Bifidobacterium adolescentis supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of the metabolic syndrome

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Abstract

The aim of the present study was to investigate the effects of Bifidobacterium adolescentis (Bif) supplementation on visceral fat accumulation and insulin sensitivity of the metabolic syndrome in HF-diet-fed rats. Adult male Wistar rats (n 10 per group) were fed four different experimental diets for 12 weeks as follows: standard diet; high-fat (HF) diet; a mix of HF diet and Bif; a mix of standard diet and Bif. Liver, mesenteric fat, epididymal fat, retroperitoneal fat, and inguinal fat, pancreas and triceps surae in all four groups of the rats were weighed, while liver steatosis and insulin sensitivity were evaluated at the end point of the study. As the number of intestinal Bifidobacterium species decreased obviously, fat pad weight and body weight increased significantly in the HF group compared with in the other three groups (P < 0.05). Addition of Bif led to a reduction in body weight and fat pad weight (P < 0.05). With an increase in liver weight, more severe steatosis of hepatocytes was observed in the HF group compared with in the other three groups. A significant decrease of the glucose infusion rate and pancreas weight was found in the HF group (P < 0.05). This deleterious effect was alleviated when Bif was added to the diets. Bifidobacterium supplementation ameliorated visceral fat accumulation and insulin sensitivity of the metabolic syndrome in HF-diet-fed rats.

Key words: Bifidobacterium; Metabolic syndrome; Insulin sensitivity; Visceral fat; Rats

The metabolic syndrome is a significant clinical problem characterised by insulin resistance, hyperinsulinaemia, dyslipidaemia, hypertension and impaired glucose tolerance(1,2). According to recent estimates, approximately 215 million people worldwide suffer from diabetes and 80–90% of them from type 2 diabetes(3). The modern lifestyle of increased intake of a high-energy-dense diet associated with decreased energy expenditure also contributes to the current rising prevalence of obesity and type 2 diabetes(4). Recent epidemiological studies also revealed that 90% of all patients with type 2 diabetes are or have been overweight, and indicated that obesity is a strong risk factor and cause of type 2 diabetes and associated metabolic disturbances(5,6). The events of hyperglycaemia and hyperlipidaemia, and their association present major risk factors for the development of diabetic and cardiovascular complications(7). To reduce these serious complications and negative outcome of the metabolic syndrome, the control not only of blood glucose but also of lipids is necessary(8). Therefore, new medicinal agents with dual properties related to controlling both blood glucose and lipids are in great demand. The currently available therapeutic options such as dietary modification or a combination of synthetic antidiabetic, hypolipidaemic drugs have their own limitations and undesirable side-effects(7). Hence, there is an increased demand to search and evaluate traditional approaches for the treatment of metabolic disorders.

An innovative hypothesis was recently proposed: the gut microbiota could be an important factor affecting energy disposal and could be implicated in metabolic disease associated with obesity(9–13). It has been recently reported that high-fat...
(HF) feeding in mice induced a low-grade inflammatory tone and that insulin resistance was associated with reduced numbers of Bifidobacterium species (spp.) in caecal content (14). At the same time, Backhed’s study (11) suggested that a bacterially related factor was responsible for HF-diet-induced obesity. Bifidobacterium is one of the predominant micro-organisms in the gut which can reduce intestinal endotoxin levels and improve mucosal barrier function (15–17). However, the role of Bifidobacterium in the prevention of the metabolic syndrome has not emerged clear yet. In the present study, we investigated the effects of Bifidobacterium adolescentis (Bif) supplementation on visceral fat accumulation and insulin sensitivity of the metabolic syndrome in HF-diet-fed rats.

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. They were kept individually in polypropylene cages in an environmentally controlled room of the departmental animal house. They were maintained at 25 ± 2°C with a 12 h dark–12 h light cycle and 40–70% humidity. Rats were acclimatised to the laboratory conditions for 1 week before experimentation and provided ad libitum with standard diet and water.

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, China. Rats (n 10 per group) were fed four different experimental diets for 12 weeks as follows: (1) standard diet (control, C group) contained (in weight %) approximately 60% carbohydrate, 25% protein, 5% fat, 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% carbohydrate (Chinese Academy of Sciences, Shanghai, China); (3) a mix of HF diet and Bif (HF + Bif group) containing 5 × 10^6 colony-forming units/ml of live Bif (Livzon Pharmaceutical Group, Inc., Zhuhai, China) in 1 ml of normal saline by oral administration once daily for 12 weeks; (4) a mix of standard diet and Bif (C + Bif group).

Real-time fluorescence quantitative PCR for quantification of Bifidobacterium species

The microbiota of the rectum was collected and extracted genome’s DNA of faecal bacteria with the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany) at the beginning and end of this study, respectively. PCR 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 mM Tris–HCl (pH 8.3), 50 mM KCL, 4 mM MgCl2, 200 μM concentration of each deoxynucleoside triphosphate, 0.5 μM each primer, 1:30 000 dilution of SYBR Green I (Molecular Probes, Eugene, OR, USA) and 0.025 units of Taq DNA polymerase (Promega, Shanghai, China) per μl, 1 μl of respective bacterial template DNA. The amplification program for Bifidobacterium comprised of activation of polymerase (95°C for 5 min), followed by forty cycles of 10 s at 95°C, 31 s at 61°C and 30 s at 72°C. The temperature transition rate was 20°C/s for all steps. The genus-specific 16S rRNA-targeted primer sets used for quantitative real-time PCR and the other details were referred to the previous study (19).

Body weight data

Daily body weight was recorded in all the groups of rats every day between 16.00 and 16.30 hours and continued for up to 12 weeks.

Insulin sensitivity (hyperinsulinaemic – euglycaemic clamp)

After 12 weeks of the respective diets, forty animals (n 10 per group) in an overnight-fasted state (18 h) were submitted to a hyperinsulinaemic – euglycaemic clamp. 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) anaesthesia (previously described by Lavoie et al. (19)). The catheters were filled with sterile heparinised physiological saline solution (500 units/ml) and heat-sealed. Subsequently, rats housed individually were allowed to recover for 4 d. During the clamp experiment, one catheter was used for insulin infusion (human insulin from Actrapid, Novo Nordisk, China) and the other for glucose (25%) infusion. Four days after surgery, the clamp was conducted according to a modified technique developed by De Fronzo et al. (20). The animals were weighed and the catheters of the jugular vein were connected to tubing extensions for glucose or insulin infusions. Rats were put in their cages for a 30 min stabilisation period. Afterwards, blood samples were taken every 10 min during 30 min to assess the basal blood glucose level for each animal. Blood glucose was measured with a blood glucose meter (Ascensia Elite; Bayer Corporation, Mishawaka, IN, USA) from samples (3-5 μl) collected from the tail vein. Insulin was then infused at a rate of 4 mU/kg per min for approximately 3 h (infusions were made with a syringe pump (KD Scientific, Hollister, CA, USA)). Insulin concentrations were determined from the tail vein using an enzymatic immunoassay kit (Rat Insulin Elisa; Mordcoa, Uppsala, Sweden). After 1 min of insulin infusion, infusion of 25% glucose was initiated and a blood sample was taken to assess glycaemia. To monitor euglycaemia, blood was drawn every 5 min during the first 30 min and every 10 min thereafter. Euglycaemia was maintained by gentle adjustment of the glucose infusion rate (GIR in ml/min). The hyperinsulinaemic – euglycaemic clamp measure of insulin sensitivity was obtained by the average of GIR during the last 30 min of the test.
Tissue sampling

The abdominal cavity was quickly opened after overnight fasting and euthanised with fluothane anaesthesia, and organs, muscles and fat deposits were excised and weighed: liver, mesenteric fat, epididymal fat, retroperitoneal fat, and inguinal fat, pancreas, triceps surae muscles (soleus, plantaris, medial and lateral gastrocnemius). All tissues were weighed, immediately frozen in liquid N2 and stored at −80°C until analyses. Mesenteric fat pad was comprised of the adipose tissue surrounding the gastrointestinal tract from the gastroesophageal sphincter to the rectum. Special care was taken in distinguishing and excluding pancreatic cells. The epididymal fat pad included adipose tissue surrounding male internal genital organs. The retroperitoneal, epididymal and inguinal fat pads were taken from the right side of each animal.

Hepatic histology

A small part of the right hepatic lobe was fixed in 10% formalin. Paraffin-embedded samples were sliced, stained with haematoxylin–eosin and studied under a light microscope. All the samples were observed and evaluated by a qualified pathologist who was blinded to the group assignment.

Statistical analyses

Copy numbers of 16S rRNA genes of *Bifidobacterium* per g of faeces were transformed into logarithms and normal distributed data were subjected to statistical analysis. All data were expressed as means and standard deviation, and were evaluated using Fisher’s least significant differences post hoc test after one-way ANOVA. Significance was set at *P*<0.05. All statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago, IL, USA).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>Mean (SD)</th>
<th>C+Bif</th>
<th>Mean (SD)</th>
<th>HF</th>
<th>Mean (SD)</th>
<th>HF+Bif</th>
<th>Mean (SD)</th>
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<tr>
<td>Bifidobacterium (log no./g)*</td>
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<tr>
<td>Before</td>
<td></td>
<td>5.33 a 0.72</td>
<td>5.28 a 0.59</td>
<td>5.46 a 0.61</td>
<td>5.29 b 0.66</td>
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<td>After</td>
<td></td>
<td>5.24 a 0.81</td>
<td>6.01 b 1.00</td>
<td>4.75 a,b 0.42</td>
<td>5.91 b 1.03</td>
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<td>Weight (g)</td>
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<td>Before</td>
<td></td>
<td>177 a 7</td>
<td>171 a 9</td>
<td>170 a 10</td>
<td>176 a 8</td>
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<td>After</td>
<td></td>
<td>429 a 12</td>
<td>416 a 11</td>
<td>502 a 13</td>
<td>459 a,b 11</td>
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<tr>
<td>Fat pad weight (g/100 g body weight)†</td>
<td></td>
<td>4.51 a 0.32</td>
<td>4.27 a 0.24</td>
<td>6.82 a 0.19</td>
<td>5.63 a,b 0.38</td>
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<td>Muscle weight (mg/100 g body weight)‡</td>
<td></td>
<td>663 a 10</td>
<td>671 a 8</td>
<td>573 a 12</td>
<td>615 a,b 11</td>
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<tr>
<td>Liver weight (g/100 g body weight)‡</td>
<td></td>
<td>2.92 a 0.18</td>
<td>2.89 a 0.20</td>
<td>3.69 a 0.26</td>
<td>3.01 a 0.18</td>
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<tr>
<td>Pancreas weight (mg/100 g body weight)</td>
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<td>280 a 19</td>
<td>288 a 17</td>
<td>225 a 20</td>
<td>278 a 19</td>
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* C, control diet; C+Bif, control diet and *Bifidobacterium adolescentis* supplementation; HF, high-fat diet; HF+Bif, high-fat diet and *Bifidobacterium adolescentis* supplementation.

* Mean values with unlike superscript letters were significantly different (*P*<0.05, according to the post hoc ANOVA statistical analysis).
† Copy numbers of 16S rRNA genes of *Bifidobacterium* per g of faeces were transformed into logarithms.
‡ Fat pad weight was the sum of mesenteric, inguinal, retroperitoneal and epididymal weight.

Results

The number of faecal bifidobacteria in all rats was measured before and after the experiment and is shown in Table 1. The initial number of faecal bifidobacteria (baseline data) was not significantly different in all four groups (*P*>0.05). After 12 weeks of specific diets, the number of faecal bifidobacteria in the C+Bif and the HF+Bif rats obviously increased than in the C or the HF rats with a significant difference (*P*<0.05). There was a significant decrease in the number of faecal bifidobacteria in the HF rats at the same time (*P*<0.05).

Body weight of all rats was measured before and after the experiment and is shown in Table 1. The initial weight of rats (baseline data) was not significantly different in all four groups (*P*>0.05). After 12 weeks of specific diets, the HF rats gained more weight than the C or the C+Bif rats with a significant difference (*P*<0.05). The weight gain of HF+Bif rats was not significantly different from those of the other three groups (*P*>0.05).

There were significant diet-induced alterations in relative body composition expressed as tissue weight/100 g body weight. The fat pad weight (mesenteric, inguinal, retroperitoneal and epididymal fat; Table 1) was significantly higher in the HF than in the C and C+Bif groups (*P*<0.05). With the changes of body weight, the fat pad weight between the HF+Bif group and the other three groups had no significant difference (*P*>0.05). In contrast, the weight of muscles (soleus, plantaris and gastrocnemius; Table 1) was significantly lower in HF rats than in C and C+Bif rats (*P*<0.05). There were no significant differences in the fat pad weight between the HF+Bif rats and the other three groups (*P*>0.05). Addition of Bif to the HF diet led to a reduction in white fat accumulation while significant mesenteric white fat accumulation was also observed in HF rats (Fig. 1).

Moreover, liver weight was increased by 26% in the HF group *v.* the C group, by 28% *v.* the C+Bif group and by 23% *v.* the HF+Bif group (*P*<0.05; Table 1). The liver weight of rats had
no significant differences between HF + Bif, C and C + Bif groups ($P > 0.05$). Data from hepatic pathology showed no obvious steatosis changes in the C + Bif group. Mild steatosis of hepatocytes was observed in the C group. The microscopic findings presented moderate to severe hydropic degenerative and steatosis changes in the HF group. In the HF + Bif group, light microscopy demonstrated the presence of mild to moderate steatosis of hepatocytes (Fig. 2).

Pancreas weight was decreased by 20% in the HF group vs. the C group, by 22% vs. the C + Bif group and by 19% vs. the HF + Bif group ($P < 0.05$; Table 1). In the HF + Bif group, Bifidobacterium counteracted the pancreas weight reduction induced by the HF diet and the pancreas weight was similar to those of the control animals. A significant decrease of the GIR in rats fed the HF diet was observed upon data of insulin sensitivity from hyperinsulinemic euglycemic clamp (Fig. 3). In the HF group, GIR was decreased by 55·28%. In the HF + Bif group, the decrease of GIR was limited to only 17·22%. Addition of Bif to the HF diet brought back the GIR to 15·05 (SD 3·01) vs. 18·18 (SD 2·05) mg/kg per min in control rats.

**Discussion**

In a globalised world, over-consumption of refined carbohydrates and saturated fat is the commonest cause of insulin resistance that dramatically increases the incidence of the metabolic syndrome, diabetes and CVD(1). It is well documented that insulin resistance is associated with alterations of glucose utilisation, decreased glycogen synthesis and increased abdominal fat(21). Recent studies have highlighted key mammalian host–gut microbial relationships, suggesting that the gut microbiota might play an important role in energy metabolism(9). It has also been reported that HF feeding increased plasma lipopolysaccharide concentration and reduced quantities of the dominant Gram-positive groups *Bifidobacterium* spp. in the mice with the metabolic

**Fig. 2.** Light microscopic histology of liver stained with haematoxylin–eosin (400 x). (a) Control (C) group, mild steatosis of hepatocytes. (b) C + *Bifidobacterium adolescentis* (Bif) group, no obvious steatosis of hepatocytes. (c) High fat (HF) group, diffuse steatosis of hepatocytes. (d) HF + Bif group, mild to moderate steatosis of hepatocytes, ⤷, Lipid droplets in hepatocytes.
syndrome, compared with controls fed a standard diet\(^\text{(12)}\). At the same time, \textit{Bifidobacterium} spp. has been shown to reduce intestinal endotoxin levels and improve mucosal barrier function\(^{(15,16,22)}\). So we decided to specifically increase the gut bifidobacterial content in order to examine the effects of Bif supplementation on the development of the metabolic syndrome in HF-diet-treated rats.

In the present study, addition of Bif to the diets did increase quantities of \textit{Bifidobacterium} in the intestine of the experimental animals. The HF feeding reduced quantities of \textit{Bifidobacterium} as previously reported. We found that body weight and total fat pad weight of the rats were significantly higher in the HF group while the muscle weight was lower than in the other three groups after 12 weeks of specific diets. In contrast, no significant differences in the previous indexes were found between the HF + Bif rats and the control animals. This indicated that addition of Bif to the HF diets did not change the obesity induced by the HF diet to some extent. Moreover, Bif supplementation in the HF diets counteracted the increased liver weight and the serious steatosis of hepatocytes induced by the HF diets. Some studies have recently demonstrated that obese and diabetic mice \((ob/ob)\) and \(db/db)\) exhibited significantly higher plasma endotoxin levels positively correlated with glucose intolerance and mesenteric fat negatively correlated with the plasma lipopolysaccharide concentration\(^{(14,23)}\). In another study about the relationship between intestinal bacterial overgrowth and translocation and parenteral nutrition-associated liver complications, \textit{Bifidobacterium} was observed to improve the steatosis of hepatocytes via lowering plasma lipopolysaccharide levels\(^{(24)}\). We speculated that the diffuse steatosis of hepatocytes and the increased liver weight in the HF group were one outcome of systemic metabolic inflammation induced by the HF diets and that Bif supplementation relieved the effects through lowering bacterial translocation and endotoxaemia. Moreover, Bif supplementation in the HF diets led to a reduction in white fat accumulation while significant mesenteric white fat accumulation was observed in HF rats. Mesenteric fat is a major determinant of hepatic insulin action and insulin resistance and strongly related to a higher risk of incident diabetes\(^{(25,26)}\). However, the relationship between visceral fat accumulation (liver steatosis and mesenteric fat) and hepatic insulin sensitivity and the exact mechanism of the beneficial effects of \textit{Bifidobacterium} still need to be further explored.

In addition, the significant decreases in the GIR in HF rats confirmed that consumption of an HF diet leads to insulin resistance and alterations in glucose utilisation in agreement with similar data obtained from previous studies\(^{(27,28)}\). In contrast, \textit{Bifidobacterium} supplementation counteracted insulin resistance induced by the HF diet and GIR of animals receiving Bif returned to those of the control animals. \textit{Bifidobacterium} supplementation improved diabetes and insulin sensitivity, which may be achieved by a mechanism promoting synthesis and secretion of the incretin, glucagon-like peptide \(^1\text{(29,30)}\). We observed that a reduction in pancreas weight induced by the HF diet was counteracted through Bif supplementation. Recent studies have described a decrease of functional \(\beta\)-cell mass related to the loss of insulin sensitivity in insulin-independent pancreas, kidney recipients and in people with diabetes\(^{(31,32)}\). The restoration of the pancreas weight in rats fed HF plus Bif may be related to the improvement of insulin sensitivity in these animals.

In conclusion, dietary modulation of gut microbiota with a view to increasing bifidobacteria reduced visceral fat accumulation (liver steatosis and mesenteric fat), as well as improved insulin sensitivity in the HF-diet-fed rats. Thus, specific strategies for modifying gut microbiota in favour of bifidobacteria could be useful tools for reducing the impact of HF feeding on the occurrence of the metabolic syndrome.

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