Original Research *Featured Article*

Structure moderation of gut microbiota in liraglutide-treated diabetic male rats

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Impact statement

Our findings suggest that significant changes in gut microbiota are associated with liraglutide treatment on the diabetic male rats, including enrichment of short-chain fatty acid producers and probiotic bacteria. This may help alleviate systemic inflammation and contribute to the beneficial effects of liraglutide against diabetes.

Abstract

The change of gut microbiome is associated with a serious of metabolic disorders, such as diabetes. As a glucagon-like peptide 1 analogue, liraglutide is a potent antidiabetic drug in clinical practice. However, the effect of liraglutide on the community of gut microbiota is still unknown. We aimed to determine the influence of liraglutide on fecal microbiota in diabetic male rats. Five-week-old male Sprague-Dawley rats were fed with a control diet or a high-fat diet for four weeks. By injecting streptozotocin, the diabetic rat model was performed. Diabetic male rats were injected subcutaneously with a low dose of liraglutide (liraglutide 0.2 mg/kg/day), a high dose of liraglutide (liraglutide 0.4 mg/kg/day), or normal saline for 12 weeks. Our data showed that liraglutide effectively prevented the development of diabetes in male rats. Pyrosequencing of the V3-V4 region of 16S rRNA genes manifested a remarkable transfer of gut microbiota construction in liraglutide-treated male rats compared with that of the diabetic male rats. Further analysis identified 879 liraglutide-treated specific operational taxonomic units. Some short-chain fatty acid (SCFA)-producing bacteria, including *Bacteroides*, *Lachnospiraceae*, and probiotic bacteria, *Bifidobacterium*, were selectively enhanced in liraglutide-treated diabetic male rats. *Lactobacillus* was negatively correlated with fasting blood glucose. To sum up, our findings propose that the prevention of diabetes by liraglutide in the diabetic male rats may be associated with the structural change of the gut microbiota, inflammation alleviation, and abundantly elevated SCFA-producing bacteria in the intestine.

Keywords: Diabetes, gut microbiota, glucagon-like peptide 1, short chain fatty acid, probiotic bacteria

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Introduction

The prevalence of type 2 diabetes (T2D) is extended dramatically during recent decades. Besides genetic and environmental factors, thriving evidence shows that gut microbiota also affects the incidence and development of T2D. Changes in terms of diversity and richness have been involved with T2D. Several human studies showed T2D patients had a moderate gut microbiota disorder, characterized by a decrease in butyrate-producing bacteria.[1] More inspiringly, germ-free mice are not prone to obesity, when they are exposed to a high-fat diet. Transferring the gut microbial community from obese mice to these germ-free mice causes a significant increase in total body fat content.[2,3] Shaping the microbial composition is a potential therapeutic approach in T2D.[4]

Liraglutide, one type of glucagon-like peptide 1 (GLP-1) analogues, was approved by the U.S. Food and Drug Administration in 2010 for treating T2D. GLP-1 is a hormone which is secreted from intestinal L-cells. It can stimulate the glucose-induced insulin response, promote pancreas β-cells survival, delay gastric emptying, and regulate energy expenditure and body weight. Besides these functions, it also exerts neuroprotective, cardioprotective, and anti-inflammatory effects.[5]

However, the study of liraglutide on gut microbiota in diabetic rats is limited. We hypothesized that liraglutide

could moderate glucose metabolism through normalizing the structure of gut microbiota. The purpose of this investigation was to find out the leading beneficial bacteria in the gut for ameliorating blood glucose. Therefore, we used 454 pyrosequencing to test the change of gut microbiota from liraglutide treatment.

Materials and methods

Animals

Five-week-old male Sprague-Dawley rats (174.7 ± 15.3 g) were bought from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China, Permit No. SCXK-2014–0013). The rats were kept in cages and maintained at 23–25°C with 12-h-light and 12-h-dark cycles. All procedures were done with the approval of the Animal Care Committee of the Peking Union Medical Hospital Animal Ethics Committee (Project XHDW-2015–0051, 15 February 2015), and all efforts were made to minimize suffering.

Induction of T2D in rats

The rats were randomly divided into two groups. The control rats were fed on a standard diet (kcal %: 10% fat, 20% protein, and 70% carbohydrate; 3.85 kcal/g). Other rats were fed with a high-fat diet (kcal %: 45% fat, 20% protein, and 35% carbohydrate; 4.73 kcal/g, Research Diet, New Brunswick, NJ, USA) for four weeks and then were injected intraperitoneally (i.p.) with streptozotocin (STZ; 30 mg/kg body weight) to induce diabetes. The animals were fed continuously on the high-fat diet throughout the remaining time of the study. Fasting blood glucose (FBG) >11.1 mmol/L was determined to the T2D model.

Animal groups and experimental design

Normal rats were administered with normal saline. Diabetic rats were randomly subdivided into three subgroups: diabetic group (DM, normal saline, hypodermic injection (i.h.), n = 6), low dose of liraglutide (liraglutide 0.2 mg/kg/day, i.h., n = 6), and high dose of liraglutide (liraglutide 0.4 mg/kg/day, i.h., n = 6). After fasting for 12 h, fresh stool samples were collected by stimulating the anus in normal, DM, and liraglutide 0.4 group and immediately stored at −80°C.

Measurements of body weight and FBG

Body weight and FBG were measured every four weeks with Bayer Contour TS glucometer (Bayer, Hamburg, Germany).

Oral glucose tolerance test

After depriving food for 12 h, an oral glucose tolerance test (OGTT) was performed. The rats were orally administered with glucose (2 g/kg body weight). The area under the curve (AUC) was calculated by linear trapezoid method.[6]

Measurement of serum insulin, interleukin 6, and homeostasis model assessment of insulin resistance

Serum insulin and interleukin 6 (IL-6) levels were assayed by an ELISA method (Millipore, Bellerica, MA, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was established by the following formula: FBG (mmol/L) × fasting serum insulin (μIU/mL)/22.5.

Fecal DNA extraction and 454 pyrophosphate sequencing

Genomic DNA was obtained from fecal samples by using QIAmp DNA Stool mini Kit (Qiagen, Valencia, CA, USA). 16S rRNA gene amplicons of 250 bps were generated from the V3-V4 region with specific primers (341F 5′-CCTAYGG GRBGCASCAG-3′ and 806R 5′-GGACTACNNGGGTATC TAAT-3′) using Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). PCR product purification was performed by using Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA). Pyrosequencing was performed on an Illumina HiSeq, 2500 platform (Norcross, GA, USA).

Bioinformatics analysis

Paired-end reads were connected using FLASH (John Hopkins University School of Medicine, Baltimore, MD, USA).[7] To gain the higher quality data, raw reads were performed quality filtering according to specific filtering conditions by using QIIME version 1.7.0.[8] Sequence analysis was performed by Uparse software version 7.0.1001.[9] Sequences which have ≥97% similarity were considered as the same operational taxonomic units (OTUs).

For each representative sequence, the GreenGene Database [10] was used based on Ribosomal Database Project (RDP) classifier version 2.2[11] algorithm to annotate taxonomic information. Alpha diversity was analyzed to describe the complexity of species diversity, including Chao1 and Shannon index. For the evaluation of differences of samples in species complexity, beta diversity was calculated. Both of weighted and unweighted UniFrac were figured out.[12] Principal Coordinate Analysis (PCoA) was performed to get the principal coordinates and visualize from complex, multidimensional data. Unweighted Pair-group Method with Arithmetic Means Clustering was performed as hierarchical clustering method to interpret the distance matrix. Also, linear discriminant analysis of the effect size (LEfSe) was performed to assess OTU abundance and find the differences between the groups.[13]

Data analysis

The data are shown as the mean ± SD. When the data were normal and variances were equal, differences among the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Otherwise, a Kruskal–Wallis with Mann–Whitney test was applied. Spearman's correlation coefficient was utilized to compare the bacterial abundance and metabolic parameters.

Bacterial taxa that were differentially abundant were identified by using the Kruskal–Wallis nonparametric test, followed by the Wilcoxon tests. The identified features were then subjected to the linear discriminant analysis (LDA) model with a threshold logarithmic LDA score set at 3.0 and ranked. A P value ≤ 0.05 was considered statistically significant. All data analyses were conducted by using Prism version 5.0 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Effect of liraglutide on body weight in diabetic male rats

Compared to the normal control rats, the diabetic male rats had reduced body weight ($P < 0.01$; Figure 1(a)). Liraglutide treatment increased the body weight in the diabetic male rats slightly (about 3%, $P < 0.01$; Figure 1(a)).

Effect of liraglutide on FBG and glucose tolerance in diabetic male rats

Diabetic male rats showed dramatically higher FBG than the normal control rats ($P < 0.01$; Figure 1(b)). Liraglutide treatment has dose-dependently reduced FBG ($P < 0.01$; Figure 1(b)). In OGTT, blood glucose and AUC in the diabetic male rats were also higher than the normal control rats ($P < 0.01$; Figure 1(c) and (d)). Liraglutide prevented the increase of blood glucose and AUC in a dose-dependent way ($P < 0.01$; Figure 1(c) and (d)).

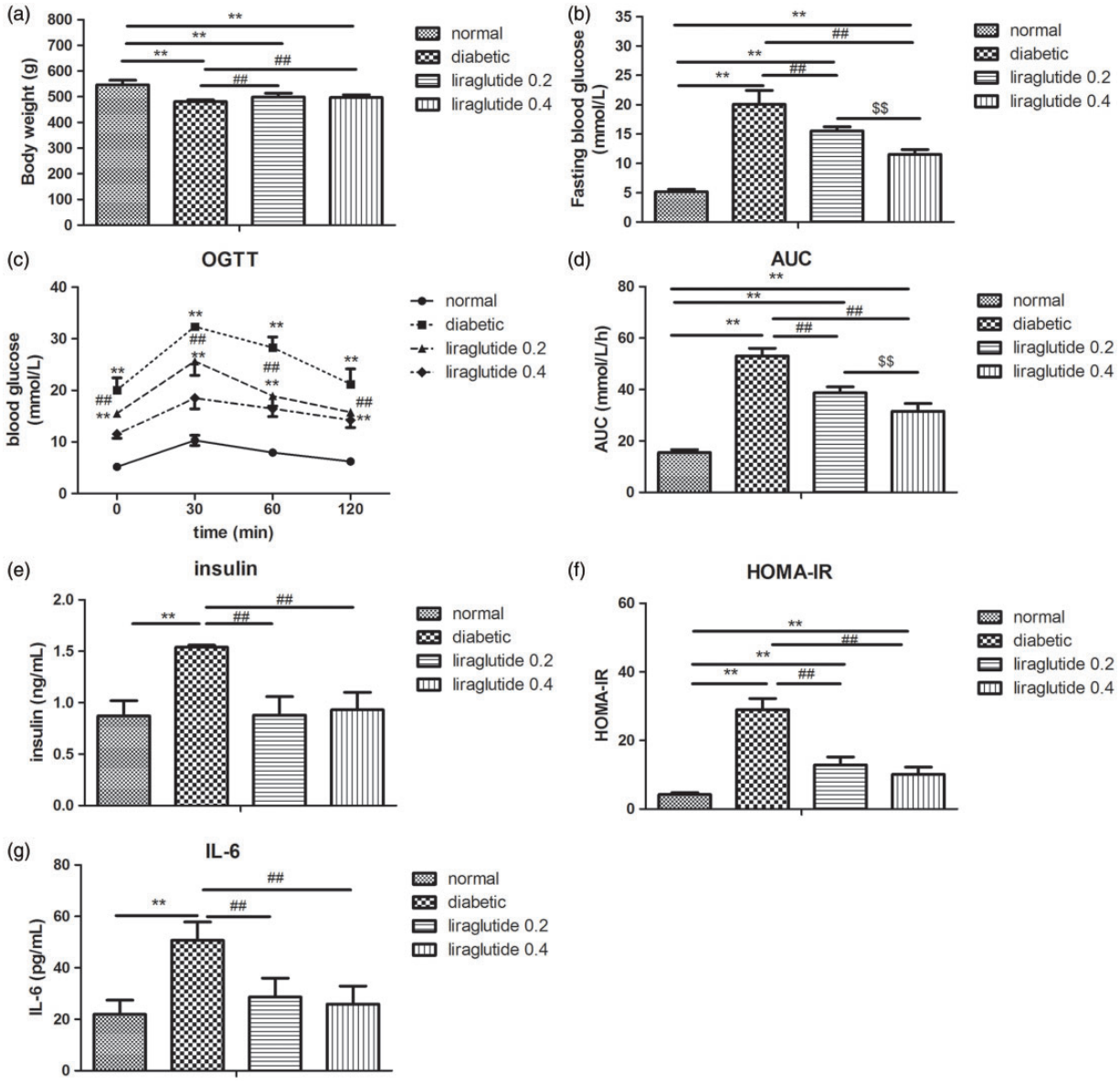

Figure 1. Effects of liraglutide on body weight, blood glucose, serum insulin, HOMA-IR, and IL-6 in diabetic male rats. (a) Body weight, (b) fasting blood glucose, (c) blood glucose in OGTT, (d) AUC in OGTT, (e) serum insulin, (f) HOMA-IR, and (g) IL-6. Values are expressed as means ± SD. n = 6 in each group. $^{**}P < 0.01$ versus normal control group; $\#\#P < 0.01$ versus diabetic group; $\$\$P < 0.01$ versus liraglutide 0.2 group.
OGTT: oral glucose tolerance test; HOMA-IR: homeostasis model assessment of insulin resistance; IL_6: interleukin 6; AUC: area under the curve.

Effect of liraglutide on fasting insulin, HOMA-IR, and serum IL-6 in diabetic male rats

Diabetic male rats exhibited significantly higher serum insulin level, HOMA-IR, and IL-6 than normal control rats ($P < 0.01$; Figure 1(e) to (g)). Liraglutide significantly decreased serum insulin level, HOMA-IR, and IL-6 ($P < 0.01$; Figure 1(e) to (g)).

Characteristics of pyrosequencing results

Because liraglutide 0.4 group had better glucose reduction effect on the diabetic male rats than liraglutide 0.2 group, only genomic DNA of fecal samples from liraglutide 0.4 group, diabetic group, and normal group were tested in 16S pyrophosphate sequencing (n = 6 in each group). A total of 790,755 high-quality reads and 879 OTUs (97% similarity) were obtained from 18 samples through a 454 pyrosequencing analysis, with an average of 2583 unique sequences and 554 OTUs in each sample. The 16S sequence data generated in this study were submitted to the NCBI Sequence Read Archive database (accession number SRP095710). Figure 2 shows the differences in gut microbiota diversity among the normal group, diabetic group, and liraglutide 0.4 group. The alpha diversities of the gut microbiota in these three groups decreased significantly in the order of the normal group, diabetic group, and liraglutide 0.4 group (Figure 2). The results of PCoA based on an unweighted UniFrac distance matrix showed that there was a substantial rearrangement of the bacterial structure among the normal, diabetic, and liraglutide-treated male rats (Figure 3).

Taxonomic shifts due to liraglutide treatment

We explored the similarities and distinctions of species distribution among the different groups. As shown in Figure 4, we found that there were 606 species shared among three groups, accounting for around 3–4 of the OTUs in each group. It is noteworthy that 91 species only found in normal group, 29 OTUs in the diabetic group, and 30 OTUs in the liraglutide 0.4 group. At the species level, the unique OTU in the liraglutide 0.4 group included *Lactobacillus_mucosae*, *Brevundimonas_vesicularis*, *Moraxella_osloensis*, and *Anaerofustis_stercorihominis* (Table 1).

In accordance with the previous studies, the rat microbiome was profoundly dominated by the phyla *Firmicutes* and *Bacteroides*. *Firmicutes*/*Bacteroidetes* ratio in the diabetic rat cecal was greatly changed. Liraglutide-treated group

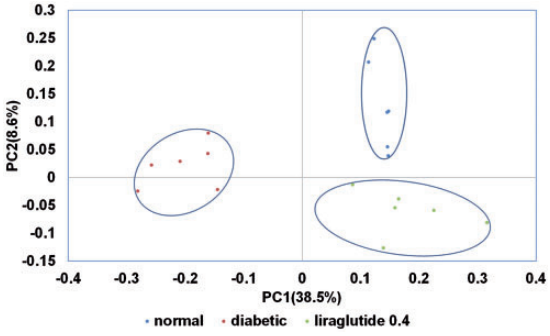

Figure 3. PCoA plots based on unweighted UniFrac metrics of the gut microbiota.
PC: principle coordinate.

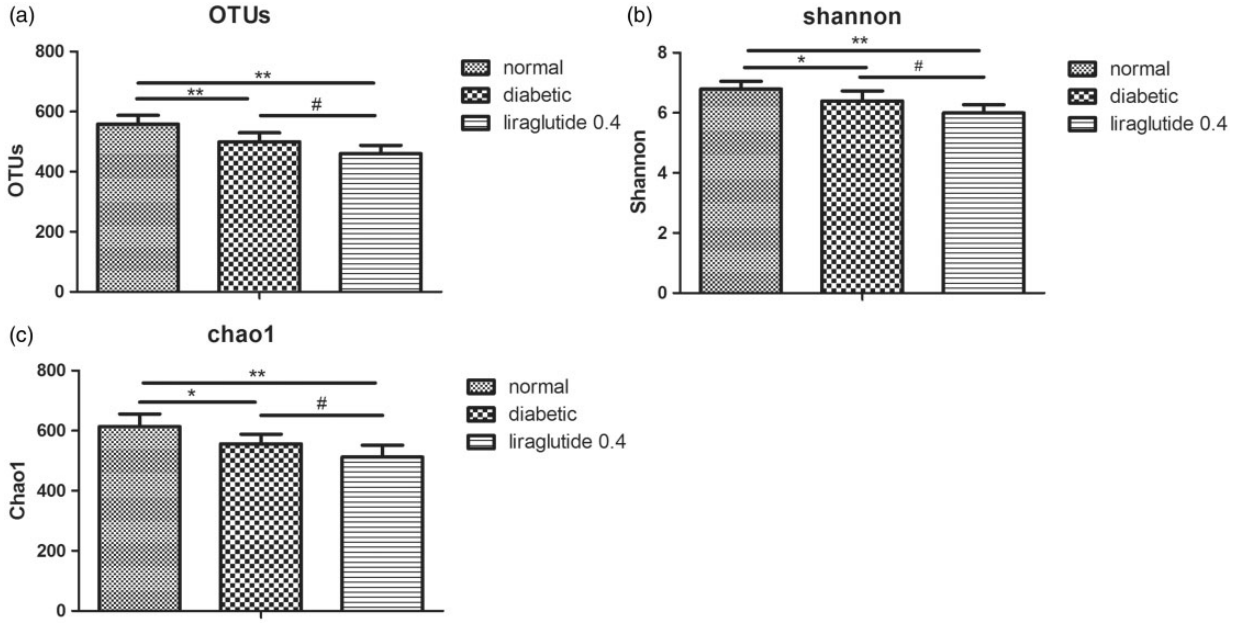

Figure 2. Effects of liraglutide on alpha diversity analysis of gut microbiota in diabetic male rats. OTU (a), Shannon index (b), and Chao1 index (c). Values are expressed as means ± SD. n = 6 in each group. *$P < 0.05$ and **$P < 0.01$ versus normal control group; #$P < 0.05$ versus diabetic group.
OTU: operational taxonomic unit.

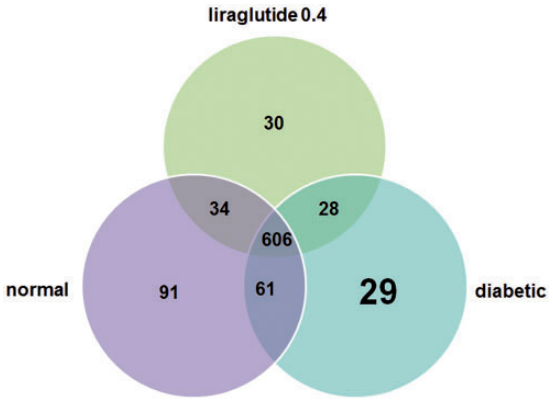

Figure 4. Shared OTU analysis of the different groups. Venn diagram showing the unique and shared OTUs (3% distance level) in the different groups. (A color version of this figure is available in the online journal.)

Table 1. Unique OTU in liraglutide 0.4 group.

Bacterial group	Level
Bacteria	Kingdom
Bacteroidales	Order
Mollicutes_RF9	Order
Bacteroidales_S24-7_group	Family
Clostridiales_vadinBB60_group	Family
Porphyromonadaceae	Family
Ruminococcaceae	Family
Alistipes	Genus
Bifidobacterium	Genus
Cellulosilyticum	Genus
Christensenellaceae_R-7_group	Genus
Desulfovibrio	Genus
Enterorhabdus	Genus
Gardnerella	Genus
Lachnospiraceae_UCG-010	Genus
Oxalobacter	Genus
Parabacteroides	Genus
Peptoniphilus	Genus
Sneathia	Genus
Sphingomonas	Genus
Anaerofustis_stercorihominis	Species
Brevundimonas_vesicularis	Species
Lactobacillus_mucosae	Species
Moraxella_osloensis	Species

OTU: operational taxonomic unit.

had a reduced *Firmicutes*/*Bacteroidetes* ratio and relative abundance of *Tenericutes* ($P < 0.01$; Figure 5).

LEfSe analysis showed multiple differences at lower taxonomic levels among the three groups (Figure 6). Fifty-eight bacteria changed significantly among the normal, diabetic, and liraglutide 0.4 group ($P < 0.05$). Among these bacteria, 11 bacteria showed a statistical difference between the liraglutide 0.4 group and diabetic group ($P < 0.05$). The genera *Flavonifractor* ($P < 0.05$), genera *Lachnoclostridium* ($P < 0.01$), species *Ruminococcus_gnavus* ($P < 0.05$), species *Flavonifractor_plautii* ($P < 0.05$), and species *Bacteroides_acidifaciens* ($P < 0.05$) were elevated significantly in liraglutide-treated group than the diabetic group. The reduced bacteria were mainly in the family *Christensenellaceae* ($P < 0.01$), genera *Christensenellaceae_R_7_group* ($P < 0.01$), the genera *Ruminococcaceae_UCG_010* ($P < 0.001$), the genera Ruminoclostridium_6 ($P < 0.01$), the genera *Prevotella_9* ($P < 0.01$), and the class *Mollicutes* ($P < 0.01$; Figure 7).

Correlations between key bacteria and glucose metabolic index

At the genus level, 11 bacteria showed a significant positive correlation with FBG. They were *Lachnospiraceae_UCG_005*, *unidentied_Rumnococcaceae*, *Lchnospiraceae_UCG-010*, *Anaerotruncus*, *Adlercreutzia*, *Eubacterium_nodatum_group*, *Family_XIII_AD3011_group*, *Anaerovorax*, *Bilophila*, *Kurthia*, and *Parvibacter* ($P < 0.01$). Nine bacteria showed a significant negative correlation with FBG. They were *Lactobacillus*, *Prevotellaceae_NK3B31_group*, *Ruminococcus_2*, *Prevotellaceae_UCG-001*, *Ruminoclostridium_5*, *Lachnospiraceae_UCG-001*, *Acetatifactor*, *Family_XIII_UCG-001*, and *Lachnospiraceae_UCG-006* ($P < 0.01$; Table 2).

Discussion

In this study, we used high-fat diet and low-dose-STZ-injected rats as an animal model for T2DM. Similar to previous studies,[14,15] we found that the body weight in the diabetic male rats was lower than that in the control group. Liraglutide-treated male rats had a slight increase in body weight (about 3%) than that in diabetic male rats in this experiment, probably due to the symptom relief of diabetes. To avoid the influence of estrogens and estrous cycle on insulin secretion and insulin sensitivity, we only used male rats in our study. We found that liraglutide treatment moderated glucose intolerance and insulin sensitivity dose-dependently in diabetic male rats. Our study demonstrated that diabetic state and administration of liraglutide profoundly change the composition of gut microbiota. First, we observed marked declines in microbial richness and diversity in diabetic male rats. Denou *et al*. found lower alpha diversity within the phylum *Bacteroidetes* in both the colon and feces of high-fat-diet-fed mice.[16] Second, by using unweighted UniFrac PCA analysis, we confirmed that the gut microbiota of the normal, diabetic, and liraglutide-treated male rats were structurally separated from each other in the two principal components.

Furthermore, our result presented that compared with the normal rats, diabetic rats had increased *Firmicutes*-to-*Bacteroidetes* ratio in the fecal microbiota. Liraglutide-treated group had a lower ratio of *Firmicutes/Bacteroidetes* in the gut microbiota. More and more evidence shows some structure changes in the gut microbiota during metabolic disease, such as obesity and diabetes, including a diminished relative abundance of *Bacteroidetes* and a relative enlargement of *Firmicutes*.[16–19]

Additionally, at the phylum level, diabetic group had a slight increase in the relative abundance of *Tenericutes*. Liraglutide-treated group had a reduced relative abundance of *Tenericutes*. Yan *et al*. found that there was a higher abundance of *Tenericutes* in obese rats than lean groups.[18] The class *Mollicutes* is the only class within the phylum *Tenericutes*. Consuming low-fat or low-carbohydrate diets

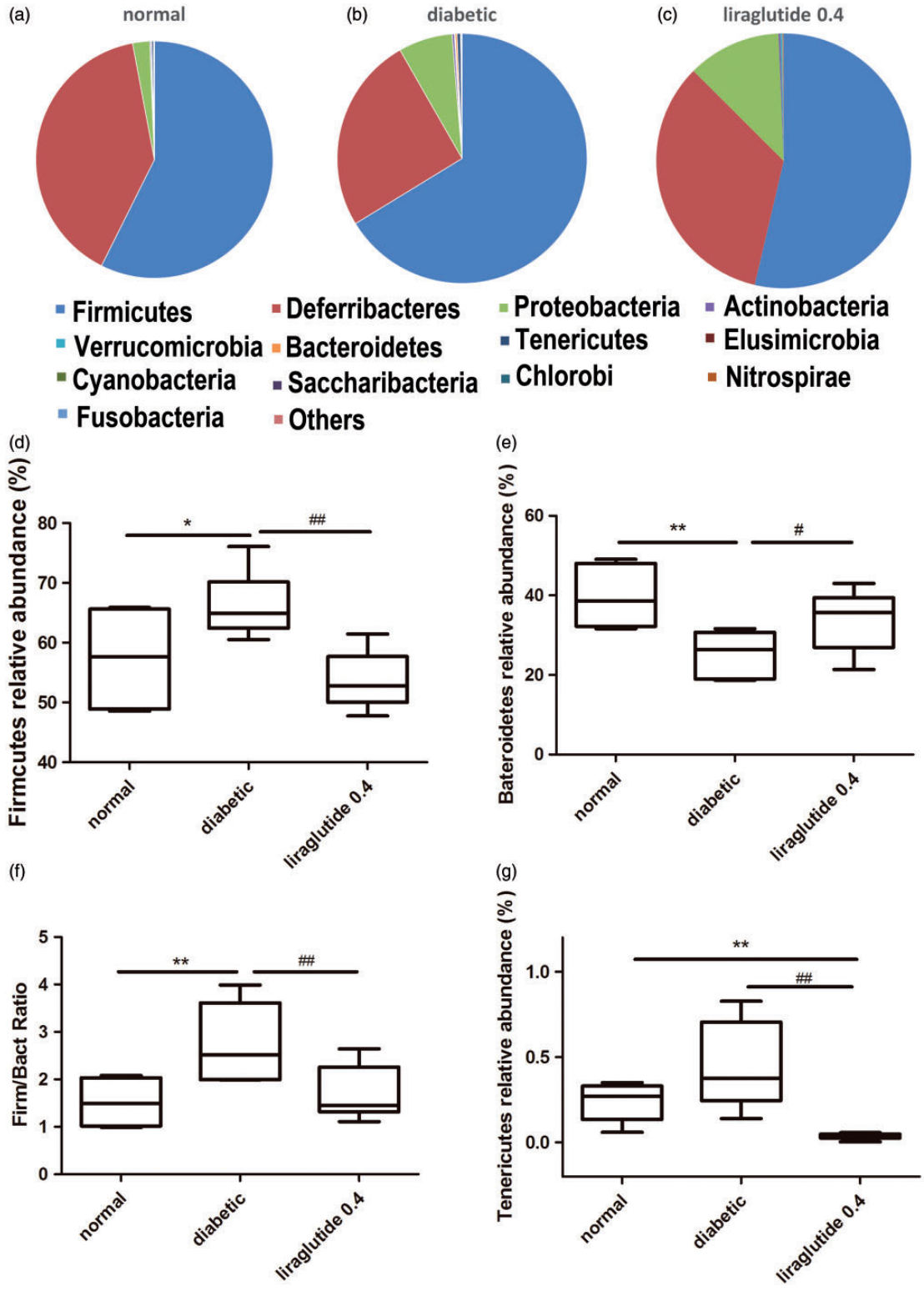

Figure 5. Diabetic and liraglutide treatment affect the proportions of different phyla. The composition of abundant bacterial phyla identified in the gut microbiota of normal (a), diabetic (b), liraglutide 0.4 (c) group; the relative abundance of *Firmicutes* (d), *Bacteroidetes* (e), *Tenericutes* (g); and the ratio of *Firmicutes* and *Bacteroidetes* (f). n = 6 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus normal control group; #$P < 0.05$, ##$P < 0.01$ versus diabetic group.

for a year can normalize the higher relative abundance of *Mollicutes* in the fecal microbiota of obese human.[20]

In addition, Venn figure identified that *Bifidobacterium* was the unique genera in liraglutide 0.4 group compared to the normal and diabetic group. Correlation analysis also showed that *Lactobacillus* was negatively related to FBG. *Bifidobacterium* and *Lactobacillus* were probiotic bacteria. Probiotics can modify the overall microbial population,

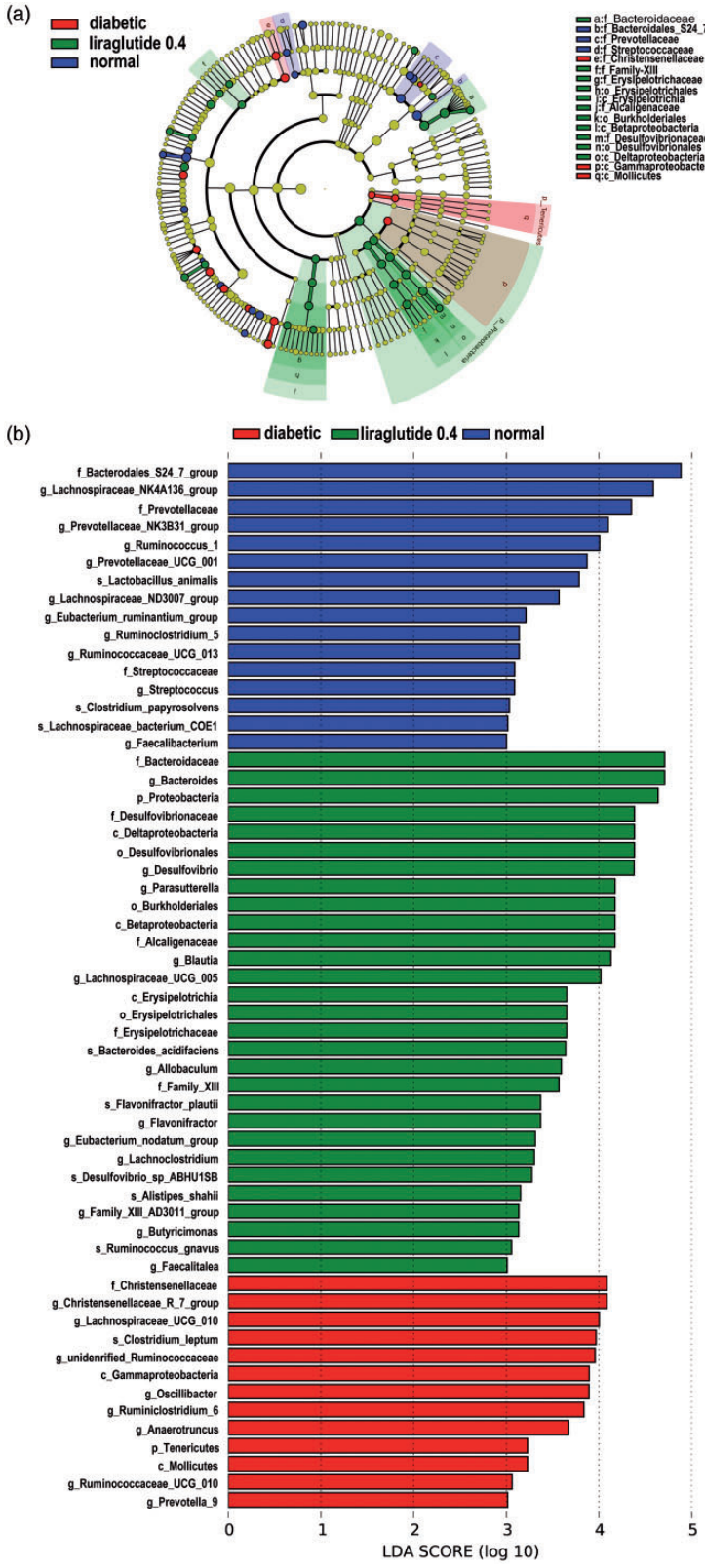

Figure 6. Taxonomic representation of statistically and biologically consistent differences among normal, diabetic, and liraglutide 0.4 group. (a) Differences are represented by the color of the most abundant class (red indicating diabetic group, green liraglutide 0.4 group, and blue normal group). The diameter of each circle is proportional to the taxon's abundance. (b) LDA values in LEfSe analysis.
LDA: linear discriminant analysis.

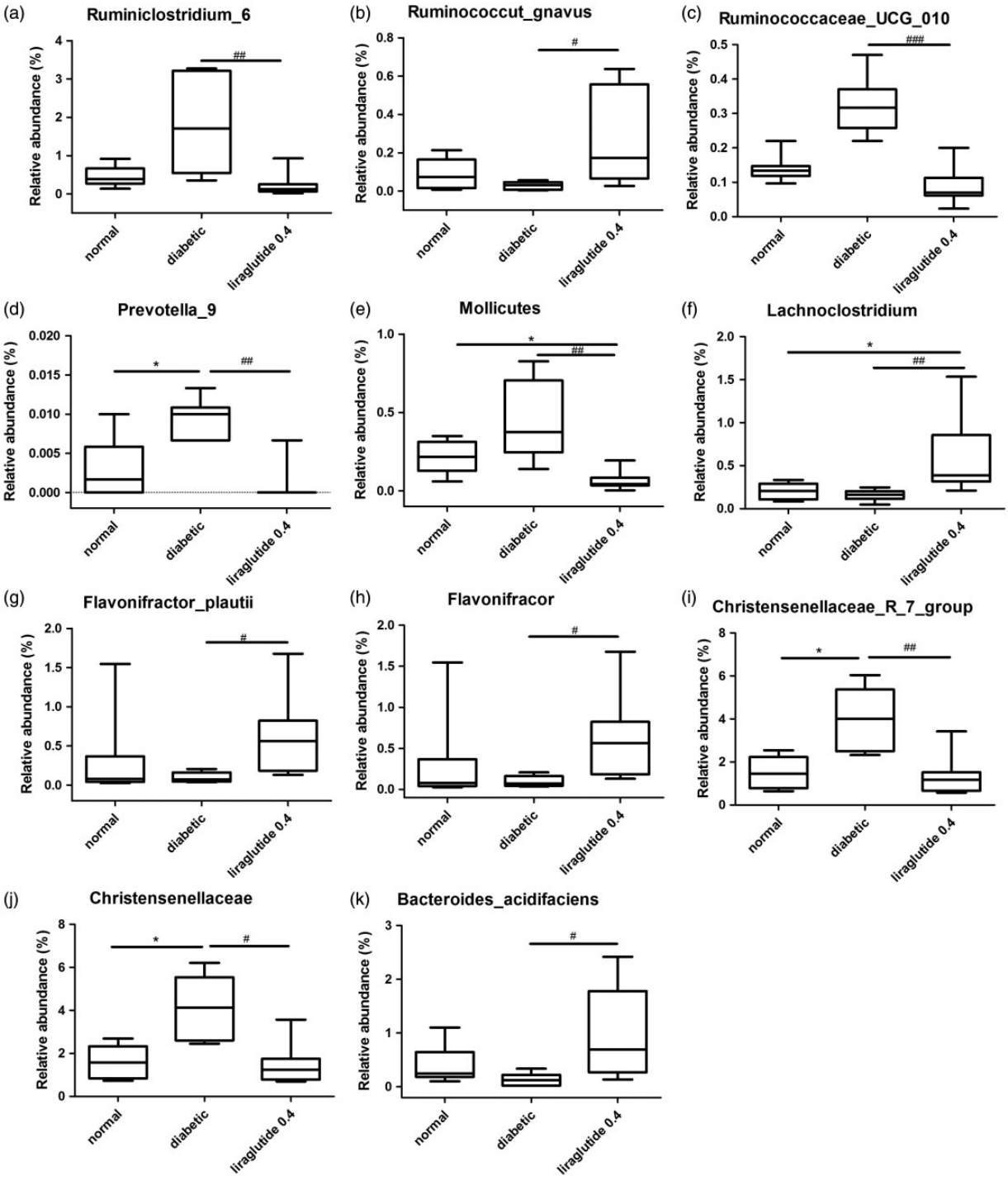

Figure 7. The boxplots of key bacterial groups in liraglutide-treated group significant differed with diabetic group. *Ruminiclostridium_6* (a), *Ruminococcut_gnavus* (b), *Ruminococcaceae_UCG_010* (c), *Prevotella_9* (d), *Mollicutes* (e), *Lachnoclostridium* (f), *Flavonifractor_plautii* (g), *Flavonifractor* (h), *Christensenellaceae_R_7_group* (i), *Christensenellaceae* (j), and *Bacteroides_acidifaciens* (k). Kruskal–Wallis nonparametric test, followed by the Wilcoxon tests. The data were shown as median (min–max). n = 6 in each group. *$P < 0.05$ versus normal group; #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ versus diabetic group.

promoting for the host health.[21] Previous studies revealed that *Lactobacillus* decreased in the diabetic rats [18] and high-fat-diet rats.[22] Moreover, probiotics treatment could reduce plasma inflammatory cytokines, such as lipopolysaccharide, Tumor necrosis factor-α, and IL-6 in Zucker obese rats.[23] Wang *et al.* found that *Lactobacillus* could prevent high-fat-diet-induced metabolic syndrome and improve inflammatory reactions in mice.[24] Fontana *et al.* found that *Lactobacillus* could reduce the plasma inflammatory cytokines on Zucker obese rats.[23] Particularly, our data also showed that liraglutide could reduce serum IL-6 in the diabetic male rats. This moderation may be involved with increasing amount of probiotic bacteria in the gut. Some food intervention could increase *Lactobacillus* in fecal microbial. Administration of feruloylated oligosaccharides from maize bran to normal diet rats could increase *Lactobacillus*

Table 2. Spearman's correlation between identified OTUs at genera level and FBG ($P < 0.01$).

Parameter	Spearman *r*	*P* value
Lachnospiraceae_UCG-010	0.7526	0.0002
Lachnospiraceae_UCG-005	0.7333	0.0004
Eubacterium_nodatum_group	0.714	0.0006
Family_XIII_AD3011_group	0.6848	0.0012
Anaerotruncus	0.6807	0.0013
Anaerovorax	0.6394	0.0032
Bilophila	0.638	0.0033
Unidentified_Ruminococcaceae	0.6345	0.0035
Adlercreutzia	0.5902	0.0078
Kurthia	0.5847	0.0086
Parvibacter	0.5776	0.0096
Acetatifactor	−0.5777	0.0096
Ruminiclostridium_5	−0.5897	0.0079
Lachnospiraceae_UCG-001	−0.6009	0.0065
Prevotellaceae_NK3B31_group	−0.6315	0.0037
Family_XIII_UCG-001	−0.6617	0.002
Ruminococcus_2	−0.6889	0.0011
Prevotellaceae_UCG-001	−0.6927	0.001
Lactobacillus	−0.7684	0.0001
Lachnospiraceae_UCG-006	−0.8067	<0.0001

OTU: operational taxonomic unit; FBG: fasting blood glucose.

in fecal microbiota.[25] Whole wheat consumption was associated with a three times higher abundance of *Lactobacillus* compared to both obese and lean control mice.[26]

At the genus level, liraglutide-treated group had an increased level of *Bacteroides_acidifaciens* and *Lachnoclostridium*. Correlation analysis revealed the negative correlation between *Lachnospiraceae_UCG-001* and FBG. *Bacteroides* and *Lachnospiraceae* are short-chain fatty acid (SCFA)-producing bacteria. SCFA may directly prevent low-grade inflammatory response,[27] enhance the secretion of peptide YY (PYY) and GLP-1,[28] and increase insulin sensitivity.[29] Previous study showed that the abundance of *Bacteroides* in the T2DM Chinese patients was just half than that of the normal glucose tolerance subjects and prediabetes.[30] Wu *et al*. also found the genera *Bacteroide vulgatus* were less represented in the microbiota of the diabetic group than the non-diabetic group.[31] Both berberine and metformin could increase *Bacteroides* in high-fat-diet-induced obese rats.[32] Main SCFAs include acetate, propionate, and butyrate. Especially, *Lachnospiraceae* is the key buryrate-producing taxa. Butyrate is a beneficial energy source for colonic epithelial cells to maintain colonic health.[33] Moreover, butyrate can increase fatty acid oxidation and energy expenditure in humans.[34] Recent data raises the linkage of reduced levels of butyrate-producing bacteria and T2D. Moreover, the alteration of microbiota structure was considered as a leading cause in improving metabolic phenotype, when transferring the microbiota from obese subjects to mice. Physically fit healthy subjects showed increased abundance of *Lachnospiraceae* in fecal microbiota and increased production of fecal butyrate. Also, *Lachnospiraceae* exhibited a positive relationship with peak oxygen uptake, the gold standard measure of cardiorespiratory fitness.[35] Metformin induced the growth of butyrate-producing gut taxa to activate gut hormones, such as GLP-1 and PYY, to reduce blood glucose.[36,37]

Besides, our data showed *Prevotella* genera was much reduced in liraglutide-treated male rats. *Prevotella*, Gram-negative bacteria, is a known mucin degrader.[38] Mucin serves as the carbon and nitrogen source for *Prevotella*. There was an increase in *Prevotella* in abundance in patients with T2D comparing with the healthy control group.[19] Another group showed increased *Prevotella* genus in abundance in patients with glucose intolerance.[39]

We also found that *Ruminococcaceae* abundance was lower in liraglutide-treated male rats than the diabetic male rats. In another study, there was a reduction in the abundance of *Ruminococcaceae* in a bitter melon formulation-treated male rats (could reduce FBG) compared to the diabetic rats.[40]

In conclusion, our findings suggest that significant changes in gut microbiota are associated with liraglutide treatment on the diabetic male rats, including enrichment of SCFA producers and probiotic bacteria. This may help alleviate systemic inflammation and contribute to the beneficial effects of liraglutide against diabetes. Further research is required to elucidate the mechanisms by which liraglutide affects gut microbiota of diabetes status and to reveal the specific bacteria which moderate diabetes.

Author contributions: XHX conceived and designed the experiments; QZ, JZ, TW, and XJW performed the experiments; MY, ML, and FP analyzed the data; XHX contributed reagents, materials, and analysis tools; and QZ wrote the paper.

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DECLARATION OF CONFLICTING INTERESTS

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