Insoluble Dietary Fiber from Pear Pomace Can Prevent High-Fat Diet-Induced Obesity in Rats Mainly by Improving the Structure of the Gut Microbiota

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**Introduction**

The long-term intake of a high-fat diet seriously affects human health and results in metabolic disorders and chronic diseases such as overweight, fatty liver, cardiovascular disease, hypertension, hypercholesteremia, insulin resistance, and leptin resistance [1]. The prevalence of these diseases is increasing in the current society and the pathogenesis is interrelated in a complex manner [2]. Supplement of dietary fiber (DF) in the diet is believed to be one of the most effective approaches to prevent the chronic diseases caused by over-intake of fat. Studies have proved that DFs can reduce the risk of hyperlipidemia, hypercholesterolemia, hyperglycemia, and colon cancer by modulating food ingestion, digestion, absorption, and metabolism [3]. The American Dietetic Association suggested that the daily diet of the public should contain more than 25 g DFs for adult women and 38 g DFs for adult men to reduce the risk of cardiovascular disease [4]. The health benefits of DF has been widely accepted and applied.

DF is generally classified into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) according to their solubility in water. Previously, it was considered that SDF contributed to the reduction of serum lipids and cholesterol,

Supplement of dietary fibers (DF) is regarded as one of the most effective way to prevent and relieve chronic diseases caused by long-term intake of a high-fat diet in the current society. The health benefits of soluble dietary fibers (SDF) have been widely researched and applied, whereas the insoluble dietary fibers (IDF), which represent a higher proportion in plant food, were mistakenly thought to have effects only in fecal bulking. In this article, we proved the anti-obesity and glucose homeostasis improvement effects of IDF from pear pomace at first, and then the mechanisms responsible for these effects were analyzed. The preliminary study by real-time PCR and ELISA showed that this kind of IDF caused more changes in the gut microbiota compared with in satiety hormone or in hepatic metabolism. Further analysis of the gut microbiota by high-throughput amplicon sequencing showed IDF from pear pomace obviously improved the structure of the gut microbiota. Specifically, it promoted the growth of Bacteroidetes and inhibited the growth of Firmicutes. These results are coincident with previous hypothesis that the ratio of Bacteroidetes/Firmicutes is negatively related with obesity. In conclusion, our results demonstrated IDF from pear pomace could prevent high-fat diet-induced obesity in rats mainly by improving the structure of the gut microbiota.

**Keywords:** Insoluble dietary fiber, anti-obesity, gut microbiota, amplicon sequencing
level, whereas the effect of IDF was mainly fecal bulking [3]. Thus, previous studies have paid more attention to the effects and mechanisms of SDF. Suggestions regarding the mechanisms responsible for these effects of SDF include the regulation of the expression of key genes involved in hepatic metabolism (such as fatty acid synthase, acetyl-CoA carboxylase, cholesterol 7 α-hydroxylase, and AMP-activated protein kinase) [5–8], alterations in satiety hormone secretion (such as ghrelin, peptide YY, and leptin) [9, 10], delayed gastric emptying, energy dilution, increased energy expenditure, and regulation of the gut microbiota [9]. Regarding IDF, fewer studies have been conducted to assess the relationship between IDF and overweight. Isken et al. [11] reported that supplementing the diet with insoluble cereal fiber resulted in significantly lower weight gain and improved insulin sensitivity. Tan et al. [12] also found the associations of IDF intake and glycemic index value with subsequent HbA1c levels. In spite of these efforts, whether and how IDF affects the gut microbiota, key lipometabolism, and satiety hormone secretion is largely unknown.

For SDF, many studies indicated its supplement could alter the composition of the microbiota, thus preventing obesity [6, 9, 13]. Compared with SDF, IDF is less possible to be absorbed, thus it remains in the intestinal tract and has interaction with the gut microbiota for a longer time. The gut microbiota is highly influenced by the host diet and the fermentability of ingested fibers [14]. Recently, many studies reported that obesity was related to disorder of the gut microbial community. Generally, people who possess less Bacteroidetes and more Firmicutes in their gut microbiota are more likely to be obese [15]. With the development of high-throughput sequencing and metagenomics, increasing evidence has indicated the role of the gut microbiota in the etiology of host chronic metabolic diseases such as type II diabetes [16] and symptomatic atherosclerosis [17]. As IDF could hardly be absorbed, the gut microbiota could be a most possible intermediary between IDF and human health.

Pomace from fruits is a good source of IDF [18–20], and thus the reasonable use of pomace could be of great benefit to human health and the protection of the environment. Pears are popular fruits worldwide, particularly in juices and soft drinks. Thousands of tons of pear seeds, pulp, and peels are generated as agricultural byproducts and are typically discarded and wasted. Natural IDF extracted from pear pomace was added to high- and normal-fat diets in the present study to evaluate the functional effects of the extracted IDF on high-fat diet-challenged rats. The purpose of the present study was to evaluate the functional effects of the IDF extracted from pear pomace on rats fed a high-fat diet. The gut microbiota, key lipometabolism gene expression, and satiety hormone secretion in these rats were determined by real-time PCR and ELISA. After finding the potential effect of IDF on the gut microbiota, the change in pattern of the gut microbiota was further analyzed by high-throughput amplicon sequencing. Our results suggested IDF from pear pomace could prevent high-fat diet-induced obesity in rats mainly by improving the structure of the gut microbiota.

Materials and Methods

Animal Model

Eight-week-old, specific pathogen-free male Sprague-Dawley rats were purchased from Vital River Laboratories (China; license number SCXK (Beijing) 2012-0001). These rats were housed at a constant room temperature (22 ± 2°C) and humidity (55% ± 10%) and maintained on a 12-h light/dark cycle in an experimental animal room in the Supervision, Inspection and Testing Center for Genetically Modified Organisms of the Ministry of Agriculture (Beijing, China; license number SYXK (Beijing) 2010-0036). Food and water were provided ad libitum throughout the test. All animal experiments were approved by the Animal Experimental Welfare and Ethical Inspection Committee (No. 100034) of the Supervision, Inspection and Testing Center for Genetically Modified Organisms of the Ministry of Agriculture (Beijing, China).

Pear Pomace Samples and Diets

The alkali-catalyzed hydrolysis method was applied to extract the IDF from the pear pomace. The pear pomace was prepared by removing the juice from the fruit and grinding the fruit to a granularity of 60 mesh using a high-speed disintegrator followed by the addition of ethyl acetate. After 3 h, the pear pomace was washed with water and dried with hot air at 55°C overnight. Sodium hydroxide was added at 20 times the volume of the pomace, and the mixture was then centrifuged at 4,000 rpm for 15 min. The collected matter was then deposited and washed with water. The IDF was recovered from the residue after the deposit was washed with 76% ethanol, 95% ethanol, and acetone at 4 times the volume of the pomace and dried with hot air at 55°C overnight. The IDF content in the final pear pomace extract was approximately 40%, and the other 60% of the extract was nitrogen-free extract, which is also a common composition in normal rat chow, providing energy only. The content of IDF was determined according to GB 5009.88-2014.

Before the test, the animals were acclimatized to the environment for 7 days. The rats were then randomly divided into four groups (8 rats per group) according to average body weight, and the groups were fed with a normal chow diet (NCD), a high dietary fiber diet (HD) containing 8% fiber supplement in total, a high-fat diet (HD) containing 8% fiber supplement in total, and a high-fat diet (HD) containing 8% fiber supplement in total.
diet (HF) that provided 45% of its energy from fat, or a high-fat and dietary fiber diet (HFD). The rats were observed every day and weighed every week, and the food intakes were recorded for each group throughout the experiment. All of the diets were manufactured by KeAoXieLi Feed Co. Ltd. (China; license number SCXK (Beijing) 2009-0015) and exposed to 60Co for sterilization. The main nutritional ingredients in the HD diets were adjusted to levels similar to those of the normal Chow diet, and the main nutritional ingredients in the HFD diet were adjusted to levels similar to those of the HF diet.

Oral Glucose Tolerance Test (OGTT) and Serum Hormone Analysis

The OGTT was performed at the end of the 5th week. To determine the blood glucose levels, the blood was collected from the tip of the tail vein, and then submitted to a glucose meter (Roche Diagnostics, Switzerland). First, the blood glucose levels of the overnight-fasted (16 h) rats were determined. Next, the rats were given glucose by oral gavage (2 g/kg body weight, 50% solution) [10], and the blood glucose levels were determined at 30, 60, 120, and 180 min post-oral administration. Blood was collected for the hormone analyses at each of the OGTT time points. The blood samples were collected from the orbital venous sinuses into tubes containing 10 μl of anticoagulant (EDTA-K2, 0.04 g/ml). These samples were maintained at 4°C for 2 h and then centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was stored at −80°C for serum hormone analysis. The ghrelin, insulin, peptide YY (PYY), and leptin concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) kits. All of the ELISA kits were purchased from Beijing North Institute of Biological Technology (China). The homeostasis model assessment-insulin resistance (HOMA-IR) values were calculated as the fasting serum glucose (mmol/l) × the fasting serum insulin (mmol/l)/22.5 [21].

Dissection and Tissue Collection

After the OGTTs, all of the rats were anesthetized with chloral hydrate, and venous blood was collected from the orbital vein after overnight fasting (16 h) for hematology and blood biochemical analyses. Then, the rats were killed via an aortic cut and immediately dissected. The liver and small intestine were collected, flash frozen in liquid nitrogen, and stored at −80°C for further analysis. Liver, spleen, and epididymal adipose tissues were fixed overnight in 10% formalin for morphometric analyses.

Hematology and Blood Biochemical Analyses

For the hematology analyses, the blood samples were analyzed to obtain the white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (HCT) level, hemoglobin (HGB) level, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), blood platelet (PLT) count, red cell volume distribution (RDW), and mean platelet volume (MPV) by a HEMAVET 950FS animal blood cell counter (Drew Scientific, Inc., USA). For the biochemical analyses, the blood samples were maintained 4°C for 2 h and then centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was stored at −80°C. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), blood urea nitrogen (BUN), creatinine (CRE), total cholesterol (CHO), triglyceride (TG), lactate dehydrogenase (LDH), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were measured using an automatic Biochemical Analyzer 7020 (Hitachi, Japan).

Hepatic RNA Extraction, Gut Microbiota DNA Extraction, and Real-Time PCR

Total RNA was extracted from the livers of rats using TRNzol reagent (Tiangen Biotech, China). Reverse transcription was performed with an approximate input of 2 μg RNA using the first-strand cDNA FastQuant RT kit (Tiangen Biotech). The resultant complementary DNA was amplified using primers synthesized by Sangon Biotech, China. Fecal samples were obtained before the rats were killed at 5 weeks. Bacterial genome DNA was extracted from about 150 mg of fecal sample using a previously described method [22].

The PCR for hepatic genes involved heating for 1 min at 95°C, followed by 40 cycles at 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, using a CFX96™ real-time system (Bio-Rad Laboratories, Inc., USA). The resulting melting curve revealed the melting point of the PCR product of interest. β-Actin primers were used as an internal control for hepatic gene expression analysis, and the primer sequences of hepatic genes used in this study are all listed in Table 1. The real-time PCR for the gut microbiota was

Table 1. Primer sequences for real-time PCR of liver genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>TCGTGGCGTGACATTAAAGGAG</td>
<td>AGGAAGAAGGGCCTGGAAGAG</td>
</tr>
<tr>
<td>FAS</td>
<td>GAGGACTTGGTGCCGCATTAC</td>
<td>GCTGGATATGTTGATGATAGAC</td>
</tr>
<tr>
<td>ACC</td>
<td>CCTCTTCTTACTGGCGACTGAG</td>
<td>TAAGCCTTCACTGTGCCTTC</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>GGCTTGAGGAGGATGCGAATTAC</td>
<td>GCTGGATATTCAGGATGC</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>TCATCAACACACACAGACAGT</td>
<td>AGAGAACACCGAGAAGAGAAG</td>
</tr>
<tr>
<td>AMPKα1</td>
<td>GCCCGACACACCCCTAGATG</td>
<td>TCAACTGCTTGATTGCTAC</td>
</tr>
</tbody>
</table>

performed as described previously [9]. The universal primer pair for the 16S rRNA gene was used as an internal control for gut microbiota analysis. Primer pairs for specific gut microbial groups [14, 23–26] are listed in Table 2. Primer specificity, amplification efficiency, and the limit of detection were determined according to Louie et al. [27] using serial dilutions of the standards. The 2−ΔΔCT method was utilized for the data analysis [2].

**Table 2.** Primer sequences for real-time PCR of gut bacteria.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rDNA</td>
<td>TCCTACGGGAGGCAGCAGT</td>
<td>GGACTACCAGGGTATCTAATCTGT</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>CAGACCTGAAGGTTGGGAC</td>
<td>CTTGCGGTTGCTTCAAGAAGAAG</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>TCGGTCCGGTGAAGAAC</td>
<td>CCACATCCAGCRTCAC</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>GGACTTCCCAAATGGAAG</td>
<td>CTCTTGCCGTTGCAAGTCAAA</td>
</tr>
<tr>
<td>Clostridium leptum (group IV)</td>
<td>GCACAAGCAGTGGGACTTCAAG</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>CATTGACGTTACCGCAGAAGAAG</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Blood biochemical analysis results.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT U/l</th>
<th>CHO mmol/l</th>
<th>TG mmol/l</th>
<th>LDL mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCD</td>
<td>46.25 ± 5.54</td>
<td>2.06 ± 0.25</td>
<td>0.84 ± 0.39</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>HD</td>
<td>39.88 ± 5.58a</td>
<td>2.09 ± 0.44</td>
<td>1.29 ± 0.38a</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>HF</td>
<td>34.00 ± 5.96</td>
<td>3.42 ± 0.74</td>
<td>0.84 ± 0.12</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>HFD</td>
<td>39.5 ± 6.56</td>
<td>2.38 ± 0.41a</td>
<td>1.31 ± 0.31a</td>
<td>0.41 ± 0.11a</td>
</tr>
</tbody>
</table>

*a*Represents significant difference (*p* ≤ 0.05) between the NCD and HD groups or HF and HFD groups. The values are the mean ± SD (n = 8).

**Table 4.** Relative abundance of several gut microbial population following different diets.

<table>
<thead>
<tr>
<th>Microbial population</th>
<th>NCD</th>
<th>HD</th>
<th>HF</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>9.67 ± 0.52</td>
<td>47.77 ± 4.36a</td>
<td>5.48 ± 0.39</td>
<td>37.34 ± 3.00a</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>0.09 ± 0.01</td>
<td>2.56 ± 0.51a</td>
<td>0.03 ± 0.0</td>
<td>0.04 ± 0</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>140.44 ± 24.18</td>
<td>44.27 ± 6.2</td>
<td>160.86 ± 10.75</td>
<td>168.04 ± 32.83</td>
</tr>
<tr>
<td>Clostridium leptum (group IV)</td>
<td>3.06 ± 0.11</td>
<td>2.02 ± 0.16</td>
<td>4.78 ± 0.44</td>
<td>1.79 ± 0.10</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.59 ± 0.06</td>
<td>1.13 ± 0.29</td>
<td>0.76 ± 0.07</td>
<td>0.84 ± 0.14</td>
</tr>
</tbody>
</table>

*a*Represents significant difference (*p* < 0.05) between the NCD and HD groups or HF and HFD groups. The values are the mean ± SD (n = 6). In order to fit in the table, all values were multiplied by 1,000.

**Gut Microbiota Amplicon Sequencing and Bioinformatic Analysis**

The amplicon sequencing was conducted by PCR amplification of the V4 region of 16S rDNA of bacterial genomes and sequencing on a MiSeq platform (Illumina, USA). Raw data were filtered and then clustered to form operational taxonomic units (OTUs) at a similarity of 97% by QIIME pipeline [28]. Each of these OTUs was annotated to taxonomic unit by the RDP classifier [29] and the GreenGene database [30]. PAST 2.17 software was used to calculate the α diversity and plot non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity as the distance measure [31]. The abundance of annotated OTUs was submitted to the LEfSe online tool [32] to discover high-dimensional biomarkers between the NCD and HD groups, as well as HF and HFD groups.

**Statistical Analysis**

The data in this study were analyzed with one-way analysis of variance using SPSS ver. 18.0 (SPSS Inc., USA). Differences were considered significant at *p* < 0.05. Data were presented as means ± SD. In Figs. 1–4 and Tables 3–4, not all the significant differences were labeled with an asterisk. In these figures and tables, the asterisk means that there was significant difference between the NCD and HD groups or HF and HFD groups. Food intake of each cage was measured. As a result, the food intake data of individual rats could not be acquired. In some studies, the formula [Food intake per animal per day] = [total food intake per cage]/[animals per cage]/[days of food consumption] was used to get food intake.
data of individual rats and make the statistics analysis of food intake data feasible. We think this strategy would artificially reduce the deviation and get inaccurate statistics result, so we did not adopt this method. As a result, the data of food intake were presented (Fig. 1C) without statistics analysis.

Results

Body Weight, Energy Intake, and Fat Accumulation

The body weight of each rat was recorded every week (Fig. 1A), and the final body weights are displayed in Fig. 2.
Fig. 1B. After 5 weeks, the rats fed with high-fat diet (HF group) showed a significant higher weight gaining than that of rats fed with normal chow diet (NCD group). When IDF from the pear pomace was supplemented into the high-fat diet (HFD group), the extra weight gain caused by the high-fat diet was relieved ($p < 0.05$), with the final body

Fig. 3. Serum satiety hormone concentrations and corresponding area under the curve (AUC) values during the oral glucose tolerance test (OGTT).

(A–C) represent the peptide YY (PYY), leptin, and ghrelin concentrations and corresponding AUCs in the OGTT, respectively. The error bars indicate the standard deviation for each group. *Represents a significant difference ($p < 0.05$) between two groups.
weight of HFD rats being at a level similar to that of the NCD group. Rats fed with IDF-supplemented normal chow diet (HD group) showed an even lower final body weight compared with NCD rats. The food intake of the HFD group was slightly higher than that of the HF group, and the food intake of the HD group was slightly higher than that of the NCD group (Fig. 1C, significant analysis was not conducted because food intake was measured by group); thus the effect of IDF on body weight loss was not due to reduced food consumption. In Fig. 1D, adipocyte size is shown at a magnification of 200 times. We observed that HF feeding for 5 weeks led to significant increases in epididymal fat accumulation, compared with the NCD group. After IDF supplement in the HFD group, the

**Fig. 4.** Hepatic gene expression results. (A–E) show the ACC, CYP, FAS, AMPK, and SREBP-1C gene expression levels, respectively. The error bars indicate the standard deviation for each group. No significant difference was observed between the NCD and HD groups or between the HF and HFD groups. The relative mRNA expression levels of the genes of interest are expressed in arbitrary units relative to the internal control gene (β-Actin).
adipocyte size decreased obviously.

**OGTT, HOMA-IR, and Hematology and Blood Biochemical Analyses**

To assess the effects of IDF on obesity prevention and glucose intolerance, we used glucose tolerance tests to examine glucose homeostasis in the rats. As expected, the HF rats exhibited an increased AUC (\(p < 0.05\)) for the OGTT test and an increased HOMA-IR index (\(p < 0.05\)) compared with the NCD group (Fig. 2), which indicated an increased incidence of fasting hyperglycemia [33]. In contrast, the HFD rats with fiber supplement in the exhibited a significantly lower AUC (\(p < 0.05\)) and HOMA-IR index (\(p < 0.05\)) compared with the HF rats. These results demonstrated that the IDF had an anti-hyperglycemic effect and improved glucose homeostasis in the high-fat diet-fed rats. The HD rats also exhibited a lower OGTT AUC (not significant) compared with their NCD counterparts. These results indicated that supplemental pear pomace IDF in the high-energy diet improved glucose tolerance and glucose homeostasis.

No differences between the NCD and HD groups or between the HF and HFD groups were observed in the hematological analyses (Table S1). In the blood biochemical analyses, ALT was significantly lower in the HD group than in the NCD group, but this difference was not observed in the comparison between the HF group and HFD group (Table 3). The cholesterol and LDL concentrations were significantly lower in the HFD group than in the HD group, but the TG levels increased significantly in the IDF supplement groups (HD and HFD) compared with the NCD and HF groups (Table 3). No other significant differences were observed in the blood biochemical analyses (Table S2).

**Preliminary Detection of the Mechanisms of IDF Responsible for Weight Loss**

**Satiety hormone analysis.** Satiety hormones can regulate appetite [10], and some of them play a significant role in regulating the distribution and rate of energy use [34]. Although the serum PYY concentrations in the HD and HFD groups were lower than those in the NCD and HF groups, respectively, no significant differences were observed (Fig. 3A). The leptin concentration in the HFD group was significantly lower than that in the HF group (\(p < 0.05\), Fig. 3B). Besides these, there were no significant differences in ghrelin concentrations (Fig. 3C). The serum hormone analysis indicated that IDF intake only elicited limited changes in the satiety hormone levels.

**Hepatic metabolism gene expression.** FAS expression was 39% lower and SREBP-1c was 20% lower in the HFD group than in the HF group. CYP was 32% higher and AMPK was 15% higher in the HFD group than in the HF group. However, no significant differences due to diet were observed (Fig. 4).

**Gut microbiota.** In order to explore the mechanism of preventing obesity effects on high-fat diet-fed rats resulted by supplementing IDF, the relative abundance of several bacterial groups was determined by RT-PCR. As shown in Table 4, *Bacteroides* was increased significantly after IDF supplementation in both the HD and HFD groups (\(p < 0.05\)). *Bifidobacterium* and *Lactobacillus* were only altered in the HD group, but no significant changes were observed between the HD and HFD groups. *Clostridium leptum* was decreased significantly in both the HD and HFD groups (\(p < 0.05\)). There were no differences in Enterobacteriaceae between NCD and HD or between HF and HFD rats. Compared with the aspects of satiety hormone and hepatic metabolism, IDF showed more effects on the gut microbiota.

**Further Analysis of Gut Microbiota by High-Throughput Sequencing**

The high-throughput amplicon sequencing resulted in 648 OUTs from 24 fecal samples of the four groups. The structure of the gut microbiota in each group was analyzed by NMDS plots (Fig. 5A). The result showed obvious segregation of each group. The HF group was the most distantly apart from the NCD group. The HFD group was between the middle of the NCD and HF groups, indicating that the supplement of IDF compensated the gut microbiota structure changes caused by the high-fat diet. However, in the aspect of \(\alpha\) diversity, there was no significant difference (Fig. 5B).

LEfSe analysis showed the significantly changed gut microbial taxa. Comparison between NCD and HD, and between HF and HFD rats showed very similar change patterns (Figs. 5C and 5D), which means the supplement of IDF caused similar effects in the normal diet and high-fat diet. In both comparisons, IDF caused significant decrease of Firmicutes and significant increase of Bacteroidetes. At the genus level, IDF caused 16 more presented genera and 9 less presented genera in the HD group compared with the NCD group, and 13 more presented genera and 10 less presented genera in the HFD group compared with the HF group. However, there were some differences between the effects of IDF supplementation in normal chow diet and in high-fat diet. As shown in Supplementary Fig. S1, IDF promoted the growth of *Bifidobacterium* in the normal chow.
diet, whereas it promoted the growth of *Akkermansia* in the high-fat diet. According to the Pathogenic Bacteria Database (http://www.globalrph.com/bacterial-strains.htm), *Acinetobacter*, *Clostridium*, and *Streptococcus* are potential pathogenic genera, and they were reduced by IDF supplementary. Thus, on the whole, IDF improved the structure of the gut microbiota.

**Discussion**

To clarify the functional effects of IDF supplementation in terms of preventing obesity elicited by a high-energy diet in rats, 8% extra IDF was added to normal chow and high-fat chow. As expected, the final body weight of the HF group was significantly higher than that of the NCD group, and the body weight of the IDF-supplemented groups was significantly decreased. According to the food intake of each group, the effect of IDF on body weight loss was not due to reduced food consumption, which was different from SDF [9]. The addition of high fat into the diet may affect the palatability of the feed and then reduce the appetite of rats. As a result, the food intake of rats in the HF group was 27.4% lower than that of NCD rats. The less food intake of HF rats might be the reason why plasma TG concentration was not elevated compared with NCD rats. We observed significantly lower plasma CHO and LDL,
but higher plasma TG concentrations in the HFD groups compared with those in the HF group. The lower plasma 
CHO and LDL is beneficial for health. LDL is occasionally referred to as bad cholesterol because it can transport 
fat molecule contents to artery walls, which attracts macrophages and thus drives atherosclerosis. Moreover, 
high-fat diets increase LDL and cholesterol in the body. As 
expected, the LDL and cholesterol serum concentrations in 
the HF group were higher (Table 2), and these concentrations 
were significantly decreased in the HFD group compared 
with the HF group. However, the triglyceride concentrations 
were significantly higher in the IDF-supplemented groups, 
and the explanation for this phenomenon requires further 
study. One possible reason might be that on the one hand, 
IDF might accelerate fat metabolism; on the other hand, the 
reduced FAS gene expression and reduced lipogenesis led 
to increased levels of triglycerides remaining in the blood. 
IDF decreased the LDL and cholesterol serum concentrations, 
which reduced the obesity risks associated with the high-
fat diet. Higher plasma TG in the HFD group was 
unexpected, but fat did not accumulate in adipocytes 
according to the morphometric analyses. Contrarily, the 
adipocyte size decreased obviously in the HFD group 
compared with the HF group. Thus, the significantly 
higher plasma TG in HFD might be caused by less 
absorption of TG by adipocytes. It could be conjectured 
that IDF supplementation might prevent the TG from 
entering and accumulating in adipocytes. More experiments 
should be conducted to verify this conjecture.

The appetite of rats is inhibited by leptin and stimulated 
by ghrelin. These hormones act together to regulate appetite 
to achieve energy homeostasis [35]. In our study, the leptin 
concentration in the HFD group was significantly lower 
than that in the HF group. IDF contains less energy, which 
means more fodder is needed to maintain daily energy 
consumption. Therefore, a lower serum leptin concentration 
is needed to resist hunger to achieve balanced energy 
homeostasis. The serum PYY concentrations in the HD and 
HFD groups were lower those in the NCD and HF groups. 
PYY is a short peptide that is released by cells in the ileum 
and colon in response to feeding and acts to reduce appetite. Thus, we suggest that the same mechanism 
responsible for the changes in leptin concentrations was 
responsible for the changes in PYY. These results also 
confirmed that the effect of IDF on body weight loss was 
not due to reduced food consumption.

Many studies indicated the association of long-term SDF 
diet with the changes of gut microbiota towards a lean 
microbiota type [33]. In this study, we used two methods to
analyze the effects of IDF on the composition of the gut 
microbiota: real-time PCR-based method and high-
throughput sequencing-based method. The results of the 
two methods were largely coincident. For the IDF 
supplement, we observed similar alterations for most 
microbial populations. We hypothesized that IDF may alter 
the composition of the gut microbiota to reduce obesity 
risk. The diversity and composition of gut microbiota could effectected by diet and other factors, and then have effects 
on the health of the host [5]. Previous studies showed 
Bacteroides would decrease in obese rats and increase after 
weight loss [33], which is consistent with our work. The 
benefits of Bifidobacterium have been reported in many 
aspects, including improvement of glucose metabolism and 
reduction of diabetes risk [8, 36]. Bifidobacterium and 
Lactobacillus only changed in HD, but not in HFD rats. 
High-fat diet might be against for IDF to increase 
Bifidobacterium or decrease Lactobacillus in the HFD group; 
this needed further study. According to Geurts et al [37], 
Odoribacter and Alistipes were more abundant in obese and diabetic leptin-resistant 
mice (db/db) compared with lean mice. However, the 
effects of these two genera on obesity and adipose tissue 
seem to be very complicated, as contradictory results were 
also widely reported. For example, in a study on diet-
induced obesity mice, Odoribacter was less presented in 
obesity mice than in lean mice [38]. Odoribacter was 
increased in the bitter melon powder-treated high-fat diet-
induced rats, in which the inflammatory status was 
relieved compared with untreated rats [39]. In the results of 
Shin et al [40], significant reductions in the proportions of 
Alistipes and Akkermansia were observed in high-fat diet-
fed mice, and were reversed by metformin treatment. With 
these contradictory results, it is difficult to make conclusions 
on the effects of increased Odoribacter and Alistipes caused 
by IDF.

In summary, the present study revealed that IDF could 
prevent obesity and reduce the damage caused by a high-
fat diet. The final body weights and cholesterol and LDL 
concentrations in serum were significant decreased in the 
IDF-supplemented HFD groups than in the HF group. IDF 
also improved glucose homeostasis in the IDF-supplemented 
groups. Compared with the aspects of satiety hormones
and hepatic metabolism, IDF showed more effects on the gut microbiota. Further analysis of the gut microbiota by high-throughput amplicon sequencing showed that IDF from pear pomace obviously improved the structure of the gut microbiota. Specifically, it promoted the growth of Bacteroidetes and inhibited the growth of Firmicutes. Our results suggested IDF from pear pomace could prevent high-fat diet-induced obesity in rats mainly by improving the structure of the gut microbiota.

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