PAPER

Lean rats gained more body weight from a high-fructooligosaccharide diet

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Fructooligosaccharides (FOS) are believed to be beneficial to the host growth and its gut health. This article is intended to investigate the different influences of a high-fructooligosaccharide (FOS) diet on the growth and gut microbiota of lean and obese rats. Diet-induced lean and obese rats were fed a high-FOS diet for 8 weeks. Rats' body weight (BW) and feed intake were recorded weekly, and their gut microbiota was analyzed by 16S rDNA sequencing. The results showed that the lean rats gained more BW than the obese ones from the high-FOS diet. In the meanwhile, the gut microbiota in both lean and obese rats was altered by this diet. The abundance of Bacteroidetes was increased significantly ($P < 0.05$) in the lean rats, while no significant alteration in Firmicutes was observed in all rats after the consumption of a high-FOS diet. In conclusion, this study first reported that the lean rats gained more body weight from a high-FOS diet than the obese ones, and the increase of Bacteroidetes might help rats harvest more energy from the high-FOS diet.

Introduction

Humans harbor more than 10^{14} microbes in the gut.[1,2] The human and mice gut microbial communities are similar at the division level, with dominant Firmicutes and Bacteroidetes;[3,4] besides, low proportions of Proteobacteria, Actinobacteria, Fusobacteria, and Verruca bacteria are also found in the human intestine.[3] Numerous studies have revealed that intestinal microbiota is associated with the host health status,[5,6] metabolic phenotype,[7] nutrient absorption or production,[1,8,9] and development and regulation of the immune system.[10] The dysbiosis of intestinal microbiota has been linked to several disorders such as obesity,[11] type 1 and type 2 diabetes,[12,13] colonic cancer[14] *etc*. Moreover, intestinal microbiota contributes to maintaining the immune,[15] intestinal,[16] and energy metabolism homeostasis.[11]

Diet plays a crucial role in shifting the intestinal microbiota.[17] The intestinal microbial composition is altered when the diet is switched from a low-fat and high-polysaccharide diet to a western diet.[18–20] A high-fat diet can increase the proportion of Firmicutes and decrease the proportion of Bacteroidetes, while a high-fiber diet can induce the decrease of Firmicutes and the increase of Bacteroidetes.[11,15]

Fructooligosaccharide (FOS), a kind of dietary fiber, is a well-established prebiotic.[21] Many studies have shown that

FOS in diet can be utilized by *Bifidobacterium* species, causing the alteration of the intestinal microbial composition.[21–24] Researchers conducted their animal experiments for one month or less to report the healthy function of FOS;[25] however, numerous studies have researched on the healthy function of other prebiotics like oligofructose and inulin in a longer duration (*e.g.*, two months).[26] Thus, this study aimed to investigate the effect of ingesting a high-FOS-diet for a longer period (2 months) on gut microbiota of obese and lean individuals.

Materials and methods

Animals, diets and sample preparation

Twenty male Sprague Dawley rats (5–6 weeks old) (Guangdong Medical Laboratory Animal Center, Guangdong, China) were housed in a temperature-controlled room (23 ± 2 °C) with 12 h-light/12 h-dark cycles. The rats had free access to standard chow diet and water. The rats were fed a low-fat diet for one-week adaption period (week 1) after they were brought from the Animal Center. Then the animals were randomly assigned to two experimental groups: (a) 10 rats were fed the low-fat diet for 10 weeks (from week 2 to week 11); then the 5 rats that gained less weight were defined as the lean ones (FL group) and were fed a high-FOS diet for 8 weeks (from week 12 to week 19); (b) 10 rats were fed the high-fat diet for 10 weeks; then the top 5 weight gainers were defined as the obese ones (FO group) and fed the high-FOS diet for 8 weeks. The feeds were formulated according to AIN-93 diet and Research Diets

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Table 1 The formulae of the low-fat feed, high-fat feed and high-FOS feed

	Low-fat feed[a]		High-fat feed[b]		High-FOS feed[a]	
	g %	kcal %	g %	kcal %	g %	kcal %
Protein	19.4	20.0	26.6	20.0	19.4	22.2
Carbohydrate	67.9	70.0	26.8	20.1	58.3	66.7
Fat	4.3	10.0	35.3	59.9	4.3	11.1
Energy (kcal g^{-1})	3.88		5.31		3.50	
Ingredient	G	kcal	g	kcal	g	kcal
Casein	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn starch	315	1260	0	0	315	1260
Maltodextrin	35	140	125	500	35	140
Sucrose	350	1400	68.8	275.2	250	1000
Soybean fiber[c]	50	0	50	0	0	0
FOS[c]	0	0	0	0	150	0
Soybean oil	25	225	25	225	25	225
Lard	20	180	245	2205	20	180
Mineral mix AIN-93	35	0	35	0	35	0
Vitamin AIN-93	10	40	10	40	10	40
Choline bitartrate	2.5	0	2.5	0	2.5	0
Total	1045.5	4057	764.3	4057.2	1045.5	3657

[a] The feeds were formulated according to AIN-93 diet and D12450B of Research Diets with modification. [b] The feeds were formulated according to AIN-93 diet and D12492 of Research Diets with modification. [c] The dietary fiber used in these feeds was soybean fiber or FOS.

(D12450B for low-fat feed and D12492 for high-fat feed) with modification[27–29] (Table 1). All animals were housed in independent ventilated cages. Food intake and body weight (BW) were recorded weekly. Fresh fecal specimens were collected individually at the end of week 11 (defined as I), week 15 (defined as II) and week 19 (defined as III). Each fecal specimen was packaged separately and frozen directly after collection. The specimens were stored at −80 °C. All rats were sacrificed by decapitation at the end of week 19. The animal experiments were approved by the Research Animal Administration Center at Jinan University (Guangzhou, China).

Fecal bacterial DNA extraction

The fecal bacterial DNA of each sample was extracted by a TIANamp Stool DNA kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The total DNA samples were characterized by 1% agarose gel electrophoresis for integrity and size. The DNA extracts were stored at −80 °C before being used as templates for 16S rDNA analysis.

16S rDNA gene PCR amplification and sequencing

The primers F515 (59-CACGGTCGKCGGCGCCATT-39) and R806 (59-GGACTACHVGGGTWTCTAAT-39)[30] were used to amplify the V$_4$ domain of bacterial 16S rDNA. PCR reactions contained 5–100 ng DNA template, 1 × GoTaq Green Master Mix (Promega, Madison, WI), 1 mM MgCl$_2$, and 2 pmol of each primer. Reaction conditions consisted of an initial 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension of 72 °C for 10 min. All samples were amplified in triplicate and combined prior to purification. Amplicons were purified using the Qiaquick 96

kit (Qiagen), quantified using the PicoGreen dsDNA reagent (Invitrogen, Grand Island, NY), all according to the manufacturers' instructions. Purified libraries were sequenced on the Illumina GAIIx platform.

16S rDNA gene analysis

Raw Illumina fastq files were demultiplexed, quality-filtered, and analyzed using Quantitative Insights Into Microbial Ecology (QIIME).[31] Sequences that were shorter than 55 bp, contained primer mismatches, ambiguous bases or uncorrectable barcodes, were removed. 16S rDNA gene sequences were assigned to operational taxonomic units (OTUs) using UCLUST with a threshold of 97% pair-wise identity,[32] and then classified taxonomically using the Ribosomal Database Project (RDP) classifier 2.0.1.[33]

Alpha diversity estimates were calculated with the Shannon value. Principal Coordinates Analysis (PCA) and heat map were performed to present differences between the gut microbial communities of the two groups. These analyses were conducted by Gene Denovo Co. (Guangzhou, China).

Statistical analysis

Results are expressed as mean values and standard deviations. The statistical analysis was performed with SPSS 17.0 software (SPSS Inc., Chicago, IL). t-Tests were conducted to compare the phenotypes of the lean and obese rats and all statistical tests were two-tailed. Statistical significance was set at $P < 0.05$. All data are presented in the text as the means ± SD.

Table 2 BW and BW gain of lean and obese rats when ingesting the high-FOS diet

Group	BW (week 11)		BW (week 15)		BW (week 19)		Total BW gain[c]	
	Mean[a]	SD[a]	Mean	SD	Mean	SD	Mean	SD
FL[b] (g)	400.84[a]	15.99	410.08[a]	20.00	429.76[a]	17.70	28.92[a]	2.58
FO[b] (g)	459.18[b]	34.58	458.38[b]	23.70	473.88[b]	26.57	14.70[b]	9.04

[a] Results are expressed as mean values and standard deviations (SD). [b] FL means the group of lean rats ($n = 5$); FO means the group of obese rats ($n = 5$). 'a,b,' mean values within a column with unlike superscript letters were significantly different ($P < 0.05$). [c] Total BW gain means the BW gained from week 11 to week 19.

Results

Animals' feed consumption and growth

We used a low-fat diet and a high-fat diet to build models of lean and obese rats. The rats of the FL group were fed the low-fat diet from week 2 to week 11, while the rats of the FO group were fed the high-fat diet. At the end of week 1, the BW of the FL rats was 230.80 ± 14.24 g, and the BW of the FO rats was 232.78 ± 16.35 g. The BW of these two groups of rats had no significant difference ($P > 0.05$). At the end of week 11, the BW of the FO rats increased to 459.18 ± 34.58 g, higher than 400.84 ± 15.99 g of the FL rats with significant difference ($P < 0.05$) (Table 2), indicating that the models of lean and obese rats were successfully built. When the diets were changed to the high-FOS diet, animals of all groups experienced a period of adaption to the new diet. At the end of week 15, the BW of the FL rats increased to 410.08 ± 20.00 g and that of the FO rats was 458.38 ± 23.70 g. From week 15 to week 19, the BW of the rats increased slowly. Finally, the BW of the FO rats reached 473.88 ± 26.57 g, higher than 429.76 ± 17.70 g of the FL rats ($P < 0.05$) (Table 2). In the meantime, the FL rats gained more BW than the FO ones with significant difference (28.98 ± 2.58 g *vs.* 14.70 ± 9.04 g, $P < 0.01$) (Table 2). These results indicated that when fed the high-FOS diet, lean rats could gain more BW than the obese rats.

Variation of fecal microbial communities in lean and obese rats

The average Shannon value of the FL rats was significantly lower than that of the FO rats at the end of week 11 (Fig. 1, $P < 0.01$). After ingesting the high-FOS diet for eight weeks, the Shannon values of both FL and FO rats were increased significantly ($P < 0.01$). The Shannon value of FL rats was increased at the end of week 15 (from 3.42 to 4.39); whereas it was increased until the end of week 19 for FO rats (from 4.39 to 5.02).

Principal Coordinates Analysis (PCA) was performed to determine the influence of diets on the similarity between samples (Fig. 2). The points of FL-I and FO-I samples (sampled at the end of week 11) can be distinguished, indicating the difference between these two gut microbial communities. Besides, the points of FL-II and FO-II samples (sampled at the end of week 15) can be distinctly separated from the points of
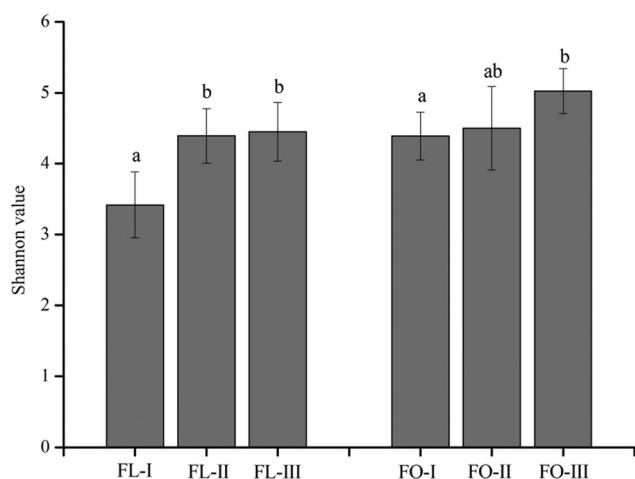

Fig. 1 Shannon values of the gut microbiota in lean and obese rats. The Shannon values were presented with the column diagram. FL means the group of lean rats; FO means the group of obese rats. The characters, I, II, and III, respectively represent the end of week 11 (FL-I, $n = 5$; FO-I, $n = 5$), week 15 (FL-II, $n = 5$; FO-II, $n = 5$), and week 19 (FL-III, $n = 5$; FO-III, $n = 5$).

FL-I and FO-I samples, suggesting that the rats' bacterial community was altered by the FOS diet. Either the points of the FL-II and FL-III samples (sampled at the end of week 19), or the points of the FO-II and FO-III samples, were hardly separable.

The relative abundances of bacterial phylum in different groups are presented in Fig. 3. The FO-I samples had higher abundance of Firmicutes ($P < 0.05$) and Bacteroidetes ($P < 0.01$), and lower abundance of Proteobacteria ($P < 0.01$) than the FL-I samples. Specifically, the ratio of Firmicutes to Bacteroidetes in the FL-I samples was as high as 31.23, while that of the FO-I samples was only 9.23. After the ingestion of a high-FOS diet, an increase in Bacteroidetes ($P < 0.01$) and a reduction in Proteobacteria ($P < 0.01$) were observed in the FL rats; however, no significant variations in the abundances of Firmicutes, Bacteroidetes and Proteobacteria were observed in the FO rats. Furthermore, the ratio of Firmicutes to Bacteroidetes was decreased sharply from 31.23 to 5.65 in the FL-II samples and then 3.26 in the FL-III samples. This ratio was also reduced slightly from 9.23 to 6.97 in FO-II samples, but was increased to 7.63 in FO-III samples (data not shown).
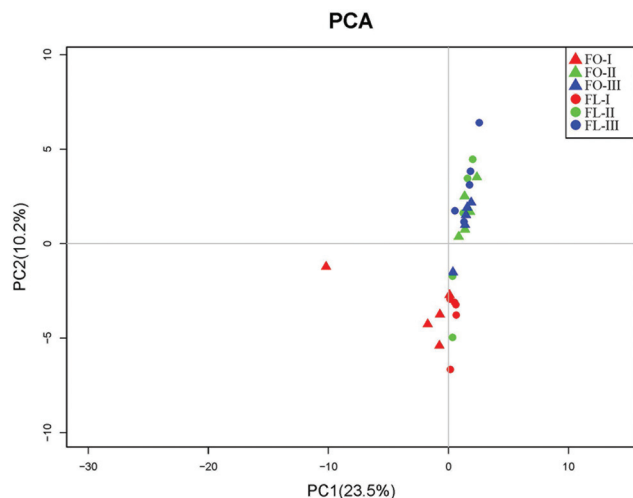
PCA

Fig. 2 Principal Coordinates Analysis (PCA) of the gut microbiota in lean and obese rats. PCA was plotted based on the family level. FL means the group of lean rats (circles); FO means the group of obese rats (triangles). The characters, I, II, and III, respectively represent the end of week 11 (red icons; FL-I, $n = 5$; FO-I, $n = 5$), week 15 (green icons; FL-II, $n = 5$; FO-II, $n = 5$), and week 19 (blue icons; FL-III, $n = 5$; FO-III, $n = 5$).

samples, the abundances of several species like Bifidobacteriaceae, S24-7, Bacteroidaceae and Prevotellaceae, which were seldom found in the FL-I samples, were significantly increased ($P < 0.05$). Besides, the abundance of Alcaligenaceae was increased too ($P < 0.05$), but the abundance of Desulfovibrionaceae decreased drastically to a small abundance ($P < 0.01$). As for the obese rats, their microbial community was less influenced by the high-FOS diet. At the end of week 19, the abundance of Ruminococcaceae was found to be increased significantly ($P < 0.01$), while the abundances of Desulfovibrionaceae and Lactobacillaceae were significantly decreased ($P < 0.05$). The abundances of other species like Prevotellaceae, S24-7, Bacteroidaceae and Lachnospiraceae were maintained in a steady level.

The variation of some dominant bacterial family was presented with a heat map to figure out their contribution to the variation of the bacterial community (Fig. 5). According to the results of the heat map, Bacteroidaceae, Prevotellaceae, S24-7 and Ruminococcaceae were enriched in the FL-II and FL-III samples and contributed most to the separation of these communities; besides, Bifidobacteriaceae and Alcaligenaceae were enriched in the FO-II samples, and Ruminococcaceae was enriched in the FO-III samples.

Discussion

In this study, models of lean and obese rats, successfully built by two different diets, were utilized to research the effect of two-month FOS consumption on their BW and gut microbiota. In the experiment, the proportion of dietary fiber in the FOS diet was elevated from 5% to 15%, in order to singularize the interaction between FOS and gut microbiota. According to the results, lean rats obtained more BW gain than the obese ones
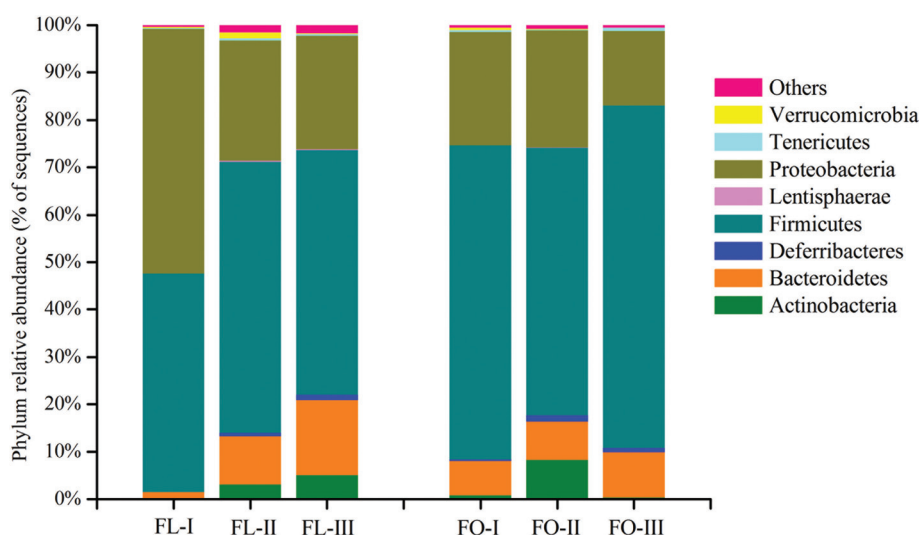
The bacterial family composition also presented an obvious alteration in response to the diet shift (Fig. 4). First, the bacterial family of FO-I samples was much more multiple than FL-I samples. The bacterial family composition of FO-I samples mainly consisted of Bacteroidaceae, S24-7, Lactobacillaceae, Lachnospiraceae, Peptostreptococcaceae, Ruminococcaceae, Alcaligenaceae and Desulfovibrionaceae; however, Bacteroidaceae, S24-7 and Lactobacillaceae were seldom detected in the FL-I samples. After the rats were fed the FOS diet, the bacterial composition totally changed. In the FL-III

Fig. 3 Relative abundances of the gut microbiota at the bacterial phyla level in lean and obese rats. FL means the group of lean rats; FO means the group of obese rats. The characters, I, II, and III, respectively represent the end of week 11 (FL-I, $n = 5$; FO-I, $n = 5$), week 15 (FL-II, $n = 5$; FO-II, $n = 5$), and week 19 (FL-III, $n = 5$; FO-III, $n = 5$).
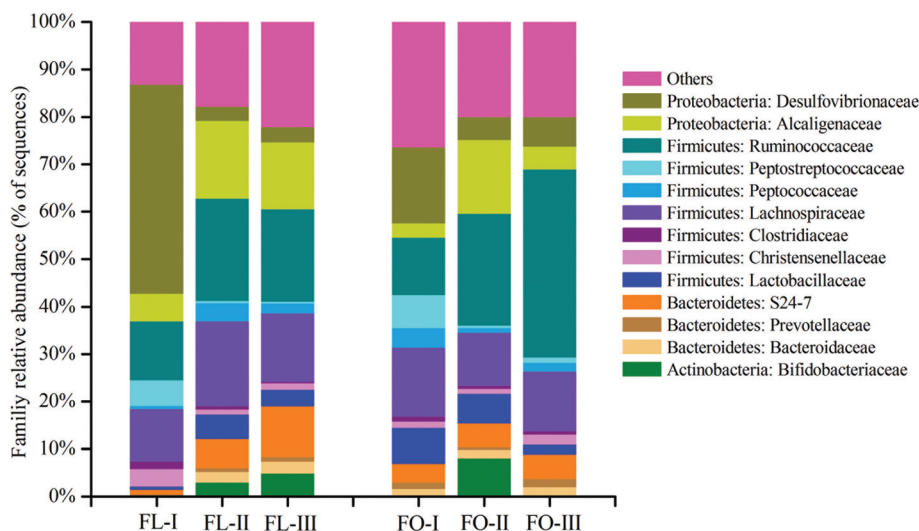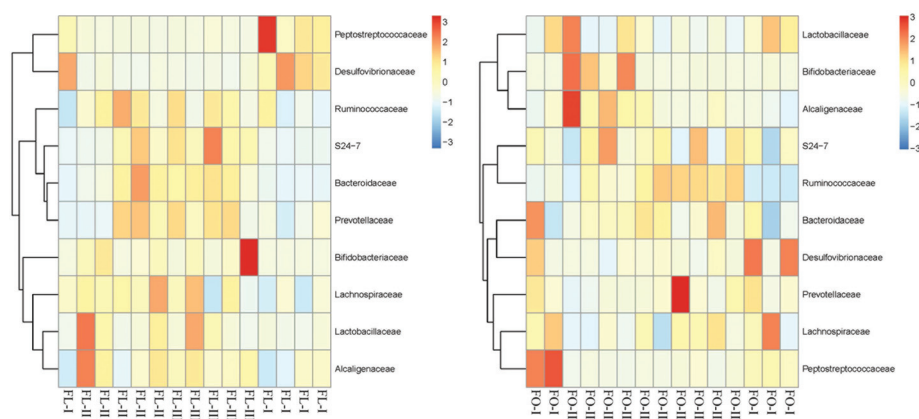
Fig. 4 Relative abundances of gut microbiota at the bacterial family level in lean and obese rats. FL means the group of lean rats. FO means the group of obese rats. The characters, I, II, and III, respectively represent the end of week 11 (FL-I, *n* = 5; FO-I, *n* = 5), week 15 (FL-II, *n* = 5; FO-II, *n* = 5), and week 19 (FL-III, *n* = 5; FO-III, *n* = 5).

Fig. 5 Heat maps of the dominant bacterial family in lean and obese rats. Columns present, for each rat, the abundances of the selected bacterial family. The abundances were clustered using unsupervised hierarchical clustering (blue, low abundance; red, high abundance). FL means the group of lean rats; FO means the group of obese rats. The characters, I, II, and III, respectively represent the end of week 11 (FL-I, *n* = 5; FO-I, *n* = 5), week 15 (FL-II, *n* = 5; FO-II, *n* = 5), and week 19 (FL-III, *n* = 5; FO-III, *n* = 5).

(Table 2); the gut microbiota of lean and obese rats was both altered by the high-FOS diet (Fig. 1–5). Particularly, the abundance of Bacteroidetes (mainly family Bacteroidaceae and S24-7) increased in the lean rats.

Turnbaugh *et al.* have reported that the obese-associated microbiota has an increased capacity to harvest energy from the diet than the "lean microbiota".[11,34] Gut microbiota can serve as an important environmental factor that affects energy intake from the diet and energy storage in the host.[35] They believe that "obese microbiota" is associated with the increased energy intake from the residue of food. However, these findings are all based on the bacterial conventionalization on germ-free mice under a conditionally experimental environment. There is no report on how effectively the "obese

microbiota" or "lean microbiota" will exert their influence on the original host. So, this experiment was designed to study the interaction between the gut microbiota and the host with a diet switch.

As a kind of dietary fiber, consumed FOS is delivered to the large intestine and utilized by intestinal bacteria as an energy source, which is found significantly helpful for the improvement of gut health[36,37] and the maintenance of body weight.[38,39] Recently, a study on the interaction of oligofructose (OFS) and obesity reported that supplemental OFS in the diet is able to reduce body weight and fat mass in both obesity prone and obesity resistant rats, and OFS-induced alterations in gut microbiota and gut hormones may contribute to the lowered body weight.[40] In our study, the FOS supplement was

also found to be able to maintain both rats' body weight; furthermore, lean rats gained more body weight from the FOS diet than the obese rats after the ingestion of the high-FOS diet for two months. It is reported for the first time that lean individuals can gain more body weight from a high-FOS diet. In the meantime, the gut microbial community of lean rats was found to be more sensitive to the change of high FOS diet (Fig. 1 and 2).

In this study, the relative abundances of various bacterial phyla showed a significant alteration, such as Firmicutes, Bacteroidetes and Proteobacteria (Fig. 3). Firmicutes and Bacteroidetes are two dominant divisions of gut microbiome. Previous studies have shown that obese mice have a significantly higher ratio of Firmicutes to Bacteroidetes compared with their respective lean counterparts.[11,41] Conversely, other studies have reported that the microbial energy extraction is not correlated with the proportions of Firmicutes or Bacteroidetes in high-fat-fed and genetically obese mice.[2,42] As more and more discrepant results are observed in different studies,[43] the reciprocity between gut microbiome and host energy intake seems to be far more complicated than that we have initially thought about. We obtained different results too in this study; the ratio of Firmicutes to Bacteroidetes was extremely high in the lean rats compared with the obese ones, as Bacteroidetes were seldom detected in the feces of the lean rats (FL-I samples). After the high-FOS diet was ingested, the abundance of Bacteroidetes in lean rats increased substantially, and the ratio of Firmicutes to Bacteroidetes correspondingly reduced. As the lean rats gained more body weight after ingesting the high-FOS diet, we inferred that the ratio of Firmicutes to Bacteroidetes might be negatively related with the energy harvest from this diet.

The results in this study suggested that the higher abundance of Bacteroidetes could have contributed to their higher BW gain from the high-FOS diet. The connection between gut microbes and their energy harvesting capacity has been discussed in many other studies. A survey of carbohydrate-active enzymes encoded by the genomes of human colonic bacteria reveals that members of the Bacteroidetes phylum carry the largest numbers of glycoside hydrolases and polysaccharide lyases. Thus, Bacteroidetes can make a better utilization on FOS. This finding strongly suggests that Bacteroidetes have a larger carbohydrate substrate range than the other organisms like Firmicutes.[44] Many anaerobic bacteria of Firmicutes from the rumen and human colon are also found to be able to degrade polysaccharide, but the numbers of encoded glycoside hydrolases are much less than polysaccharide-degrading bacteria of Bacteroidetes. Typically, encoded glycoside hydrolases of Firmicutes are limited to the hydrolases like xylanases, cellulases, amylases and glycosidases. However, genomes of Bacteroidetes phylum contain many other encoded glycoside hydrolases, including fructan hydrolase that degrades FOS.[44,45] Our results were in accordance with these genomic studies on encoded glycoside hydrolases of gut bacteria; as Bacteroidetes were far more capable of utilizing FOS than Firmicutes, the ratio of Firmicutes to Bacteroidetes in all rats was drastically reduced due to the consumption of the high-FOS diet. The most common genera of Bacteroidetes in the human gut microbiota are *Bacteroides* and *Prevotella*, and they dominate in individuals with a habitually high intake of dietary fibers. Species of *Bacteroides* and *Prevotella* show much higher diversity of glycan-cleaving enzymes than species of the other bacterial genera and are capable of utilizing non-cellulosic polysaccharides, such as FOS, as energy sources.[20,44–47] As it was shown in this study, the abundance of Bacteroidaceae family in lean rats were significantly increased from week 11 to week 19; that is, the gut microbiota of lean rats developed more species of the Bacteroidaceae family (*e.g.*, *Bacteroides*) after FOS consumption. This could possibly be beneficial to FOS degradation and energy release, and its mechanisms deserve further exploration.

Conclusion

It is the first time to report that the lean rats can gain more BW than the obese ones from a high-FOS diet. This study posted that the lower ratio of Firmicutes to Bacteroidetes could help rats harvest more energy from the high-FOS diet. Furthermore, Bacteroidetes induced by the high-FOS diet might make primary contribution to this alteration. Besides, when the rats kept ingesting the high-FOS diet for two months, their gut microbiota became homogeneous and relatively constant, and their BW gain was maintained at a low level.

Acknowledgements

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