DOI: 10.26599/FSHW.2026.9251261

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Effective probiotics alleviate obesity induced by high-fat diet by regulating bile acid and short-chain fatty acid metabolism

Jiahe Li[a#], Ziyuan Wang[b,c#], Jinyu Gao[a#], Jiaquan Yan[a], Xin Wang[b,c], Yuxin Wang[b,c], Biyan Ouyang[b,c], Lanxiu Yu[b,c], Jiachao Zhang[a], Hui Xue[a*]

[a] *School of Food Science and Engineering, Key Laboratory of Food Nutrition and Functional Food of Hainan Province, Hainan University, Haikou 570228, China.*

[b] *Classykiss Yogurt (Suzhou) Co., Ltd, Suzhou 215144, China.*

[c] *ClassyKiss Dairy (Shenzhen) Co., Ltd, Shenzhen 518105, China*

ABSTRACT: High-fat diet can cause individuals to metabolic syndrome, including obesity, dyslipidemia, nonalcoholic fatty liver, fat accumulation, and chronic inflammation. The purpose of this study was to identify effective probiotic strains that mitigate high-fat diet–induced obesity and elucidate their underlying mechanisms. Golden hamsters, which exhibit lipid metabolism profiles most similar to humans, were used as the experimental model. In this study, we evaluated the efficacy of four *Bifidobacterium* in alleviating obesity induced by high-fat diet. Three effective probiotics, *Bifidobacterium animalis* subsp. *lactis* C-1 (*B. lactis* C-1), *Bifidobacterium animalis* subsp. *lactis* C-2 (*B. lactis* C-2), and *Bifidobacterium longum* subsp. *longum* C-5 (*B. longum* C-5) effectively reduced weight gain, improved serum lipid, decreased fat accumulation, and attenuating hepatic injury and systemic inflammation. Mechanistically, the effective probiotics enhanced bile salt hydrolase (BSH) activity and *Clostridium scindens*, promoting fecal bile acid excretion, reducing intestinal lipid absorption and bile acid reabsorption, which was alleviating obesity induced high-fat diet. Moreover, the effective probiotics alleviated high-fat diet–induced systemic inflammation and hepatic injury by increasing short-chain fatty acids (SCFAs) in the gut. In contrast, *Bifidobacterium longum* subsp. *infantis* CK2010 (*B. infantis* CK2010) showed limited effects. The study provided an in-depth mechanistic insight, detailing the gut microbes and metabolites which mediate the effects of the effective probiotics.

Keywords: *Bifidobacterium*, Probiotic, Gut microbiome, High-fat diet, Bile acids, Short-chain fatty acids

1. Introduction

High-fat foods may predispose individuals to metabolic syndrome, including obesity[1], diabetes[2], dyslipidemia[3], nonalcoholic fatty liver disease[4], fat accumulation, and sustained inflammatory responses[5]. High-fat Western diet was associated with markedly increased serum triglyceride levels, elevated LDL cholesterol accumulation, and the initiation of atherosclerotic cardiovascular disease[6,7]. A prior study reported that, following an eight-week on high-fat diet, mice exhibited significant gains in body and liver, noticeable fat deposition in both abdominal and epididymal tissues, and enhanced lipid storage within liver

***Corresponding author**
xuehui@hainanu.edu.cn (H. Xue)
[#]Co-first authors.

Received 4 February 2026
Received in revised from 23 March 2026
Accepted 13 April 2026

and fat cells[8]. Moreover, evidence suggested that consumption of high-fat diet enhanced the permeability of the intestinal barrier, upregulated pro-inflammatory cytokines, and enhanced susceptibility to experimental colitis, while also contributing to progressive liver injury ranging from steatosis to cirrhosis and hepatocellular carcinoma[9,10,11]. At the same time, high-fat diet quickly disrupted microbial homeostasis, promoting the expansion of harmful bacteria and significantly modifying both microbial metabolites and metabolic pathways[12].

Roughly 100 trillion microorganisms resided within the human digestive system, constituting a highly intricate microbial community commonly known as the intestinal microbiome[13,14]. Growing scientific data indicated that disruption of intestinal microbial equilibrium induced by consumption of high-fat diet, played a pivotal role in the development of obesity, abnormal lipid profiles, elevated blood glucose levels, insulin resistance, and other aspects associated with metabolic syndrome[15,16]. For example, *myo*-inositol markedly reduced the intestinal uptake in fatty acids, while the gut-resident bacterium *Megamonas rupellensis* possessed genes involved in *myo*-inositol catabolism, which promoted lipid assimilation in the intestine and contributed to the development of obesity and abnormal lipid metabolism[17]. *Enterobacter cloacae*, a species capable of generating endotoxins and originally isolated in the intestinal tract of an obese individual, has been shown to induce excessive weight gain, elevated blood glucose, and impaired insulin sensitivity in germ-free mice fed high-fat diet[18]. High-fat feeding increased the abundance of lipopolysaccharides (LPS), gut bacterial metabolites that contributed to the aggravation of obesity through chronic low-grade inflammation[19]. Therefore, alterations in intestinal microbial communities and their metabolic byproducts, triggered by consumption of fat-rich diets, played a pivotal role in driving the onset of obesity and related metabolic disorders.

Probiotics can provide physiological advantages to the host by modulating gut microbiota and their metabolites[20]. Therefore, probiotic supplementation represented an important strategy for preventing obesity and metabolic syndrome induced by high-fat diet. *Bifidobacterium adolescentis* protected mice against high-fat diet-induced obesity by affecting intestinal ANGPTL4 which functions as the principal intestinal lipase[21]. However, not all probiotics were capable of effectively mitigating the adverse effects of a high-fat diet. Western-style diet led to fat and hepatic lipid accumulation, while oral supplementation with *Lactobacillus rhamnosus* GG did not significantly alter body weight or liver fat deposition compared with saline-treated controls[22]. In high-fat diet-fed mice, supplementation with *Bifidobacterium animalis* subsp. *lactis* BB12 did not significantly reduce serum TC or TG levels compared with the HFD group[23].

This study evaluated the efficacy of *Bifidobacterium* in alleviating obesity induced by high-fat diet. Based on the functional and safety characterization of probiotics, four potential *Bifidobacterium* isolated from fermented dairy products were selected, including *B. infantis* CK2010, *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5. High-fat diet intervention was supplemented with *B. infantis* CK2010, *B. lactis* C-1, *B. lactis* C-2, *B. longum* C-5, or saline vehicle control. Fecal samples were collected for shotgun metagenomic sequencing, and serum and liver tissues were subjected to biochemical analyses. Histopathological

assessments of liver and adipose tissues were also performed to evaluate tissue-level alterations. This research aimed to comprehensively investigate the beneficial effects of four representative *Bifidobacterium* in the context of a high-fat diet. In addition, this study elucidated the underlying mechanisms through which they help counteract metabolic dysfunctions associated with obesity and metabolic syndrome induced by high-fat diet.

2. Materials and methods

2.1 Probiotics and animal experiments

B. infantis CK2010, *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 were provided by Classy Kiss Yogurt (Suzhou) Co., Ltd (Suzhou, China). All four *Bifidobacterium* strains were originally isolated from fermented dairy products.

A total of forty-eight golden hamsters, aged eight weeks, were obtained through Beijing Viton Lihua Laboratory Animal Technology Company (Beijing, China). The animals were housed in a specific pathogen-free (SPF) environment, with a regulated 12-hour light/dark schedule, room temperature kept at 25 ± 2 ℃, and humidity levels controlled at 55% ± 10%. During the six weeks of intervention, the hamsters were provided ad libitum access to regular chow and drinking water. The golden hamsters were randomly allocated into 6 experimental groups, including normal-fat diet group (NFD, n=8), high-fat diet group (HFD, n=8), high-fat diet supplemented with *B. infantis* CK2010 group (*B. infantis* CK2010, n=8), high-fat diet supplemented with *B. lactis* C-1 group (*B. lactis* C-1, n=8), high-fat diet supplemented with *B. lactis* C-2 group (*B. lactis* C-2, n=8), and high-fat diet supplemented with *B. longum* C-5 group (*B. longum* C-5, n=8). Randomization was performed using a computer-generated random number sequence to ensure unbiased group assignment. The normal-fat diet was composed of 22.8% proteins, 13.8% lipids, and 63.4% carbohydrate content. Conversely, the high-fat diet included 19.23% proteins, 46.53% lipids, 0.5% added cholesterol, and 33.74% carbohydrate content. The study procedures involving animals received formal approval from the Ethics Committee of Hainan University (China), in compliance with the institution “Laboratory Animals Protection and Utilization Guidelines of Hainan University”. The study was conducted under ethical clearance no.HNUAUCC-2025-00243, as authorized for animal experimentation.

2.2 Experimental design

Following a one-week acclimatization period, each golden hamster was individually labeled. Animals in the NFD group received normal-fat diet for six weeks, whereas the remaining groups were fed high-fat diet. The probiotic-treated cohorts—including *B. infantis* CK2010, *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5—were administered daily by gavage with 1×10^9 CFU suspended in 300 μL of saline, along with the high-fat diet. Golden hamsters consuming either normal-fat diet or high-fat diet received a daily dose of saline in equal volume. Body weight was recorded weekly throughout the experiment.

2.3 Blood and tissue sample collection

Following six weeks of intervention, all golden hamsters underwent a 12-hour fasting period during which food was withheld, though sterile distilled water remained available ad libitum. At the end of the experiment, biological samples—including serum, hepatic tissue, fecal matter, and adipose depots (inguinal, perinephric, and epididymal) were collected. The animals were euthanized via cervical dislocation. Blood specimens were left at ambient temperature for one hour before being centrifuged at 4 ℃, 3500 rpm for 45 minutes to separate serum, which was divided into portions and preserved at −80 ℃ for subsequent analytical procedures. Epididymal adipose and liver tissues were washed using ice-cold 0.2 M phosphate buffer solution (pH 7.2). Portions of the liver were preserved in 4% paraformaldehyde for histological processing and staining. Additional liver tissue was used for biochemical assays to assess pathological features, including triglycerides (TG), total cholesterol (TC), malondialdehyde (MDA), superoxide dismutase (SOD), and alanine aminotransferase (ALT). Remaining hepatic and fat samples were immediately frozen in liquid nitrogen and kept at −80 ℃ for subsequent analysis. Fecal samples of BSH activity and bile acid were assessed using enzyme-linked immunosorbent assay (ELISA) kits obtained from Nanjing Jiancheng Bioengineering Institute Co., Ltd.

2.4 Quantification of serum biochemical indicators

Serum concentrations of TG, TC, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free fatty acids (FFAs), and ALT were assessed using enzyme-linked immunosorbent assay (ELISA) kits obtained from Nanjing Jiancheng Bioengineering Institute Co., Ltd. Additionally, serum levels of interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were determined using assay kits supplied by Shanghai Xinyu Biotechnology Co., Ltd.

2.5 Histological analysis

Samples from the liver, colon, and epididymal adipose tissue were preserved in 4% neutral-buffered formaldehyde solution. Following the dehydration process, the specimens were paraffin-embedded and sliced into 5 μm-thick sections. The tissue slices were subsequently subjected to hematoxylin and eosin (H&E) staining for histological assessment of structural alterations in the colon and adipose cells. Additionally, hepatic lipid deposition was assessed through oil red O staining, with the visualized outcomes examined and documented using a microscope (Olympus Corporation, Tokyo, Japan).

2.6 Determination of short-chain fatty acids (SCFAs) in colonic contents of golden hamsters

Lyophilized fecal samples were accurately weighed, suspended in saturated sodium chloride solution, and homogenized on ice. The mixture was acidified by incorporating 10% (v/v) sulfuric acid and subsequently mixed by vortexing. Subsequently, 800 μL of hexane was added to the solution, which was then left undisturbed for half an hour. Following this step, the specimens underwent centrifugation at 12,000 × g for 15 minutes at 4 ℃, after which the upper phase was carefully retrieved for subsequent chromatographic examination. The separation was performed using an Agilent HP-INNOWAX gas chromatography column

(30 ×0.320 mm×0.5 μm). Injection was carried out in split mode (5:1) with a 1 μL sample volume, employing high-purity nitrogen as the carrier gas at a flow rate of 1 mL/min. Both the injection port and flame ionization detector were maintained at a constant temperature of 220 ℃. The temperature of the column oven was first stabilized at 60 ℃ for half an hour, subsequently increased at a gradient of 3.5 ℃ /min until reaching 180 ℃, and then sustained at this final temperature for an additional 30 minutes. Short-chain fatty acid (SCFA) concentrations were quantified using an external standard calibration method.

2.7 Shotgun metagenomic sequencing and data analysis

Genomic DNA was isolated from fecal samples utilizing the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany). To evaluate quality, the isolated DNA was examined for purity and structural integrity using 0.8% (w/v) agarose gel electrophoresis, while its concentration was measured at OD 260/280 with a NanoDrop 2000 spectrophotometer (Novogene Co., Beijing, China). High-throughput metagenomic sequencing was carried out on the Illumina HiSeq 6000 system, provided by Beijing Novogene Technology Co., Ltd. Library preparation yielded DNA fragments around 300 base pairs long. The sequences were then filtered using Sickle to eliminate low-quality regions, and host-derived genetic material was subsequently excluded. MetaPhlan3 was employed to identify microbial species and calculate their proportional representation within the metagenomic datasets. Functional pathway annotation was conducted using HumanN3, referencing the UniRef90 protein database for metabolic profiling.

2.8 Statistical analysis

Statistical computations and data analyses were carried out utilizing both GraphPad Prism and the R software. Data are presented as mean ± SEM. The ANOVA test was used to calculate the significance of data, and the Tukey test was used for pairwise comparisons thereafter. If data does not meet the normal distribution or homogeneity of variance, the significance of differences was analyzed using the Kruskal–Wallis test, and the Dunn test was used for post hoc pairwise comparisons. Microbial α-diversity metrics were computed in R using the "vegan", "permute", and "lattice" packages. Gut microbiota composition differences were assessed via Bray–Curtis dissimilarity and tested with the ADONIS function. Heatmaps were generated with the "pheatmap" package. Graphical representations, including bar charts and violin plots, were created using GraphPad Prism version 8.0. The predefined criteria used to evaluate statistical relevance were as listed below: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

3. Results

3.1 Bifidobacterium exhibited a strain-specific effect in alleviating weight gain, dyslipidemia, and hepatic changes induced by high-fat diet

To evaluate the effects of dietary supplementation with four *Bifidobacterium* in high-fat diet, we compared their impacts on body weight, serum lipid levels, and hepatic morphology in a golden hamster model (Fig. 1A). Golden hamster exhibited lipid metabolism profiles most similar to those observed in humans[24].

After 6 weeks of ad libitum high-fat diet feeding, the average body weight increased to 186.79 g, which was 24.33% higher than that of normal-fat diet (150.24 g), indicating that high-fat diet intake leads to rapid weight gain (Fig. 1B-C). Supplementation with 1×10^9 CFU of *B. infantis* CK2010 did not lead to a significant change in body weight compared with high-fat diet-fed golden hamsters receiving saline. However, high-fat diet supplementation with *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 elicited significantly lower body weight to 169.69 g, 169.26 g, and 170.85 g. In addition, *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 supplementation significantly improved the abnormal serum lipids induced by the high-fat diet, including TG, TC, LDL-C, HDL-C and FFAs, with TG reduced by more than 36%, TC reduced by more than 32%, and LDL-C reduced by more than 49% (Fig. 1D-H). By contrast, we did not detect that *B. infantis* CK2010 could effectively alleviate the abnormal serum lipids induced by high-fat diet.

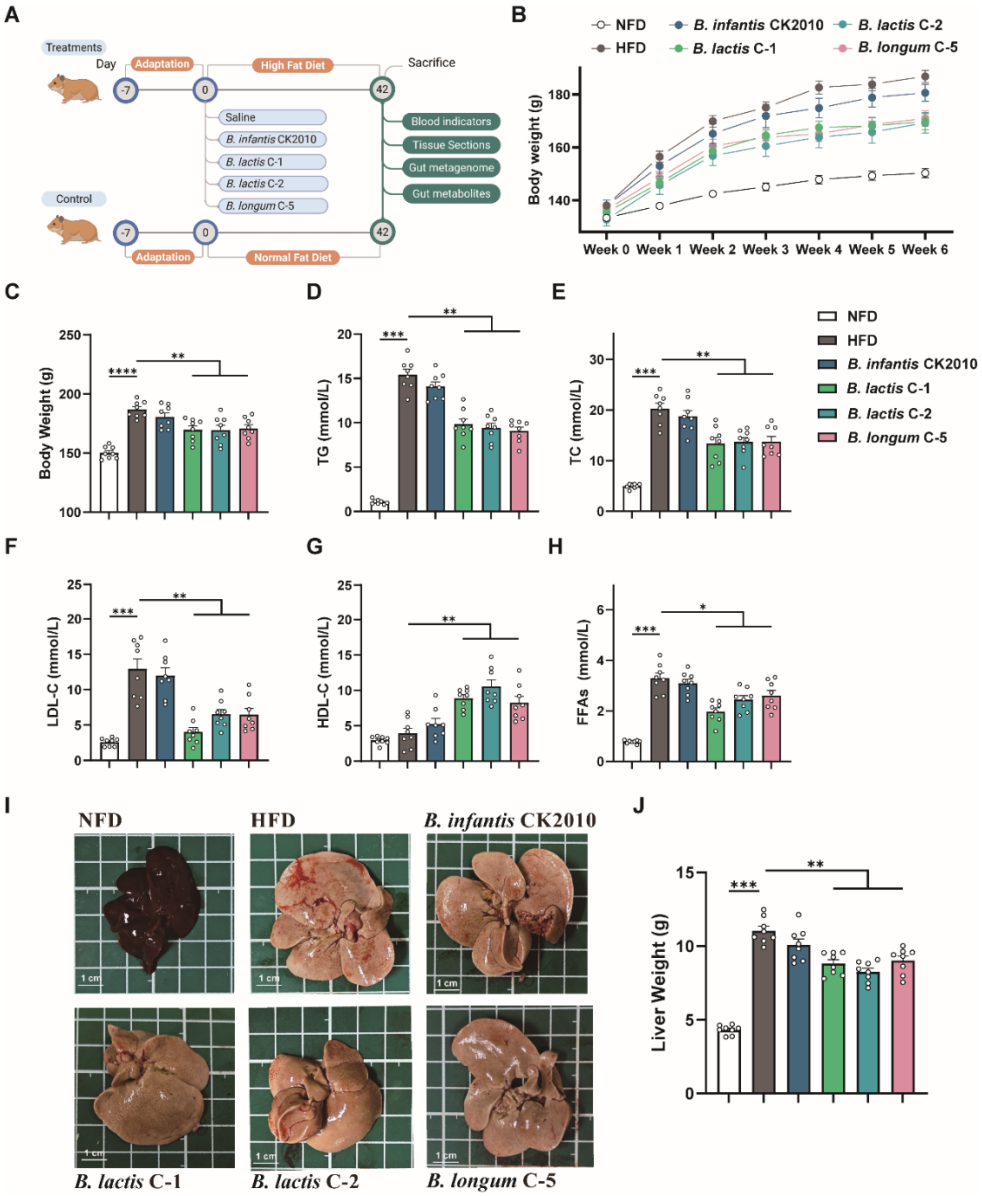


Fig. 1. Four *Bifidobacterium* exhibited divergence in high-fat diet-induced weight gain and serum lipid levels. (A) The experimental design. The golden hamsters in the NFD group received normal-fat diet, whereas the remaining groups were fed high-fat diet. The probiotic-treated cohorts were respectively administered daily oral with *B. infantis* CK2010, *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5, along with the high-fat diet. (B) Body weight from week 0 to week 6. (C) Body weight at the end of the sixth week. (D-G) Serum TG, TC, LDL-C and HDL-C concentrations measured by ELISA. (H) Serum FFAs. (I) Representative macroscopic images of livers in golden hamster. (J) Liver weight of golden hamster.

High-fat diets also led to fat accumulation in the liver, which can subsequently cause non-alcoholic fatty liver. We found that administration of *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 led to a reduction in liver size and a visibly darker red coloration of the liver, suggesting improved hepatic condition (Fig. 1I). Compared with liver weight in golden hamster fed high-fat diet, supplementation of *B. infantis* CK2010 did not result in any significant changes (Fig. 1J). By contrast, *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 obviously reduced liver weight by 2.24 g, 2.79 g, and 2.04 g, corresponding to decreases of 20.24%, 25.27%, and 18.43%, respectively. In summary, these data demonstrated the successful development of a mixed hyperlipidemia model. Furthermore, *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 displayed pronounced anti-obesity effects, significantly improved serum lipid, and alleviated hepatic hypertrophy. By contrast, *B. infantis* CK2010 failed to alleviate obesity symptoms induced by high-fat diet.

3.2 Bifidobacterium exhibited a strain-specific effect in lower fat accumulation in adipose tissue and liver induced by high-fat diet

Given the increased lipid concentrations observed in the serum of golden hamsters fed high-fat diet, the potential of four *Bifidobacterium* to mitigate fat deposition in hepatic and adipose tissues was subsequently assessed. Consumption of high-fat diet resulted in greater fat accumulation across multiple regions of the body, notably within the inguinal, perinephric, and epididymal fat depots, in contrast to normal-fat diet (Fig. 2A). Supplementing high-fat diet with *B. lactis* C-1, *B. lactis* C-2, or *B. longum* C-5 significantly decreased adipose tissue mass relative to the group receiving the high-fat diet alone. Furthermore, we also observed a slight decrease in the fat tissue of *B. infantis* CK2010 group, although these changes were not statistically significant. In addition, histopathological alterations in epididymal adipose tissue were assessed and measured using H&E-stained sections (Fig. 2B-C). After 6 weeks on high-fat diet, adipose cells exhibited an enlarged morphology indicative of hypertrophy (Fig. 2B). Quantitative analysis of adipocyte diameter observed that, except for *B. infantis* CK2010, treatment with *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 decreased the size of adipocytes in epididymal fat tissue by 12.17%, 10.99%, and 10.81%, respectively (Fig. 2C). These results suggested that *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 effectively attenuated fat accumulation induced by high-fat diet, whereas *B. infantis* CK2010 did not prevent fat accumulation under the same conditions.

Liver was a key organ affected by high-fat diet, it is also essential to evaluate and quantify hepatic lipid deposition. Histological examination of hepatic tissue stained with Oil Red O, which selectively highlighted neutral lipids such as triglycerides, demonstrated that golden hamsters receiving either the vehicle or *B. infantis* CK2010 alongside high-fat diet exhibited substantial lipid droplet accumulation in the liver, occupying approximately 90% and 83% of the tissue area, respectively (Fig. 2D-E). At the same time, supplementation of high-fat diet with *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 significantly reduced hepatic lipid accumulation compared to the high-fat diet alone, with lipid droplet areas decreased by 21.04%, 32.35%, and 18.74%, respectively (Fig. 2D-E). Moreover, the level of hepatic TG and TC further revealed that *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 supplementation was able to attenuate lipid accumulation

in liver caused by high-fat diet (Fig. 2F). In contrast, hepatic TG and TC levels were not markedly improved by *B. infantis* CK2010 supplementation. In conclusion, high-fat diet supplementation with *B. lactis* C-1, *B. lactis* C-2 or *B. longum* C-5 significantly mitigated the lipid accumulation in adipose and liver tissue, whereas *B. infantis* CK2010 did not exhibit a comparable effect. This further confirmed that *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 effectively alleviated obesity induced by high-fat diet, while *B. infantis* CK2010 did not.

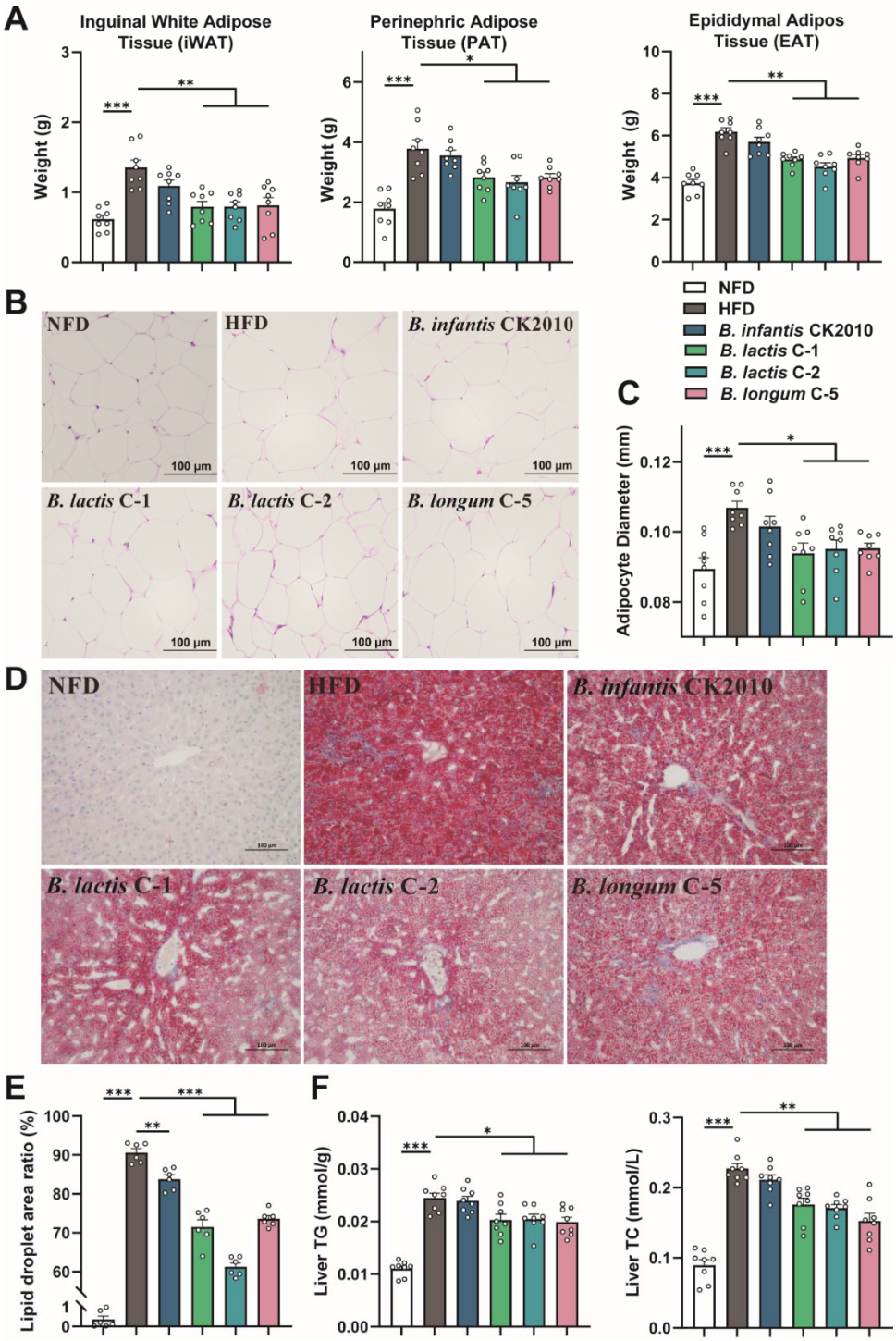


Fig. 2. Four Bifidobacterium exhibited divergence in lower fat accumulation in adipose tissue and liver induced by high-fat diet. (A) Weight of inguinal white adipose tissue (iWAT, g), perinephric adipose tissue (PAT, g) and epididymal adipose tissue (EAT, g). (B) H&E staining of epididymal adipose tissue. Scale bars, 100 μm. (C) Diameter of adipocyte in epididymal adipose tissue (mm). (D) Oil Red O-staining in the liver, where red signifies lipid accumulation. Scale bars, 100 μm. (E) Measurement of the liver lipid droplet area expressed as a percentage of the total area. Lipid droplet area ratio (%) = Lipid droplet area / total area × 100%. (F) Liver TG and TC concentrations measured by ELISA.

3.3 Bifidobacterium exhibited a strain-specific effect in reducing markers of liver injury and systemic inflammation induced by high-fat diet

In addition to promoting lipid accumulation, high-fat diet also induced liver injury and systemic inflammatory responses. To assess liver injury, we measured ALT levels in both liver tissue and serum, along with hepatic oxidative stress markers MDA and SOD. Compared with normal-fat diet, high-fat diet had significantly increased ALT levels in liver and serum, elevated MDA, and decreased SOD, collectively indicating liver injury (Fig. 3A-D). By contrast, supplementation with *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 markedly decreased ALT, reduced MDA, and restored SOD in the liver, while *B. infantis* CK2010 did not significantly ameliorate hepatic injury induced by high-fat diet.

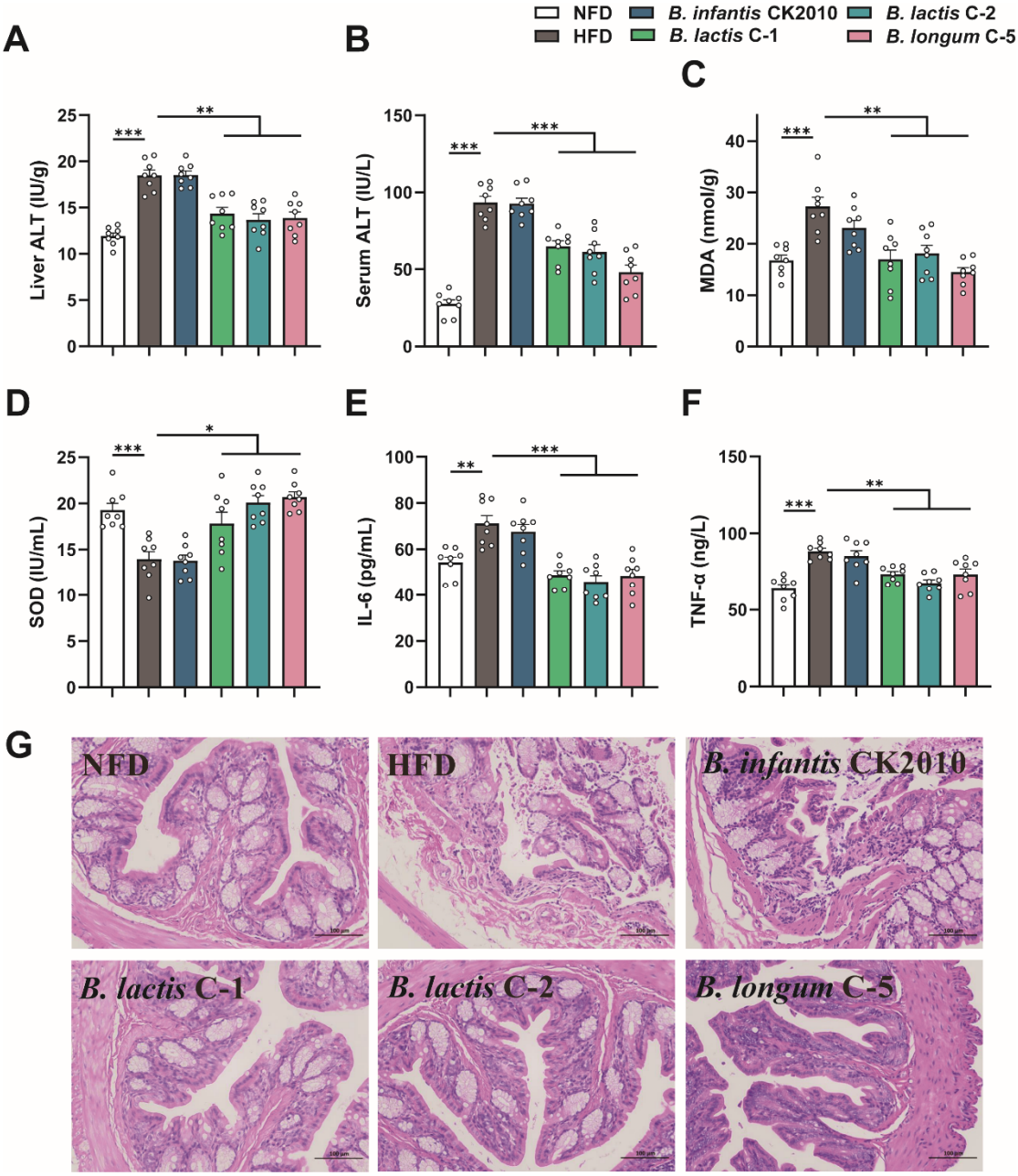


Fig. 3. Four Bifidobacterium exhibited divergence in liver injury and systemic inflammation induced by high-fat diet. (A) ALT in liver. (B) ALT in serum. (C-D) Hepatic oxidative stress markers MDA and SOD. (E) IL-6 in serum. (F) TNF-α in serum. (G) H&E staining of colon tissues (100 μm).

Beyond hepatic injury, high-fat diet consumption is also known to induce systemic inflammation. The results showed that high-fat diet significantly increased IL-6 and TNF-α levels, while supplementation with *B. infantis* CK2010 had no significant effect on these pro-inflammatory cytokines (Fig. 3E-F). However, *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 reduced IL-6 levels by more than 30% and TNF-α levels by more than 16%. In the colon tissue, consumption of high-fat diet resulted in a reduction of goblet cells accompanied by increased infiltration of inflammatory cells (Fig. 3G). In receiving *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5, goblet cell counts were elevated, while immune cell infiltration in the tissue was notably diminished. However, pronounced inflammatory cell presence remained evident in the group receiving *B. infantis* CK2010 treatment. Collectively, these findings indicated that *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 alleviated pro-inflammatory cytokines and intestinal inflammation induced by high-fat diet, whereas *B. infantis* CK2010 showed limited protective effects.

In summary, *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 were effective probiotics against high-fat diet challenge, alleviating obesity, improving serum lipid, reducing fat accumulation, attenuating hepatic injury and systemic inflammation induced by high-fat diet. In contrast, supplementation of *B. infantis* CK2010 did not exhibit comparable benefits.

3.4 The effective probiotics B. lactis C-1, B. lactis C-2, or B. longum C-5 modulated the composition in gut microbiota

To elucidate the mechanisms by which the effective probiotics *B. lactis* C-1, *B. lactis C-2*, or *B. longum* C-5 alleviated obesity induced by high-fat diet, we performed an in-depth analysis of the gut microbiome. Following high-fat diet consumption, the relative abundance of the phylum Firmicutes markedly increased, while that of Bacteroidota significantly decreased, indicating that high-fat diet altered the ratio between Firmicutes and Bacteroidota (Fig. 4A). Compared with high-fat diet, no notable alterations were detected in α-diversity of the intestinal microbiota in groups of supplementing with *Bifidobacterium* (Fig. 4B). However, we found that based on Bray−Curtis distance, the microbial community structure revealed significant differences among the six groups (Fig. 4C). Further analyzing the gut microbiota structure, supplementation with different *Bifidobacterium* resulted in distinct gut microbial compositions (Fig. 4D-G). Interestingly, supplementation with each probiotic (*B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5) significantly increased in the gut abundances of *Clostridium scindens* and *Peptostreptococcus russellii*. Meanwhile, administration of *B. lactis* C-2 and *B. longum* C-5 was also associated with a reduction in *Megamonas rupellensis*. Notably, *Peptostreptococcus russellii* and *Megamonas rupellensis* were two key gut microbes closely associated with obesity[17,25,26]. In contrast, *B. infantis* CK2010 neither significantly enriched *Peptostreptococcus russellii* nor *Megamonas rupellensis* and showed limited effects on gut microbial composition.

In addition, each *Bifidobacterium* also induced distinct species-specific changes in the gut microbiota. Specifically, *B. lactis* C-1 increased the abundance of *Ligilactobacillus salivarius*. *B. lactis* C-2 promoted the enrichment of *Faecalibacterium prausnitzii*, *Ligilactobacillus ruminis* and *Eubacterium ventriosum*. *B. longum* C-5 elevated the levels of *Bifidobacterium catenulatum* and *Lacticaseibacillus rhamnosus*. In

addition, *B. infantis* CK2010 led to an increase in *Bifidobacterium animalis* and *Lactobacillus gasseri*. These results suggested that the beneficial effects of the three probiotics (*B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5) may be attributable to their ability to modulate key obesity-associated gut microbes, thereby contributing to the alleviation of high-fat diet-induced obesity in the host. In contrast, *B. infantis* CK2010 did not markedly alter key gut microbes and showed limited effects on gut microbial composition.

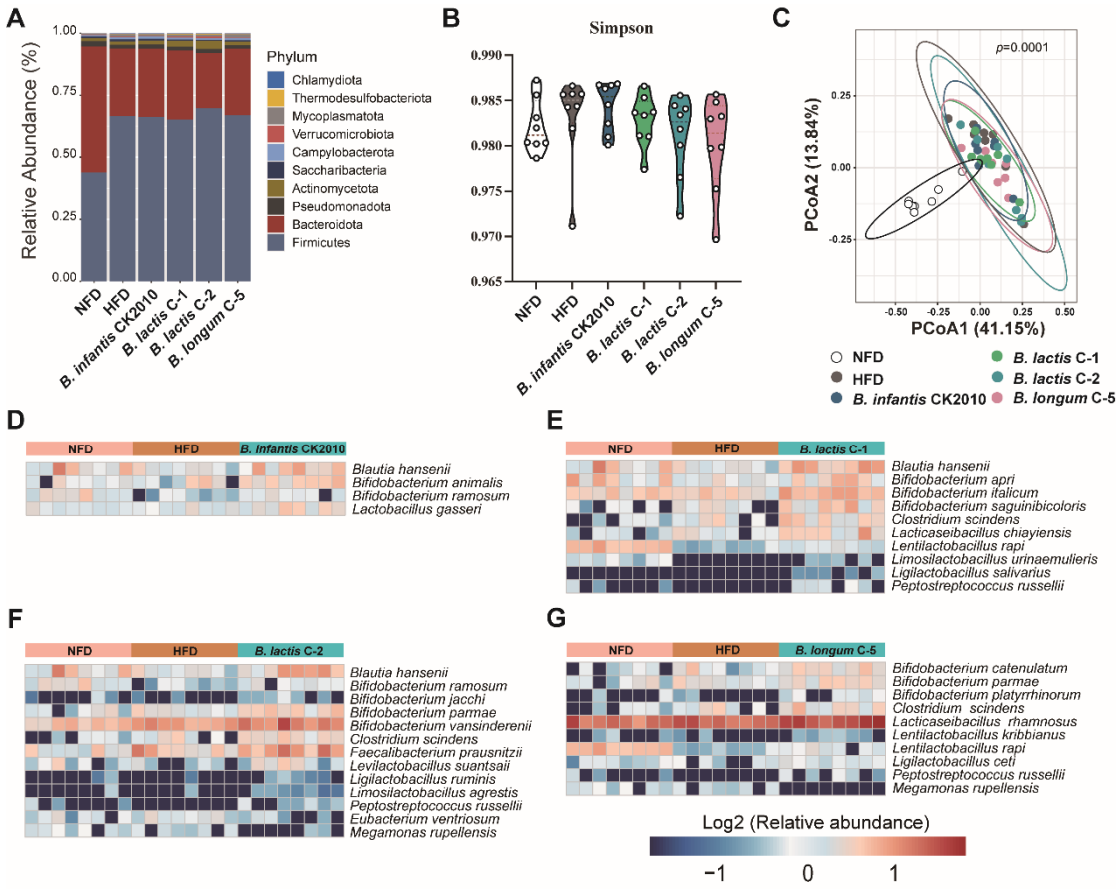
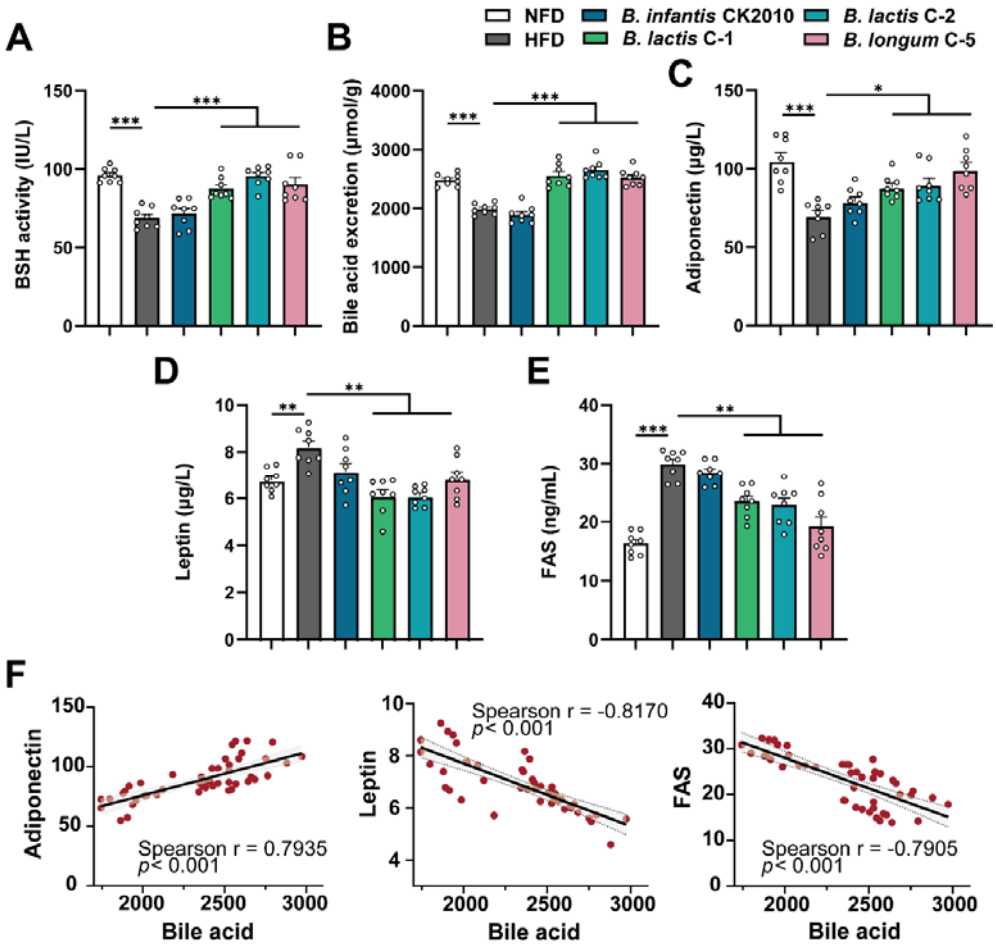


Fig. 4. Effects of four Bifidobacterium on intestinal microbiota in high fat diet. (A) The relative abundances of gut microbiota in phylum. (B) The Simpson index. (C) PCoA analysis, utilizing the Bray-Curtis distance, showed variations in the composition of microbial communities across different groups. (D-G) The microbial species that showed significant variations in the gut between each group. The depth of color represents the relative abundance of the species (red represents high abundance and blue represents low abundance).

3.5 The effective probiotics B. lactis C-1, B. lactis C-2, or B. longum C-5 modulated bile acid metabolism to decrease lipid accumulation induced by high-fat diet

After establishing that probiotic supplementation under high-fat diet conditions reshaped key gut microbes, we further assessed changes in gut metabolites, focusing first on bile acid metabolism due to its close relevance to high-fat diet. The results showed that high-fat diet feeding reduced gut BSH activity and decreased fecal bile acid excretion (Fig. 5A-B). In high-fat diet, supplementation with *B. lactis* C-1, *B. lactis* C-2, or *B. longum* C-5 significantly restored gut BSH activity and increased fecal total bile acid excretion. However, *B. infantis* CK2010 did not significant affect BSH activity or bile acid excretion relative to the HFD group. The results suggested that supplementation with the effective probiotics led to a restoration of gut microbial metabolic functions. Bile acid metabolism can influence host metabolic homeostasis by

adipocytokines and hepatic lipid metabolism[27]. Imbalances in adipocytokines, including adiponectin (ADP) and leptin, played a pivotal role in the development of obesity[28]. The findings revealed that consumption of high-fat diet resulted in a decrease in ADP and an increase in leptin (Fig. 5C-D). However, the effective probiotic *B. lactis* C-1, *B. lactis* C-2, or *B. longum* C-5 were able to reverse these disturbances, restoring adipokine levels to those comparable with the normal-fat diet group. Moreover, the effective probiotics reduced FAS by over 20%, thereby potentially attenuating lipid accumulation and lipogenesis, as FAS was a key enzyme catalyzing the synthesis of long-chain fatty acids (Fig. 5E). However, *B. infantis* CK2010 failed to significantly improve the dysregulation of adipokines and FAS. Correlation analysis further revealed that fecal bile acid excretion was significantly and positively correlated with serum ADP, while showing significant negative correlations with leptin and FAS (Fig. 5F). These findings suggested that supplementation with the effective probiotics *B. lactis* C-1, *B. lactis* C-2, or *B. longum* C-5 enhanced BSH activity and increased the abundance of *Clostridium scindens* in the gut, thereby promoting total bile acid excretion in the gut (Fig. 5G). In parallel, bile acids function as signaling molecules and were associated with changes in adipokines and hepatic fatty acid synthase[30]. Taken together, these findings suggested that the effective probiotics modulated gut microbial metabolites, particularly bile acids, to restore host metabolic balance, thereby alleviating lipid accumulation and obesity-related metabolic disturbances.



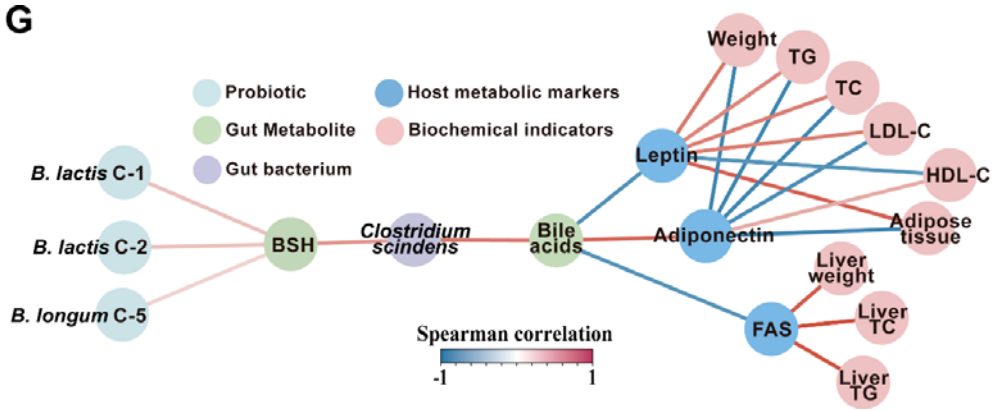


Fig. 5. The effective probiotics modulated bile acid metabolism to decrease lipid accumulation. (A) BSH activity levels in intestinal contents. (B) Fecal bile acid excretion. (C) Adiponectin in serum. (D) Leptin in serum. (E) FAS in liver. (F) Correlation relationships between bile acid and adiponectin, leptin, and leptin. (G) Correlation network illustrates the relationships among effective probiotics, gut metabolite, gut bacterium, host metabolic markers and biochemical indicators. Edge color (red: positive correlation; blue: negative correlation) is proportional to the strength of the Spearman correlation.

3.6 The effective probiotics B. lactis C-1, B. lactis C-2, or B. longum C-5 modulated SCFAs to influence host metabolism

In addition to bile acids, SCFAs were important gut metabolites that played beneficial roles in host, whereas high-fat diet feeding was associated with a reduction in SCFA production[29]. The results showed that high-fat diet disrupted the production of SCFAs in the gut, whereas supplementation with three effective probiotics significantly enhanced SCFA production, including propionic acid, butyric acid, isovaleric acid and valeric acid (Fig. 6A). High-fat diet feeding resulted in a decrease in total SCFAs (Fig. 6B). Compared with the HFD group, effective probiotics increased the total SCFAs in the gut, while *B. infantis* CK2010 supplementation did not significantly increase SCFAs (Fig. 6B). These results indicate that high-fat diet decreased the levels of SCFAs in the gut, while supplementation with effective probiotics significantly restores SCFAs levels. In the correlation analysis, the total SCFAs was negatively correlated with ALT, MDA, IL-6 and TNF-α, while showing positive correlations with SOD (Fig. 6C). This suggests a close relationship between SCFAs and hepatic injury as well as inflammation. Combined with changes in gut microbiota abundance, correlation network analysis revealed that *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 were positively correlated with *Ligilactobacillus salivarius*, *Ligilactobacillus ruminis*, *Faecalibacterium prausnitzii*, and *Lacticaseibacillus rhamnosus* in the gut, respectively (Fig. 6D). Moreover, *Ligilactobacillus salivarius*, *Ligilactobacillus ruminis*, *Faecalibacterium prausnitzii*, and *Lacticaseibacillus rhamnosus* showed positive correlations with short-chain fatty acids. Overall, these results suggest that the effective probiotics increase short-chain fatty acid levels through modulation of the gut microbiota, thereby alleviating hepatic injury and inflammation induced by a high-fat diet.

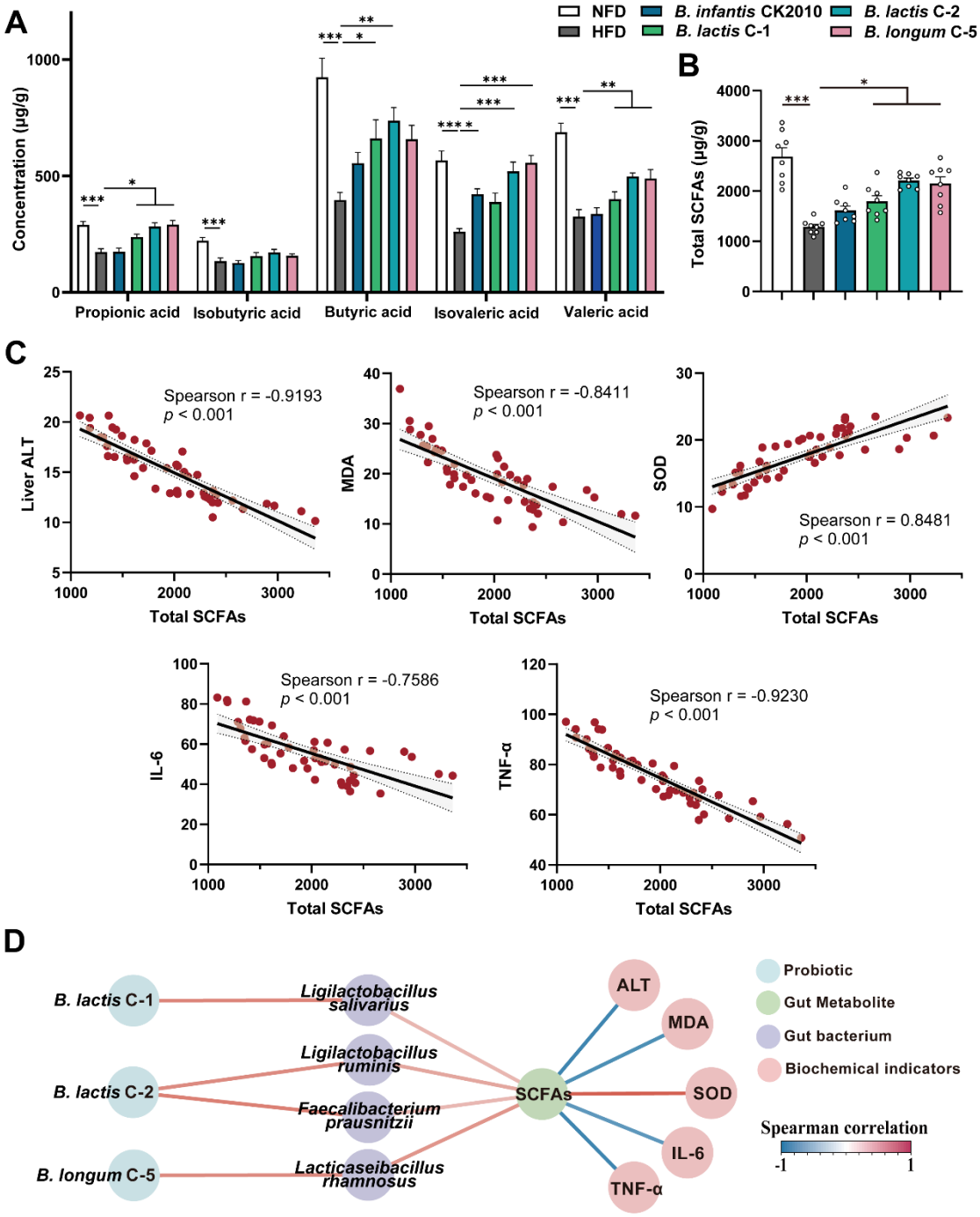


Fig. 6. The effective probiotics alleviate hepatic injury and inflammation induced by high-fat diet through increased SCFAs. (A) The content of SCFAs in gut was measured, such as propionic, isobutyric, butyric, isovaleric and valeric acid. (B) Total SCFAs concentration. (C) Correlation relationships between total SCFAs and hepatic injury (ALT, MDA, and SOD), inflammation (IL-6, and TNF-α). (D) Correlation network illustrates the relationships among effective probiotics, gut bacterium, SCFAs and biochemical indicators. Edge color (red: positive correlation; blue: negative correlation) is proportional to the strength of the Spearman correlation.

4. Discussion

High-fat diet intake was known to metabolic syndrome, including obesity, hyperlipidemia, hepatic steatosis, and low-grade inflammation[3,4]. Our results confirmed that high-fat diet led to significant increases in body weight, serum triglycerides, and liver lipid accumulation. *B. longum* CCFM1077 was a probiotic shown to reduce TC by 13.86% and LDL-C by 13.88% in individuals with hyperlipidemia[31]. Compared with

high-fat diet-fed mice, supplementation with *Bifidobacterium animalis* subsp. *lactis* BB12 did not significantly reduce serum TC or TG levels[23]. In this study, *B. lactis* C-1, *B. lactis* C-2 and *B. longum* C-5 significantly improved the abnormal serum lipids induced by the high-fat diet, with TG reduced by more than 36%, TC reduced by more than 32%, and LDL-C reduced by more than 49%. By contrast, *B. infantis* CK2010 failed to alleviate obesity symptoms induced by high-fat diet. In the liver, prolonged intake of high-fat diet led to significantly elevated levels of TC, TG, and MDA, ALT, SOD, indicating the occurrence of hepatic damage[32,33]. Administering high-fat diet to mice induced pathological features resembling human non-alcoholic fatty liver disease (NAFLD), such as hepatic enlargement, fat accumulation, hepatocellular inflammation, and fibrotic changes[34]. *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 obviously reduced liver weight by 2.24 g, 2.79 g, and 2.04 g, respectively. Supplementation with *B. animalis* subsp. *lactis* BB12 reduced liver weight by 0.85 g[23]. In addition, *B. lactis* C-1, *B. lactis* C-2, or *B. longum* C-5 can attenuate hepatic injury induced by high-fat diet, as reflected by decreased ALT and MDA levels, and restored SOD activity, indicating reduced hepatic damage and oxidative stress. These results highlighted the hepatoprotective potential of three effective probiotics supplementation in high-fat diet. Moreover, multiple investigations have demonstrated elevated concentrations of systemic inflammatory mediators, including TNF-α and IL-6, in obese individuals and in animal models subjected to high-fat diet[35,36,37]. In this study, the observed improvements in inflammatory markers (IL-6 and TNF-α) and colon histology further supported the role of three effective probiotics in suppressing systemic and intestinal inflammation. In summary, we found that, among the four *Bifidobacterium*, three effective probiotics (*B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5) showed superior efficacy in alleviating weight gain, improving serum lipid, reducing fat accumulation in both adipose and hepatic tissues, and attenuating markers of hepatic injury and systemic inflammation. In contrast, *B. infantis* CK2010 failed to produce significant improvements across most parameters, highlighting the strain-specific nature of probiotic efficacy in metabolic regulation.

Based on our results demonstrating that *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 exerted significant anti-obesity effects, whereas *B. infantis* CK2010 showed limited efficacy, we further compared gut microbiome among groups to explore the potential mechanisms by which effective probiotics ameliorate high-fat diet-induced obesity. Brief exposure to high-fat diet markedly shifted microbial composition in the gut, whereas prolonged consumption leads to broader structural modifications of the microbiome[38,39]. In this study, analysis at the phylum classification revealed that high-fat diet led to an increased Firmicutes/Bacteroidota ratio. In bile acid metabolism, compared with *B. infantis* CK2010, the effective probiotics *B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5 not only enhanced BSH activity but also increased the abundance of *Clostridium scindens* in the gut. BSH, an enzyme commonly found in the intestinal microbiota of both humans and other mammals, catalyzed the breakdown of conjugated bile salts into unconjugated bile acids and their corresponding amino acids[40,41]. *Clostridium scindens* possessed the 7α-dehydroxylation capability, enabling the conversion of unconjugated primary bile acids into secondary bile acids[42,43]. Unconjugated bile acids and secondary bile acids reduced host intestinal lipid absorption and, due

to their higher hydrophobicity, were less efficiently reabsorbed in the intestine, thereby promoting their elimination through fecal excretion.[44] In this study, we observed that supplementation with effective probiotics significantly increased bile acid excretion compared with the HFD group, whereas *B. infantis* CK2010 did not induce significant changes. Increased bile acid excretion promoted hepatic conversion of cholesterol into bile acids, helping maintain enterohepatic balance[40]. Collectively, these findings indicated that the effective probiotics reshape gut bile acid metabolism by enhancing BSH activity and increasing the abundance of *Clostridium scindens*, thereby promoting fecal bile acid excretion. Increased fecal bile acid loss can reduce intestinal lipid absorption and diminish bile acid reabsorption, thereby alleviating high-fat diet-induced obesity.

Bile acids were not only important metabolites involved in lipid digestion and absorption but also act as systemic regulatory molecules and signaling factors with hormone-like functions[30]. In this study, the effective probiotics elevated bile acid levels in the intestine. Elevated bile acid levels can activate FXR signaling, which acts in the intestine–liver axis and peripheral tissues such as adipose tissue to modulate lipid metabolism and maintain metabolic homeostasis[45,46]. Supplementation with effective probiotics normalized the HFD-induced adipokine imbalance and reduced hepatic FAS levels, thereby suppressing lipogenesis and decreasing lipid accumulation in serum, adipose tissue, and liver. In contrast, *B. infantis* CK2010 did not significantly ameliorate adipokine or FAS dysregulation. Besides, effective probiotics reversed the HFD-induced changes of key bacteria closely associated with obesity, such as *Peptostreptococcus russellii* and *Megamonas rupellensis*. *Peptostreptococcus russellii* is capable of converting tryptophan into indole-3-propionic acid (IPA), which has been shown to enhance leptin sensitivity by targeting the STAT3 pathway, thereby exerting protective effects against diet-induced obesity[25,26]. Both in vitro and in vivo studies have identified that *Megamonas rupellensis* harbors genes involved in myo-inositol degradation. Since myo-inositol has been shown to effectively inhibit fatty acid absorption in intestinal organoids, the presence of *Megamonas rupellensis* may enhance intestinal lipid uptake, thereby contributing to obesity[17].

SCFAs, functional gut microbial metabolites, were produced through the fermentation of undigested dietary fibers and providing various benefits to host metabolism[47,48]. Meanwhile, high-fat diet significantly increased IL-6 and TNF-α. Beyond promoting inflammation, high-fat diet feeding also induced liver injury and dysfunction. SCFAs have been shown to reduce inflammation by modulating the production of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor[49], and to alleviate hepatic injury[50,51]. In groups of effective probiotics, there was a notable elevation in the concentrations of SCFAs within the intestine. Meanwhile, effective probiotics alleviated high-fat diet-induced systemic inflammation and hepatic injury. Compared with the HFD group, *B. infantis* CK2010 supplementation did not significantly increase SCFAs levels of the gut and no effect on pro-inflammatory cytokines or liver injury. Following effective probiotics supplementation, a marked elevation was detected in the abundance of *Ligilactobacillus salivarius*, *Ligilactobacillus ruminis*, *Faecalibacterium prausnitzii*, and *Eubacterium ventriosum*. These microbes were recognized SCFA producers that contribute to intestinal SCFA generation[52]. These results indicate that the

effective probiotics alleviated high-fat diet–induced systemic inflammation and hepatic injury by enriching gut bacteria capable of producing short-chain fatty acids, thereby increasing intestinal SCFA levels.

5. Conclusions

This study evaluated the efficacy of four *Bifidobacterium* in alleviating obesity induced by high-fat diet. Our results found that *Bifidobacterium* exhibited a strain-specific effect, three effective probiotics (*B. lactis* C-1, *B. lactis* C-2, and *B. longum* C-5) showed superior efficacy in alleviating weight gain, improving serum lipid, reducing fat accumulation in both adipose and hepatic tissues, and attenuating markers of hepatic injury and systemic inflammation. In contrast, *B. infantis* CK2010 failed to alleviate obesity induced by high-fat diet. We therefore further explored the potential mechanisms underlying the protective effects of the effective probiotics. Mechanistically, compared with *B. infantis* CK2010, the effective probiotics enhanced BSH activity and *Clostridium scindens*, promoting fecal bile acid excretion, reducing intestinal lipid absorption and bile acid reabsorption, which was associated with alleviating obesity induced high-fat diet. In addition, the effective probiotics modulated bile acid signaling, helping maintain adipokine balance and reducing hepatic FAS levels, thereby suppressing lipogenesis and decreasing lipid accumulation in serum, adipose tissue, and liver. In contrast, *B. infantis* CK2010 did not significantly ameliorate adipokine or FAS dysregulation. Moreover, the effective probiotics alleviated high-fat diet-induced systemic inflammation and hepatic injury by increasing SCFAs in the gut.

Conflicts of interest

The authors declare that there is no conflict of interests.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (32402144), the Research Fund for the International Science & Technology Cooperation Program of Hainan Province (GHYF2023001) and the Research Fund for Hainan Province Postgraduate Innovative Research Projects (Qhyb2024-26).

Data availability

The datasets generated during the current study are available in the National Center for Biotechnology Information (NCBI) repository (PRJNA1284449).

References

[1] X. Wang, Q. Wu, M.J. Zhong, et al., Adipocyte-derived ferroptotic signaling mitigates obesity, Cell Metab. 37 (2025) 673-691. https://doi.org/10.1016/j.cmet.2024.11.010.

[2] K. Hosomi, M. Saito, J. Park, et al., Oral administration of *Blautia wexlerae* ameliorates obesity and type 2 diabetes via metabolic remodeling of the gut microbiota, Nat. Commun. 13 (2022) 4477. https://doi.org/10.1038/s41467-022-32015-7.

[3] H. Shimizu, J. Miyamoto, K. Hisa, et al., Sucrose-preferring gut microbes prevent host obesity by producing exopolysaccharides, Nat. Commun. 16 (2025) 1145. https://doi.org/10.1038/s41467-025-56470-0.

[4] B. Zhou, Y.C. Luo, H.Q. Bi, et al., Amelioration of nonalcoholic fatty liver disease by inhibiting the deubiquitylating enzyme

RPN11, Cell Metab. 36 (2024) 2228-2244. https://doi.org/10.1016/j.cmet.2024.07.014.

[5] Y.G. Sun, Q.X. Nie, S.S. Zhang, et al., *Parabacteroides distasonis* ameliorates insulin resistance via activation of intestinal GPR109a, Nat. Commun. 14 (2023) 7740. https://doi.org/10.1038/s41467-023-43622-3.

[6] J. Borén, M.J. Chapman, R.M. Krauss, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 41 (2020) 2313-2330. https://doi.org/10.1093/eurheartj/ehz962.

[7] P. Libby, The changing landscape of atherosclerosis, Nat. 592 (2021) 524-533. https://doi.org/10.1038/s41586-021-03392-8.

[8] C.J. Chang, C.S. Lin, C.C. Lu, et al., Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota, Nat. Commun. 6 (2015) 7489. https://doi.org/10.1038/ncomms8489.

[9] Z. Al Nabhani, S. Dulauroy, E. Lécuyer, et al., Excess calorie intake early in life increases susceptibility to colitis in adulthood, Nat. Metab. 1 (2019) 1101-1109. https://doi.org/10.1038/s42255-019-0129-5.

[10] H. Tilg, T.E. Adolph, A.R. Moschen, Multiple Parallel Hits Hypothesis in Nonalcoholic Fatty Liver Disease: Revisited After a Decade, Hepatology 73 (2021) 833-842. https://doi.org/10.1002/hep.31518.

[11] S.V. Raj, M. Ismail, W.K. Chan, et al., A systematic review on factors associated with non-alcoholic fatty liver disease (NAFLD) among adolescents, Clin. Nutr. ESPEN 57 (2023) 131-137. https://doi.org/10.1016/j.clnesp.2023.06.014.

[12] L. Xiong, S. Diwakarla, R. Chatzis, et al., Acute exposure to high-fat diet impairs ILC3 functions and gut homeostasis, Immun. 58 (2025) 1185-1200. https://doi.org/10.1016/j.immuni.2025.03.017.

[13] G. Rook, F. Backhed, B.R. Levin, et al., Evolution, human-microbe interactions, and life history plasticity, Lancet 390 (2017) 521-530. https://doi.org/10.1016/s0140-6736(17)30566-4.

[14] A.M. Valdes, J. Walter, E. Segal, et al., Role of the gut microbiota in nutrition and health, BMJ 361 (2018) k2179. https://doi.org/10.1136/bmj.k2179.

[15] R.E. Ley, P.J. Turnbaugh, S. Klein, et al., Microbial ecology: human gut microbes associated with obesity, Nat. 444 (2006) 1022-1023. https://doi.org/10.1038/4441022a.

[16] P.J. Turnbaugh, R.E. Ley, M.A. Mahowald, et al., An obesity-associated gut microbiome with increased capacity for energy harvest, Nat. 444 (2006) 1027-1031. https://doi.org/10.1038/nature05414.

[17] A.C. Wu, F.M. Yang, H.Z. Zhong, et al., Obesity-enriched gut microbe degrades myo-inositol and promotes lipid absorption, Cell Host Microbe 32 (2024) 1301-1314. https://doi.org/10.1016/j.chom.2024.06.012.

[18] N. Fei, L.P. Zhao, An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice, ISME J. 7 (2013) 880-884. https://doi.org/10.1038/ismej.2012.153.

[19] J.L. Chen, Y.H. Xiao, D.M. Li, et al., New insights into the mechanisms of high-fat diet mediated gut microbiota in chronic diseases, Imeta 2 (2023) e69. https://doi.org/10.1002/imt2.69.

[20] M.E. Sanders, D.J. Merenstein, G. Reid, et al., Probiotics and prebiotics in intestinal health and disease: from biology to the clinic, Nat. Rev. Gastroenterol. Hepatol. 16 (2019) 605-616. https://doi.org/10.1038/s41575-019-0173-3.

[21] H.T. Li, L. Zhang, J. Li, et al., Resistant starch intake facilitates weight loss in humans by reshaping the gut microbiota, Nat. Metab. 6 (2024) 578-597. https://doi.org/10.1038/s42255-024-00988-y.

[22] C.R. Naudin, K. Maner-Smith, J.A. Owens, et al., *Lactococcus lactis* Subspecies *cremoris* Elicits Protection Against Metabolic Changes Induced by a Western-Style Diet, Gastroenterol. 159 (2020) 639-651. https://doi.org/10.1053/j.gastro.2020.03.010.

[23] T. Li, J.J. Yang, H.X. Zhang, et al., *Bifidobacterium animalis* subsp. *lactis* A12 prevents obesity- associated dyslipidemia by modulating gut microbiota-derived short-chain fatty acid production and energy metabolism in high-fat diet-fed mice, Food Nutr. Res. 66 (2022) 8670. https://doi.org/10.29219/fnr.v66.8670.

[24] S. Shen, J. Wang, C. Ma, et al., Understanding the "individual drug reaction" from the perspective of the interaction between probiotics and lovastatin in vitro and in vivo, Microbiome. 11 (2023) 209. https://doi.org/10.1186/s40168-023-01658-z.

[25] H.M. Roager, T.R. Licht, Microbial tryptophan catabolites in health and disease, Nat. Commun. 9 (2018) 3294. https://doi.org/10.1038/s41467-018-05470-4.

[26] Z.W. Wang, S.Y. Yang, L.J. Liu, et al., The gut microbiota-derived metabolite indole-3-propionic acid enhances leptin sensitivity by targeting STAT3 against diet-induced obesity, Clin. Transl. Med. 14 (2024) e70053. https://doi.org/10.1002/ctm2.70053.

[27] K.C. Lee, P.S. Wu, H.C. Lin, Pathogenesis and treatment of non-alcoholic steatohepatitis and its fibrosis, Clin. Mol. Hepatol. 29 (2023) 77-98. https://doi.org/10.3350/cmh.2022.0237.

[28] Y.Q. Han, Q.W. Sun, W. Chen, et al., New advances of adiponectin in regulating obesity and related metabolic syndromes, J. Pharm. Anal. 14 (2024) 100913. https://doi.org/10.1016/j.jpha.2023.12.003.

[29] F. Siracusa, N. Schaltenberg, Y. Kumar, et al., Short-term dietary changes can result in mucosal and systemic immune depression, Nat. Immunol. 24 (2023) 1473–1486. https://doi.org/10.1038/s41590-023-01587-x.

[30] A. Schmid, T. Karrasch, A. Schäffler, The emerging role of bile acids in white adipose tissue, Trends Endocrinol. Metab. 34 (2023) 718-734. https://doi.org/10.1016/j.tem.2023.08.002.

[31] C.Q. Chu, J.C. Jiang, L.L. Yu, et al., *Bifidobacterium longum* CCFM1077 Attenuates Hyperlipidemia by Modulating the Gut Microbiota Composition and Fecal Metabolites: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial, Eng. 28 (2023) 193-205. https://doi.org/10.1016/j.eng.2023.04.010.

[32] T. Xiao, F.L. Huang, Z.H. Guo, et al., Black Raspberry Polyphenols Shape Metabolic Dysregulation and Perturbation in Gut Microbiota to Promote Lipid Metabolism and Liver Regeneration, J. Agric. Food Chem. 73 (2025) 7833-7856. https://doi.org/10.1021/acs.jafc.5c00702.

[33] N.Y. Tang, X.G. Zhao, T. Lin, et al., Amelioration of *Kluyveromyces marxianus* GY-8 on high-fat diet-induced obesity and gut microbiota dysbiosis, Food Biosci. 68 (2025) 106649. https://doi.org/10.1016/j.fbio.2025.106649.

[34] M. Yang, X.Q. Qi, N. Li, et al., Western diet contributes to the pathogenesis of non-alcoholic steatohepatitis in male mice via remodeling gut microbiota and increasing production of 2-oleoylglycerol, Nat. Commun. 14 (2023) 228. https://doi.org/10.1038/s41467-023-35861-1.

[35] T. Kawai, M. Autieri, R. Scalia, Adipose tissue inflammation and metabolic dysfunction in obesity, Am. J. Physiol. 320 (2021) C375-C391. https://doi.org/10.1152/ajpcell.00379.2020.

[36] P. Wang, Y. Ma, D. Wang, et al., Protective Effects of Dietary Resveratrol against Chronic Low-Grade Inflammation Mediated through the Gut Microbiota in High-Fat Diet Mice, Nutrients 14 (2022) 1994. https://doi.org/10.3390/nu14101994.

[37] A. Christ, P. Günther, M.A.R. Lauterbach, et al., Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming, Cell 172 (2018) 162-175. https://doi.org/10.1016/j.cell.2017.12.013.

[38] M.W. Rohr, C.A. Narasimhulu, T.A. Rudeski-Rohr, et al., Negative Effects of a High-Fat Diet on Intestinal Permeability: A Review, Adv. Nutr. 11 (2020) 77-91. https://doi.org/10.1093/advances/nmz061.

[39] V. Las Heras, A.G. Clooney, F.J. Ryan, et al., Short-term consumption of a high-fat diet increases host susceptibility to *Listeria monocytogenes* infection, Microbiome 7 (2019) 7. https://doi.org/10.1186/s40168-019-0621-x.

[40] K. Chen, D. Dang, H. Li, et al., *Lactobacillus johnsonii* CCFM1376 improves hypercholesterolemia in mice by regulating the composition of bile acids, Microbiome Res. Rep. 4 (2025) 6. https://doi.org/10.20517/mrr.2024.38.

[41] Z.W. Song, S. Feng, X.C. Zhou, et al., Taxonomic identification of bile salt hydrolase-encoding lactobacilli: Modulation of the enterohepatic bile acid profile, Imeta 2 (2023) e128. https://doi.org/10.1002/imt2.128.

[42] Y. Sato, K. Atarashi, D.R. Plichta, et al., Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians, Nat. 599 (2021) 458-464. https://doi.org/10.1038/s41586-021-03832-5.

[43] M. Funabashi, T.L. Grove, M. Wang, et al., A metabolic pathway for bile acid dehydroxylation by the gut microbiome, Nat. 582 (2020) 566-570. https://doi.org/10.1038/s41586-020-2396-4.

[44] F. Stellaard, D. Lütjohann, Dynamics of the enterohepatic circulation of bile acids in healthy humans, Am. J. Physiol. 321 (2021) G55-G66. https://doi.org/10.1152/ajpgi.00476.2020.

[45] T.H. Won, M. Arifuzzaman, C.N. Parkhurst, et al., Host metabolism balances microbial regulation of bile acid signalling, Nat. 638 (2025) 216-224. https://doi.org/10.1038/s41586-024-08379-9.

[46] B.L. Clifford, L.R. Sedgeman, K.J. Williams, et al., FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption, Cell Metab. 33 (2021) 1671-1684. https://doi.org/10.1016/j.cmet.2021.06.012.

[47] D.J. Morrison, T. Preston, Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism, Gut Microbes 7 (2016) 189-200. https://doi.org/10.1080/19490976.2015.1134082.

[48] S. Sanna, N.R. van Zuydam, A. Mahajan, et al., Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases, Nat. Genet. 51 (2019) 600-605. https://doi.org/10.1038/s41588-019-0350-x.

[49] I. Mukhopadhya, P. Louis, Gut microbiota-derived short-chain fatty acids and their role in human health and disease, Nat. Rev. Microbiol. 23 (2025) 635-651. https://doi.org/10.1038/s41579-025-01183-w.

[50] M.R. Gallop, R.F.L. Vieira, P.D. Mower, et al., A long-term ketogenic diet causes hyperlipidemia, liver dysfunction, and glucose intolerance from impaired insulin secretion in mice, Sci. Adv. 11 (2025). https://doi.org/10.1126/sciadv.adx2752.

[51] Z. Zhang, T. Zhu, Y. Li, et al., Butyrate Regulates Intestinal DNA Virome and Lipopolysaccharide Levels to Prevent High-Fat Diet-Related Liver Damage in Rats, J. Agric. Food Chem. 73 (2025) 8277-8289. https://doi.org/10.1021/acs.jafc.4c07966.

[52] E.R. Mann, Y.K. Lam, H.H. Uhlig, Short-chain fatty acids: linking diet, the microbiome and immunity, Nat. Rev. Immunol. 24 (2024) 577-595. https://doi.org/10.1038/s41577-024-01014-8.