Original Research

Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression

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Abstract

Recent evidence suggests that intestinal *Bifidobacterium* species (spp.) positively correlates with improved insulin resistance and obesity, and this might be linked to metabolic inflammation. The expression of intestinal REG (regenerating) family proteins which are widely involved in inflammatory bowel disease and diabetes are still unknown in metabolic syndrome. Hence, we investigated the effects of *Bifidobacterium longum* (BIF) supplementation on metabolic parameters, intestinal function and expression of Reg family genes in a rat model of metabolic syndrome induced by a high-fat (HF) diet. We specifically increased the gut bifidobacterial content of HF-fed rats through BIF supplementation. Compared with the normal chow-fed control rats, HF feeding significantly reduced intestinal *Bifidobacterium*. As expected, BIF supplementation fed rats had totally restored quantities of *Bifidobacterium*. HF diet-fed rats showed significant increase in body weight, fat deposits, systolic blood pressure, fasting glucose, fasting triglycerides and reduced insulin sensitivity, while increases of intestinal *Bifidobacterium* did improve HF-diet-induced metabolic disorders. HF feeding led to significantly higher levels of the plasma lipopolysaccharide, interleukin-1β and intestinal myeloperoxidase, as well as intestinal inflammatory activity index, while these parameters were normalized to the control levels in the HF + BIF-treated rats. The levels of RegI mRNA and protein in the HF + BIF group were significantly higher than the control and the HF groups. Increasing *Bifidobacterium* in the gut improved HF-fed-induced metabolic syndrome by reducing metabolic endotoxin concentrations and intestinal inflammation, as well as upgrading the expression of intestinal Reg I as a regulator of growth factor.

Keywords: *Bifidobacterium*, metabolic syndrome, rat, regenerating gene, diet

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Introduction

Metabolic syndrome is a significant clinical problem characterized by insulin resistance, hyperinsulinemia, dyslipidemia, hypertension and obesity.[1,2] In a globalized world, over-consumption of refined carbohydrates and saturated fat is the commonest cause of insulin resistance that dramatically increases the incidence of metabolic syndrome, which is a risk factor for diabetes and cardiovascular diseases.[1] Evidence suggests that obesity and metabolic disorders (type 2 diabetes and insulin resistance) are tightly linked to inflammation.[3,4] An innovative hypothesis was recently proposed: the gut flora could be an important factor affecting energy disposal and could be implicated in metabolic disease associated with obesity.[5–9] It has been recently reported that *Bifidobacterium* spp. significantly and positively correlated with improved glucose tolerance, glucose-induced insulin secretion and lowered plasma lipopolysaccharide (LPS) concentration (metabolic endotoxin concentrations).[10–12] However, the role of *Bifidobacterium* on the prevention of metabolic syndrome has not yet been clarified.

Bifidobacterium is one of the most important microorganisms in the gut which reduces intestinal endotoxin concentrations and improves mucosal barrier function, according to previous studies related to stress.[13–16] Recently, it has been observed that the intestinal inflammatory tone increased in obese mice, induced by a high-fat (HF) diet.[17]

Therefore, we speculated that *Bifidobacterium* might improve metabolic syndrome by reducing metabolic endotoxin concentrations and intestinal inflammation.

REG (regenerating) family proteins, involved in liver, pancreatic, gastric and intestinal cell proliferation, belong to a conserved protein family sharing structural and functional properties.[18] The increase of REG expression in inflammatory bowel disease and autoimmune diabetes could promote cell growth.[19–21] To date, the members of this family can be grouped into four subclasses, type-I, -II, -III and -IV, according to their structural similarities.[22] One type-I gene (RegI) and three type-III genes (PAP I, PAP II and PAP III) have been cloned in rats.[23] They play a wide range of roles in researching mammal physiology and human diseases.[18–21] However, the expression of intestinal REG in metabolic syndrome and the effects of *Bifidobacterium* supplementation on its expression are still unknown.

In this study, we investigated the effects of *Bifidobacterium longum* (BIF) supplementation on metabolic parameters, intestinal function and the expression of Reg family genes in a rat model of metabolic syndrome induced by HF feeding.

Materials and methods

Animals

The experimental protocol was approved by the Animal Ethics Committee of Shanghai Jiao Tong University and was in accordance with the Guiding Principles in the Care and Use of Animals.

Animals (adult male Wistar rats weighing 160–185 g) were procured from the Animal Science Department of Shanghai Institutes for Biological Science (Chinese Academy of Sciences [CAS], Shanghai, China). They were kept individually in polypropylene cages in an environmentally controlled room of the departmental animal house. They were maintained at $25 \pm 2^{\circ}C$ with a 12 h dark/light cycle and 40–70% humidity. Rats were acclimatized to the laboratory conditions for one week before experimentation and were provided with a standard diet and water *ad libitum*.

Diet and experimental groups

Both the standard and HF diets were supplied by Shanghai Institutes for Biological Science following the recommendations of the National Institute of Nutrition, Beijing, China. Rats ($n = 10$ per group) were fed three different experimental diets for 12 weeks as follows: (1) standard diet (control, C group) containing (in weight percent) approximately 60% carbohydrate, 25% protein, 5% fat and 7% crude fibre; (2) HF diet (HF group) containing 49.5% fat – corn oil and lard – (g/100 g of total dry diet), 37% protein, 10% cellulose, 3.5% mineral/vitamin mixture. The energy content of the HF diet was 72% fat, 28% protein and <1% carbohydrates (CAS); and (3) a mix of the HF diet and BIF (HF + BIF group) containing 2.0×10^9 of live BIF CGMCC NO.2107 (BL88-Onlly, Shanghai, China) in 1 mL of normal saline by oral gavage once daily for 12 weeks. The other two groups (control group and HF group) were given normal saline without BIF by oral gavage once daily.

Body weight and fat mass data

The daily body weights were recorded in all the groups of rats every day between 04:00 and 16.30 and continued up to 12 weeks. At the end of the experiment, the fat mass in the abdominal cavity was excised and weighed (mesenteric fat, epididymal fat, retroperitoneal fat and inguinal fat), and stored at $-80^{\circ}C$ until analyses.

Systolic blood pressure measurement

Systolic blood pressure (SBP) was monitored indirectly in conscious rats (prewarmed and restrained slightly in animal nose-cone acrylic holders) by the tail-cuff method and recorded on a Grass Model 7 polygraph (Grass Instruments, West Warwick, RI, USA). Rats were accustomed to the apparatus several times before actual measurements were taken.

Biochemical analyses

For fasting blood analyses, the samples were collected after an overnight fast. Blood was collected from the tail vein of the rats and plasma was separated. Fasting plasma insulin was assayed by an ACS:180 SE automated chemiluminescence system (Bayer, Leverkusen, Germany). Plasma glucose and plasma triglyceride concentrations were analyzed using an Automatic Biochemistry Analyzer (Beckman LX20; Beckman Coulter Inc, Phoenix, AZ, USA). Plasma LPS determinations in the rat were performed using a kit based upon a Limulus amoebocyte extract (LAL kit; Cambrex BioScience, Walkersville, MD, USA).

Insulin sensitivity (hyperinsulinemic euglycemic clamp)

After 12 weeks of the respective diets, 30 animals (10/group) in an overnight-fasted state (18 h) were submitted to a hyperinsulinemic euglycemic clamp (HIEC). Four days before these clamps, two catheters were inserted into the right jugular vein through a small incision in the neck under sodium pentobarbital (50 mg/kg) anesthesia. Subsequently, rats housed individually were allowed to recover for four days. During the clamp experiment, one catheter was used for insulin infusion (human insulin from Actrapid, Novo Nordisk, Beijing, China) and the other for glucose (25%) infusion, which were previously described by Lavoie *et al.*[24] Euglycemia was maintained by gentle adjustment of the glucose infusion rate (GIR in mL/min). The HIEC's measure of insulin sensitivity was obtained by the average of GIR during the last 30 min of the test.

Quantification of *Bifidobacterium* spp.

The flora was collected and genome DNA of fecal bacteria was extracted with the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany) at the beginning and end of this study, respectively. Polymerase chain reactions (PCRs) were performed in 20-μL final volumes in capillary tubes in an ABI Prism 7300 (Applied Biosystems, Foster City, CA, USA). Reaction mixtures contained 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 4 mmol/L $MgCl_2$, 200 μmol/L concentration of each deoxynucleoside triphosphate,

0.5 μmol/L of each primer, 1:30,000 dilution of SYBR Green I (Molecular Probes, Eugene, OR, USA), and 0.025 U of Taq DNA polymerase (Promega, Shanghai, China) per μL, 1 μL of respective bacterial template DNA. The amplification program and the primers were referred to our previous study.[25]

Inflammatory parameters of intestinal injuries

For assessment of proinflammatory agents, the production of interleukin-1β (IL-1β) and myeloperoxidase (MPO) in the small intestine tissues was measured by an enzyme immunoassay (EIA). The amounts of IL-1β and MPO were assessed by using EIA kits for IL-1β (R&D Systems, Minneapolis, MN, USA) and MPO (Hycult Biotechnology, Uden, Netherlands), respectively.

Gut histology and histological inflammatory activity index

Histological sections were obtained from the ileum and fixed in 10% formalin. Transverse 5-lm-thick sections were prepared in a standardized fashion, washed with pure ethanol and embedded in paraffin. The slides thus prepared were stained with hematoxylin and eosin. Histological sections were observed and photographed using an Axio-Cam digital-camera (Carl Zeiss, Oberkochen, Germany) installed on a light microscope (Olympus, Tokyo, Japan).

The histological inflammatory activity index was graduated using a previous proposed scale.[26] The degree of inflammation of each slide was scored as follows: absent (0), no crypt atrophy, no epithelial infiltration by polymorphonuclear cells (PMN), no erosions or ulcers; low (1–3), no epithelial erosions or ulcers, slight atrophy of crypts and PMN of $<50\%$ in five contiguous crypts or $<50\%$ of three different analyzed fields; moderate (3–6), no epithelial erosions or ulcers, severe atrophy of crypts and PMN infiltration of $>50\%$ in five crypts or $>51\%$ of three different analyzed fields; severe (7–8) epithelial erosion or ulceration, irrespective of the other features.

Realtime quantitative reverse transcription-PCR

The small intestinal mucosal layer was scraped off and stored in RNAlater (Qiagen). Total RNA was extracted with TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions, and 1 μg of total RNA was reverse transcribed into complementary DNA (cDNA) by using the random primer from an affinity script QPCR cDNA synthesis kit (Stratagene, La Jolla, CA, USA). Realtime PCR was performed using an ABI 7900 System (Applied Biosystems) using SYBR Green primers for Reg I, PAP I, PAP II, PAP III and Gapdh, which were listed in Table 1. Relative values (RVs) were determined by normalizing the expression for each gene of interest to a housekeeping gene (Gapdh).[27]

Western blot

The tissue was homogenized in buffer containing 125 mmol/L mannitol, 40 mmol/L sucrose, 5 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L PIPES-Tris (pH 6.7) and one tablet of protease inhibitor cocktail per 50 mL of buffer. Total protein concentrations were determined using the Bio-Rad protein assay. Electrophoresis was performed using equal amounts of total protein (10 mg/well) in a 100 g/L sodium dodecyl sulfate (SDS) polyacrylamide gel. The electrophoresis was carried out at 80 V for the first 15 min and then at 130 V for 45 min in SDS electrophoresis buffer. The protein was transferred onto nitrocellulose membrane (Micron Separations, Inc, Westborough, MA, USA) at 100 V for one hour. The membrane was blocked overnight in 50 mL/L milk. A dilution of 1:5000 was used for the rat anti-reg (Reg I, PAP I, PAP II, PAP III) monoclonal antibody and incubated for one hour with the membrane. After washing with Tris-Tween buffered saline solution, the membrane was incubated for 45 min with a 1:2000 dilution of horse radish peroxidase-linked anti-rat IgG. Detection was done using the ECL Western Blotting Detection System (Amersham Pharmacia, Piscataway, NJ, USA).

Statistical analysis

All data were expressed as mean $\pm$ standard deviation (SD) and were evaluated using least significant differences *post hoc* tests after one-way analysis of variance. A P value less than 0.05 was considered to be statistically significant.

Results

Effects of the diets on quantity of intestinal *Bifidobacterium* spp

The number of intestinal *Bifidobacterium* in all rats was measured before and after the experiment and is shown in Table 2. The initial number of rats (baseline data) was not significantly different in all three groups ($P = 0.651$ between groups). After 12 weeks of specific diets, the number of fecal *Bifidobacterium* in the HF + BIF rats obviously increased compared with the C or HF rats with a significant difference ($P_C < 0.001$, $P_{HF+BIF} < 0.001$). There was a significant decrease in the number of fecal *Bifidobacterium* for the HF rats compared with the control rats ($P < 0.001$).

Effects of the diets on parameters related to metabolic syndrome

Chronic administration of HF induced several alterations in the cluster of risk factors that characterize metabolic syndrome. Body weights of all rats were measured before and after the experiment and are shown in Table 2. The initial weights of rats (baseline data) were not significantly different in all three groups ($P = 0.472$ between groups). After 12 weeks of specific diets, the HF rats gained more weights than the control animals with a significant difference ($P = 0.001$). The weight gain of HF + BIF rats was not significantly different from the control group ($P = 0.124$). Compliance with the change of weight, the fat pad weights (mesenteric, inguinal, retroperitoneal and epididymal fat) (Table 2) were significantly higher in the HF than in the control group ($P < 0.001$). Also, the fat pad weights

Table 1 Primer pairs for the reverse transcription-polymerase chain reaction (PCR) analysis in detecting rat Reg family members

Rat Reg family member	Accession no.	PCR primers used in this study	References
Reg I	M18962	Sense 5′ GCATCCTAAGCAGAAGACAGT 3′ Antisense 5′ TTCTTGTCTGGCTCTGTATGAC 3′	55
PAP I	M55149	Sense 5′ AAGATGTTGCATCGCTTGGCC 3′ Antisense 5′ CTAAAGCTGTTTGCTGTCTGG 3′	56 46,57
PAP II	D23676	Sense 5′ ATCCCAGATCACTGCAAGGC 3′ Antisense 5′ AAGGTCTCTTCTGGCAGGCC 3′	58 59
PAP III	L20869	Sense 5′ GGAAGATGTGCCCACTTCACG 3′ Antisense 5′ GTTGTTGATCTTCCCATTGGG 3′	60

between the HF + BIF group and the control group had no significant difference ($P = 0.263$). Table 2 also shows the changes in SBP between the baseline and at the end of the experimental period. By week 12, the SBP of the HF group was significantly increased compared with that in the control group ($P < 0.001$). Daily administration of BIF to HF partially reversed the increment in SBP ($P = 0.004$ compared with HF group); however, SBP in the HF + BIF group remained greater than that in the control group ($P < 0.001$). Addition of BIF to the HF diet caused a significant reduction in both fast glucose and fast triglycerides ($P_{glucose} < 0.001$, $P_{triglycerides} < 0.001$, compared with HF group; Table 2). A significant decrease of the GIR in rats fed the HF diet was observed in the data of insulin sensitivity from the HIEC ($P < 0.001$, compared with control group; Table 2). Addition of BIF to the HF diet brought back the GIR to the level of control rats ($P_{HF} < 0.001$, $P_C = 0.078$; Table 2).

Effects of the diets on inflammatory parameters and intestinal injures

After 12 weeks of specific diets, the data of plasma LPS concentration showed that HF feeding caused a high level of endotoxemia ($P < 0.001$, compared with control group; Figure 1a), and was defined as a metabolic endotoxemia.[11] *Bifidobacterium* supplementation obviously reduced the level of metabolic endotoxemia induced by the HF diet ($P = 0.006$, compared with the HF group; Figure 1a). As to the proinflammatory agents, the IL-1β and MPO concentrations in the small intestine tissues were significantly higher in HF rats than in the control rats ($P_{IL-1\beta} < 0.001$, $P_{MPO} < 0.001$, compared with control group; Figures 1b and c), and the addition of BIF to the HF diet caused a significant reduction in both IL-1β and MPO ($P_{IL\text{-}1\beta} < 0.001$, $P_{MPO} < 0.001$, compared with HF group, Figures 1b and c). Consistent with the changes in inflammatory factors, the HAI also showed more severe intestinal inflammatory injuries induced by the HF diet than in the other two groups, with significantly higher scores in the HF group ($P_C < 0.001$, $P_{HF+BIF} < 0.001$; Figure 1d). At the same time, daily supplementation of BIF to HF partially relieved the intestinal inflammation ($P_{HF+BIF} < 0.001$, $P_C = 0.292$; Figure 1d). In addition, Figure 2 shows representative pictures of small intestines dissected from the experimental rats. Histological examinations revealed prominent inflammatory cell infiltration, regional villous sloughing and villus necrosis present in HF rats (Figure 2b). In contrast, there was no active inflammation in the control group (Figure 2a). *Bifidobacterium* supplementation obviously reduced the intestinal inflammation induced by the HF diet (Figure 2c).

Effects of the diets on expression of Reg mRNA and protein

We examined small intestinal Reg I, PAP I, PAP II and PAP III mRNA levels in different groups of experimental rats by realtime quantitative reverse transcription PCR (Figure 3). We observed the highest mRNA expression of Reg I in the HF + BIF rats, which was 3.8- and 3.0-fold of the levels in the control rats and the HF rats, respectively ($P < 0.001$ compared with the control group and the HF group). No

Table 2 Effects of the diets on intestinal *Bifidobacterium*; parameters related to metabolic syndrome

	C	HF	HF + BIF
Bifidobacterium (Log no. of cells/g of feces)			
Before	7.36 ± 0.15^a	7.35 ± 0.13^a	7.34 ± 0.22^a
After	7.33 ± 0.20^a	6.65 ± 0.21^b	8.01 ± 0.10^{ab}
Weight (g)			
Before	176.41 ± 4.35^a	174.38 ± 3.81^a	179.84 ± 4.01^a
After	425.04 ± 21.35^a	476.40 ± 39.36^b	447.19 ± 29.58^a
Fat pad weights (g/100 g body weight)	4.26 ± 0.33^a	6.71 ± 0.21^b	4.77 ± 0.36^a
Systolic blood pressure (mmHg)			
Before	103.8 ± 1.48^a	103.9 ± 1.66^a	104.3 ± 1.49^a
After	109.00 ± 1.05^a	122.01 ± 3.52^b	118.5 ± 2.32^{ab}
Fasting glucose (mmol/L)	4.87 ± 0.14^a	7.23 ± 0.56^b	5.46 ± 0.35^{ab}
Fasting triglycerides (mmol/L)	0.97 ± 0.06^a	1.46 ± 0.29^b	0.96 ± 0.15^a
GIR (mg/kg/min)	18.18 ± 2.05^a	8.13 ± 1.11^b	15.95 ± 5.04^a

Data are mean ± SD

Data with different superscript letters are significantly different; $P < 0.05$, according to analysis of variance statistical analysis

C, control diet; HF, high-fat diet; HF + BIF, high-fat diet and *Bifidobacterium longum* supplementation; GIR, glucose infusion rate

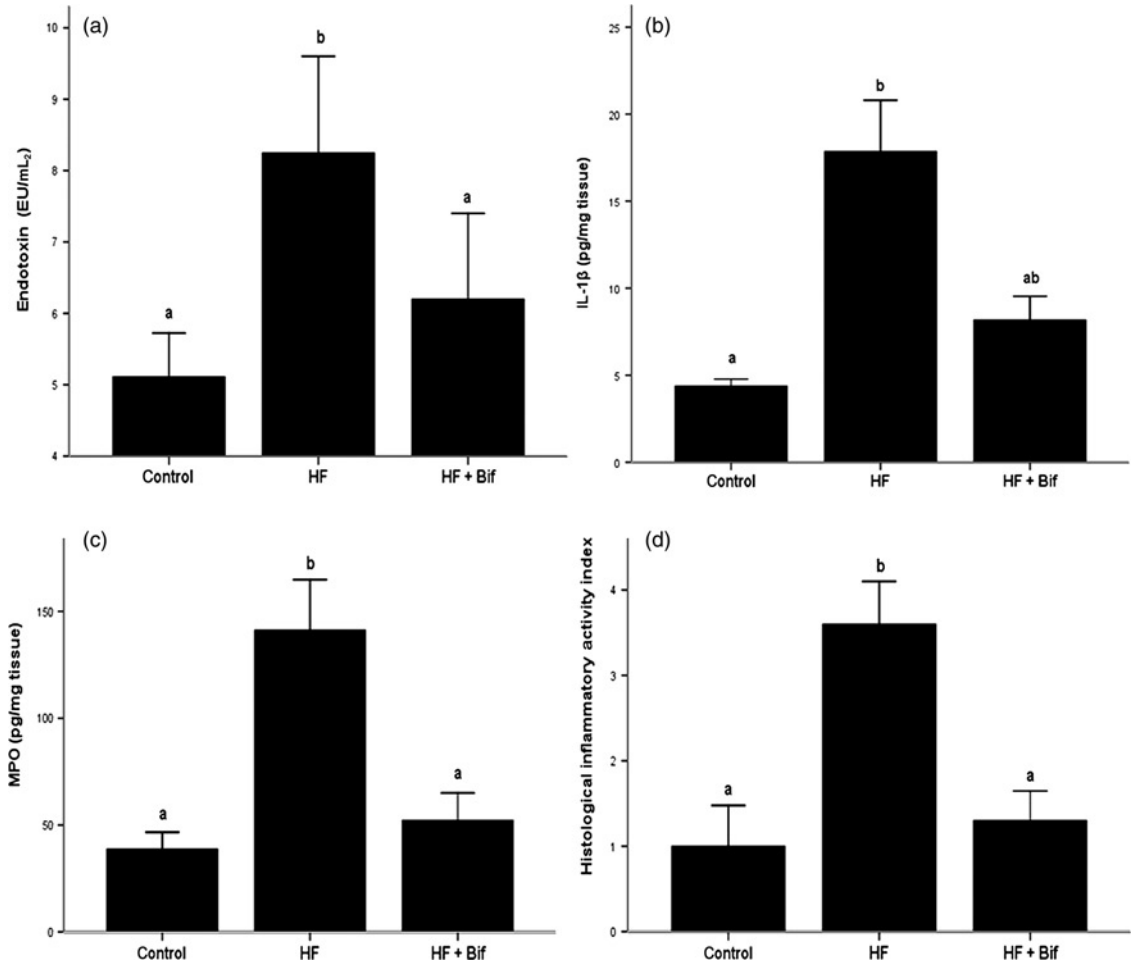

Figure 1 Effects of *Bifidobacterium longum* supplementation on high-fat-diet-induced systemic inflammation and intestinal inflammatory injuries in rats. The concentration of plasma lipopolysaccharide (a), inflammatory parameters in the small intestinal tissue (b and c) and histological inflammatory activity index (d) are shown. Control, Control diet; HF, high-fat diet; HF + BIF, high-fat diet and *B. longum* supplementation; IL-1β, interleukin-1β; MPO, myelaperoxidase. Values not sharing a same letter are statistically different, $P < 0.05$

significant difference on Reg I mRNA expression was observed between the control group and the HF group ($P = 0.612$). In contrast, expression of the other three genes' (PAP I, PAP II and PAP III) mRNA in the small intestine was continuously at very low levels in all three groups (Figure 3). The mRNA expression of the three genes, either in the HF rats or in the HF + BIF rats was no significantly different from the control rats ($P_{\text{PAP I}} = 0.737$, $P_{\text{PAP II}} = 0.830$, $P_{\text{PAP III}} = 0.155$, between groups). To verify the Reg expression observed by realtime quantitative reverse transcription-PCR, we investigate the Reg protein concentrations by Western blot analysis. We observed similar patterns for Reg mRNA and protein expression in three groups of experimental animals. In line with the faint mRNA expression, no protein expression of PAP I, PAP II and PAP III was detected in either the control or the experimental rats. Importantly, we found that Reg I protein concentration in the HF + BIF group ($\text{RV}_{\text{Reg I/GAPDH}} = 1.17 \pm 0.03$) was also the highest when compared with the control group ($\text{RV}_{\text{Reg I/GAPDH}} = 0.25 \pm 0.07$) and the HF

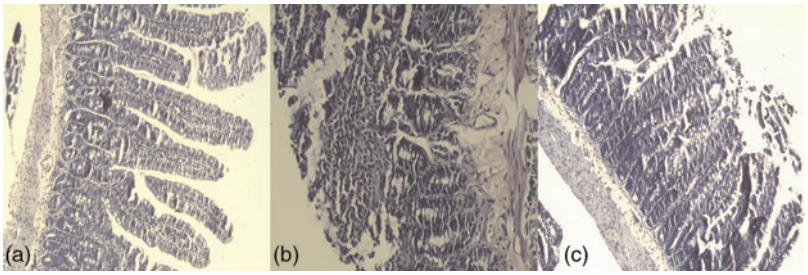

Figure 2 Representative histological images of terminal ileum (hematoxylin and eosin stained, magnification: ×200). (a) Control group, villi of generally normal morphology. (b) HF group, severe inflammatory cell infiltration, regional villous sloughing and villus necrosis. (c) HF + BIF group, mild to moderate inflammatory cell infiltration (A color version of this figure is available in the online journal)

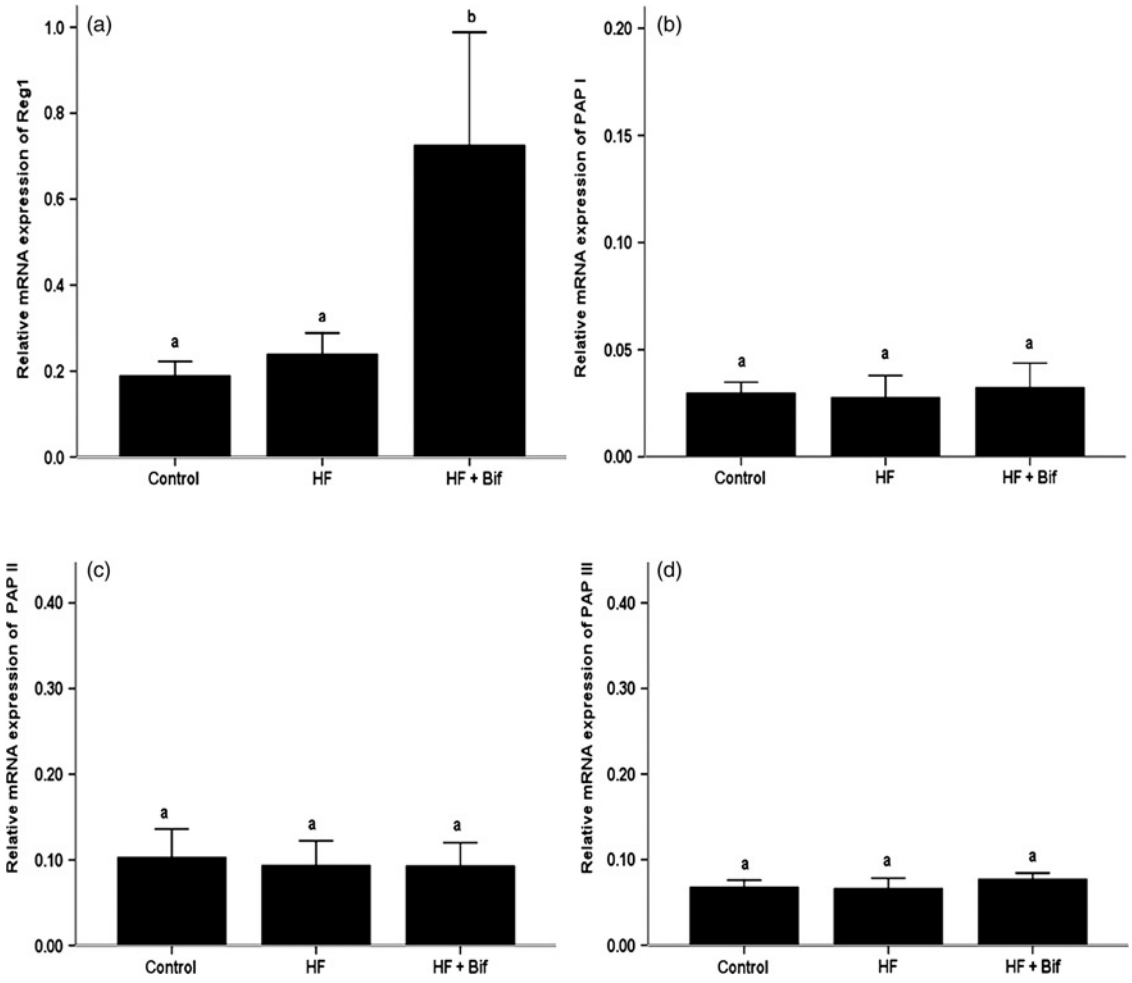

Figure 3 Histogram of quantitative reverse transcription-polymerase chain reaction for Reg I, PAP I, PAP II and PAP III mRNA expression in the small intestine from three groups of the experimental rats. Results of Reg I (a), PAP I (b), PAP II (c) and PAP III (d) expressed as the relative mRNA expression of Reg to Gapdh. The $2^{-\Delta\Delta Ct}$ method was followed. Control, Control diet; HF, high-fat diet; HF + BIF, high-fat diet and *Bifidobacterium longum* supplementation. Values not sharing a same letter are statistically different, $P < 0.01$

group ($RV_{Reg\ I/GAPDH} = 0.31 \pm 0.06$; Figure 4), which indicated that addition of BIF to the HF diet caused a higher expression of intestinal Reg I at both the mRNA and protein levels.

Discussion

Metabolic syndrome is a complex polygenic disorder resulting in part from the contribution of impaired insulin secretion and/or impaired insulin action on its receptors.[28] When carbohydrates are in low supply, or their breakdown is incomplete, fats become the preferred source of energy.[29]

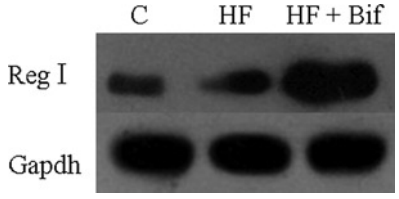

Figure 4 Protein expression of Reg I in the small intestine from three groups of experimental rats. Gapdh served as a loading control. C, Control diet; HF, high-fat diet; HF + BIF, high-fat diet and *Bifidobacterium longum* supplementation

As a result, fatty acids are mobilized into the general circulation, leading to secondary triglyceridemia, in which the total serum lipids, including triglycerides, cholesterol and phospholipids, increase, leading to life-threatening lipid disorders.[30] The development of metabolic syndrome is influenced by a combination of genetic and environmental factors. Among the environmental factors, long-term HF intake is most intensively studied because of its contribution to the development of metabolic syndrome in human beings and rodents.[31] Nowadays, a high proportion of daily energy derived from the fat component is a common situation in current lifestyles in most societies. The high prevalence of metabolic disorders is probably related to abnormal blood lipid profiles that may be due to long-term effects of HF intake.[32] In our study, HF-diet-fed rats showed significant increases in body weight, SBP, fasting glucose, fasting triglycerides and reduced insulin sensitivity.

The growing prevalence and high-risk nature of metabolic syndrome highlights the need to treat it with an assertive, multitargeted approach.[33–35] Recent studies have highlighted key mammalian host–gut microbial relationships, suggesting that the gut microbiota play an important role in energy metabolism.[5] In the present study, we found

that HF feeding did reduce quantities of intestinal *Bifidobacterium* as previously reported.[10] At the same time, some researchers have found that selective increases of *Bifidobacterium* can protect the host from diabetes induced by HF diets.[10] Another research indicated that *Bifidobacterium* supplementation ameliorates HF-nutrition-induced lipid metabolism disorder.[36] Based on the above findings, we investigated the role of *Bifidobacterium* in the prevention of metabolic syndrome which has not been reported before. Evidence provided by this present study revealed that increases of *Bifidobacterium* in gut microflora did improve HF-diet-induced metabolic syndrome, while BIF supplementation effectively suppressed body weight gain, reduced fat deposits, lowered SBP, serum glucose and triglycerides, and increased insulin sensitivity upon concomitant administration along with the HF diet.

Extensive research is dedicated to the effects of an inflammatory reaction on energy metabolism. For example, obesity and insulin resistance were associated with low-grade chronic systemic inflammation,[3] and expression and content of proinflammatory cytokines such as IL-1 and IL-6[37,38] increased in models of diet-induced and genetic obesity. Metabolic concentrations of plasma LPS modulated by fat food content were a sufficient molecular mechanism for triggering the HF diet-induced metabolic obesity/diabetes.[11] Moreover, previous studies demonstrated that probiotics, especially *Bifidobacterium* species, could effectively maintain the integrity of the gut barrier[39–41] and reduce intestinal endotoxin concentrations.[13,14,42] Hence, we speculated that the protective effects of *Bifidobacterium* on metabolic syndrome observed in the present study were also achieved through reducing the inflammatory tone of intestine and the level of metabolic endotoxemia. The current study showed that HF feeding led to significantly higher levels of plasma LPS and IL-1β and MPO contents in the small intestine tissues, as well as intestinal inflammatory activity index. Daily supplementation of BIF to HF partially relieved the above changes, which supported our previous hypothesis. It might be of help to understand the protective effects of BIF on metabolic syndrome; Cani *et al.*[12] found that selective increases of *Bifidobacterium* spp. lowered intestinal permeability by a mechanism involving glucagon-like peptide-2 (GLP-2), thereby improving inflammation and metabolic disorders during obesity and diabetes. However, whether the GLP-2 or the other tight junction proteins are also involved in the probiotic protective effects on metabolic syndrome needs to be confirmed in further.

Reg and Reg-related genes constitute a family belonging to the calcium-dependent lectin (C-type lectin) gene superfamily.[43–46] It represents a group of small secretory proteins, which can function as acute phase reactants, lectins, antiapoptotic factors or growth factors for pancreatic β-cells, neural cells and epithelial cells in the digestive system.[47,48] Considerable attention has been focused on the Reg family and its structurally related molecules. In the current study, we observed the highest mRNA and protein expression of Reg I in the HF + BIF rats, while there was no significant difference between the control group and the HF group. In contrast, expression of the other three genes' (PAP I, PAP II and PAP III) mRNA in the small intestine was continuously at very low levels in any group of rats. In line with the faint mRNA expression, no protein expression of these three genes was detected in either the control or the experimental rats by Western blot. This might be due to the different location and function of these genes, where PAP II is often over-expressed in the regenerator islets and over-expression of PAP I/PAP III is usually detected in the injured neural cells.[49,50] However, it has been reported that Reg I mRNA in the gastrointestinal tract was expressed at the highest levels in the small intestine of wild-type murine, suggesting a critical role in this tissue.[51] In the small intestine of the adult Reg I-knockout mice, less densely packed, round-shaped aberrant morphology of the absorptive cells was observed light microscopically, and electron microscopical examination revealed a strikingly loose connection of these cells to the basement membrane.[52] Thus, Reg I was considered to be a regulator of cell growth that was required to generate and maintain the villous structure of the small intestine.[52] At the same time, researchers also found that mild to moderate injury to tissues stimulated the synthesis of Reg/PSP-protein, whereas more severe injury tended to depress it.[53,54] This might explain the high expression of Reg I in the HF + BIF rats in our study, for the intestinal inflammation ameliorated by *Bifidobacterium* supplementation stimulated the high expression of Reg I. The present data have suggested that there is a link between modulation of gut microbiota, metabolic endotoxemia, intestinal inflammation and Reg I expression. However, the relationship between Reg I and the tight junction proteins such as GLP-2 is still unknown, although both of them have been found to play a critical role in improvement of the intestinal permeability.[12,52] This merits further investigation. In the future, the precise delineation of the Reg-signaling pathway in intestinal cells would clarify this issue. In addition, it also needs to be further attested whether Reg and Reg-related molecules can offer novel molecular targets for metabolic syndrome therapeutics.

In conclusion, dietary modulation of gut microbiota with a view to increasing *Bifidobacterium* improved HF-fed-induced metabolic syndrome by reducing metabolic endotoxin concentrations and intestinal inflammation, as well as upgrading the expression of intestinal Reg I as a regulator of growth factor. Thus, specific strategies for modifying gut microbiota in favor of *Bifidobacterium* could be useful tools for reducing the impact of HF feeding on the occurrence of metabolic syndrome.

Author contributions: JJC and RW designed the research; JJC, RW, X-fL and R-lW performed the research; JJC, RW and X-fL analyzed the data; and JJC and RW wrote the paper. JJC and RW are joint first authors.

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