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Basic nutritional investigation

Changes of serum lipopolysaccharide, inflammatory factors, and cecal microbiota in obese rats with type 2 diabetes induced by Roux-en-Y gastric bypass

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ABSTRACT

Objectives: Previous studies have shown that Roux-en-Y gastric bypass (RYGB) leads to rapid regression of obesity and type 2 diabetes (T2D). However, the underlying mechanism remains unclear. This study aimed to investigate the effect of RYGB on serum lipopolysaccharide (LPS), interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α), and cecal microbiota in obese rats with T2D.

Methods: Obese Sprague-Dawley rats with T2D were randomly divided into RYGB diabetes operation (DO; n = 8), diabetes sham operation (DS; n = 8), and diabetic control (DC; n = 8) groups. Healthy Sprague-Dawley rats were grouped as normal control (NC; n = 8). Fasting plasma glucose and body weight were measured. The levels of peripheral serum LPS, IL-1, IL-6, and TNF-α were measured by enzyme-linked immunosorbent assay. The rats were sacrificed 12 wk after operation. Subsequently, a superior mesenteric venous blood sample was taken to measure serum LPS levels by enzyme-linked immunosorbent assay. The cecal contents of the DO and DS groups were taken to extract metagenomic DNA per the genomic DNA standardization procedure. The V4 region of the 16 S rRNA was sequenced with the Illumina Hiseq sequencing platform to compare the structure and relative abundance of cecal microbiota between the DO and DS groups.

Results: Twelve weeks after operation in the DO group, fasting plasma glucose and body weight showed a significant decrease ($P < 0.05$). Moreover, the levels of peripheral serum LPS, IL-1, IL-6, and TNF-α were obviously decreased ($P < 0.05$). A change in the LPS level of superior mesenteric venous blood also revealed a dramatic decrease ($P < 0.05$). Additionally, RYGB resulted in a shift of cecal microbiota in obese rats with T2D.

Conclusions: Hypoglycemic effects after RYGB may be associated with improved levels of LPS, IL-1, IL-6, and TNF-α. Changes in the structure of cecal microbiota may also play an important role.

Introduction

The dual epidemic of obesity and type 2 diabetes (T2D) is a challenge in the global health care arena, and 80% of severely obese patients suffer from T2D [1,2]. These grim situations require an increased understanding of potential pathophysiology to continually explore new and effective treatment options.

Low-grade chronic inflammation is one of the main features of T2D and characterized by high levels of inflammatory markers [3]. The imbalance of gut microbiota was found to be associated with low inflammation [4]. In obese patients with T2D, intestinal microbiota is disordered and causes elevated levels of lipopolysaccharide (LPS) in the blood and tissues. LPS is transported largely to the circulatory system, which induces the secretion of inflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α); promotes low-grade inflammation; and induces insulin resistance [5].

Currently, Roux-en-Y gastric bypass (RYGB) is one of the most frequently performed bariatric surgeries in the world, which is a treatment option for obese patients with T2D and provides a good model to better understand the pathogenesis of obesity and T2D.

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The weight loss and antidiabetic effect of RYGB are reliable [6], but the mechanisms of RYGB are not completely clear. A greater understanding of these mechanisms is essential for the development of noninvasive interventions in T2D.

Human studies have explored the relationship between inflammatory factors and microbiota after RYGB [7]. However, LPS, inflammatory factors, and intestinal microbiota changes after RYGB have not been well studied in animal experiments. Compared with human models, animal models can better avoid the effects of dietary preference, living environments, and medication on experimental results. We set up a diabetes sham operation group (DS) to avoid changes of the digestive tract caused by the operation itself and avoid affecting the experimental results.

As a target tissue of insulin, insulin resistance of the liver significantly promotes the development of T2D [8]. Our previous study found that duodenal-jejunal exclusion improved glycemic control by upregulating hepatic insulin signaling [9]. The superior mesenteric vein and splenic vein meet at the back of the pancreatic neck to form the hepatic portal vein, and the lesion of the pancreas can often involve the hepatic portal vein. LPS can be transported through blood circulation, so in our study, we reserved blood samples from the superior mesenteric vein (main branch of hepatic portal vein) to measure changes in LPS levels in the serum. To our knowledge, our study is the first to demonstrate serum LPS in the superior mesenteric vein after RYGB, and we hope to indirectly reflect changes of serum LPS levels in the hepatic portal vein after surgery.

Studies have shown that RYGB causes significant anatomic reorganization of the gastrointestinal system, leading to changes in the gut microbiota, which may be a mechanism for the rapid improvement of blood glucose and long-term significant weight loss [10]. Yet, the results of the study on the changes of gut microbiota after surgery are often inconsistent, and some are even contrary. Most existing studies on the changes of gut microbiota caused by RYGB concentrate on feces, and there are a few researches on altered microbiota along the length of the intestine. Different intestinal microbiota may transmit different metabolic signals. However, in the few colonization studies of different locations in the intestine after RYGB [11], cecal microbiota has been shown to play an important role. Recently, in a diet-induced weight loss model of mice, the abundance of several cecum bacterial populations in inguinal white adipose tissue was shown to be associated with selected levels of inflammation and metabolic gene expression [12].

Therefore, in this study, we explored cecal microbial composition after RYGB in Sprague-Dawley (SD) rats. We want to draw more attention to and research on the cecal bacteria through our study. In addition, the relationship between blood glucose reduction and weight loss after RYGB remains controversial and was further clarified and validated in our study as well.

SD rats are very important for experimental animal models because they have great similarities in their genetic content with humans [13]. SD rats are suitable to study the relationship between human diseases and gut microbiota, and can provide important reference data for future research [14].

Herein, we studied the effects of RYGB on glucose homeostasis and body weight in obese SD rats with T2D and changes in serum LPS, IL-1, IL-6, and TNF-α levels, and cecal microbiota. We investigated: 1) the relationship between the decrease of fasting plasma glucose (FPG) and weight loss after RYGB; 2) how does RYGB affect changes of serum IL-1, IL-6, and TNF-α levels; 3) changes of serum LPS levels in peripheral and superior mesenteric venous blood caused by RYGB; and 4) how cecal microbiota is affected by RYGB.

Methods

Animals

The experiments used 10-wk-old, clean-grade, healthy male SD rats, weighing 260 g to 350 g, that were purchased from the Animal Laboratory of Xuzhou Medical University. Rats were individually housed in ambient temperature of 22°C and humidity at 60% in a 12 h light/dark cycle. All procedures were approved by the Animal Use Ethics Committee of Xuzhou Medical University.

Animal model and experimental groups

After adapting to the environmental conditions 1 wk later, 40 rats were randomly divided into one of two groups: normal rats (n = 10) and diabetes-induced model group (n = 30).The normal group was fed the basic feed, and the model group was fed a high-sugar and high-fat diet (18% lard, 20% sucrose, 3% vitellus, and 59% basal feed; Experimental Animal Center of Xuzhou Medical University). All rats had free access to drinking water and the diet. Two months later, the rats in the model group were fasted overnight and injected with 1% streptozotocin (35 mg/kg; Sigma) by intraperitoneal injection.

After 72 h and 1 wk, the random blood glucose levels of the tail vein of the rats were measured using a hand-held glucose meter (Daltai Industrial CoLtd,Tianjin, China). Rats with blood glucose readings of ≥16.7 mmol/L at both timepoints were considered successful diabetic models and used for the experiments. These T2D model rats were randomly allocated to the T2D RYGB diabetes operation (DO), T2D control (DC), and T2D sham operation (DS) groups, with eight rats in each group, and the remaining six rats were spared. Eight of 10 normal rats were randomly selected as the normal control (NC) group without any surgery, and the remaining two rats were spared. Table 1 shows the preoperative weight, food intake, and FPG for the four groups.

Surgical procedures

Rats were fasted overnight before surgery and then anesthetized with 3% sodium pentobarbital(1 mL/kg) by intraperitoneal injection during surgery. In the midabdominal incision into the abdomen, a small stomach pouch was created around the cardia, and the distal residual stomach was sutured in the same way. The jejunum was transected 10 cm below the Treiz ligament, and the small gastric sac was connected with the distal jejunum by side-to-side gastrointestinal anastomosis. The proximal end of the jejunum was connected with the Roux limb at 15 cm below the gastrointestinal anastomosis with a side-to-side jejunal-jejunum anastomosis. All anastomoses were performed with a 7 to 0 suture in an interrupting fashion, and the abdomen was irrigated with 2 mL gentamicin (80 000 U/L) and closed (Fig. 1). Rats in DS group underwent the same laparotomy, transverse gastrointestinal transection at the same position as the RYGB operation, and anastomosed at the original position, keeping the operation time close to those in the DO group.

During the first 24 h after surgery, the rats were given free access to water, followed by a liquid diet (10% glucose) for 2 d, after which an unlimited normal diet was provided.

Body weight

Electronic scale (Nancheng long association electronic products, Dongguan, China) was used to measure the rats'· body weight before surgery and 1, 2, 4, 8, and 12 wk after surgery.

Table 1
Comparisons of preoperative weights, food ration, and fasting plasma glucose ($\chi \pm s$)

Index	DO group	DC group	DS group	NC group
Weight (g)	323.62 ± 16.42*	328.84 ± 8.16*	323.45 ± 13.79*	267.42 ± 15.11
Food ration (g/d)	207	212	200	199
Fasting plasma glucose (mmol/L)	9.10 ± 0.88*	9.20 ± 1.50*	10.74 ± 2.98*	5.88 ± 0.94

DO, diabetes operation; DC, diabetes control; DS, diabetes sham; NC, normal control.
The data were presented as mean ± standard deviation. The DO, DC, and DS groups were compared with the NC group.
*$P < 0.05$.

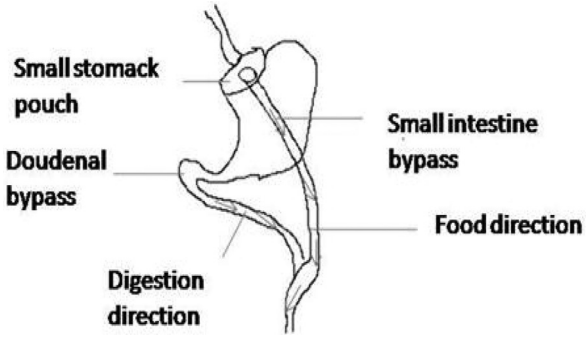

Fig. 1. Roux-en-Y gastric bypass.

Fasting plasma glucose

FPG was measured via the tail vein using a Glucometer Elite (Daltai Industrial Co., Ltd., China) before surgery and 1, 2, 4, 8, and 12 wk after surgery.

Interleukin-1, -6, and tumor necrosis factor alpha

An enzyme-linked immunosorbent assay kit (Jin Yibai Biological Technology Co., Ltd., Nanjing, Jiangsu, China) was used to measure the levels of serum IL-1, IL-6, and TNF-α in peripheral venous blood samples via venae angularis before and 4, 8, and 12 wk after surgery per the manufacturer 's instructions.

Lipopolysaccharide

An enzyme-linked immunosorbent assay kit (Jin Yibai Biological Technology Co., Ltd., Nanjing, Jiangsu, China) was used to measure the levels of serum LPS in peripheral venous blood samples via venae angularis before and 4, 8, and 12 wk after surgery per the manufacturer's instructions. The rats were sacrificed 12 wk after surgery, and superior mesenteric venous blood was collected rapidly to measure the LPS level. The method is the same as described earlier.

Cecal microbiota analysis

The rats were sacrificed 12 wk after surgery, and the contents of the cecum were taken from the DO and DS groups and snap frozen in a -80°C refrigerator. Metagenomic DNA in the cecal contents was extracted according to the genomic DNA standardization procedure and the manufacturer's protocol (High speed refrigerated centrifuge, Thermomixer comfort, Sample crushing system; Eppendorf, Qiagen). Concentration testing was done with a fluorometer or microplate reader.

Sample integrity testing was conducted by Agarose Gel Electrophoresis (concentration of agarose gel: 1 %; voltage: 150 V; electrophoresis time: 40 min). The V4 region of the 16 S rRNA gene was amplified using 515 F and 806 R primers [15] (515 F: GTGCCAGCMGCCGCGGTAA [upstream primer]; 806 R: GGACTACHVGGGTWTCTAAT [down-stream primer]). Polymerase chain reactions (PCRs) were performed for each sample under the following conditions: One cycle: 3 min at 98°C; 30 cycles: 45 s at 98°C, 45 s at 55°C, 45 s at 72°C, and 7 min at 72°C. The PCR products were purified with AmpureXPbeads (Agencourt) to remove unspecified products.The final library was quantitated in two ways: Determination of average molecule length using the Agilent 2100 bioanalyzer instrument (Agilent DNA 1000 Reagents), and quantification of the library by real-time quantitative PCR (EvaGreen). The Illumina MiSeq (Illumina RTA v1.17.28; MCS v2.5) platform was used for the analysis of the qualified libraries.

Statistical analysis

SPSS version 20.0 statistical software was used to analyze the data. All data are presented as mean ± standard deviation. One-way analysis of variance was used to compare data between the groups. The two-sided paired *t* test was used for the comparison between the two groups. Wilcoxon tests were used to confirm significant changes in the relative abundance of genera between the DS and DO groups after RYGB. The repeated measures were analyzed by repeated measures analysis of variance. A $P < 0.05$ was considered statistically significant.

Results

Body weight

All operations were successful. The body weight levels were measured before surgery and then at 1, 2, 4, 8, and 12 wk after surgery. Mean body weight in the DO group was 323.62 ± 16.42 g before surgery and 264.38 ± 10.89 g 12 wk after surgery ($P < 0.05$), which was markedly decreased ($P < 0.05$). The decrease in weight in the DO group was significantly higher than that in the DS and DC groups at each timepoint 2 wk after surgery ($P < 0.05$). There were no significant changes in body weight in the DC, DS, and NC groups before and after surgery ($P > 0.05$; Fig. 2).

Fasting plasma glucose levels

FPG levels were measured before surgery and then 1, 2, 4, 8, and 12 wk after surgery. Mean FPG levels in the DO group, which were 9.10 ± 0.88 mmol/L before surgery, decreased to 5.89 ± 1.09 mmol/L 12 wk after surgery ($P < 0.05$). FPG levels markedly declined at 1, 2, 4, 8, and 12 wk after surgery ($P < 0.05$). These

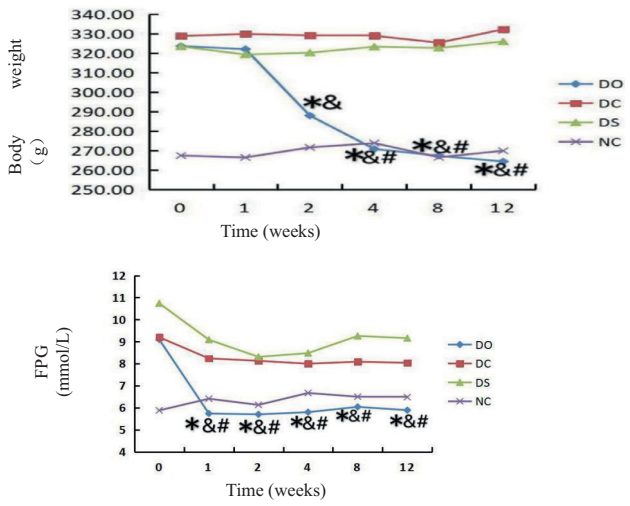

Fig. 2. Body weight of rats in the four groups before and after surgery. Diabetes operation group compared with the preoperative values ($^{*}P < 0.05$) compared with the diabetes sham and diabetes control groups at the same time ($^{\&}P < 0.05$) compared with the normal control group at the same time ($^{\#}P > 0.05$). The data are presented as mean ± standard deviation.

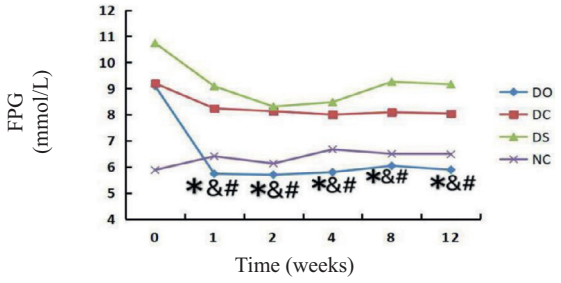

Fig. 3. Fasting plasma glucose of the four groups at different timepoints. Diabetes operation group compared with the preoperative values ($^{*}P < 0.05$) compared with the diabetes sham and diabetes control groups at the same time ($^{\&}P < 0.05$) compared with the normal control group at the same time ($^{\#}P > 0.05$). The data are presented as mean ± standard deviation.

levels were significantly lower than those in the DS and DC groups at each timepoint 2 wk after surgery ($P < 0.05$). In contrast, no significant changes in FPG levels were observed in the DC, DS, and NC groups before and after surgery ($P > 0.05$; Fig. 3).

Interleukin-1, -6, and tumor necrosis factor alpha levels

Levels of serum IL-1, IL-6, and TNF-α were measured before surgery and then at 4, 8, and 12 wk after surgery. Levels in the DO group decreased markedly after surgery ($P < 0.05$). In the DO group, IL-1 levels decreased from 106.88 ± 6.25 ng/L before surgery to 90.42 ± 5.01 ng/L ($P < 0.05$). IL-6 levels decreased from 107.88 ± 2.01 ng/L before surgery to 77.23 ± 4.18 ng/L ($P < 0.05$), and TNF-α levels decreased from 116.67 ± 5.27 ng/L before suregry to 78.39 ± 8.81 ng/L ($P < 0.05$) 12 wk after surgery. These levels were significantly lower than those in the DS and DC groups at each timepoint 4 wk after surgery ($P < 0.05$). There were no significant changes in levels of IL-1, IL-6, and TNF-α in the DC, DS, and NC groups before and after surgery ($P > 0.05$; Fig. 4 A-C).

Lipopolysaccharide levels

Peripheral venous serum LPS levels in the DO group, which were 44.35 ± 2.41 mmol/L before surgery, decreased to 32.19 ± 1.24 ng/L 12 wk after surgery ($P < 0.05$). The decrease was significant at 4, 8, and 12 wk after surgery ($P < 0.05$). These levels were significantly lower than those in the DC group at each timepoint 4 wk after surgery ($P < 0.05$). There were no significant changes in LPS level in the DC, DS, and NC groups before and after surgery ($P > 0.05$; Fig. 5A).

LPS levels in superior mesenteric venous blood samples from the DO group were 34 ± 1.41 ng/L 12 wk after surgery, which was significantly lower than in the DC and DS groups ($P < 0.05$). In comparison, the NC group showed no significant difference (Fig. 5B).

Changes in cecal bacterial population modified by RYGB

Subsequently, we examined cecal microbial components 12 wk after RYGB surgery. At the phylum level of the DO and DS groups, firmicutes, bacteroidetes, proteobacteria, and actinobacteria accounted for >92%. Compared with the DS group, in the DO group, although the relative abundance of proteobacteria, actinobacteria, fusobacteria, and verrucomicrobia did not reach significant differences, they all showed an increasing trend. Deferribacteres showed a decreasing trend, but did not reach significant difference (Table 2; Fig. 6A).

Compared with the DS group, at the genus level of the DO group, the relative abundance of bifidobacterium, streptococcus, fusobacterium, veillonella, enteroco, and so on, showed an increasing trend, and the relative abundance of coprococcus, and so on, showed a decreasing trend (Fig. 6B). The relative abundance of bifidobacterium emerging in the DO group was significantly different from that in the DS group ($P < 0.05$; Fig. 6G).

In addition to comparing the DO and DS groups at the phylum and genus levels, we also compared the two groups with regard to class, order, family, and species levels. Compared with the DS group, at the

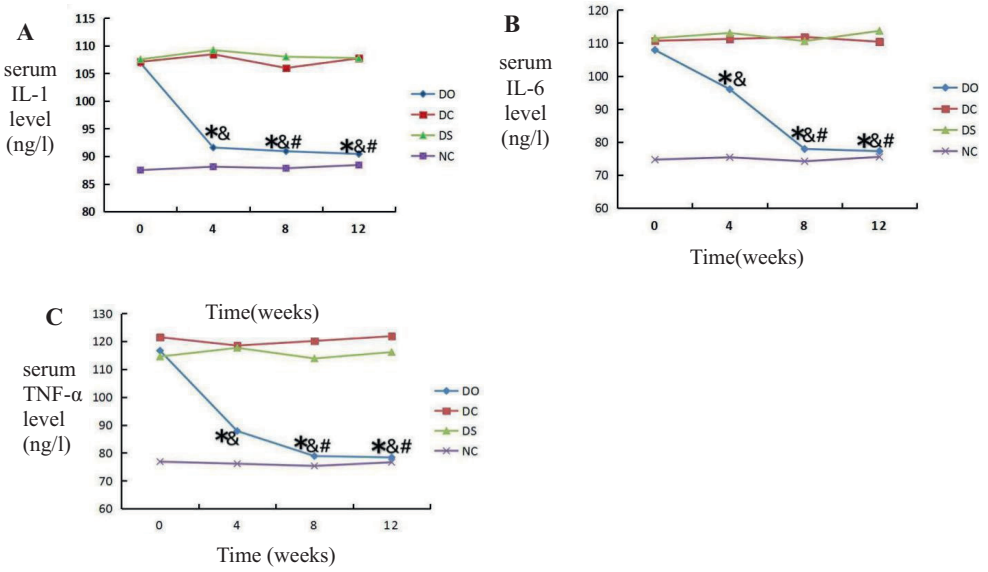

Fig. 4. Improvements in the peripheral serum inflammatory factors levels of the four groups before and after surgery. (A) Peripheral serum Interleukin-1 changes in the groups. (B)Peripheral serum Interleukin-6 changes in four groups. (C) Peripheral serum tumor necrosis factor alpha changes in the groups. The diabetes operation group compared with the preoperative values ($^{*}P < 0.05$) compared with the diabetes sham and diabetes control groups at the same time ($^{\&}P < 0.05$) compared with the normal control group at the same time ($^{\#}P > 0.05$). The data are presented as mean ± standard deviation.

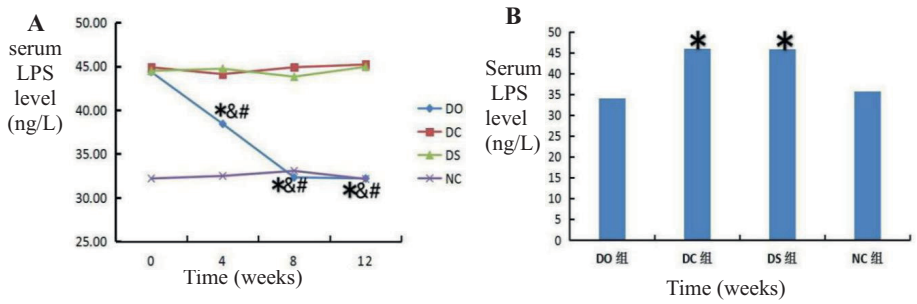

Fig. 5. (A) Improvements of levels of serum lipopolysaccharide in peripheral venous blood samples in the four groups before and after surgery. Diabetes operation group compared with the preoperative values ($^{*}P < 0.05$) compared with the diabetes sham and diabetes control groups at the same time ($^{\&}P < 0.05$) compared with the normal control group at the same time ($^{\#}P > 0.05$). (B) Lipopolysaccharide levels of mesenteric venous blood samples in the four groups were performed 12 wk after surgery. Diabetes operation and normal control groups compared with the diabetes sham and diabetes control groups ($^{*}P < 0.05$). The data are presented as mean ± standard deviation.

Table 2
Comparison of relative abundance of cecal microbiota at phylum level (%)

group	Proteobacteria	Actinobacteria	Fusobacteria	Verrucomicrobia	Deferribacteres
DO	7.134 ± 3.568	0.765 ± 1.203	0.246 ± 0.408	0.020 ± 0.029	0.009 ± 0.01
DS	4.559 ± 0.278	0.114 ± 0.039	0.001 ± 0.002	0.008 ± 0.010	0.033 ± 0.026

DO, diabetes operation; DS, diabetes sham.
The data were presented as mean ± standard deviation.
DO vs DS group: $P > 0.05$.

class level of the DO group, actinobacteria, verrucomicrobiae, and fusobacteriia showed an increasing trend and deferribacteres showed a decreasing trend, but none reached significant differences (Fig. 6C). In addition, we only found that the differences in newly emerged vifidobacteriales (order level), bifidobacteriaceae (family level), and bifidobacterium adolescentis ATCC 15703 (species level) were statistically significant between the DS and DO groups ($P < 0.05$; Fig. 6D-F).

Discussion

Our study resulted in several major findings. First, RYGB significantly reduced the FPG and weight levels of obese rats with T2D. Of note, FPG improved earlier than weight loss. Second, IL-1, IL-6, and TNF-α levels were obviously decreased after RYGB. Third, LPS levels of peripheral and superior mesenteric venous serum samples were dramatically reduced. Finally, after RYGB, bifidobacterium appeared in cecum microbiota, which increased significantly.

Our previous study showed that duodenal-jejunal exclusion effectively improved insulin resistance [9]. Furthermore, we confirmed that RYGB is a more effective treatment for T2D. RYGB can cause rapid improvements in glucose metabolism and sustained weight loss [16]. However, the relationship between postoperative blood glucose reduction and weight loss is still up for debate. The traditional view is that weight loss surgery mainly causes a loss of calories in the feces through gastric restriction or malabsorption, and thus continues to reduce body weight to improve metabolic symptoms [17,18]. However, in recent years, several studies have shown that the underlying mechanisms for glycemic control improvement may not be affected by weight loss [19–22].

We performed RYGB on obese rats with T2D, which achieved a sustained and stable hypoglycemic effect and weight loss. Our data showed that in the DO group, FPG levels decreased from 9.10 ± 0.88 mmol/L to 5.74 ± 1.30 mmol/L the first week after RYGB ($P < 0.05$), and to 5.89 ± 1.09 mmoL/L the 12th week after RYGB ($P < 0.05$). Body weight decreased from 323.62 ± 16.42 g to 287.95 ± 14.12 g ($P < 0.05$) the second week after RYGB, and to 264.38 ± 10.89 g ($P < 0.05$) the 12th week after RYGB. Our study suggested that RYGB not only significantly reduces body weight and blood glucose levels, but also improves blood glucose earlier than weight loss. We demonstrated that blood glucose reduction is independent of weight loss after RYGB.

Proinflammatory factors, such as LPS, can affect host metabolism through host-derived proteins to mediate immune responses [23,24]. Our data showed that serum LPS levels in the peripheral serum and superior mesenteric veins of rats after RYGB were significantly decreased ($P < 0.05$). We speculate that RYGB effectively reduced LPS levels in rats with T2D and thereby alleviated endotoxemia and promoted the homeostasis of blood glucose. We hypothesize that the reduction of superior mesenteric vein LPS in turn decreased LPS levels in the hepatic portal vein, and thereby reduced insulin sensitivity in the liver. However, this assumption requires more studies in support.

Inflammatory markers such as IL-1, IL-6, and TNF-α are the major cytokines that mediate inflammatory responses, and levels in patients with T2D with insulin resistance are elevated [3]. Our study found that RYGB significantly reduced IL-1, IL-6, and TNF-α levels in the serum of rats with T2D ($P < 0.05$), which indicates that RYGB also plays an antiinflammatory role. RYGB can improve the level of inflammatory markers after surgery.

Interestingly, RYGB causes profound alterations in the gut microbiota that benefit host metabolism [25]. The transfer of microbiota after RYGB from both mice [26] and humans [23] to germ-free mice recipients reduced adiposity and improved metabolic phenotype [27]. Of note, the colonization of the gut microbiota of each intestinal segment after RYGB showed an important role in the cecal microbiota. The reduction of adiposity after RYGB can be transferred to germ-free mice by transplanting cecal microbiota [28]. Studies have also shown that the higher biomass and more metabolically active cecal microbiota changes after RYGB promote glucose tolerance, and metabolic phenotypes can be transferred by transplanting the cecal microbiota [11]. These findings prompted us to study more about cecal microbiota.

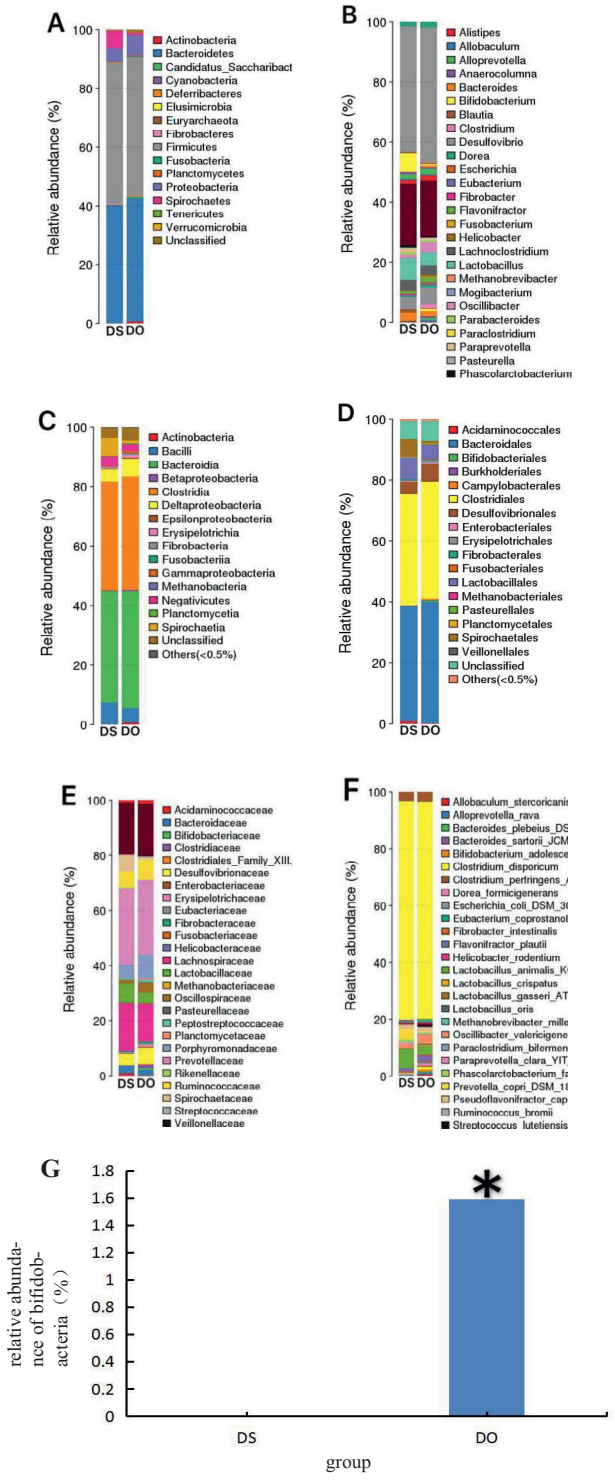

Fig. 6. Diabetes sham (DS) group, type 2 diabetes sham operation group; diabetes operation (DO) group, type 2 diabetes Roux-en-Y gastric bypass operation group. (A) Relative abundance of phylum level in the DO and DS groups. (B) Relative abundance of genus level in the DO and DS groups. (C) Relative abundance of class level in the DO and DS groups. (D) Relative abundance of order level in the DO and DS groups. (E) Relative abundance of family level in the DO and DS groups. (F) Relative abundance of species level in the DO and DS groups. (G) Comparison of relative abundance of bifidobacterium (genus level) in the DO and DS groups. $^{*}P < 0.05$. The data are presented as mean ± standard deviation.

Our study suggests that in rats with T2D, cecal bifidobacterium increased significantly after RYGB, which indicates that cecal bifidobacterium may play a role in the improvement of metabolism, such as hypoglycemia, and reflects the importance of cecal microbiota. Our results are similar to those from the study by Chen et al. [6], who reported that bifidobacterium increased significantly after RYGB. In contrast, Kong et al. [7] observed a reduction in bifidobacterium after RYGB in their initial trial using the reverse transcription PCR method. On the basis of this study, the researchers extended their previous results by using a pyrosequencing method, and also observed a decrease in Bifidobacterium [29]. We speculate that the difference among these results might be related to the difference in postoperative feeding, probiotic administration, experimental models, and sample selection.

Although not a dominant genus in gut bacteria, bifidobacterium plays a key role in host metabolism. Bifidobacterium is a Gram-positive bacterium associated with antiinflammatory properties and insulin, and is negatively correlated with inflammatory markers [7] with lower levels in obese and diabetic patients [30,31]. Bifidobacterium can improve mucosal barrier function, and reduce endotoxin levels [32–34]. Obese mice treated with bifidobacterium as probiotic agents had a higher expression of tight junction/barrier protein and lower level of LPS, resulting in improved intestinal permeability and metabolic parameters [35]. At the same time, bifidobacterium as a probiotic bacteria has been used in some disease prevention and treatment with good results [36,37].

We speculate that changes in cecal bacteria, especially the emergence of bifidobacterium, may lead to a decrease in LPS, and thereby alleviate metabolic endotoxemia and ultimately reduce low-grade chronic inflammation and improve diabetes. Causal mechanisms remain unknown, but changes in cecal bacteria may be one of the mechanisms of T2D relief.

Conclusions

Our study suggests that the improvement of glucose metabolism and weight loss in rats with T2D after RYGB may be related to the reduction of serum LPS, IL-1, IL-6, and TNF-α levels, asd well as attributed to the adjustment of the cecum bacterial population. Futhermore, our study provides animal experimental data for the treatment of T2D, and the regulation of gut microbiome may represent a new treatment for diabetic inflammation; however, the exact mechanism is not clear and further research is needed. These findings stimulate deeper explorations of the mechanisms that link T2D.

Limitations

Owing to the high cost and complexity of the sampling of the contents of the cecum, the final sample size is small, so our research is mostly exploratory, and provides only a preliminary evaluation in animal models, which need to be confirmed and validated in a larger sample size.

Acknowledgments

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