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Research on sleeve gastrectomy for the treatment of rats with type 2 diabetes mellitus and the regulation of ghrelin and intestinal lesions

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Abstract. – OBJECTIVE: To explore the effect of sleeve gastrectomy on type 2 diabetes mellitus (T2DM) in regulating ghrelin and intestinal lesions.

MATERIALS AND METHODS: Specific pathogen free (SPF) Wistar rats were injected with streptozotocin (STZ) after giving a high-sugar and high-fat diet, to establish a T2DM rat model. The rats were randomly divided into a sleeve gastric excision group, a non-surgical group and a fake surgical group, with 10 rats in each group. The weight, blood glucose, glucose tolerance and ghrelin hormone of rats were compared. The feces of rats in each group at the 8th week after surgery were collected, to extract the total bacterial deoxyribonucleic acid (DNA). The bacterial 16S universal primer was used to expand the 16SrRNA V46 conserved region. The total Polymerase Chain Reaction (PCR) products were sequenced by PE101-bp to classify the gene and genera.

RESULTS: The weight of the rats after sleeve gastrectomy significantly decreased (p <0.05). The area under the blood glucose curve and the area under the insulin curve were significantly smaller than those in the non-surgical group and the fake surgical group (p <0.05). Compared with the sleeve gastric excision group, the abundance of *Phylum Firmicutes* was higher, that of Bacteroidetes was lower. Compared with the sleeve gastric excision group, there were more genera in the fake surgical group and the non-surgical group. The genera with higher abundance in the three groups were *Lactobacillus* and *Bacteroidetes*. Compared with the sleeve gastric excision group, the fake surgical group and the non-surgical group had higher abundance of *Phylum Firmicutes* (p <0.05) and lower abundance of *Bacteroidetes* (p <0.05).

CONCLUSIONS: To sum up, sleeve gastrectomy can reduce the weight of rats in T2DM rat model, lower blood glucose levels of rats in the model and improve insulin resistance levels. The related mechanism may be related to the upregulation of ghrelin and intestinal flora.

Key Words:
Sleeve gastrectomy, Type 2 diabetes mellitus, Ghrelin, Intestinal flora.

Introduction

According to statistics from the International Diabetes Federation (IDF), the number of patients with type 2 diabetes mellitus (T2DM) has reached 366 million in 2011 in the world and by 2030 and this number will reach 552 million[1]. At present, the main treatment of T2DM includes keeping nutritional balance, doing exercises, taking oral medication, and insulin. Anyway, there are still 60% of T2DM patients failing to meet the standard[2]. In addition, some oral hypoglycemic drugs and insulin may increase the weight and further impair blood glucose control. Therefore, there has been an urgent need for a new way to control blood glucose and lose weight. Sleeve gastrectomy is one of the most common weight-loss surgery at present, which can effectively lose weight and improve metabolic symptoms by losing weight and changing hormone. Lee[3] showed that weight-loss and T2DM relief may be related to ghrelin, bile acid, and sleeve gastrectomy. Sleeve gastrectomy can reduce blood glucose of rats with T2DM and increase ghrelin levels in the model[4].

Ghrelin, growth hormone releasing peptide, is an endogenous ligand of growth hormone secre-

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tagogue receptor type 1a (GHS-R1a), a 28 amino acid peptide hormone, which is mainly secreted by gastric X/A-like endocrine cells. It is also secreted in the colon, small intestine, gonads, placenta, brain, and kidney, and expressed in the hypothalamus and islets. Ghrelin has two main molecular forms, including acylated ghrelin (AG) and unacylated ghrelin (UAG)[5-7]. Ghrelin mostly exists in the form of UAG in human body. UAG does not activate GHS-R1a and has low activity. UAG has also been reported to have activity; it can promote insulin secretion by islet cells and is used to prevent T2DM. When UAG is converted to AG, AG can activate GHS-R1a. Apart from regulating growth hormone release, food intake and energy homeostasis, AG also has effects on gastrointestinal, pancreatic, cardiovascular, pulmonary, immune, neurological function, myogenesis, and fat production[8-10]. A dynamic balance was maintained between AG and UAG in the body. The AG/(UAG+AG) value is often used as an indicator to assess ghrelin activity, and UAG+AG is total ghrelin (TG). The enzyme responsible for converting UAG to AG was discovered in 2008 and named Ghrelin O-acyltransferase (GOAT)[11]. The wide expression of GOAT corresponds to the wide distribution of AG expression in tissues, such as pancreas, stomach, and hypothalamus. Previous studies[12-14] have shown that both Ghrelin and GOAT played an important role in regulating obesity, blood glucose and insulin. Ghrelin and insulin secretion participate in the regulation of blood glucose together, and there may be a negative feedback mechanism between them. However, it is unclear if sleeve gastrectomy takes part in maintaining blood glucose concentration by regulating the pancreatic islets express Ghrelin/GOAT axis and insulin secretion apart from restricting food intake. In addition, obesity and T2DM were related to changes in intestinal flora[14] and the mechanism of treating T2DM with gastrointestinal surgery may be involved in the regulation of intestinal flora[15]. It was found in animal models that after gastric bypass surgery, the number of *Phylum Firmicutes* decreased and the number of *Bacteroidetes* increased[16]. However, it is inconclusive whether sleeve gastrectomy affects blood glucose and is related to changes in intestinal flora. Therefore, this experiment aims to verify the hypoglycemic effect of sleeve gastrectomy on rats with T2DM and further explore the related mechanism of sleeve gastrectomy affecting ghrelin levels and regulating intestinal flora, to provide a new idea for the treatment of T2DM.

Materials and Methods

Experimental Animals and Reagents

Thirty specific pathogen free (SPF) male Wistar rats (4-5 weeks old, 90-100 g in weight), purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., were selected [Animal License Number: SCXK (Beijing, China) 2014-0004]. The sources of reagents and instruments are as follows: Electronic Analytical Balance (Libror AEL-200, Japan); CR2032 Roche Blood Glucose Meter; Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA, Art. No.: S0130); Total Ghrelin in Rats (TG, Art. No.: RF(m)14769, Qiming Biotechnology Co., Ltd., Shanghai, China); AG (Art. No.: ML-elisa-A10163, Shanghai Enzyme Biotechnology Co., Ltd.); TG (Art. No.: ML-elisa-E607, Enzyme Biotechnology Co., Ltd., Shanghai); Insulin Enzyme-linked Immunosorbent Assay (ELISA) kit (Art. No.: QY-VN3310, Qiaoyu Biological Technology Co., Ltd., Shanghai, China); Sodium Taurocholate (Art. No.: 145-42-6, Sigma-Aldrich, St. Louis, MO, USA); TIA Namp Stool DNA Kit (Art. No.: D4015-0, Beinuo Biotechnology Co., Ltd., Shanghai, China).

Experimental Grouping and Surgical Methods

Wistar rats were randomly fed with high-sugar and high-fat diet. High-sugar and high-fat diet consisted of 65% of maintenance feed, 10% of lard, 20% of sucrose, 2.5% of cholesterol, 1% of sodium cholate, and 1.5% of mixed preparations (eggs, sesame oil, and peanuts). All rats had free food and drink for 6 weeks. After the weight was 187 g or more, a single intraperitoneal injection of 45 mg/kg streptozotocin was used to cause non-insulin-dependent diabetes mellitus (NIDDM). After the model was formed, keep high-sugar, and high-fat diet. Blood from the tail veins was taken once a day for the first 3 days after streptozotocin injected and blood glucose levels were measured by Roche blood glucose. When the random concentration of blood glucose was 11.1 mmol/L or more, the T2DM rat model was successfully modeled[16].

Thirty rats in the T2DM model were randomly divided into a non-surgical group, a fake surgical group and a sleeve gastric excision group, with 10 in each group (n = 10). The non-surgical group received no treatment. The three groups fasted for 12 hours before surgery. In the sleeve gastric excision group, the rats were anesthetized with 0.3% pentobarbital sodium injection

(50 mg/kg) and the corneal response and pain response were observed. 0.9% sodium chloride solution (20 ml) was injected subcutaneously and penicillin sodium (800,000 IU) was injected muscularly before surgery, to prevent dehydration and infection. A 3 cm incision was made at 1 cm below the xiphoid. After opening the abdominal cavity and exposing the stomach, the fracture of ligaments in gastric and gastric tissues were made a 3-5 mm cross-section along the greater curvature of the stomach to empty stomach contents. After emptying the stomach, the bottom of the stomach and most of the stomach (70% of the total stomach) were removed and then absorbable wire was used to suture the remaining stomach one single layer by one. 0.9% sodium chloride solution (20-30 ml) was used to do peritoneal lavage and then the skin was sutured. The surgery to the rats in the fake surgical group was similar to that in the sleeve gastric excision group, which included an open abdominal transection to the stomach after anesthesia, cleaning the stomach contents but not cutting the stomach, and then, sutured. Rats that underwent surgery were free to drink water after anesthesia and awakened, and then, they were given a liquid diet 24 hours later. Then, they were gradually transitioned to the ordinary diet within 1 week after surgery and returned to the original high-sugar and high-fat diet after defecation.

Testing Indicators and Methods

Weight

All rats were weighed during 3 days before surgery and every day in 1 week after surgery, and they were weighed weekly for 8 following consecutive weeks.

Glucose tolerance test

Blood from orbital veins was collected at 0 min, 15 min, 30 min, 60 min, 90 min and 120 min after all the rats fasted for 16 hours. Oral administration of a 50% glucose solution (1 g/kg) was performed at baseline d1, 3 days before surgery and 8 weeks after surgery. Blood glucose concentration was measured by Roche blood glucose meter (Basel, Switzerland) and insulin concentration was measured by ELISA method. The area under the glucose curve, the area under the insulin curve and Homa insulin resistance index (HOMA-IR) (fasting insulin × fasting blood glucose/22.5) were calculated by the receiver operating characteristic curve (ROC) of SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Ghrelin test

Blood was collected from orbital veins after all rats fasted for 16 hours at baseline d1, 3 days before surgery and the 8th week after surgery. The content was measured by ELISA kit. The serum ghrelin level was represented by AG/TG ratio of 450 wavelength absorptivity values.

Intestinal flora

At the 8th week after operation, 1-2 feces samples of rats before and after intervention were collected from each group and the fecal genomic DNA extraction kit (Art. No.: EX1752-100T, Golden Clone Biotechnology Co., Ltd., Beijing, China) was used to extract DNA of fecal samples. Sequence pretreatment was performed using BIPES and the sequence similarity was 97%, which could be classified into the same classification operation unit and then they were classified using RDP. 16SrDNA V3 region universal primers and functional flora-specific primers (*Bacteroidetes* and *Phylum Firmicutes*) were used to determine the gene sequence of intestinal bacteria. The number of copies of the total bacterial gene sequence was detected by Real-Time fluorescence quantitative PCR. The relative abundance of each phylum was represented by the percentage of the total bacterial copy number[17].

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and images were edited by GraphPad Prism 6.0c (La Jolla, CA, USA). The data were expressed as mean ± standard deviation. Univariate analysis of variance was used for comparison between groups and then LSD multiple comparisons were made. Pairwise comparisons were made by pairing sample *t*-test to get specific *p*-value. When $p<0.05$, it was considered to be statistically significant.

Results

Changes in Weight

There was no significant difference in the weight of rats of the baseline and before surgery in the three groups ($p>0.05$). The weight in the sleeve gastric excision group remarkably decreased, and then, gradually increased after about 7 days after surgery, but the weight was still significantly reduced compared with that of the non-surgical group and the fake surgical group at the 8th week after surgery ($p=0.041$). The weight

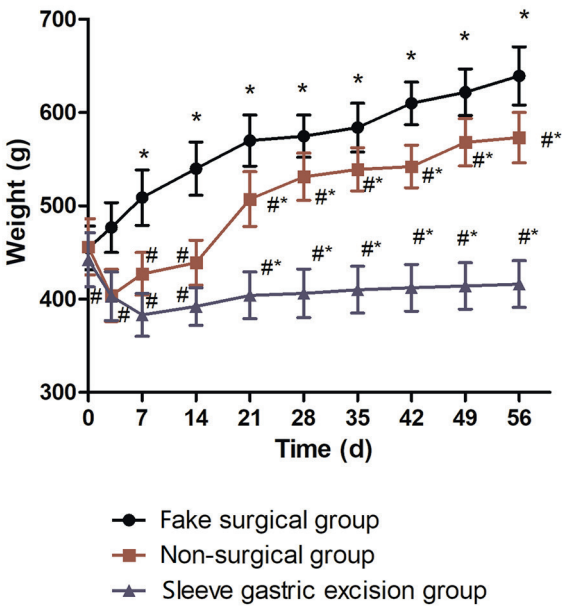

Figure 1. Changes in Weight of Rats (Mean ± SD, g).

in the non-surgical group gradually increased and the weight in the fake surgical group decreased first, and then, increased gradually about 3 days after surgery and the weight increased significantly at 8 weeks after surgery (Figure 1).

Blood Glucose AUC, Insulin AUC, HOMA-IR and AG/TG Values in Different Groups

The rats of the three groups were compared, and there were no statistical differences of blood glucose AUC, insulin AUC, HOMA-IR and AG/TG values of the baseline and before surgery ($p>0.05$). Compared with that of the baseline, blood glucose AUC, insulin AUC, HOMA-IR and AG/TG values before and after surgery increased at various degrees. Blood glucose AUC levels (non-surgical group $p=0.023$; fake surgical group $p=0.030$), insulin levels (non-surgical group $p=0.024$; fake surgical group $p=0.020$) and HOMA-IR (non-surgical group $p=0.019$; fake surgical group $p=0.021$) in the sleeve gastric excision group after surgery significantly decreased compared with those in the non-surgical group and the fake surgical group. The AG/TG level (non-surgical group $p=0.035$; fake surgical group $p=0.041$) significantly increased compared with that of the non-surgical group and the fake surgical group (Figure 2).

Changes in Glucose Tolerance Test and Insulin Glucose Tolerance Test in Different Groups

Insulin levels were significantly different from that of the baseline and before surgery (baseline $p=0.038$; before surgery $p=0.022$). The highest insulin level in the sleeve gastric excision group appeared in 60 minutes after oral glucose, it was earlier than that in the non-surgical group and the fake surgical group. After surgery, the area under the insulin curve in the sleeve gastric excision group was significantly lower than that in the non-surgical group and the fake surgical group ($p<0.05$). There were statistical differences in the area under the insulin curve between the three groups and the baseline before and after surgery ($p<0.05$), suggesting the successful models and significant effects of surgical intervention on the level of insulin resistance (Figure 3).

Changes of Intestinal Flora

In the heat map, there were no significant differences of phylum in the three groups. The abundance of *Phylum Firmicutes* and *Bacteroidetes* was higher. Compared with the sleeve gastric excision group, the abundance of *Phylum Firmicutes* in the fake surgical group and the non-surgical group was higher and that of *Bacteroidetes* were lower. The genera of the three groups were significantly different. Compared with the sleeve gastric excision group, there were more genera in the fake surgical group and the non-surgical group. The genera with high abundance in the three groups were Lactobacillus and *Bacteroidetes*. Compared with the sleeve gastric excision group, the abundance of *Phylum Firmicutes* in the fake surgical group and the non-surgical group was higher ($p<0.05$) and that of *Bacteroidetes* was lower ($p<0.05$). It can be seen that after T2DM modeled successfully, it has an impact on the flora of the rats (Figure 4 and 5).

Discussion

Weight-loss surgery was only used as a surgical treatment for severe obesity at first. Since a retrospective study of 608 people in 1998 demonstrated that weight-loss surgery can improve blood glucose level in obese patients with T2DM[17], the role of weight-loss surgery in the treatment of metabolic lesions has been studied. The latest research showed that weight-loss surgery is more effective than traditional medicine in controlling hyperglycemia in patients with severe obesity and T2DM[18].

There are currently four methods of weight-loss surgery, including vertical gastroplasty, adjustable gastric band surgery, sleeve gastrectomy, and Roux-en-Y gastric bypass surgery. Adjustable gastric band surgery uses laparoscopy, which has become the standard weight-loss surgery procedure in Europe and Australia. Roux-en-Y gastric bypass surgery combines the dual advantages of limiting dietary intake and limiting absorption, which can quickly lose weight, but there are still some drawbacks, such as complicated surgical procedures, more postoperative complications and patients often need long-term treatment. Sleeve gastrectomy is a surgical method that has appeared in recent years, which is operated with laparoscope and relatively simple with fewer complications. It is currently recognized as an effective surgical treatment for severe obesity[19,20]. The success rate of weight loss is as high as 80% in the first year after sleeve gastrectomy was performed and it is stable at about 50% in 6-8 years. Compared with changes in the diet plan, sleeve gastrectomy has a longer lasting effect on weight loss[21]. Apart from restricting calories, sleeve gastrectomy can regulate the plasma levels of the hormone ghrelin that promotes food intake and the leptin that inhibits food intake, maintaining a unique hormone balance, thereby achieving the goal of weight loss. Sleeve gastrectomy not only significantly loses weight, but also effectively improves the metabolic complications caused by T2DM and obesity[22]. With the increase of sleeve gastrectomy, the technology is more and more mature and the understanding of surgeons to the sleeve gastrectomy has also changed from obesity surgery to metabolic surgery. However, the effect of sleeve gastrectomy on blood glucose is quite controversial. This experiment found that apart from losing the weight of rats in the T2DM rat model, sleeve gastrectomy has an effect on fasting blood sugar, posterior blood sugar, hypoglycemic area of curve, the insulin area of the curve, and the insulin resistance index of rats. This suggests that sleeve gastrectomy can reduce blood glucose levels in rats with T2DM, increase islet β-cell regulation, and improve insulin resistance.

The mechanism of sleeve gastrectomy for treating T2DM is not clear. Apart from reducing food calorie intake, it may be related to complex hormonal changes. One of the hormonal changes is a significant reduction in ghrelin levels after gastric

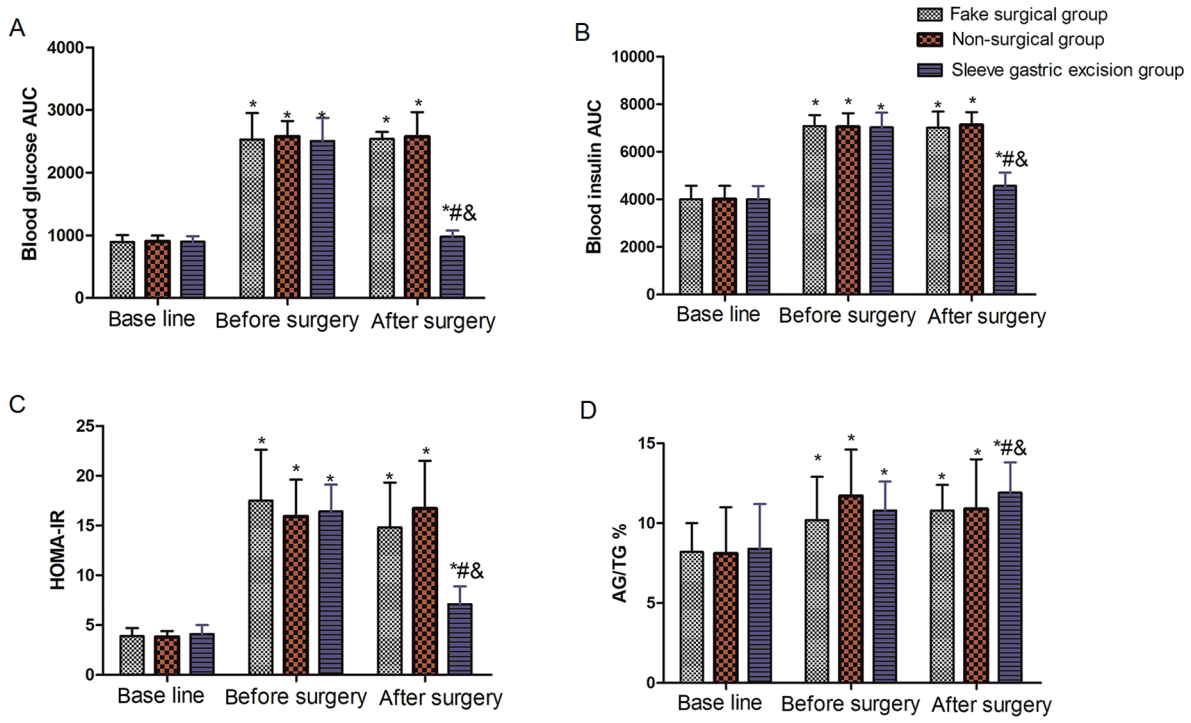

Figure 2. Glucose AUC, Insulin AUC, HOMA-IR and AG/TG Values in Different Groups. **(A)** Glucose AUC; (**B**) Insulin AUC; (**C**) HOMA-IR; (**D**) AG/TG Value (*p<0.05, Comparison with preoperative; #p<0.05, Comparison with non-surgical group; \$$p$<0.05, Comparison with fake surgical group).

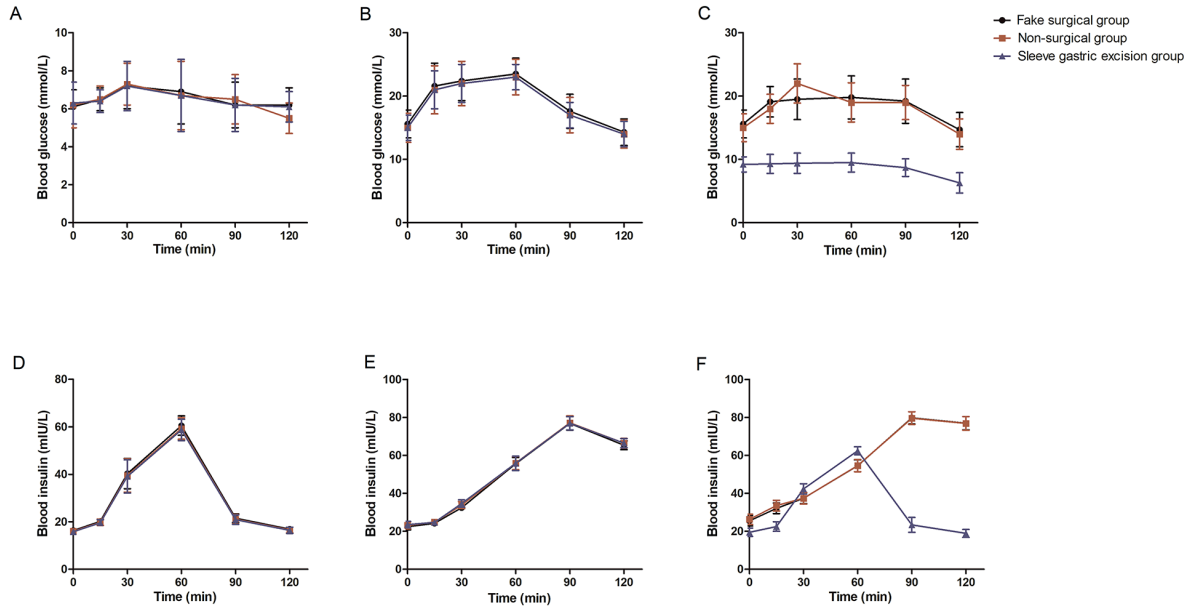

Figure 3. Changes in Glucose Tolerance Test and Insulin Glucose Tolerance Test in Different Groups. **(A)** Changes in baseline glucose tolerance test; (**B**) Changes in glucose tolerance test before surgery; (**C**) Changes in glucose tolerance test after surgery; (**D**) Changes in baseline glucose tolerance test; (**E**) Changes in insulin glucose tolerance test before surgery; (**F**) Changes of insulin glucose tolerance test after operation.

fundectomy[23]. The balance between ghrelin promoting food intake and leptin inhibiting food intake has led to improved metabolism and changes in food preferences, i.e., shifting from high-fat foods to low-calorie foods. Low plasma ghrelin levels are beneficial for enhancing fasting insulin levels, improving hyperglycemia, insulin resistance and reducing obesity, while high ghrelin level inhibits insulin release and enhances the food intake signal of the hypothalamus[24-26]. After ghrelin gene knockout, the blood glucose of mice significantly reduces and insulin level significantly increases. AG is one of the two ghrelin forms that exist in the human body, which is considered to be the active form of ghrelin. It has a positive regulation effect on hypothalamic glucose metabolism and food intake signals[27,28], while UAG can antagonize the effect of AG[29-31]. TG levels in obese individuals were lower, but serum AG levels or AG/TG ratios did not change. Sleeve gastrectomy can further reduce serum UAG level, but cannot reduce AG level[32], thereby increasing the AG/TG ratio. This is consistent with our experimental results, showing that AG/TG level are significantly increased after sleeve gastrectomy. Ghrelin is mainly produced by the stomach. Therefore, it is believed that after sleeve gastrectomy, the ability of endocrine cells to produce gastrin decreases, but the mechanism of AG/TG ratio in the blood regulating a series of changes in blood glucose is still unclear. Ghrelin inhibits insulin release from islet β cells by limiting glucose-induced cytosolic calcium ion concentration and it is also possible to inhibit cell signaling by inhibiting glucose-induced cell membrane excitability, to reduce insulin secretion[33]. After sleeve gastrectomy, the ghrelin levels mRNA and protein expression in the hypothalamus significantly decreased and the expression of growth hormone secretagogue receptor (GHSR) protein significantly increased, which may be related to the metabolism of the special circulation of ghrelin. Hormones are usually secreted in response to contextual stimuli, but when the stimulus continues to occur, the hormones are inhibited, so that sleeve gastrectomy is considered to eliminate the cyclical effects of circulating hunger on the hypothalamus, leading to the decrease of expression ghrelin levels precursor mRNA and ghrelin proteins. GHSR is compensated and the negative energy balance of the hypothalamus is activated through the mammalian target of rapamycin (mTOR) pathway, which regulates a series of metabolic activities, such as energy homeostasis and food intake,

to affect blood glucose level[23]. In short, ghrelin plays an important role in fat metabolism and glucose homeostasis, and the decrease in ghrelin level in patients with T2DM is associated with increased abdominal obesity and insulin resistance[34]. Katsuki et al[35] have shown that there has been a positive correlation between ghrelin level and food intake, i.e., the higher the ghrelin level, the higher the food intake, metabolic rate and the linear change in the metabolic index. In this regard, the results of the pre-experimental research of this subject showed that the dietary intake of rats after sleeve gastrectomy was significantly reduced, from 20 g/d to 10-15 g/d. This result is consistent with the trend of decreased weight and decreased glucose tolerance index. During the same period, the food intake was controlled in the non-surgical group. The results showed that the reduction in food intake had no significant effect on the level of ghrelin. Whether it is related to the relatively short research time is not clear. However, the results further confirmed that ghrelin signaling plays a potential regulatory role in sleeve gastrectomy for T2DM.

Since weight-loss surgery involves the gastrointestinal tract, the interaction of intestinal flora with metabolic lesions after weight-loss surgery has become a new area of research. There are more studies about Roux-en-Y gastric bypass surgery, but less about sleeve gastrectomy on intestinal flora in patients with T2DM after surgery[36-38]. Compared with Roux-en-Y gastric bypass surgery, sleeve gastrectomy has less effect on intestinal structure and intestinal flora. Whether there is a similar effect on intestinal flora has not been reported. Therefore, part of the exploration was done in this experiment. Our experiment found that sleeve gastrectomy not only could lose weight, but also significantly improve blood glucose metabolism. Furthermore, postoperative rats were tested for intestinal flora, the results showed that there were no significant differences between phylum of the three groups in the heat map, the abundance of *Phylum Firmicutes* and *Bacteroidetes* was higher. Compared with the sleeve gastric excision group, the abundance of *Phylum Firmicutes* in the fake surgical group and the non-surgical group was higher, and that of the *Bacteroidetes* was lower. The genera of the three groups were significantly different. Compared with the sleeve gastric excision group, there were more genera in the fake surgical group and the non-surgical group. The high abundance genera in the three groups were Lactobacillus and *Bacteroidetes*. Compared with sleeve gastric excision group, the abundance of *Phylum Firmicutes* in the fake surgical group and non-operation group was higher and the abundance of *Bacteroidetes* was lower. It can be seen that the intestinal flora of rats with T2DM has changed after sleeve gastrectomy. Although there was no difference in the type of phylum, the proportion of *Phylum Firmicutes* decreased and the proportion of *Bacteroidetes* increased. Gastrointestinal hormones were involved in the formation of intestinal flora after weight-loss surgery, which were considered to be an important link to improve the degree of metabolism. It has been reported that the proportion of *Phylum Firmicutes* and *Proteobacteria* of obese and non-obese individuals significantly increased after Roux-en-Y gastric bypass surgery[39,40]. The mechanism responsible for these changes is unknown. It is speculated that changing the structure of the digestive tract, especially shortening the small intestine, is more conducive to facultative anaerobic bacteria growth, rather than strictly anaerobic bacteria, such as *Phylum Firmicutes*. In addition, sleeveless gastrectomy allows incompletely digested food to quickly enter the colon, further changing the intestinal environment. *Firmicutes* is closely related to obesity, and the proportion of *Phylum Firmicutes* in obese individuals is higher. In our experimental research,

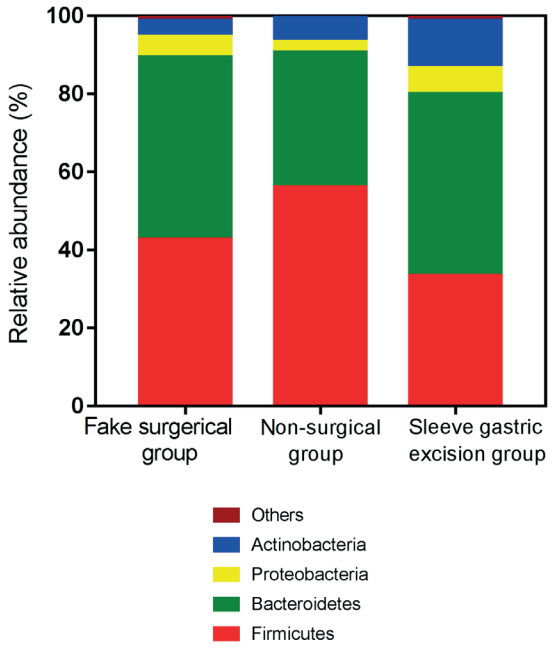

Figure 4. Phylum and abundance of intestinal flora in the three groups after surgery.

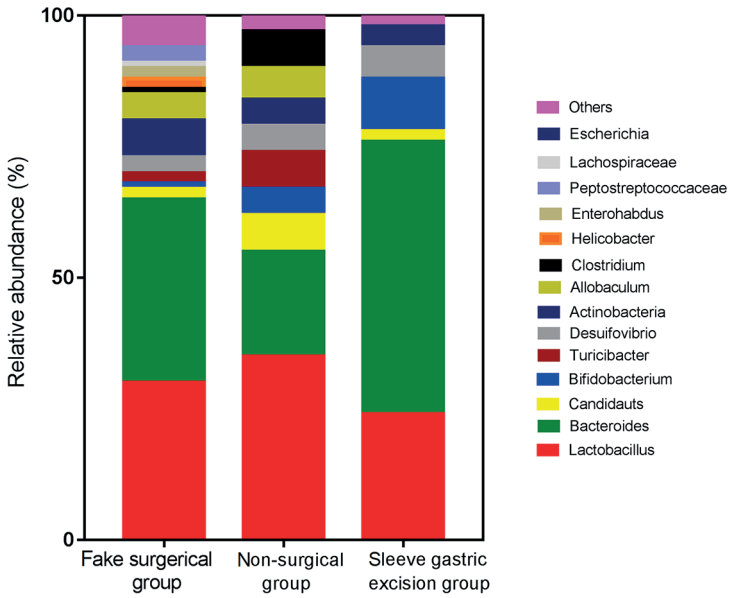

Figure 5. Genera and abundance of intestinal flora in the three groups after surgery.

after a high-fat and high-sugar diet, the relative abundance of *Phylum Firmicutes* in rats significantly increased and, at the same time, the relative abundance of *Bacteroidetes* decreased. After sleeve gastrectomy, the ratio of intestinal flora in rats was significantly adjusted. Therefore, it can be considered that changes in the intestinal flora may also be one of the important mechanisms of sleeve gastrectomy for T2DM. Ghrelin works through its receptor, the growth hormone secretory receptor (GHSR)-1, such as maintaining energy balance, food intake and metabolism[41,42]. Correspondingly, the gastrointestinal flora and its metabolites can also regulate GPCR signaling, which in turn affects ghrelin level, such as lactic acid and bacterial supernatant can reduce ghrelin level, showing that ghrelin is closely related to the intestinal flora. At present, the research on intestinal flora is mainly focused on observations, the relationship between intestinal flora changes and gastrointestinal surgery and metabolism needs further studies.

Conclusions

Apart from losing the weight of rats in the T2DM rat model, sleeve gastrectomy can also reduce blood glucose level in rats with T2DM, increase islet β-cell regulatory function and improve insulin resistance. The related mechanism may be related to upregulation of ghrelin and regulation of intestinal bacteria. Sleeve gastrectomy results in reducing food intake, and the impact of reduced food intake on the above indicators remains to be further studied.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes mellitus Atlas: global estimates of the prevalence of diabetes mellitus for 2011 and 2030. Diabetes Mellitus Res Clin Pract 2011; 94: 321.
2) Koro CE, Bowlin SJ, Bourgeois N, Fedder DO. Glycemic control from 1988 to 2000 among U.S. adults diagnosed with type 2 diabetes mellitus: a preliminary report. diabetes mellitus Care 2004; 27: 17-20.
3) Lee WJ. Recent advances in bariatric/metabolic surgery: appraisal of clinical evidence. J Biomed Res 2015; 29: 98-104.

4) Wang Q, Tang W, Rao WS, Song X, Shan CX, Zhang W. Changes of ghrelin/GOAT axis and m TOR pathway in the hypothalamus after sleeve gastrectomy in obese type-2 diabetes mellitus rats. World J Gastroenterol 2017; 34: 48-58.
5) Kijima M. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402: 656.
6) Granata R, Baragli A, Settanni F, Scarlatti F, Ghigo E. Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. J Molecul Endocrinol 2010; 45: 107-118.
7) An W, Li Y, Xu G, Zhao J, Xiang X, Ding L, Li J, Guan Y, Wang X, Tang C, Li X, Mulholland M, Zhang W. Modulation of ghrelin O-Acyltransferase expression in pancreatic islets. Cell Physiol Biochem 2010; 26: 707-716.
8) Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005; 85: 495-522.
9) Zhang W, Chen M, Chen X, Segura BJ, Mulholland MW. Inhibition of pancreatic protein secretion by ghrelin in the rat. J Physiol 2010; 537: 231-236.
10) Zhang W, Hu Y, Lin TR, Fan Y, Mulholland MW. Stimulation of neurogenesis in rat nucleus of the solitary tract by ghrelin. Peptides 2005; 26: 2280-2288.
11) Gualillo O, Lago F, Dieguez C. Introducing GOAT: a target for obesity and anti-diabetic drugs? Trends Pharmacoll Sci 2008; 29: 398-401.
12) Sun Y, Asnicar M, Smith RG. Central and peripheral roles of ghrelin on glucose homeostasis. Neuroendocrinol 2007; 86: 215-228.
13) Kouno T, Akiyama N, Ito T, Okuda T, Nanchi I, Notoya M, Oka S, Yukioka H. Ghrelin O-acyltransferase knockout mice show resistance to obesity when fed high-sucrose diet. J Endocrinol 2015; 228: 115-125.
14) Clarke SF, Murphy EF, Nilaweera K, Ross PR, Shanahan F, O'Toole PW, Cotter PD. The gut microbiota and its relationship to diet and obesity. Gut Microbes 2012; 3: 186-202.
15) Zhang H, Dibaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci 2009; 106: 2365-2370.
16) Li JV, Ashrafian H, Bueter M, Kinross J, Sands C, le Roux CW, Bloom SR, Darzi A, Athanasiou T, Marchesi JR, Nicholson JK, Holmes E. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. Gut 2011; 60: 1214-1223.
17) [No authors listed]. Total gastrectomy with Roux-en-Y anastomosis. AORN J 2018; 108: 13-15.
18) Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 2005; 102: 11070-11075.
19) Hickey MS, Pories WJ, Macdonald KG, Cory KA, Dohm GL, Swanson MS, Israel RG, Barakat HA, Considine RV, Caro JF, Houmard JA. A new paradigm for type 2 diabetes mellitus: could it be a disease of the foregut? Ann Surg 1998; 227: 637-643; discussion 643-644.
20) Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, Nanni G, Pomp A, Castagneto M, Ghirlanda G, Rubino F. Bariatric surgery versus conventional medical therapy for type 2 diabetes mellitus. N Engl J Med 2012; 366: 1577-1585.
21) Eid GM, Brethauer S, Mattar SG, Titchner RL, Gourash W, Schauer PR. Laparoscopic sleeve gastrectomy for super obese patients. Ann Surg 2012; 256: 262-265.
22) Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE, Bhatt DL. Bariatric surgery versus intensive medical therapy in obese patients with diabetes mellitus. N Engl J Med 2012; 366: 1567-1576.
23) Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. long-term persistence of hormonal adaptations to weight loss. N Engl J Med 2011; 365: 1597-1604.
24) Lee WJ. Recent advances in bariatric/metabolic surgery: appraisal of clinical evidence. J Biomed Res 2015; 29: 98-104.
25) Wang Q, Tang W, Rao WS, Song X, Shan CX, Zhang W. Changes of ghrelin/GOAT axis and m TOR pathway in the hypothalamus after sleeve gastrectomy in obese type-2 diabetes mellitus rats. World J Gastroenterol 2017; 34: 48-58.
26) Yada T, Dezaki K, Sone H, Koizumi M, Damdindorj B, Nakata M, Kakei M. Ghrelin regulates insulin release and glycemia: physiological role and therapeutic potential. Curr Diabetes Rev 2008; 4: 18-23.
27) Dezaki K, Sone H, Yada T. Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. Pharmacol Theraps 2008; 118: 239-249.
28) Khatib N, Gaidhane S, Gaidhane AM, Khatib M, Simkhada P, Gode D, Zahiruddin QS. Ghrelin: ghrelin as a regulatory peptide in growth hormone secretion. J Clin Diag Res 2014; 8: 13-17.
29) Chen CY, Asakawa A, Fujimiya M, Lee SD, Inui A. Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev 2009; 61: 430-481.
30) Chen CY, Inui A, Asakawa A, Fujino K, Kato I, Chen CC, Ueno N, Fujimiya M. Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. Gastroenterol 2005; 129: 8-25.
31) Pacifico L, Poggiogalle E, Costantino F, Anania C, Ferraro F, Chiarelli F, Chiesa C. Acylated and non-acylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome. Eur J Endocrinol 2009; 161: 861-870.
32) Kumar R, Salehi A, Rehfeld JF, Höglund P, Lindström E, Håkansona R. Proghrelin peptides: Desacyl ghrelin is a powerful inhibitor of acylated ghrelin, likely to impair physiological effects of acyl ghrelin but not of obestatin: a research of pancreatic poly-

peptide secretion from mouse islets. Regul Pept 2010; 164: 65-70.

33) Ezquerro S, Méndez-Giménez L, Becerril S, Moncada R, Valentí V, Catalán V, Gómez-Ambrosi J, Frühbeck G, Rodríguez A. Acylated and desacyl ghrelin are associated with hepatic lipogenesis, β-oxidation and autophagy: role in NAFLD amelioration after sleeve gastrectomy in obese rats. Sci Rep 2016; 6: 39942.

34) Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents. Diabetes 2004; 53: 3142-3151.

35) Katsuki A, Urakawa H, Gabazza E, Murashima S, Nakatani K, Togashi K, Yano Y, Adachi Y, Sumida Y. Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. Eur J Endocrinol 2004; 151: 573-577.

36) López-Ferreras L, Richard JE, Anderberg RH, Nilsson FH, Olandersson K, Kanoski SE, Skibicka KP. Ghrelin's control of food reward and weight in the lateral hypothalamic area is sexually dimorphic. Physiol Behav 2017; 176: 40-49.

37) Clément K. Weight-loss surgery, adipose tissue and gut microbiota. Int J Obes 2011; 35: S7-S15.

38) Aronwisnewsky J, Doré J, Clement K. The importance of the gut microbiota after weight-loss surgery. Nat Rev Gastroenterol Hepatol 2012; 9: 590-598.

39) Zhang H, Dibaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci 2009; 106: 2365-2370.

40) Li JV, Ashrafian H, Bueter M, Kinross J, Sands C, le Roux CW, Bloom SR, Darzi A, Athanasiou T, Marchesi JR, Nicholson JK, Holmes E. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. Gut 2011: 1214-1223.

41) Lach G, Schellekens H, Dinan TG, Cryan JF. Anxiety, depression and the microbiome: a role for gut peptides. Neurotherapeutics 2018; 15: 36-59.

42) Torres-Fuentes C, Golubeva AV, Zhdanov AV, Wallace S, Arboleya S, Papkovsky DB, El Aidy S, Ross P, Roy BL, Stanton C, Dinan TG, Cryan JF, Schellekens H. Short-chain fatty acids and microbiota metabolites attenuate ghrelin receptor signaling. FASEB J 2019; 33; 13546-13559.