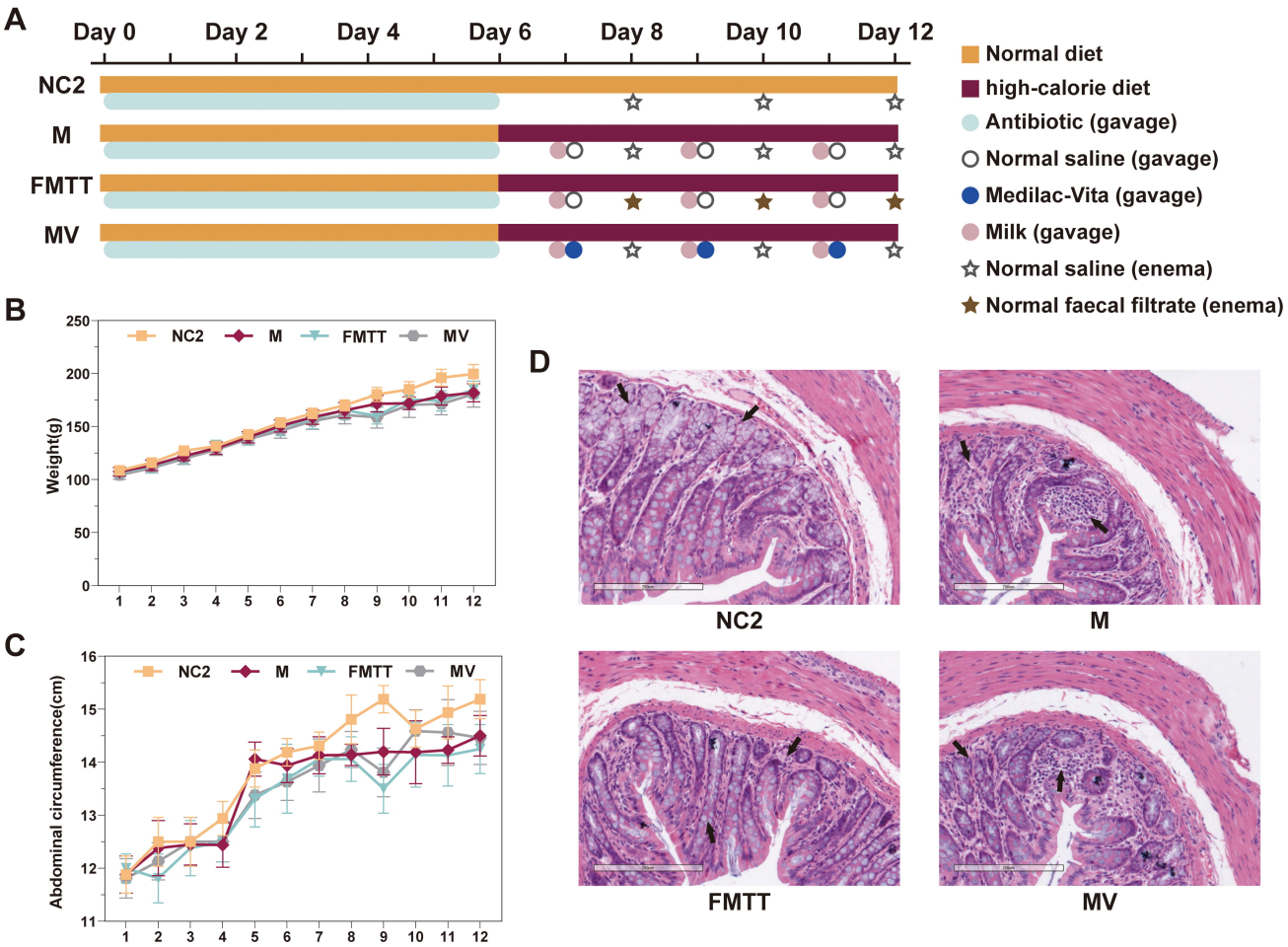

Fig. 2. Therapeutic impact of FMT on rats subjected to HCD. (A) Experimental protocol outlining FMT and MV treatments for antibiotic-induced rats subjected to HCD. (B) Body weight variations in NC2, M, FMTT, and MV groups. (C) Changes in abdominal circumference recorded in NC2, M, FMTT, and MV groups. (D) Histopathological examination through HE staining of distal colons from rats subjected to HCD. Black arrows indicate goblet cells or inflammatory infiltration. Scale bar: 200 μm. n = 6. HCD, high-calorie diet; FMT, fecal microbe transplantation; NC2, normal control 2; M, high-calorie diets model; FMTT, fecal microbe transplantation treatment; MV, Medilac-Vita; HE, hematoxylin-eosin.

pared with the NC2 group. Following FMT intervention, a noticeable reduction in pathway enrichment was observed compared to the M group (Fig. 4B).

Effect of FMT on SCFAs-GPR43-IL-18 in Rats Fed HCD

As anticipated, fatty acid degradation pathways emerged as potential targets in FMT treatment. Confirming our hypothesis, the alterations in SCFAs concentrations in the feces exhibited a significant decrease in rats subjected to HCD (Fig. 5A). Notably, an upward trend in SCFAs content was observed in the FMTT and MV groups, compared to the M group, especially in acetic acid content ($p < 0.05$). Similar trends were observed for propionic acid, butyric acid, caproic acid, isobutyric acid, isovaleric acid, and valeric acid, although some changes were not significantly different. Additionally, the change of GPR43 was consistent with the alterations in SCFAs, displaying a decreasing trend in the M group and an increasing trend in the FMTT group (Fig. 5B). Moreover, IL-18 in the serum exhibited significant improvement following FMT treatment, especially in the FMTT group rats (Fig. 5C).

FMT is Associated with Lactobacillaceae and Promotes SCFA Production

As demonstrated earlier, FMT may regulate SCFAS by modifying the relative abundances of the key families to influence the expression of the GPR43-IL-18 (Fig. 5). However, the exact mechanism remains unknown, so we explored the correlations between the top 50 families and the concentration of SCFAs, GPR43, and IL-18 to unveil their potential roles (**Supplementary Fig. 2**). Simultaneously, our investigation into the key genera regulating acetic acid-GPR43-IL-18 identified four key common differential families with a linear discriminant analysis (LDA)

Fig. 3. Comparative analysis of FAM structures and identified taxa in NC2, M, FMTT, and MV groups. (A) The Shannon index for microbial diversity. (B) PCoA of FAM at the OTU level (Bray-Curtis dissimilarity), with ANOSIM results (R = 0.6205, p = 0.001). (C) PLS-DA of FAM at the OTU level. (D) Composition of bacterial communities at the family level across samples. (E) Significant bacterial differences within groups at the family level determined by the Kruskal-Wallis test. (F) LDA score showing significant bacterial differences within the groups at the family level. Different colors represent distinct groups. (n = 6 for NC2 and M; n = 8 for FMTT and MV). NC2, normal control 2; M, high-calorie diets model; FMTT, fecal microbe transplantation treatment; MV, Medilac-Vita; LDA, linear discriminant analysis; FAM, fecal-associated microbiome; PLS-DA, partial least squares-discriminant analysis; PCoA, principal coordinates analysis; OTUs, operational taxonomic units; PC, principal coordinates; COMP, components to include in the model; ANOSIM, analysis of similarities. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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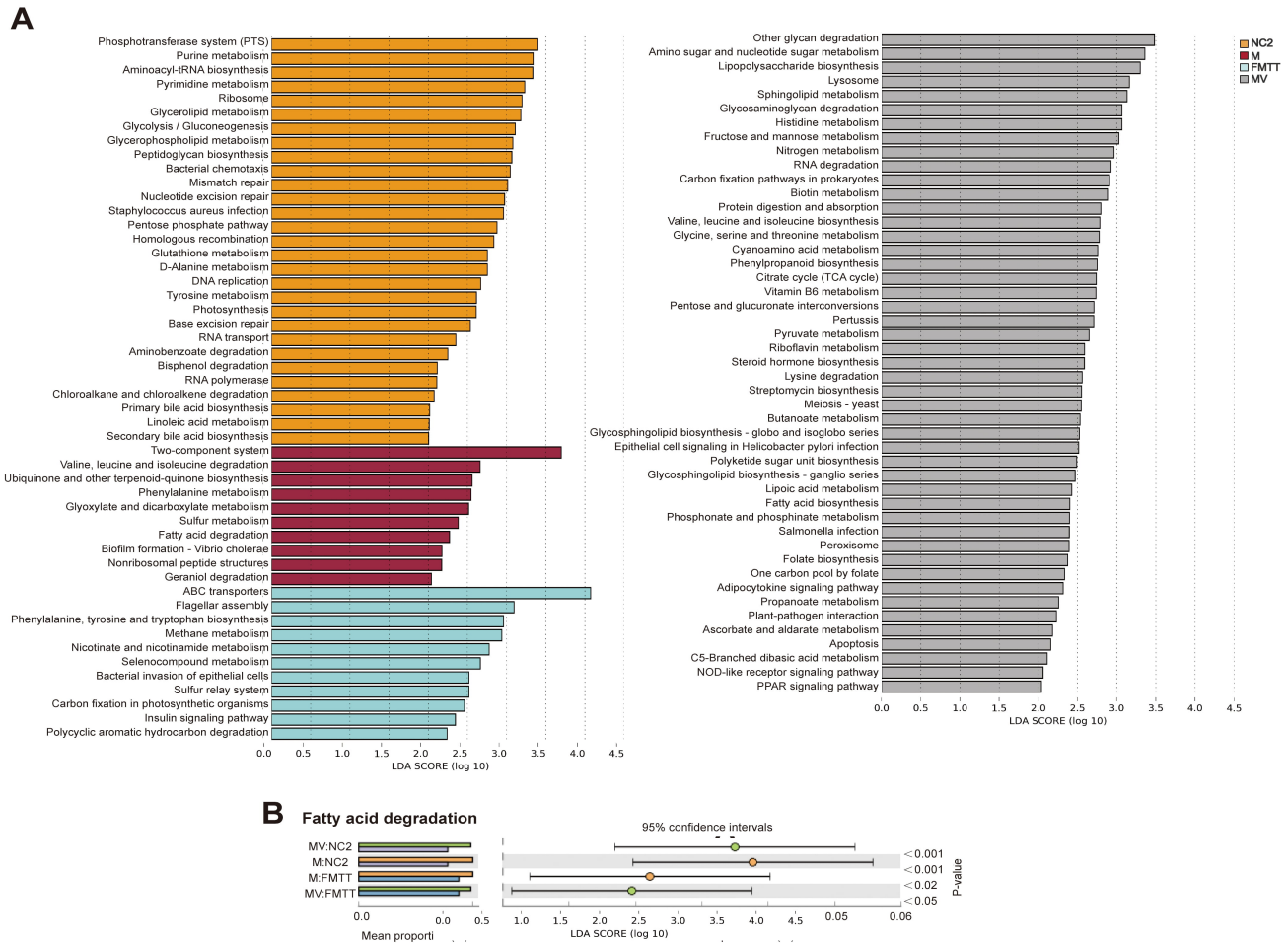

Fig. 4. Core pathways that played important roles in FMTT. (A) Significant differences revealed by LDA score in metabolic pathways within groups. (B) Outputs of Wilcoxon rank-sum test of fatty acid degradation pathway in different groups obtained by STAMP. Different colors represent distinct groups: NC2 in purple, M in orange, FMTT in blue, and MV in green. (n = 6 for NC2 and M; n = 8 for FMTT and MV). NC2, normal control 2; M, high-calorie diets model; FMTT, fecal microbe transplantation treatment; MV, Medilac-Vita; LDA, linear discriminant analysis; STAMP, Statistical Analysis of Metagenomic Profiles; NOD, Nucleotide-binding and oligomerization domain; PPAR, peroxisome proliferators-activated receptor.

score >4 and $p < 0.05$ based on LEfSe and Wilcoxon rank sum test: Lactobacillaceae, Ruminococcaceae, Christensenellacea, and Acidaminococcaceae (Fig. 5D).

As illustrated in Fig. 5D, acetic acid was positively correlated with Lactobacillaceae (R = 0.443, $p = 0.030$) and Ruminococcaceae (R = 0.426, $p = 0.038$). Moreover, Lactobacillaceae was positively correlated with GPR43 (R = 0.560, $p = 0.004$) and negatively correlated with IL-18 (R = –0.601, $p = 0.002$). Additionally, acetic acid showed a positive correlation with GPR43 (R = 0.412, $p = 0.045$). However, no significant correlation was observed between GPR43 and IL-18.

Discussion

The primary goal of FMT is to restore the diversity in quantity and species of gut microbiota, aiming to recover the normal immune function of the intestinal mucosa [28]. Extensive evidence supports that probiotics can enhance the barrier function of the intestinal mucosa, regulate the immunity of the intestinal mucosa, restore the community structure of FAMs, and mitigate chronic intestinal inflammation [29–31]. Due to experimental constraints, germ-free rats were impractical. Therefore, antibiotic gavage was employed to eliminate most intestinal microorganisms and reduce the intestinal microbial load, simulating antibiotic-induced rats [32,33]. Our findings revealed a significant reduction in the diversity and richness of the flora in antibiotic-induced rats, with diminished body weight and altered stool characteristics. Notably, FMT effectively restored the flora imbalance induced by antibiotics, suggesting the feasibility of the FMT we used and its potential to restore the gut microbiota structure in normal rats by increasing bacterial abundance and diversity.

Building on previous research, we used 16SrRNA sequencing to explore the potential impact of HCD on micro-

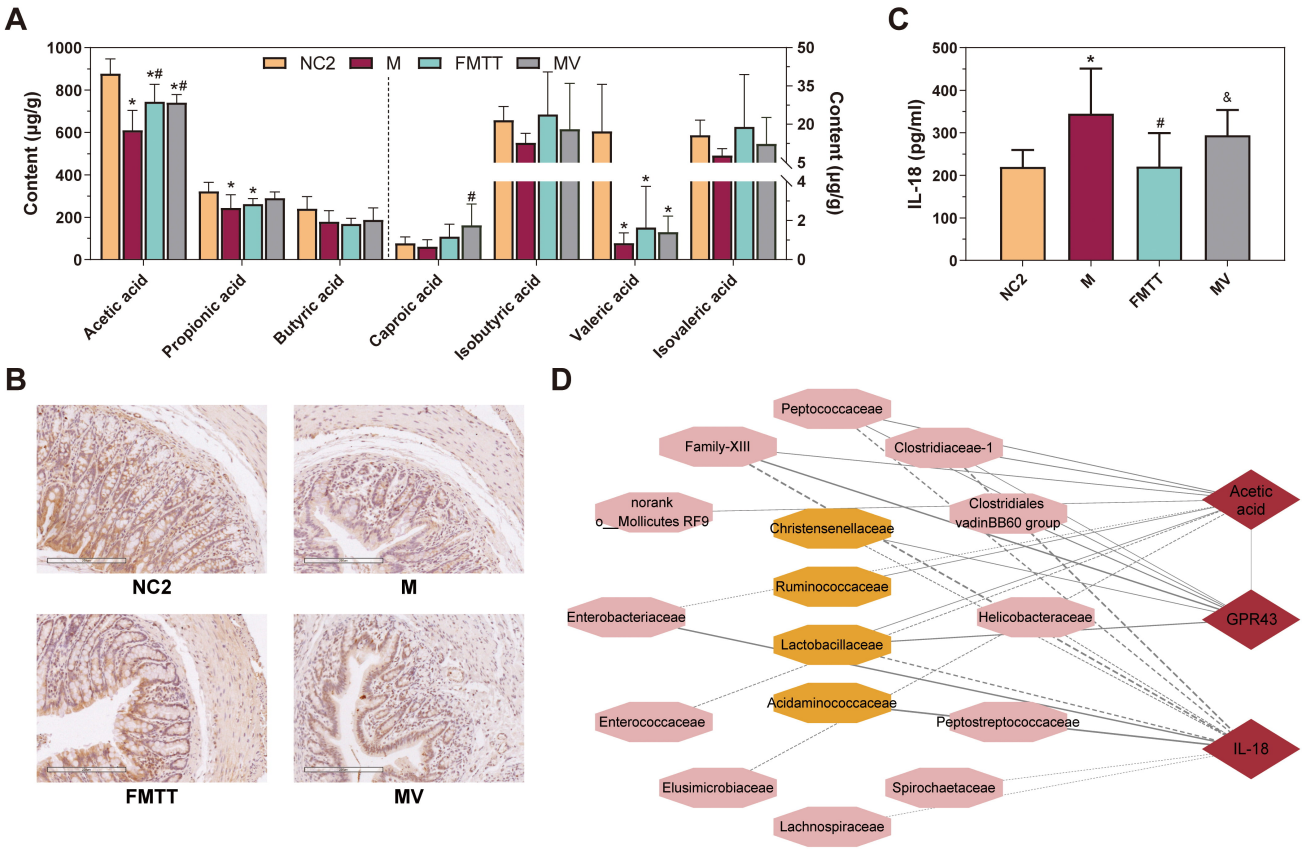

Fig. 5. Changes in levels of SCFAs and downstream metabolites in NC2, M, MV, and FMTT groups. (A) Fecal SCFA levels. (B) Expression of GPR43 in colon tissues. Scale bar: 200 μm (C) Expression of IL-18 in serum. (D) The network of the differential predominant FAM families, related SCFAs, and IL-18. Solid lines represent positive correlations, while dotted lines represent negative correlations. The thickness of the line represents the |R|. Different colors represent distinct groups (NC2, M, MV, FMTT). n = 6 for NC2 and M; n = 8 for FMTT and MV. NC2, normal control 2; M, high-calorie diets model; FMTT, fecal microbe transplantation treatment; MV, Medilac-Vita; SCFAs, short-chain fatty acids; FAM, fecal-associated microbiome; GPR43, G protein-coupled receptor 43; IL-18, interleukin-18. Compared with NC2, $^{*}p < 0.05$; Compared with M, $^{\#}p < 0.05$; Compared with FMTT, $^{\&}p < 0.05$.

bial structure and function, and the subsequent recovery of dysbiosis through FMT. Our study unveiled the significant role of HCD in gut microbiota dysregulation, characterized by reduced diversity in FAMs and alterations in abundance, including a marked decrease in Lactobacilliaceae and Bacteroidales S24-7 group and an increase in Bacteroidaceae. Moreover, FMT induced comprehensive changes in the gut microbiota structure, influencing the abundance of several families that might act as pivotal bacteria in alleviating HCD-induced dysbiosis. The FMTT group had higher predominance in Lactobacillaceae and Christensenellaceae, and lower predominance in Verrucomicrobiaceae and Bacteroidaceae, while the MV group had lower predominance in Verrucomicrobiaceae, without significant increases in dominant flora. These findings suggest that, compared to probiotics, FMT may present a more robust approach to manipulate the gut microbiota as a therapeutic strategy for HCD susceptible diseases [34].

Among the bacterial families, Lactobacillaceae and Christensenellaceae are two important SCFA-producing bacteria, and their reduction may directly lead to SCFA deficiency in the intestine [35]. In a KEGG functional prediction analysis, it was observed that FMT regulates fatty acid degradation pathways. SCFAs, integral components of fatty acids, constitute the primary sources of free fatty acids (FFAs) in the metabolic network [36]. Their expression throughout the intestines, including intestinal and adipose cells, closely correlates with the overall intestinal function and body fat metabolism. Recent studies have demonstrated that SCFAs play a pivotal role in maintaining the regulation of energy metabolism, regulating inflammation and immunity, and enhancing epithelial cell repair function [37,38]. Acetic acid not only regulates adipocyte differentiation, but also inhibits fat deposition in the body [39,40]; and propionic acid contributes to hepatic gluconeogenesis and is a known precursor for glucose synthesis in the liver [41]. Additionally, butyric acid, the primary energy source for intestinal mucosal cells, regulates cell proliferation and differentiation, repairs damaged cells and maintains the integrity of the colon mucosa [42].

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In our study, rats induced with HCD exhibited decreased levels of SCFAs, particularly in acetic acid. Post FMT, there was a restoration of SCFA levels, indicating the potential therapeutic impact of FMT on SCFA deficiency induced by HCD. While the changing trend of butyric acid was consistent with acetic acid and propionic acid, no significant effect of FMT on butyric acid content in HCD-induced rats was observed, possibly due to the limited sample size. This finding may suggest that HCD induces significant changes in the body through the influence of bacterial flora on lipid metabolism rather than directly affecting the integrity of the intestinal mucosa, warranting further investigation.

Extensive studies have demonstrated that GPR43 controls the energy utilization of the body and participates in lipid metabolism, regulating immune and inflammatory responses [43,44]. GPR43 is activated by SCFAs, with acetic acid and propionic acid being the most efficient activators, followed by butyric acid and other SCFAs [45,46]. Acetic acid primarily depends on binding to GPR43, compared to SCFAs-specific receptors, G protein-coupled receptor 41 (GPR41), and G protein-coupled receptor 109A (GPR109A) [47,48]. At the same time, SCFAs promote the release of IL-18, accelerating the repair of the intestinal mucosal barrier [15]. IL-18, a proinflammatory factor, regulates the immune and inflammatory responses, serving as a key link in intestinal epithelial repair [14].

Our evidence suggests that HCD-induced FAM alters SCFA levels, impacting acetic acid and leading to immune dysfunction in rats by suppressing GPR43 expression and subsequently increasing IL-18 levels. Notably, FMT intervention appears to influence the GPR43-IL-18 axis, characterized by an increase in GPR43 and a decrease in IL-18 levels. These observations suggest that exogenous normal flora can activate GPR43 in epithelial or immune cells, correcting immune disorders through this mechanism.

Given the regulatory influence of FMT on gut microbiota and SCFAs in the HCD state, it is evident that GPR43 and IL-18 are the key downstream nodes. Building upon this observation, we employed Spearman correlation analysis to construct a regulatory network encompassing the effects of FMT on gut microbiota, SCFAs, GPR43, and IL-18. Our findings revealed a positive correlation between Lactobacillaceae, a key bacterium influenced by FMT, and both acetic acid and GPR43 while displaying a negative correlation with IL-18. Numerous studies highlight the essential roles of *Lactobacillus* in regulating the intestinal mucosal immune and epithelial barriers, thus protecting intestinal mucosal function [49]. The gas chromatographic analysis of *Lactobacillus* indicated that, in addition to lactic acid, hydrogen peroxide, and bacteriocin, SCFAs were prominent metabolites with a high content of acetic acid in their extract [50]. Although no direct correlation between GPR43 and IL-18 was identified, a correlation between SCFAs and GPR43 was observed, further supporting our hypothesis that FMT influences the FAM of gut microbiota and protects HCD-induced rats through the "acetic acid-GPR43-IL-18" pathway. Moreover, enhancing the levels of Lactobacillaceae emerges as a crucial factor in regulating the imbalance of bacteria induced by high-fat diet.

Furthermore, beyond impacting the structure and function of the gut microbiota, HCD manifests gastrointestinal dysfunction, immune dysfunction, abnormal levels of neuroendocrine-related hormones, and damage to the intestinal mechanical barrier [51]. Notably, structural changes in the mucosal epithelium were inconspicuous in the M group, and only disturbed cell arrangement and inflammatory cell infiltration were observed. This observation might be linked to inflammatory inhibition resulting from antibiotic intervention factors, aligning with the findings of our study, where there was no significant difference in the changes of butyric acid, a factor closely related to the structure of intestinal mucosa integrity.

Conclusion

In conclusion, this study, conducted through animal experimental models and pharmaceutical interventions, confirms that factors associated with HCD can impact the FAM of the body. This is primarily manifested by a reduction in beneficial bacteria and an increase in conditional pathogens. Additionally, the study reveals the activation of the gut microbiota- SCFAs-GPR43-IL-18 pathway, initiating a cascade of pathological reactions in the body. Among them, the Lactobacillaceae-acetic acid-GPR43-IL-18 pathway emerges as a potential biological foundation for the pathological state observed in rats subjected to HCD. Fecal microbiota transplantation (FMT) shows promise in correcting immune disorders induced by HCD by influencing this identified pathway. However, the intricate mechanism underlying the effects of a high-calorie diet and the downstream metabolic pathways of intestinal flora remain unclear, warranting further investigations.

To gain a more comprehensive understanding of FMT mechanisms of action, future studies should consider combining analyses of transcriptomics, proteomics, and other multi-omics approaches. Such integrative methodologies can shed light on the intricate mechanisms of FMT. Furthermore, taking the gut microbiota as a focal point for investigating fecal bacterial transplantation in children presents an opportunity to prevent the onset and progression of pediatric refractory diseases. This may prove pivotal in preventing and treating refractory pediatric diseases in clinical settings.

Abbreviations

HCD, high-calorie diet; FMT, fecal microbiota transplantation; SCFAs, short-chain fatty acids; GPR43, G protein-coupled receptor 43; GPR41, G protein-coupled receptor 41; GPR109A, G protein-coupled receptor 109

A; IL-18, interleukin-18; SPF, specific pathogen-free; SD, Sprague-Dawley; GC-MS, gas chromatography-mass spectrometry; HE, hematoxylin-eosin; IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; DAB, diaminobenzidine; OD, optical density; BAs, bile acids; AAs, amino acids; PLS-DA, partial least squares-discriminant analysis; PCoA, principal coordinates analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; FAM, fecal-associated microbiome; STAMP, Statistical Analysis of Metagenomic Profiles; LDA, linear discriminant analysis; FFAs, free fatty acids; OTUs, operational taxonomic units; PC, principal coordinates; COMP, components to include in the model; ANOSIM, analysis of similarities.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the first author if needed.

Author Contributions

YL, HY and JZ conducted the analysis, summarized the result, and drafted the manuscript. TL assisted in the data analysis and the figure editing. FG, JH, LC, and YS assisted in the experiments and the data analysis. HY and XG conceived the study. XG supervised the manuscript writing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All experiments were approved by the Committee on the Ethics of Animal Experiments of Beijing Hospital of Traditional Chinese Medicine (ethics number: 2018090101).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243805.305.

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