

ORIGINAL ARTICLE

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

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Popular scientific summary

- *Bifidobacterium lactis* A12 (the fermented products of *B. lactis* A12 and live *B. lactis* A12 cells), a novel probiotic strain, isolated from the faces of breast-fed infants, in application to regulate metabolism diseases.
- *B. lactis* A12 increased SCFAs-producing bacteria abundance in the gut and eventually mediated beneficial effects on host by SCFAs-GPR43-GLP-1 pathway.
- *B. lactis* A12 may be involved in metabolism, genetic, and environment information processing-related pathway in HFD-fed mice by regulating intestinal microbiota.

Abstract

Background: *Bifidobacterium lactis* A12 (*B. lactis* A12) has been shown to have the potential to prevent obesity. However, the mechanisms by which it affects the control of energy metabolism have not been fully elucidated.

Objective: The present work aimed to clarify the mechanisms by that *B. lactis* A12 has an effect on the management of energy metabolism.

Design: Three- to five-week-old male C57BL/6J mice were randomly divided into five groups, 15 mice for each group. Low-fat diet (LFD) group and high-fat diet (HFD) group were fed with phosphate-buffered saline (PBS) on a daily basis. Cell-free supernatant (CFS), A12, and *B. lactis* BB12 (BB12) groups were fed with daily probiotics for 10 weeks (1×10^9 CFU of every strain).

Results: The results showed that A12 effectively alleviated weight gain and dyslipidemia, inhibited liver adipose accumulation, and improved leptin resistance in HFD-fed mice ($p < 0.05$). The anti-obesity effects of *B. lactis* A12 were closely related to the assembly of short-chain fatty acids (SCFAs), SCFA-downstream receptors, and glucagon-like peptide-1 (GLP-1) secretion. Additionally, high-throughput sequencing of the 16S rRNA showed that *B. lactis* A12 supplementation reversed HFD-induced gut microbiota dysbiosis, which was possible related to the augmented abundance of SCFA-producing bacterium and a minimized ratio of *Bacteroidetes* to *Firmicutes* in mice.

Conclusions: *B. lactis* A12 prevents obesity in some pathways, including the downregulation of sterol regulatory element binding protein-1 mRNA levels in the liver, modulation of the structure of gut microbiota in a gut microbiota-dependent manner, and the upregulation of the SCFA-producing bacteria-related G protein-coupled receptor 43 pathway.

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Keywords: *Bifidobacterium*; obesity; short chain fatty acids; gastrointestinal hormone; gut microbiota

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Obesity has become a worldwide health problem, and as a result, its prevalence has increased rapidly within the last decades (1). Obesity, often characterized by the consumption of a high-calorie/fat diet and lacking of physical activity and referred to as a sort of clinical metabolic syndrome, appears because it cannot fully promote the utilization of fatty acids and the imbalance of lipid metabolism when intake surpasses energy consumption (2). At present, more and more research is devoted to find the effective treatment methods to decline the obesity rate. Some synthetic drugs used to treat obesity cause side effects and secondary failure (3, 4). Moreover, prevention is the best treatment option for any disease. Therefore, it is important to seek more natural and safer materials to prevent obesity.

Generally, probiotics colonize the intestinal tract and are beneficial to the health of the host. The gut origin of chronic diseases indicates that metabolic diseases are closely associated with gut microbiota (5, 6). Recent studies have highlighted that probiotics may exert their beneficial effects through the gut microbiota (7, 8). However, numerous studies have shown that probiotic mixtures can treat metabolic diseases, and research on a single strain has mainly focused on *Lactobacillus* (9–11).

Bifidobacterium is one of the major microbiota in the human intestinal tract, and some of its members are the most commonly used human probiotics (12, 13). However, the prospects of *Bifidobacterium* application in metabolic diseases have not been extensively explored, and the molecular mechanisms have not been fully elucidated, but it may be related to the regulation of gut microbiota (14).

Our previous *in vivo* studies have shown that *B. lactis* A12, a novel probiotic strain isolated from breast-fed infant faces, had a positive influence on the management of diabetes-related parameters, like blood glucose (15) and eased constipation (16), and some studies have suggested that probiotics appear to normalize gut dysbiosis in mice (10). We hypothesized that gut microbiota may play a vital role in the improvement of obesity by *B. lactis* A12. Thus, the potential mechanisms were clarified by this study. Our results shown that two separate regulatory pathways connecting intestinal flora and energy metabolism may be associated with the anti-obesity actions of *B. lactis* A12.

Materials and methods

Bacteria strain and culture condition

Bifidobacterium animalis subsp. *lactis* A12 strain (CGMCC No. 17308) was originally isolated from the fecal matter of a 1-month-old infant (vaginal delivery and breastfed) and identified by sequencing amplified 16S rDNA regions as described previously (15).

B. lactis A12 was cultured on improved De Man-Rogosa-Sharpe (MRS) medium (1L medium contained 20 g maltose, 10 g beef extract, 5 g soy peptone, 5 g malt extract, 5 g $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$, 2 g $\text{C}_6\text{H}_5\text{O}_7(\text{NH}_4)_3$, 2 g $\text{K}_2\text{HP0}_4\cdot 3\text{H}_2\text{O}$, 1 ml Tween 80, 0.58 g $\text{Mg}\cdot \text{SO}_4\cdot 7\text{H}_2\text{O}$, 0.5 g l-cysteine hydrochloride, and 0.25 g $\text{MnSO}_4\cdot \text{H}_2\text{O}$), and pH was altered to 6.5, 37°C anaerobic culture for 12 h. The bacterial suspensions were centrifuged at 8,000 $\times g$ (10 min, 4°C), washed three times with phosphate-buffered saline (phosphate-buffered saline (PBS), pH 7.4, Gibco, Life Technologies, Ghent, Belgium), and adjusted to a live bacterial concentration of 10^9 CFU/mL. The pH of *B. lactis* A12 cell-free supernatant (CFS) was altered to 6.5 and filtered through a 0.22 μm pore-size sterile filter (Millipore, Burlington, MA, USA). *B. lactis* BB12 was used as a reference strain because it has been widely used in commercial functional foods and has the potential to prevent obesity.

Animals experimental

All experiments were approved by the Ethical Committee of the Experimental Animal Care of Beijing University of Agriculture (Beijing, China). A total of 75 (3- to 5-week-old) male C57BL/6J mice, which obtained from Xinglong Experimental Animal Factory (Beijing, China), were housed in standard cages (three mice per cage), maintained under standard laboratory conditions, at ambient temperatures of 22–25°C, under a 12 h/12 h light/dark cycle, with a relative humidity of 40–60%, and allowed to access water and food freely.

Mice were arranged in five groups at random: low-fat diet (LFD) group (LFD and PBS, LFD, 10% kcal from fat, D12450B, Research Diets Inc., New Brunswick, NJ, USA, $n = 15$); high-fat diet (HFD) group (HFD and PBS, HFD, 60% kcal from fat D12492, Research Diets Inc., $n = 15$); CFS group (HFD and CFS of *B. lactis* A12, $n = 15$); A12 group (HFD and live *B. lactis* A12 cells, $n = 15$); BB12 group (HFD and live *B. lactis* BB12 cells,

$n = 15$). All probiotics were administered daily for 10 weeks (1×10^9 CFU of each strain). In addition, we measured the weight of mice every week.

Biochemistry determination of serum

Biochemistry determination of serum was measured using enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng, Nanjing, China), including total cholesterol (T-CHO) (Code No. A111-2-1), triglyceride (TG) (Code No. A110-2-1), glucagon-like peptide-1 (GLP-1) (Code No. H294-1-2), leptin (Code No. H174-1-2), and adiponectin (Code No. H179-1-2), following the manufacturer's protocol.

Histological evaluation

The liver tissues of mice were excised and treated as previously described (17). We quickly removed liver tissues, rinsed it with saline, and then fixed a tissue mass of approximately $2.0 \text{ cm} \times 2.0 \text{ cm} \times 0.3 \text{ cm}$ size with 4% formaldehyde (Nanjing Jiancheng, Nanjing, China). After dehydration, paraffin embedding, and sectioning, liver tissue sections were stained with hematoxylin and eosin (H&E) staining. The histopathological changes of each tissue were observed with camera accessories under a high-resolution microscope (Nikon Eclipse Ti-SR, Japan).

Analysis of gene expression

Real-time polymerase chain reaction (RT-PCR) was used to analyze the relative transcript levels of β -actin, sterol regulatory element binding protein-1 (SREBP-1), peroxisome proliferator-activated receptors (PPAR- γ), G-protein coupled receptors (GPR43), glucagon (GCG), and prohormone convertase 3 (PC3). Following the manufacturer's protocol, the TRIzol reagent (Ambion, Austin, TX, USA) was used to extract total RNA in colon and liver tissues. We used the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Kyoto, Japan) to obtain complementary DNA (cDNA) through reverse transcription. The primers used are listed in Table 1. PCR reaction conditions referred to our previous studies (15). Using

the $2^{-\Delta\Delta C_t}$ method, mRNA abundance was calculated (reference gene: β -actin) and expressed as a percentage of the control group induction (calibrator) (18).

Short-chain fatty acid (SCFA) concentrations quantified using gas chromatography (GC)

Fecal samples (0.2g) of the colon were collected immediately after the animals were dissected into a covered sterile centrifuge tube and then stored at -80°C until use. The SCFAs in feces were analyzed by GC, including acetate, propionate, and butyrate. According to the method of Goossens et al., we slightly modified it to analyze the SCFA concentration (19). We added 10 mmol/L HCl (1 mL) containing heptanoic acid (internal standard, Sigma Aldrich, Merck, Germany) to 1 mL of diluted fecal samples to form free fatty acids. SCFAs were extracted using 1 mL of diethyl ether, then transferred to a tube of 1.5 mL containing 0.3 g glass beads ($\Phi = 100 \mu\text{m}$), and homogenized the mixture twice in a bead-beater chamber (Thermo Scientific, MA, USA) for 30 s each time. The solution was centrifuged ($5,000 \times g$ for 2 min, at 4°C) and then filtered through a $0.22 \mu\text{m}$ sterile filter (Millipore, Burlington, MA, USA). One microliter of the supernatant was injected for measurement. SCFA concentration was measured as the number of millimoles per gram of feces after dilution correction.

DNA extraction and high throughput sequencing

Fecal samples collected using a sterile centrifuge tube with a lid were stored at -80°C until assay. We extracted DNA using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Each 20 μL PCR mixtures was as follows: $5 \times$ TransStart FastPfu buffer 4 μL , 2.5 mM dNTPs 2 μL , forward primer (5 μM) 0.8 μL , TransStart FastPfu DNA Polymerase 0.4 μL , template DNA 10 ng, and up to 20 with ddH₂O. The reaction conditions were 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, followed by 72°C for 10 min.

PCR products were abstracted from 2.0% agarose gels. An AxyPrep DNA Gel Extraction Kit (Axygen

Table 1. Oligonucleotide primers used to amplify RNA transcripts

Gene ID	Primers name	Oligonucleotide (5'-3')	Oligonucleotide (3'-5')
11461	β -actin	TGAGAGGGAAATCGTGCGTGAC	GCTCGTTGCCAATAGTGATGACC
19013	PPAR- γ	CAGGAGCAGAGCAAAGAGGT	TGGACACCATACTTGAGCAGA
20787	SREBP-1	GGCTGTTGTCTACCATAAGC	AGGAAGAAACGTGTCAAGAA
233079	GPR43	ACAGTGGAGGGGACCAAGAT	GGGGACTCTCTACTCGGTGA
14526	GCG	GCTTATAATGCTGGTGCAAGG	CTGGGAAGCTGGGAATGAT
18548	PC3	TGGAGTTGCATATAATTCCAAAGTT	AGCCTCAATGGCATCAGTTAC

Direction: Tm = 58°C .

Biosciences, Union City, USA) was used for purifying, following the manufacturer's instructions, and the QuantiFluor™-ST Kit (Promega, USA) was used to quantify. The raw reads were stored in the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP213006). In brief, raw FASTQ files were demultiplexed and quality-filtered using QIIME (version 1.17), and operational units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1, <http://drive5.com/uparse/>); chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed using the RDP Classifier (<http://rdp.cme.msu.edu/>) against the Silva (SSU115) 16S rRNA database (Release 128 <http://www.arb-silva.de>) using a confidence threshold of 70% (20). So, we explored the changes of bacterial community structure in each experimental group from the level of phylum and genus. The linear discriminant analysis effect size (LEfSe) and the PICRUST software were used to analyze 16S rRNA data.

Statistical analysis

Data are indicated as the mean \pm standard deviation (SD). The SPSS 16.0 software was used to analyze data by one-way analysis of variance (ANOVA). GraphPad Prism software was used for graphing. The Networkx software was applied to the Spearman correlation network between bacteria at the genus level. P value < 0.05 was indicated statistically significant. Except for animal experiments, three parallel tests were carried out in the assays.

Results

Effects of *B. lactis* A12 on physiologic and metabolic parameters

Continued intake of HFD for 10 weeks caused the development of obesity-associated dyslipidemia, which was mainly manifested in increased body weight gain, serum TG, T-CHO, and leptin levels, compared with the LFD group. Body weights of all groups increased after 10 weeks (Table 2). During the experiment, although there was no clear difference in body weights among LFD, CFS, A12, and BB12 groups, the HFD group was more than all these groups. It suggested that HFD-induced body weight and weight gain are prevented in mice treated with CFS, A12, and BB12.

The CFS (2.26 g), A12 (2.65 g), and BB12 groups (2.66 g) displayed lower food intake than the HFD (3.32 g) group. In contrast, no significant difference was regarded between the LFD (3.04 g) group and the HFD group. Consistently, due to the different calories between the diets, the LFD (11.72 kcal), CFS (11.85 kcal), A12 (13.90 kcal), and BB12 groups (13.95 kcal) displayed less energy intake compared with the HFD group (17.39 kcal) (Table 2).

The liver is an important organ that participates in glucose and lipid metabolism. Epididymal fat, which is a white adipose tissue, represents the excess energy accumulated. Therefore, we examined the weight of liver and epididymal fat mass. Compared with the HFD group, the liver weight and epididymal fat mass in the LFD, CFS, A12, and BB12 groups were significantly decreased ($p < 0.05$) (Table 2).

Table 2. Effects on the morphometric and metabolic variables

	LFD	HFD	CFS	A12	BB12
Body weight (g)					
Initial body weight	17.53 \pm 1.87 ^a	18.8 \pm 0.89 ^a	18.57 \pm 1.1 ^a	18.73 \pm 0.83 ^a	18.73 \pm 0.95 ^a
Final body weight	25.39 \pm 0.97 ^a	29.85 \pm 0.23 ^b	26.35 \pm 0.43 ^a	27.6 \pm 0.17 ^a	26.96 \pm 0.40 ^a
Body weight gain	7.86 \pm 0.45 ^a	11.05 \pm 0.12 ^b	7.78 \pm 0.23 ^a	8.87 \pm 0.69 ^a	8.23 \pm 0.55 ^a
Food consumption (g/day)	3.04 \pm 0.23 ^{ab}	3.32 \pm 0.23 ^a	2.26 \pm 0.41 ^b	2.65 \pm 0.17 ^b	2.66 \pm 0.33 ^{ab}
Energy intake (kcal/mice/day)	11.72 \pm 0.70 ^a	17.39 \pm 1.70 ^{bc}	11.85 \pm 2.00 ^a	13.90 \pm 2.13 ^{ac}	13.95 \pm 1.96 ^{ac}
Organ weight (g)					
Liver	0.78 \pm 0.07 ^a	1.145 \pm 0.08 ^b	0.72 \pm 0.17 ^a	0.69 \pm 0.11 ^a	0.82 \pm 0.10 ^a
Epididymal fat	0.32 \pm 0.12 ^a	0.72 \pm 0.06 ^b	0.37 \pm 0.07 ^a	0.43 \pm 0.09 ^a	0.37 \pm 0.06 ^a
Plasma					
Triacylglycerol (mmol/L)	0.58 \pm 0.08 ^a	0.92 \pm 0.13 ^{bc}	0.48 \pm 0.08 ^a	0.62 \pm 0.15 ^{ac}	0.77 \pm 0.11 ^{ac}
Total cholesterol (mmol/L)	4.78 \pm 0.2 ^a	7.33 \pm 0.38 ^{bc}	3.44 \pm 1.60 ^a	3.30 \pm 0.85 ^a	4.89 \pm 0.86 ^{ac}
Serum					
Leptin (ng/mL)	2.27 \pm 0.17 ^a	4.04 \pm 0.36 ^{bc}	2.65 \pm 0.42 ^a	2.38 \pm 0.59 ^a	3.19 \pm 0.45 ^{ac}
Adiponectin (mg/L)	8.43 \pm 1.65 ^a	3.59 \pm 0.98 ^{bc}	7.01 \pm 0.96 ^{ac}	8.13 \pm 1.66 ^a	7.41 \pm 1.31 ^a

Data are presented as the mean \pm SD. Shoulders marked with different letters indicate significant differences ($p < 0.05$).

Changes in lipid and hormone levels were also investigated. Our results suggested that serum levels of T-CHO, TG, and leptin in the HFD group had a significant increase compared with the LFD group, while adiponectin levels showed the opposite trend. The serum concentration of T-CHO was decreased in the CFS (3.44 mmol/L) and A12 (3.30 mmol/L) groups compared with the HFD group, and there was no significant difference between the BB12 (4.89 mmol/L) group and the HFD (7.33 mmol/L) group. Likewise, the CFS (0.48 mmol/L) group decreased in TG levels compared with the HFD group. However, there was no significant difference between the A12 (0.62 mmol/L) and BB12 (0.77 mmol/L) groups compared with the HFD (0.92 mmol/L) group. Additionally, leptin levels were decreased in the CFS (2.65 ng/mL) and A12 (2.38 ng/mL) groups compared with the HFD group (4.04 ng/mL). Furthermore, A12 (8.13 mg/L) and BB12 (7.41 mg/L) groups exhibited increased serum adiponectin levels compared with the HFD group (Table 2).

In short, these results indicated that continuous intake of *B. lactis* A12 CFS, live *B. lactis* A12 cells, or live *B. lactis* BB12 cells can reduce metabolic disorders in obese and leptin resistant mice.

Effects of *B. lactis* A12 treatment on histology in liver tissue

The liver has a central role in lipid metabolism. Adipose in the liver originates from food and peripheral tissues. Compared with the LFD histological section, the section of liver in the HFD group showed a large number of fat vesicles, which characterized hepatic steatosis (red arrow).

However, the CFS, A12, and BB12 groups did not exhibit fat accumulation in the liver parenchyma (Fig. 1).

mRNA expression of genes involved in adipogenesis in liver tissue

Adipogenesis is a process in which preadipocytes differentiate into adipocytes. Several transcription factors and binding proteins, including the PPAR and SERBP families, are expressed sequentially and cooperatively during adipocyte differentiation. PPAR- γ is a key regulatory factor in adipogenesis and influences adipose accumulation. SERBP-1 regulates adipose synthesis (1).

Compared with the HFD group, all groups exhibited a decreased PPAR- γ mRNA expression in liver tissue ($p > 0.05$) (Fig. 2). *SERBP-1* mRNA levels in liver tissue decreased in the CFS, A12, and BB12 groups compared with the HFD group, but only the expression level of *SERBP-1* mRNA in the CFS group decreased significantly (Fig. 2B). Our findings showed that *B. lactis* A12 reduced the gene expression of PPAR- γ and *SERBP-1*, which led to a reduction in fat deposition in the liver.

B. lactis A12 improved SCFAs-producing capacity and enhanced mRNA levels of GPR43

It has been suggested that SCFAs are produced by insoluble carbohydrates, which are fermented in the colon by probiotics. SCFAs play a pivotal role in regulating the balance of the intestinal microecological environment and host metabolism (21, 22). To investigate the content of SCFAs regulated by *B. lactis* A12, we quantified SCFA concentrations

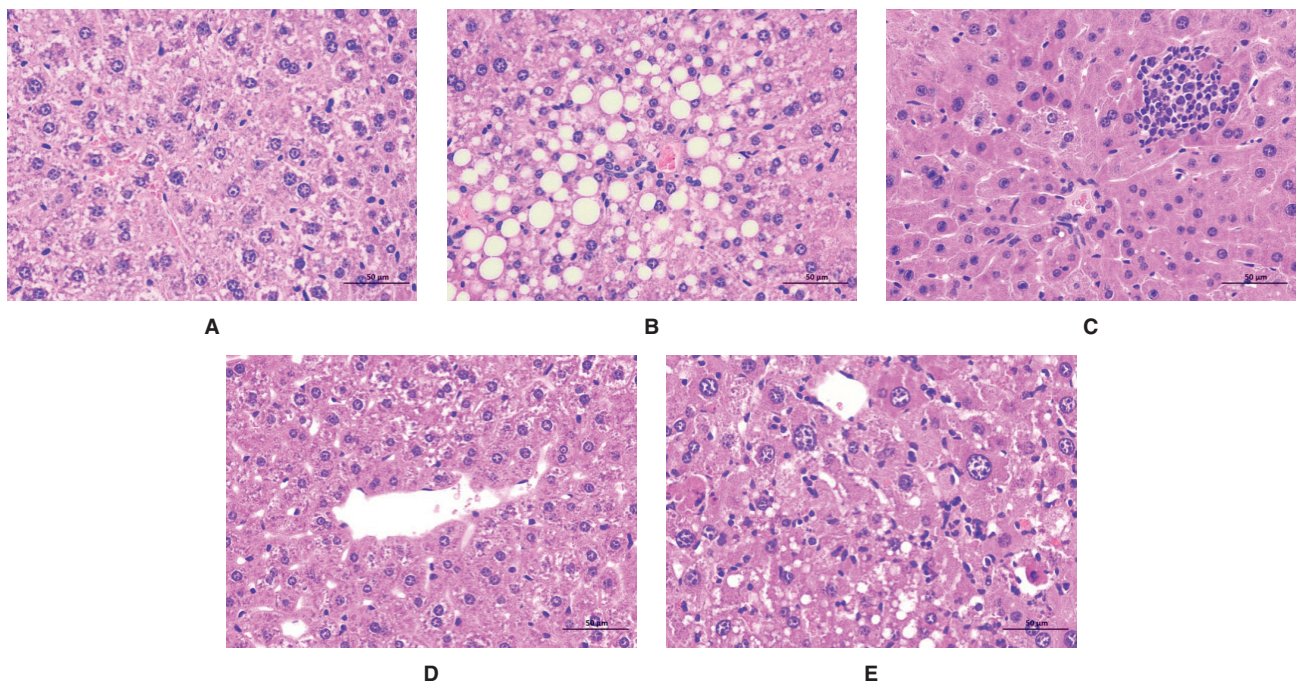


Fig. 1. Morphologic characterization of liver tissue samples. Original magnification: 400 \times , scale bar: 50 μ m. (A) LFD group. (B) HFD group. (C) CFS group. (D) A12 group. (E) BB12 group. Hepatic steatosis as indicated by the red arrow.

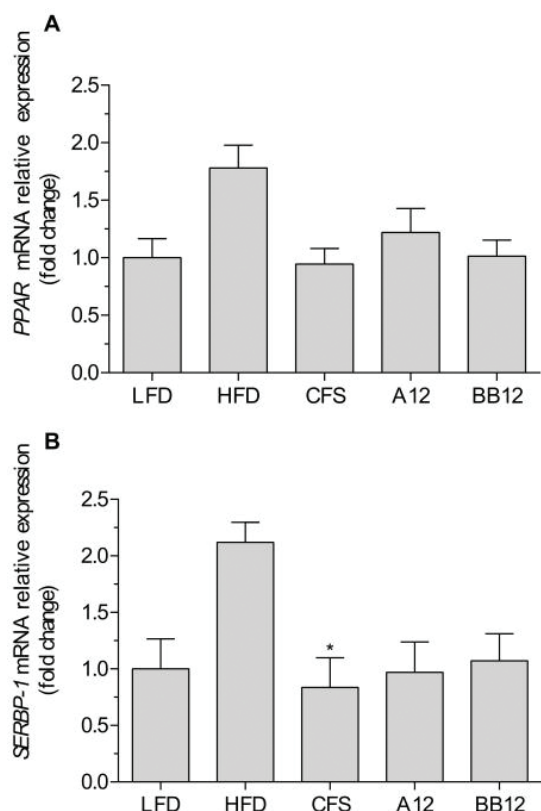


Fig. 2. mRNA abundance with the genes involved in adipocytes differentiation-related gene relative expression in the liver tissue. (A) mRNA abundance with the PPAR- γ . (B). mRNA abundance with the SERBP-1. Data are presented as means \pm SD. * $p < 0.05$ vs. HFD.

in faces by GC. The concentrations of acetic acid and propionic acid were higher in the CFS and A12 groups than in the HFD group, and there was no significant difference among the LFD, BB12, and HFD groups. Butyric acid levels were increased in the A12 (0.269 mmol/g) and BB12 (0.270 mmol/g) groups compared with the HFD group, and there was no significant difference among the LFD (0.197 mmol/g), CFS (0.233 mmol/g), and HFD (0.196 mmol/g) groups (Fig. 3A, B, and C).

SCFAs confer some of their biological effects via G protein-coupled receptors (GPRs), known as GPR43, which has beneficial roles in energy and glucose homeostasis (23, 24). Consequently, we further investigated mRNA expression of *GPR43* in colon tissue. All groups significantly increased the mRNA expression of *GPR43* compared with the HFD group. Together, these results indicated that the CFS, A12, and BB12 groups could upregulate *GPR43* mRNA levels and promote the production of SCFAs (Fig. 3D).

Secretion and expression of GLP-1 in vivo

Probiotics usually colonize and play vital roles in the intestinal tract, and some endocrine hormones in the intestinal tract may be the key targets underlying these beneficial

functions. GLP-1 is synthesized and secreted by enteroendocrine L-cells in the whole small and large intestine, and proglucagon is processed after translation by PC3 (25, 26). GLP-1 can enhance glucose and lipid homeostasis by reducing food intake and stimulating insulin secretion. Butyrate has been suggested to stimulate the secretion of GLP-1 to exhibit metabolic effects (9). Consequently, the secretion of GLP-1 and the expression of *PC3* and *GCG* genes in colonic tissue were further evaluated.

Compared with the HFD group (0.020 pmmol/L), serum GLP-1 levels in the CFS (0.040 pmmol/L), A12 (0.037 pmmol/L), and BB12 (0.062 pmmol/L) groups were significantly increased (Fig. 4). *GCG* mRNA levels were significantly increased in all tested groups compared with the HFD group, and the abundance of the *PC3* gene was noticeably higher in the A12 and BB12 groups than in the HFD group, but there was no significant difference in *PC3* mRNA expression among the CFS, LFD, and HFD groups (Fig. 4B, C). These results indicated that the CFS of *B. lactis* A12, live *B. lactis* A12, and *B. lactis* BB12 cells could increase the expression and secretion of GLP-1.

Effects of *B. lactis* A12 treatment on gut microbiota

High-throughput sequencing was performed on fecal samples to analyze the effect of *B. lactis* A12 on the composition of gut microbiota. A total of 2,066,662 high-quality sequences of the V3–V4 region of the 16S rRNA were collected from 20 fecal samples (four groups and five samples per group), and the average sequence length was 418 bp. After removing the low-quality reads, 1,033,331 OTUs at a 97% similarity level were reserved.

Firmicutes and *Bacteroidetes* were the two major bacterial phyla in all groups (Fig. 5). Compared with the LFD group, a low ratio of *Firmicutes* to *Bacteroidetes* was observed in the HFD group. Meanwhile, compared with the HFD (31.15%) group, the CFS (35.46%) and A12 (38.56%) groups increased the relative abundance of *Bacteroidetes*. A heatmap of Spearman's correlation analysis was performed at the genus level (Fig. 5B), and according to the clustering tree, compared with the HFD group, a similar community composition was shown among the CFS, A12, and LFD groups. In addition, *Romboutsia* was the dominant genus in the LFD group. *Muribaculaceae* was dominant in the CFS and A12 groups, and *Bacteroides* was the major genus in the HFD group.

The LEfSe method was used to analyze the crucial species that changed significantly after all treatments, and the threshold of logarithmic LDA score of 2 was used to distinguish features. According to the size of the LDA score (Fig. 6B), the A12 group was characterized by higher amounts of *Faecalibaculum*, *Anaeroplasmia*, *Muribaculaceae*, *Muribaculum*, *Mollicutes_RF39*, *Prevotellaceae_UCG_001*, and *GCA_900066225*. The enrichment of *Bacteroides_vulgatus*, *Alistipes_finegoldii*, *Akkermansia*,

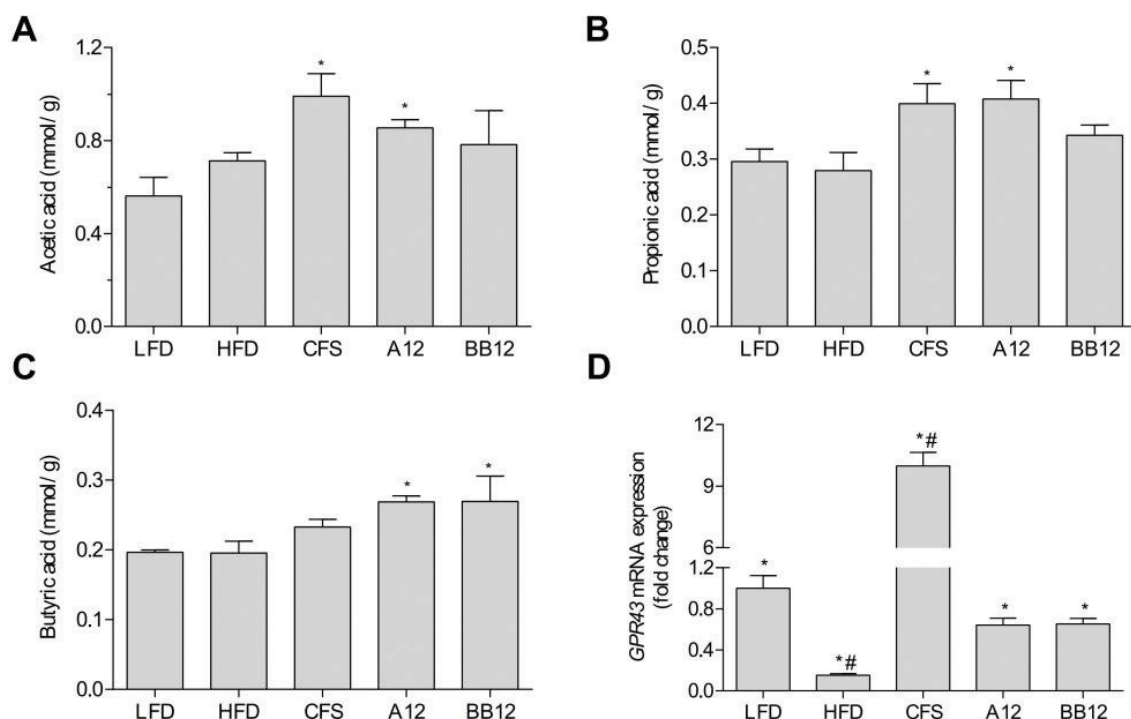


Fig. 3. SCFAs receptors gene expression in colonic tissue and SCFAs levels in fecal samples. (A) Acetic acid level. (B) Propionic acid level. (C) Butyric acid level. (D) mRNA abundance with the GPR43. Data are presented as means \pm SD. * $p < 0.05$ vs. HFD. # $p < 0.05$ vs. LFD.

Helicobacter, *Gordonibacter*, *Bacteroides uniformis*, *Bitarella massiliensis*, *Erysipelatoclostridium*, *Enterorhabdus*, *Ruminococcaceae*, and *Eggerthellaceae* significantly increased the gut microbiota of the HFD group. The dominant bacteria in the LFD group were *Lactobacillus*, *Firmicutes*, *Bifidobacterium*, *Barnesiella*, *ASF356*, and *Rhodospirillales*. The CFS group was characterized by *Coriobacteriaceae_UCG_002*, *Dubosiella*, *Candidatus_Saccharimonas*, *Marvinbryantia*, *Arcobacter*, *Mollicutes_RF39*, and *Coprococcus_3*.

Predicted metabolic profile of the fecal microbiome after *B. lactis* A12 supplementation

In view of the above structural changes, the 16S rRNA data were further analyzed using PICRUST to predict the metabolism of fecal microbiota in all groups. The Kyoto Encyclopedia of Genes and Genomes database (KEGG, <http://www.genome.jp/kegg/>) was used for determining whether *B. lactis* A12 caused obvious changes in the functional pathways of the gut microbiota in mice.

The *B. lactis* A12 could regulate metabolism, genetic information processing, and environmental information processing-related pathways (Fig. 7). The metabolic pathways involved in purine and pyrimidine metabolism (nucleotide metabolism), starch and sucrose metabolism (carbohydrate metabolism), glycolysis (carbohydrate metabolism), and amino sugar and nucleotide sugar

metabolism (carbohydrate metabolism) were characterized. The genetic information processing pathways involved in ribosome and aminoacyl-tRNA biosynthesis (translation) were characterized. The environment information processing pathway involved in ABC transporters (membrane transport).

Discussion

Few studies have focused on the effects of *Bifidobacterium*, and the molecular mechanisms have not been fully demonstrated (14). Therefore, the current study aimed to elucidate underlying mechanisms of *Bifidobacterium-mediated* prevention of obesity. Our previous *in vivo* studies elucidated that *B. lactis* A12 plays a beneficial role in the management of metabolism-related parameters, including blood glucose and insulin levels (15). In this study, we observed reduced body weight and less energy intake associated with *B. lactis* A12 administration; meanwhile, *B. lactis* A12 prevented obesity-associated dyslipidemia and reduced leptin resistance. Liver lipid accumulation is a marker of obesity induced by HFD, and we found that liver weight was noticeably increased in the HFD group, and *B. lactis* A12 decreased the weight of liver tissue. A study has shown that HFD-induced fatty liver was intervened by a probiotic mixture, consistent with our results (10). In addition, *Lactobacillus rhamnosus* LRA05 improves lipid accumulation in mice fed with a high fat

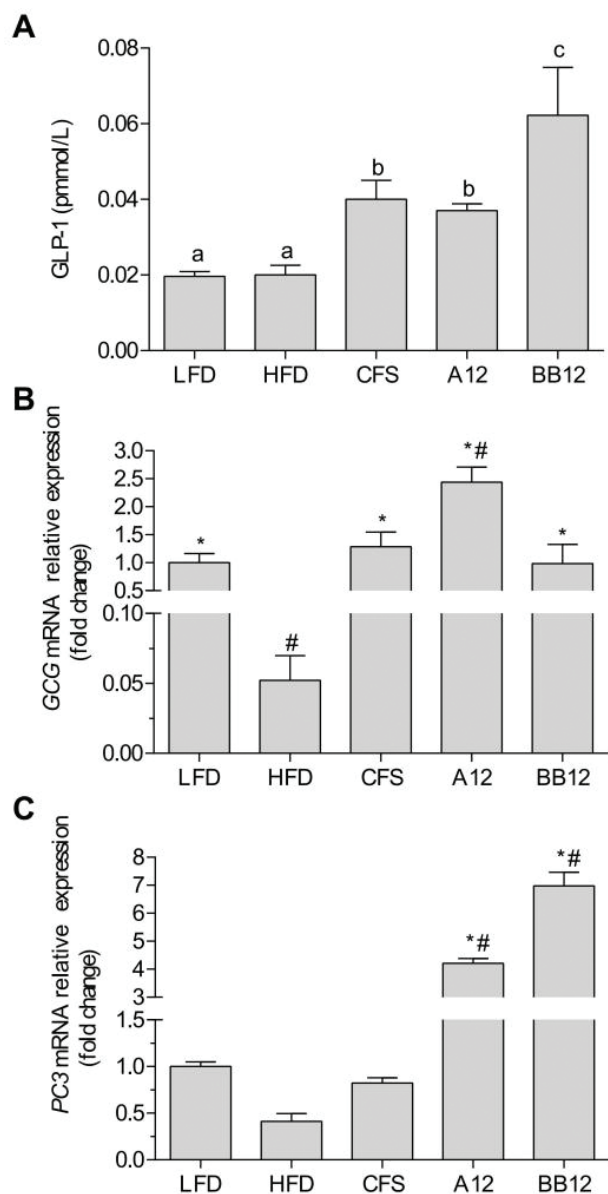


Fig. 4. Effect of *B. lactis* A12 on GLP-1 secretion and expression. (A) GLP-1 level in serum. (B) mRNA abundance with the GCG. (C) mRNA abundance with the PC3. Data are presented as means \pm SD. (A) Shoulder marked with the different letter indicates that the difference was significant. (B) and (C) $p < 0.05$. * $p < 0.05$ vs. HFD. # $p < 0.05$ vs. LFD.

diet via regulating intestinal microbiota, reducing glucose content, and promoting liver carbohydrate metabolism (27). However, we investigated mRNA expression levels of adipose accumulation- and differentiation-related genes. We observed that *B. lactis* A12 inhibited liver adipose accumulation by downregulating *SERBP-1* mRNA levels, which was our new finding on the improvement of obesity by *B. lactis* A12.

SCFAs are byproducts of non-digestible dietary components metabolized by gut microbiota and have some potential beneficial functions, including maintaining the

dynamic equilibrium for intestinal microecology (28) and regulating energy homeostasis (29), which are all closely related to obesity. Various SCFAs, including acetate, propionate, and butyrate, have differential metabolic effects (30, 31). Our results demonstrated that *B. lactis* A12 promoted the enrichment of acetate, propionate, and butyrate. Similar results concerning the beneficial effects of probiotics have also been observed in other studies (32, 33). Some studies showed that the expression of *GPR43* stimulated by SCFAs can improve glucose tolerance (11). To verify *B. lactis* A12 could prevent obesity, mediated by promoting the enrichment of SCFAs, we further investigated the mRNA expression of *GPR43* in colon tissue. Compared with the HFD group, all groups significantly increased the mRNA expression of *GPR43* (Fig. 3B). Together, these results indicated that *B. lactis* A12 could prevent obesity by upregulating *GPR43* mRNA levels and promoting the production of SCFAs. Furthermore, probiotics colonize and play vital roles in the intestinal tract, and we postulate that the intestinal tract may be a key target through which *B. lactis* A12 improves obesity-related parameters. Some studies showed that intestinal expression of *GPR43* improves glucose tolerance and reduces food intake by promoting the secretion of GLP-1 from L cells (34). Additionally, GLP-1 secretion has been related to the modulation of energy homeostasis in other studies (35). Consequently, we evaluated the secretion of GLP-1 and verified the results with the *PC3* and *GCG* expression in colonic tissue. These findings indicated that *B. lactis* A12 increased the expression and secretion of GLP-1. Taken together, the protective metabolic effects of *B. lactis* A12 were mediated by SCFA production and the SCFAs-GPR43-GLP-1 pathway.

Because the origin of several chronic diseases resides in the gut, we can conclude that metabolic diseases are closely associated with gut microbiota. Although probiotics have been used for decades, the relationship between gut microbiota, its manipulation, and metabolic syndrome has only recently been the attention. Various strategies have been suggested to adjust these imbalances of gut microbiota (also known as intestinal dysbiosis) in obesity; however, the underlying mechanisms leading to these benefits are unclear (7, 8). Consequently, we assessed the effects of *B. lactis* A12 on the composition of gut microbiota and gut microbial diversity. More importantly, we investigated the correlation between the dominant bacteria, *B. lactis* A12, and obesity and predicted the metabolic profile of the fecal microbiome. Some studies have reported that a high *Bacteroidetes*-to-*Firmicutes* ratio is related to enhanced energy harvesting from food, which suggests that these two main phyla are essential for regulating obesity (36–39). Consistently, our high-throughput sequencing analysis of gut microbiota demonstrated the prevalence

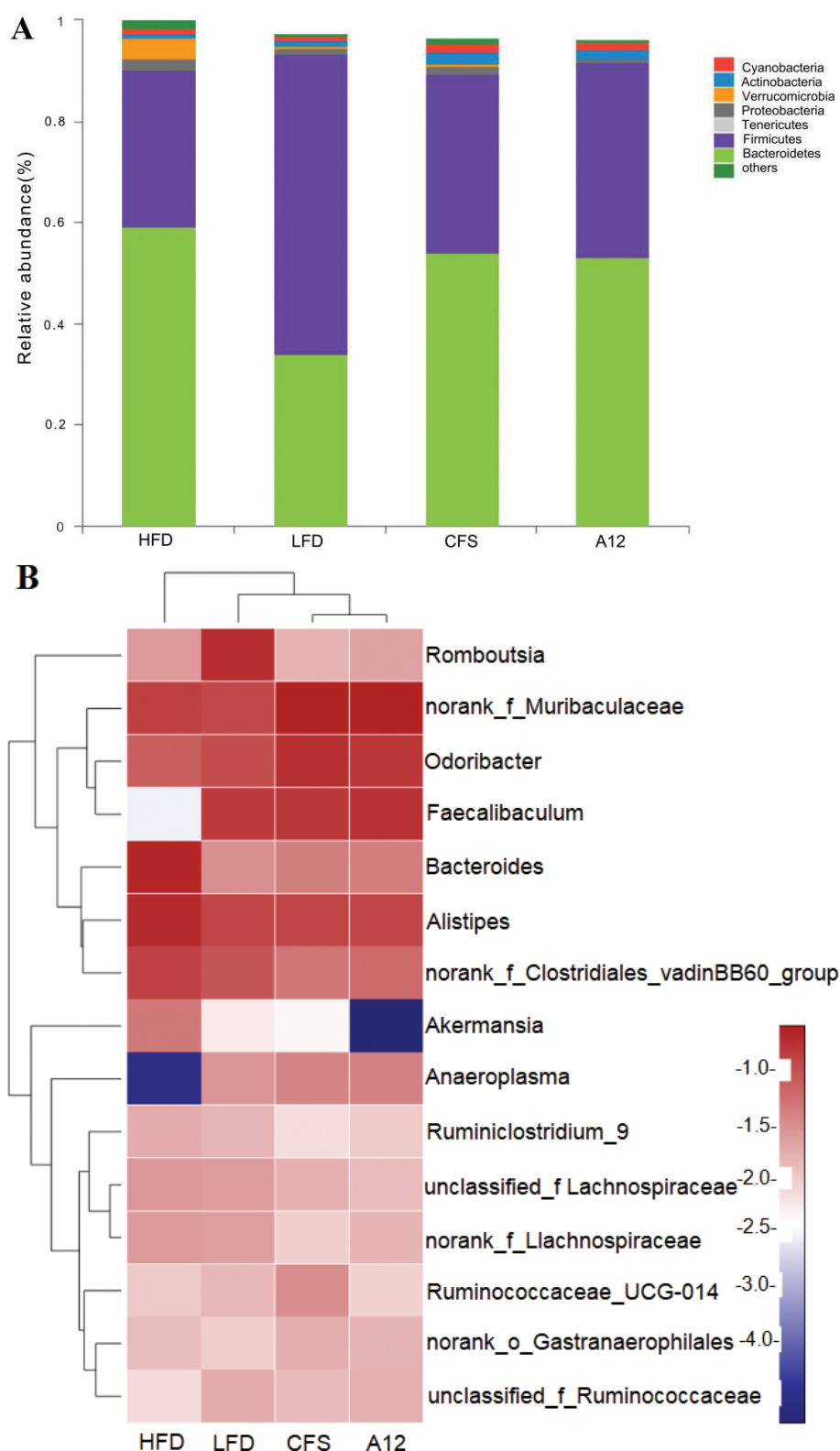


Fig. 5. The composition of gut microbiota. (A) The taxonomic composition distribution and relative abundance with phylum level (the species of which abundance was less than 0.5% in all samples were classified into 'others' in other ranks). (B) Heatmap of Spearman's correlation analysis between gut microbiota on the genus level (the relative abundances with 15 key phylotypes).

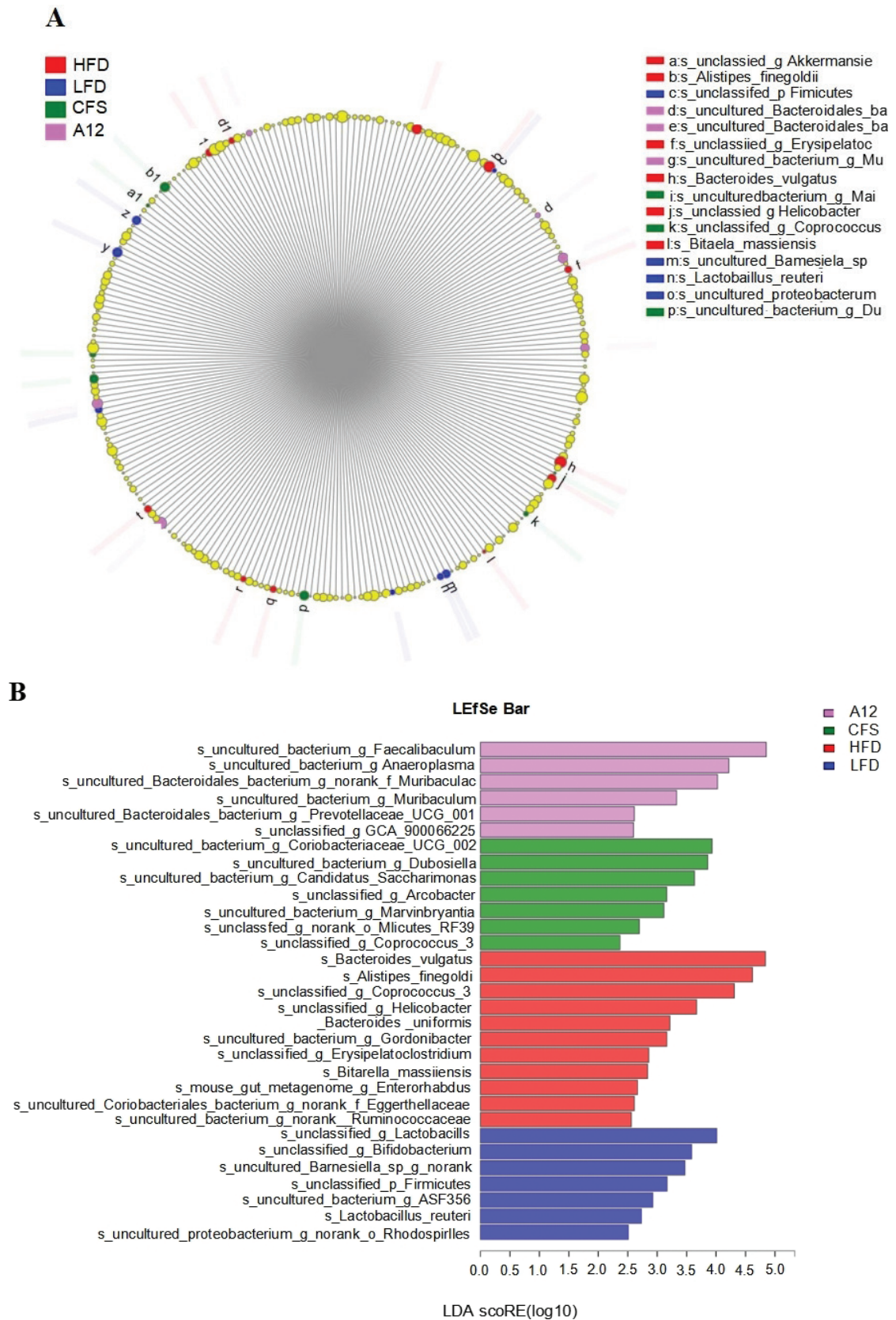


Fig. 6. Structure and key species of gut microbiota responding to high-fat diet and *B. lactis* A12 supplementation. (A) Taxonomic cladogram obtained from the LEfSe analysis of the 16S rRNA sequencing. (B) The taxa meeting an LDA significant threshold >2.

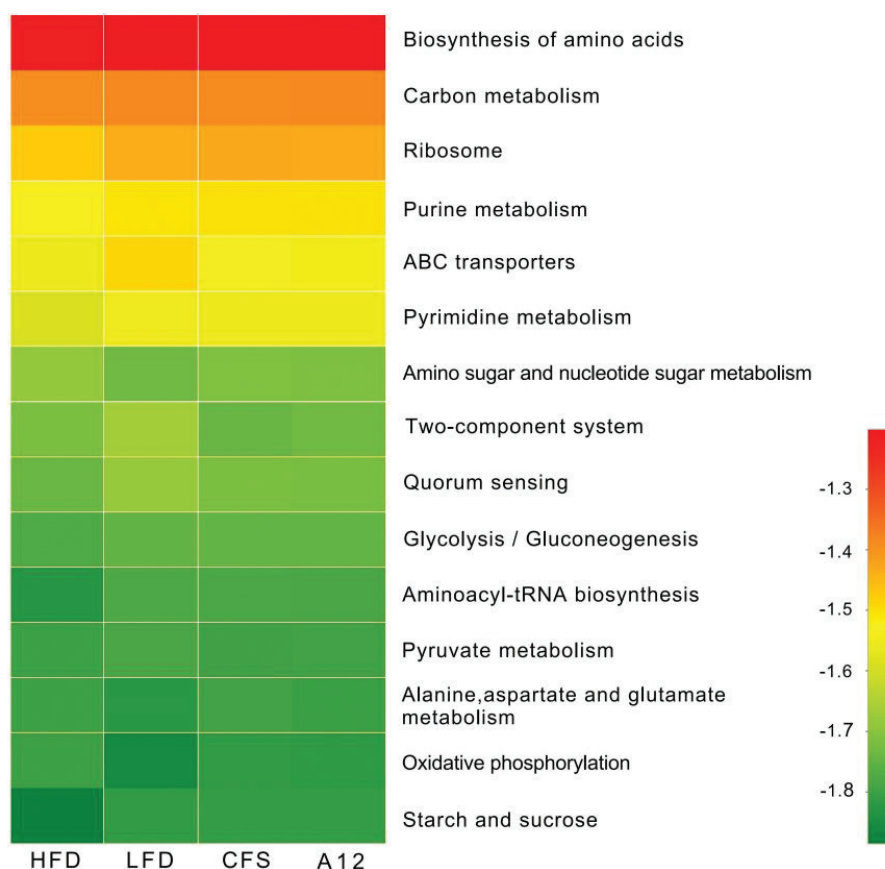


Fig. 7. Predicted metabolic profile of the fecal microbiome after the *B. lactis* A12 supplementation. The 16S rRNA data were further analyzed as indicated by the PICRUSt. Statistical significance difference was assessed using the unpaired two-tailed Student's *t*-test ($p < 0.05$) in STAMP.

of *Firmicutes* increased and *Bacteroidetes* decreased. The heatmap results (Fig. 5B) at the genus level clarified that the CFS and A12 groups exhibited clear divergences in the community structure of the gut microbiota compared with the HFD group and were similar to the LFD group. To further explore the key species that play a vital role in stimulating the production of SCFAs, the abundance of the top 15 species with significant changes was investigated. *Bacteroides vulgatus*, *Eggerthellaceae*, and *Erysipelatoclostridium* were the dominant bacteria in the HFD group, similar to previous studies (40). Notably, some beneficial bacteria, including *Faecalibaculum* (mainly produce butyrate), *Anaeroplasm* (fermentation of polysaccharides to produce SCFAs), *Muribaculaceae* (produce succinate, acetate, and propionate), *Mollicutes_RFS9* (associated with less weight gain), and *Prevotellaceae_UGG_001*, were markedly increased in the A12 group (41–43). In addition, *Coriobacteriaceae_UCG_002* (beneficial effects in type 2 diabetes) and *Coproccoccus_3* (associated with a low body mass index or high microbial richness in both animals and humans) were markedly increased in the CFS group (44, 45). Although the effects of *Dubosiella*,

Candidatus_Saccharimonas, *Marvinbryantia*, *Arco-bacter*, and *Mollicutes_RF39* on lipid and glucose metabolism are not well-documented, these might also be conducive to maintain energy homeostasis of the body. Based on this phenomenon, we concluded that *B. lactis* A12 may regulate the abundance of SCFA-producing bacteria in the gut, thereby regulating the SCFAs' production. Finally, this study predicted the metabolic profile of the fecal microbiome after the *B. lactis* A12 consumption. Interestingly, *B. lactis* A12 could regulate metabolism, genetic, and environmental information processing-related pathways in HFD-fed mice, indicating that *B. lactis* A12 may regulate the gut microbiota in a variety of ways to prevent HFD-induced obesity. However, because of the complexity of the gut microbiota, the precise regulatory mechanism of *B. lactis* A12 needs to be further elucidated.

Conclusions

In this study, our findings indicated that *B. lactis* A12, a novel probiotic strain isolated from breast-fed infant faces, can effectively suppress HFD-induced obesity. Two regulatory pathways may be involved in the anti-obesity actions of

B. lactis A12. First, *B. lactis* A12 inhibited liver adipose accumulation through downregulation of *SERBP-1* mRNA levels. Second, *B. lactis* A12 increased the abundance of SCFA-producing bacteria in the gut and, eventually, mediated beneficial effects on the host via the SCFAs-GPR43-GLP-1 pathway. Our findings indicated that *B. lactis* A12 may be potentially used to prevent obesity and metabolic syndrome. Further studies are needed to clarify the mechanisms of specific metabolic interactions modulated by *B. lactis* A12 and to determine whether these changes play a critical role in human metabolism.

Authors' contributions

Project administration and supervision: F.R. and J.J.; investigation and writing: T.L. and J.Y.; methodology: H.Z.; conceptualization and software: Y.X. and H.L.; validation: J.R. All authors have read and agreed the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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