

61032-Answering Reviewers

Dear editors and reviewers:

Thank you very much for giving us an opportunity to revise our manuscript. We appreciate the editors and reviewers very much for their constructive comments and suggestions on our manuscript entitled "Effect of Oligofructose on Resistance to Postoperative High-Fat Diet-induced Damage of Metabolism in Diabetic Rats after Sleeve Gastrectomy" (Manuscript NO: 61032).

We have studied reviewers' comments carefully. According to the reviewers' detailed suggestions, we have made a careful revision on the original manuscript. All revised portions are marked in red in the revised manuscript (Document attachment) which we would like to submit for your kind consideration. The main corrections are in the manuscript and the responds to the reviewers' comments are as follows (the replies are highlighted in blue).

Replies to the reviewers' comments:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This is an interesting study about the oligofructose on resistance to postoperative high-fat diet-induced damage of metabolism in diabetic rats after sleeve gastrectomy. In this study, the authors studied the function and mechanism of oligofructose on diabetic remission in diabetic rats after sleeve gastrectomy. The animal models are good, and the research methods are reasonable. Results are good. Minor comments: 1. The manuscript requires a minor language editing; 2. Some abbreviations in the main text should be spell out, and I suggest authors to check the manuscript

throughout. 3. The limit of the study is well discussed. Are there any plan to do further experimental about this topic? If so, please indicate it in the discussion. 4. References are updated, and well discussed. 5. Information in figures are good. The figures are too small, please provide the original figure documents.

1. The manuscript requires a minor language editing

Response: We had a native-English speaker that edited the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability to improve the manuscript. And made some changes in the manuscript, these changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

2. Some abbreviations in the main text should be spell out, and I suggest authors to check the manuscript throughout.

Response: We have checked the manuscript throughout and carefully. We spell out the abbreviations in page 3, line16, 18 and 19, page 6, line 23 and 26 of the revised manuscript.

3. The limit of the study is well discussed. Are there any plan to do further experimental about this topic? If so, please indicate it in the discussion.

Response: We indicated the further plan of this study in page 14, line 30 to 32, page 15, line 1-3 in the revised manuscript.

4. References are updated, and well discussed.

Response: We sincerely appreciate the comments.

5. Information in figures are good. The figures are too small, please provide the original figure documents.

Response: We updated the figures in the revised manuscript and finished the

figures in a powerpoint that was uploaded via the publishing system.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Bariatric surgery has been considered as the most effective treatment for type 2 diabetes mellitus. How to reduce or delay the recurrence of diabetes after surgery is a severe problem that the clinicians have to face. Prebiotics are non-digestible oligosaccharides, such as oligofructose, galactooligosaccharides, lactulose and inulin. Prebiotics can promote the loss of body weights and improve the metabolism of glucose and lipid in rodents and human. The mechanisms for these benefits may be due to the reduction in energy intake, regulation of gut microbiota, improved low-grade inflammation and increased gut hormones, which stimulates us to explore whether prebiotics can reduce or delay the recurrence of diabetes after surgery. In the present study, we conducted SG on nicotinamide-streptozotocin-HFD induced diabetic model rats with metabolic characteristics of human diabetes, and stimulated the recurrence of diabetes with postoperative HFD feeding. This study is very well designed, the methods are clearly described. Results are very interesting. In my opinion, this manuscript can be accepted after a minor editing.

1. In my opinion, this manuscript can be accepted after a minor editing.

Response: We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Science editor: 1 Scientific quality: The manuscript describes a basic study of

the effect of oligofructose on resistance to postoperative high-fat diet-induced damage of metabolism in diabetic rats after sleeve gastrectomy. The topic is within the scope of the WJD. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: This is an interesting study about the oligofructose on resistance to postoperative high-fat diet-induced damage of metabolism in diabetic rats after sleeve gastrectomy. In this study, the authors studied the function and mechanism of oligofructose on diabetic remission in diabetic rats after sleeve gastrectomy. The animal models are good, and the research methods are reasonable. The questions raised by the reviewers should be answered; and (3) Format: There are 5 figures. A total of 47 references are cited, including 13 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B and Grade B. A language editing certificate issued by AJE was provided. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by Science Fund for Creative Research Groups of the National Natural Science Foundation of China. The topic has not previously been published in the WJD. 5 Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text. 6 Recommendation: Conditional acceptance.

1.The authors did not provide the approved grant application form

Response: [We have uploaded the approved grant application form via the F6Publishing system.](#)

2. The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Response: [We updated the figures in the revised manuscript and finished the figures in a powerpoint that all graphs or arrows or text portions can be reprocessed, then it was uploaded via the F6Publishing system.](#)

3. The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text.

Response: [We added the “Article Highlights” in page 15 to 16, which was written in strict accordance with relevant requirements.](#)

Once again, thank you very much for your constructive comments and suggestions which would help us both in English and in depth to improve the quality of the paper.

Kind regards,

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Basic Study

Effect of Oligofructose on Resistance to Postoperative High-Fat Diet-induced Damage of Metabolism in Diabetic Rats after Sleeve Gastrectomy

Zhong MW *et al.* Effect of oligofructose on Diabetic Rats

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Author contributions: Zhong MW, Li Y, Liu QR, Hu SY, Zhang GY contributed to the conception of the manuscript, design of experiments, and analysis and interpretation of the data, and writing of the manuscript; Zhong MW, Li Y, Cheng YG performed the experiments, analyzed the data, and wrote the manuscript; all authors have commented on the initial and final drafts of the manuscript and are responsible for approval of the final version of the manuscript in all aspects.

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Abstract

BACKGROUND

Sleeve gastrectomy (SG) can induce prominent remission of type 2 diabetes mellitus (T2DM). However, the long-term remission rate of diabetes usually decreases over time. Oligofructose has been verified to modulate host metabolism. The aim of this study is to explore the protective effect of oligofructose on high-fat diet (HFD)-induced metabolic dysfunction after SG.

AIM

To study the effect and mechanism of oligofructose on diabetic remission in diabetic rats after sleeve gastrectomy.

METHODS

SG and SHAM operation were performed on diabetes rats induced by HFD, nicotinamide and low-dose streptozotocin (STZ). Then the rats in SHAM and SG groups were continuously provided with HFD, and the rats in **sleeve gastrectomy- oligofructose (SG-OF)** group were provided with a specific HFD containing 10% oligofructose. Body weight, calorie intake, **oral glucose tolerance test (OGTT)**, **homeostasis model assessment of insulin resistance (HOMA-IR)**, lipid profile, serum insulin, glucagon-like peptide 1 (GLP-1), total bile acids (TBA), lipopolysaccharide (LPS) and colonic microbiota levels were determined and compared at the designated time points. All statistical analysis was performed using SPSS version 19.0 (IBM, USA), and the statistically significant difference was considered at $P < 0.05$.

RESULTS

At 2 weeks after surgery, rats that underwent SG exhibited improved indexes of glucose and lipid metabolism. Compared with SG group, the rats from SG-OF group exhibited better parameters of glucose and lipid metabolism, lower

body weight (526.86 ± 21.51 vs 469.25 ± 21.84 , $P < 0.001$), calorie intake (152.14 ± 9.48 vs 129.63 ± 8.99 , $P < 0.001$), HOMA-IR (4.32 ± 0.57 vs 3.46 ± 0.52 , $P < 0.05$), and LPS levels (0.19 ± 0.01 vs 0.16 ± 0.01 , $P < 0.05$), and higher levels and secretion of insulin (1.17 ± 0.17 vs 1.58 ± 0.16 , $P < 0.001$), GLP-1 (12.39 ± 1.67 vs 14.94 ± 1.86 , $P < 0.001$), and relative abundances of *Bifidobacterium* (0.0034 ± 0.0014 vs 0.0343 ± 0.0064 , $P < 0.001$), *Lactobacillus* (0.0161 ± 0.0037 vs 0.0357 ± 0.0047 , $P < 0.001$) and *Akkermansia_muciniphila* (0.0050 ± 0.0024 vs 0.0507 ± 0.0100 , $P < 0.001$) at the end of the study. However, no difference in TBA levels was observed between the two groups.

CONCLUSION

Oligofructose partially prevents HFD-induced metabolic damage of glucose and lipid after SG, which may be due to the changes of calorie intake, insulin, GLP-1, LPS and the gut microbiota in rats.

Key words: Sleeve gastrectomy; Oligofructose; Diabetes; Gut microbiota; Lipopolysaccharide; Glucagon-like peptide 1

Core tip: Bariatric surgery is one of the important methods to treat obesity and type 2 diabetes mellitus, but the remission and recurrence of type 2 diabetes mellitus after surgery is still a hot issue. In this study, we have demonstrated that oligofructose can partially resist the HFD-induced metabolic damage of glucose and lipid after SG in rats, and the influence of oligofructose on calorie intake, insulin, GLP-1, LPS and gut microbiota may play an important role in the improving function.

INTRODUCTION

Bariatric surgery has been considered as the most effective treatment for type 2 diabetes mellitus (T2DM)^[1, 2]. Over the past decade, the total number of bariatric surgery worldwide is increasing, particularly sleeve gastrectomy (SG). Currently, SG is the most frequently used procedure in the USA/Canada and in Asian/Pacific regions^[3]. Although bariatric surgery induces rapid and prominent remission of T2DM, the long-term remission rate usually decreases over time, and the recurrence of T2DM is observed in a part of patients with initial remission after bariatric surgery^[4-7]. As a novel type of bariatric surgery, SG is **characterized by** lower complication rate, faster operation, less technical requirements, and less postoperative nutritional problems^[8], so that a marked increase in the relative application rate of SG has been observed^[3]. Many investigators have reported that SG and Roux-en-Y Gastric Bypass (RYGB) surgery have equal treatment efficacy for diabetes^[9,10]. However, long-term randomized controlled comparison for the impact of SG coupled with RYGB as the gold standard in subjects with T2DM is surprisingly limited. The long-term effect of SG on diabetes is questionable^[8]. What is more, SG has been reported to be an independent predictor of the relapse of diabetes at 48.7 months of follow-up^[5]. How to reduce or delay the recurrence of diabetes after surgery is a severe problem that the clinicians have to face.

Prebiotics are non-digestible oligosaccharides, such as oligofructose, galactooligosaccharides, lactulose and inulin. Prebiotics promote the loss of body weights and improve the metabolism of glucose and lipid in rodents and human^[11-15]. The mechanisms **underlying** these benefits may be due to the reduction in energy intake, regulation of gut microbiota, **improvements in** low-grade inflammation and increased levels of gut hormones, such as glucagon-

like peptide-1 (GLP-1) and peptide YY (PYY)^[16-19], which prompted us to explore whether prebiotics reduce or delay the recurrence of diabetes after surgery.

A high-calorie diet appears to be one of primary factors **contributing** obesity and diabetes^[20], and our previous studies have confirmed that a high-fat diet (HFD) induces the deterioration of glucose tolerance after an initial improvement in diabetic rats subjected to duodenal-jejunal bypass (DJB)^[21]. In the present study, we conducted SG on nicotinamide (NA)-streptozotocin (STZ)-HFD-induced diabetic model rats with metabolic characteristics of human diabetes^[22, 23, 24], and stimulated the recurrence of diabetes with postoperative HFD feeding. The glucose and lipid profiles, serum **levels of such as** insulin, GLP-1, total bile acids (TBA), and lipopolysaccharide (LPS) and changes **in the composition of the** gut microbiota were determined and compared.

MATERIALS AND METHODS

Animals and Diets

Forty eight-week old male Wistar rats (Laboratory Animal Center of Shandong University, Jinan, China) were individually housed in independent ventilated cages (IVC) at a constant temperature (24–26 °C), humidity (50–60%), and a light-dark cycle (12 h light: 12 h dark). All animal procedures were approved by the Institutional Animal Care Committee of the First Affiliated Hospital of Shandong First Medical University. All rats were provided a HFD (40% of calories as fat, Huafukang Biotech Company, Beijing, China) for 4 weeks to induce insulin resistance, and then treated with a single intraperitoneal injection of NA (170 mg·kg⁻¹, Sigma, St. Louis, MO, United States) followed by a single injection of STZ (65 mg·kg⁻¹, Sigma, St. Louis, MO, United States) 15 min later after 12 h of fasting to induce a diabetic state. **Two weeks after STZ injection**, 29 rats were selected as diabetic rats with a fasting blood glucose level higher than 7.1 mmol/L or a blood glucose level after gavage for 2 h higher than 11.1 mmol/L during the **oral glucose tolerance test (OGTT)**, and the rats

with extreme hyperglycemia (blood glucose level > 16.7 mmol/L) were excluded from the study^[22, 23,24]. The diabetic rats were randomly allocated to the SHAM group (n = 9), SG group (n = 10) and **sleeve gastrectomy-oligofructose (SG-OF)** group (n = 10). The rats in SHAM and SG groups were continuously provided with the HFD after the operation, and the rats in SG-OF group were provided with a specific HFD supplemented with 10% oligofructose (Huafukang Biotech Company, Beijing, China) since 2 weeks after surgery. Body weight and calorie intake were measured at baseline, and 2, 12, and 24 weeks after surgery.

Surgical Techniques

The diabetic rats were fed a low-residue diet from 48 h before the operation to 72 h after the operation. Then, they were allowed to ad libitum access to the HFD and water. Rats were anesthetized with a 10% chloral hydrate solution during surgery. The surgical processes were performed **as described in** our previous study and a published report^[25, 26].

SG surgery: (1) A 4 cm midline abdominal incision was made from the xiphoid process; (2) The gastric omentum was dissected to disclose the gastric cardium; (3) Short gastric vessels, corresponding gastroepiploic vessels, and the branches of left gastric vessels in the greater curvature were ligated and transected using a 7-0 silk suture (Ningbo Medical Needle, Ningbo, Zhejiang, China); (4) A vena caval clamp was placed to occlude stomach wall through cardium to pylorus for the prevention of bleeding; (5) The gastric fundus and a large portion of gastric body were removed (70% of the total stomach); and (6) the residual stomach was closed using a 7-0 silk suture (Ningbo Medical Needle, Ningbo, Zhejiang, China).

SHAM surgery: A similar abdominal incision was made as described for the SG procedure, and a similar process was conducted, but the gastric fundus and the gastric body were not removed. The operation time of the SHAM surgery was prolonged to the same time as SG surgery to acquire similar surgical and anesthetic stress.

OGTT

OGTT was performed at baseline and 2, 12 and 24 weeks after surgery. After 8 h of fasting, all rats were subjected to gavage with glucose at a dose of 1 g/kg. Blood glucose levels were measured in conscious rats at baseline and 10, 30, 60, 90, and 120 min after gavage.

Serum Parameters

During the OGTT conducted at baseline and 2, 12 and 24 weeks after surgery, blood samples were collected from retrobulbar venous plexus at the baseline and 10, 30, 60, and 120 min after glucose gavage. Blood samples were centrifuged (1006 ×g) at 4 °C for 15 min, and serum was immediately extracted and stored at -80 °C. Fasting serum TBA levels and serum lipid profiles were measured using automatic biochemical analyzer (Hitachi, Tokyo, Japan). Serum insulin, fasting serum GLP-1 and LPS levels were measured using enzyme-linked immuno-sorbent assay (ELISA) kits (insulin: Millipore, MA, USA; GLP-1: Uscn Life Science Inc., Wuhan, Hubei, China; LPS: Bio-Swamp, Wuhan, Hubei, China).

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

At baseline and 2, 12 and 24 weeks after the operation, HOMA-IR was calculated to evaluate insulin resistance using the following formula:

$$\text{HOMA-IR} = \text{fasting insulin (mIU/L)} \times \text{fasting glucose (mmol/L)} / 22.5^{[27]}.$$

16S rDNA Sequence Analysis of Gut Microbiota

The change in the gut microbiota may alter host metabolism partially due to the biological activity of the colonic microbiota. Thus, the colonic contents were collected at 24 weeks after surgery, and stored at -80 °C immediately. Genomic DNA was extracted from the colonic contents using standard methods^[28]. Amplicons of the 16S rRNA gene V4 region were sequenced using the Illumina MiSeq platform (BGI technology, Shenzhen, Guangdong, China).

Statistical Analysis

All data are presented as the means \pm standard deviations ($M \pm SD$). Areas under the curves for OGTT (AUC_{OGTT}) were calculated using the trapezoidal integration. The data in each group were normal distribution by Shapiro-Wilk test. Intergroup comparisons were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc comparison. The concentrations of insulin and total GLP-1 after glucose gavage were analyzed using a mixed model ANOVA followed by Bonferroni post hoc comparison. All statistical analyses were performed using SPSS version 19.0 (IBM, USA), and the statistically significant difference was considered at $P < 0.05$.

RESULTS

Oligofructose promoted the improvement of insulin resistance after sleeve gastrectomy in rats

At the end of this experiment, 9, 7 and 8 rats were alive in SHAM, SG and SG-OF groups respectively. Five rats died of residual stomach leakage after SG. As shown in Figure 1A, the AUC_{OGTT} of the three groups was not significantly different at the baseline assessment. At 2, 12 and 24 weeks after surgery, the AUC_{OGTT} in SG and SG-OF groups were both lower than that in SHAM group. The difference of AUC_{OGTT} between SG group and SG-OF group was observed since 24 weeks after surgery. At 24 weeks after surgery, rats from the SG-OF group showed significantly lower AUC_{OGTT} than rats from the SG group.

HOMA-IR showed a similar trend to the AUC_{OGTT} , as shown in Figure 1B. No significant differences of HOMA-IR among three groups were observed at baseline assessment. At 2 and 12 weeks after surgery, the rats underwent SG showed significantly lower HOMA-IR than that from the SHAM group. At 12 weeks after surgery, the HOMA-IR of the SG group appeared to be higher than that in the SG-OF group, but there was no statistic difference between two groups. At 24 weeks after surgery, the HOMA-IRs in the SHAM and SG groups were comparable, and higher than that in the SG-OF group.

The curves of serum insulin levels measured during OGTT at baseline and 2, 12 and 24 weeks after surgery are shown in Figure 1C, 1D, 1E, and 1F, respectively. No significant difference of serum insulin levels was observed among the three groups at baseline assessment. **A greater amount of insulin was secreted into the serum in the SG and SG-OF groups during the OGTT than in the SHAM group at 2, 12 and 24 weeks after surgery. A difference in the secretion of insulin into the serum was observed between the SG and SG-OF groups, and detected earlier than changes in the AUC_{OGTT} and HOMA-IR. A lower amount of insulin was secreted into the serum of the SG group than the SG-OF group beginning at 12 weeks after surgery.**

Oral oligofructose can improve postoperative lipid profiles

Fasting serum triglyceride and cholesterol levels are shown at Figure 2A and 2B. There was no significant difference in lipid profiles among the three groups at baseline assessment. And triglyceride and cholesterol in the SG and SG-OF groups were significantly lower than those in the SHAM group at 2 and 12 weeks after surgery. At 12 weeks after surgery, the SG-OF group showed a lower triglyceride level than the SG group and a comparable cholesterol level to the SG group. At 24 weeks after surgery, the SHAM group showed the highest triglyceride level among three groups and the triglyceride level in the SG group was higher than that in the SG-OF group. Cholesterol levels in the SG group were comparable to the levels in the SHAM group, but higher than that in SG-OF group.

The mechanism of diabetes remission may be related to weight loss and energy intake

As shown in Figure 3A and 3B, body weight and calorie intake exhibited a similar trend during this experiment. No significant differences in body weight or calorie intake were observed among the three groups at baseline, and all rats subjected to SG showed a lower body weight and calorie intake than the rats from the SHAM group at 2, 12 and 24 weeks after surgery. The body weight

and calorie intake in the SG-OF group were lower than those in the SG group **beginning at** 12 weeks after surgery.

Oligofructose can increase GLP-1 level and lower LPS level, but has limited effect on TBA

The fasting serum GLP-1 level at baseline and 2, 12 and 24 weeks after surgery was shown in Figure 4A, 4B, 4C and 4D, respectively. All three groups showed similar baseline levels of GLP-1, but the SG and SG-OF groups showed higher GLP-1 levels at all postoperative time points. Compared with the SG group, the SG-OF group exhibited higher GLP-1 levels beginning at 12 weeks after surgery. As shown in Figure 4E, there was no significant difference in LPS levels between groups at baseline assessment. In contrast to the GLP-1 level, the SG and SG-OF groups showed lower LPS levels at all postoperative time points than the SHAM group, and the LPS level in the SG-OF group was lower than in the SG group beginning at 12 weeks after surgery.

The fasting serum TBA levels are shown in Figure 4F. At all postoperative time points, the TBA levels in SG and SG-OF groups were significantly higher than that in the SHAM group, but no significant difference was observed between the SG group and SG-OF group.

Oligofructose can change gut microbiota

As shown in Figure 5A and 5B, *Bacteroidetes* were the predominant gut microbes in the SHAM group while *Firmicutes* were dominant in the SG and SG-OF groups. The relative abundance of *Bifidobacterium* (Figure 5C), *Lactobacillus* (Figure 5D) and *Akkermansia_muciniphila* (Figure 5E) in SG-OF group **was significantly higher than that in the** SHAM and SG groups. There was no difference in relative abundance of *Bifidobacterium* and *Akkermansia_muciniphila* between the SHAM group and SG group. The SG group showed a significantly higher relative abundance of *Lactobacillus* than the SHAM group.

DISCUSSION

Compared with nonsurgical treatment, bariatric surgery has achieved a higher remission rate of T2DM^[2, 29]. In addition, SG is **characterized by** a faster operation, less technical requirements, lower complication rate and less postoperative nutritional problems^[8]. In recent years, SG has been performed more frequently than RYGB in the USA/Canada and in Asian/Pacific regions^[3]. However, as a novel surgical approach, the long-term effect of SG on diabetes is questionable. Suboptimal loss and regain of body weight are associated with noncompliant dietary and lifestyle habits^[30]. Suboptimal loss and regain of body weight have been demonstrated as the factors for insufficient control of diabetes following bariatric surgery^[31, 32]. Prebiotics have been considered as an effective management tool to promote body weight loss and improve metabolism of glucose and lipid^[11-14]. **As shown in our previous study**, HFD reverses the improvements in diabetes after bariatric surgery^[21]. In the present study, we have conducted SG on diabetic rats, and stimulated the reversion of improvements in diabetes with sustained postoperative HFD feeding. Meanwhile, a portion of the SG rats was provided a specific HFD supplemented with 10% oligofructose (one kind of prebiotics) to explore whether oligofructose **prevented the reversion of improvements in** diabetes after SG.

In this study, at two weeks after surgery, the AUC_{OGTT} of the SG rats was significantly lower than that of the SHAM rats. Following postoperative HFD feeding, the AUC_{OGTT} of the SG rats showed a gradually increasing trend. At 24 weeks after surgery, the AUC_{OGTT} of the SG group was significantly higher than that of the SG-OF group. The changing trends of insulin, HOMA-IR, triglyceride and cholesterol levels were similar to the AUC_{OGTT}. Among these factors, the difference in insulin secretion during the OGTT and triglyceride levels (at 12 weeks after surgery) revealed an earlier appearance than others (at 24 weeks after surgery), suggesting that, at the early stage after surgery, SG improves the metabolism of glucose and lipid obviously; postoperative HFD feeding reverses these metabolic improvements, and oligofructose feeding

partially prevents the detrimental effect of HFD feeding. Factors such as body weight, calorie intake, GLP-1 levels, serum TBA levels, LPS levels and the gut microbiota were measured to elucidate the possible mechanisms underlying the protective effect of oligofructose.

The body weight and calorie intake in the SG group are obviously lower than those in the SHAM group at all postoperative time points, and a lower body weight and calorie intake were observed in the SG-OF group but not the SG group beginning at 12 weeks after surgery. Body weight and calorie intake seem to be one of primary factors contributing to diabetes^[20], so we hold the opinion that the lower body weight and calorie intake may be one of the mechanisms for the protective effect of SG and oligofructose.

As one of the most important incretin hormones, GLP-1 is secreted by L-cells that are mainly located in the epithelium of distal ileum and colon^[33]. It regulates glucose homeostasis by stimulating insulin secretion, suppressing glucagon secretion, and promoting proliferation and inhibiting apoptosis of β cells^[34]. In our study, a greater amount of GLP-1 was secreted in rats subjected to SG during the OGTT than in rats in the SHAM group at all postoperative time points, and the higher level of GLP-1 secretion in the SG-OF group than in the SG group was observed beginning at 12 weeks after surgery. GLP-1 has been considered as a mediator in the remission of diabetes after SG^[35], and oligofructose has been demonstrated as the promoter for the production of endogenous GLP-1^[36]. Based on these findings, we have deduced that the function of oligofructose for improving glucose metabolism after SG is partially attributed to the enhanced secretion of GLP-1. The mechanisms for the enhanced secretion of GLP-1 after oligofructose feeding may be due to the increased number of L-cells and colonic fermentation^[36, 37]. Short-chain fatty acids (SCFAs, butyrate, propionate and acetate) are produced through the fermentation of nondigestible carbohydrates in the colon, and it has been reported that the oral administration of butyrate and propionate in mice can significantly increase plasma levels of GLP-1, thus leading to an improvement in insulin sensitivity^[38], which is consistent with another report showing that

SCFAs stimulate free fatty acid receptor 2 (FFA2) that is colocalized in L-cells to increase the secretion of GLP-1^[39]. Unfortunately, SCFAs were not determined in our study.

Bile acids can act as signaling molecules to regulate the metabolism of lipid, glucose and energy, and these regulatory functions of bile acids are predominantly mediated by farnesoid X receptor (FXR) and the G-protein-coupled receptor TGR5^[40]. In this study, at 2 weeks after surgery, fasting serum TBA of the rats subjected to SG is significantly higher than that in SHAM group. This result is coincident with the previous report in human^[41], showing that the increased TBA levels contribute to the improvements in the metabolism of glucose and lipid after SG. However, we did not observe a significant difference in TBA levels between the SG group and SG-OF group at 12 and 24 weeks after surgery, indicating that the protective effect of oligofructose may be independent of the increased TBA levels.

LPS is a component of the cell wall in Gram-negative intestinal microbiota, that triggers low-grade inflammation and results in insulin resistance. A chronic infusion of LPS over a four-week period in chow-fed mice leads to increased adiposity, increased macrophage infiltration in adipose tissues, hepatic inflammation, and hepatic insulin resistance^[42]. **Clinical trials have shown that SG decreases LPS levels^[43], consistent with the outcome reported in our study.** What is more, we have found that LPS levels in the SG-OF group is lower than that in the SG group at 12 and 24 weeks after surgery. A similar effect of oligofructose has also been observed in obese mice without surgery^[15]. So we consider that the protective effect of oligofructose might partially be ascribed to the reduced LPS level.

The gut microbiota has been verified as an environmental factor affecting obesity and diabetes, and can modulate the metabolism of host glucose and lipid^[44]. A recent study has revealed a significantly decreased *Firmicutes* abundance in T2DM patients when compared with nondiabetic individuals^[45]. Meanwhile, a remarkably higher relative abundance of *Firmicutes* and a lower relative abundance of *Bacteroidetes* in rats receiving SG are observed. These

results are **consistent** with the report from Ryan^[46]. However, no difference in the relative abundances of *Firmicutes* and *Bacteroidetes* between SG group and SG-OF group were observed, indicating that the benefit of oligofructose is not associated with the relative abundance of *Firmicutes* and *Bacteroidetes*. Notably, the rats from SG-OF group had remarkably higher relative abundances of *Bifidobacterium*, *Lactobacillus* and *Akkermansia_muciniphila*. And the increased relative abundance of these bacteria has been observed in oligofructose-fed diabetic rodents by other research groups^[19, 47]. The advantage of oligofructose in the control of diabetes may be associated with an improvement in inflammation. Therefore, the effect of oligofructose on protecting the recurrence of diabetes after SG may be partially mediated by the increased abundance of *Bifidobacterium*, *Lactobacillus* and *Akkermansia_muciniphila*, accompanied by the decrease **in the abundance of *Bacteroides***, thus reducing the concentration of LPS.

There are still some limitations in this study. First, although oligofructose partially prevented the HFD-induced metabolic damage of glucose and lipid after SG in rats, further clinical studies are highly desired to validate these results in humans. Second, the effects of oligofructose on calorie intake, insulin levels, GLP-1 levels, TBA levels, LPS levels and the gut microbiota after SG were analyzed in this study; however, the intrinsic mechanism underlying the protective function of oligofructose should be further explored. **We plan to further study the internal mechanism of these changes, especially focusing on the changes of the gut microbiota. At the same time, we are applying for relevant clinical trials to further observe the effects and side effects of probiotics, and we hope that the oligofructose will become a novel target to reduce or delay the recurrence of diabetes after bariatric surgery.**

CONCLUSION

Oligofructose can partially prevent the HFD-induced metabolic damage of glucose and lipid in rats after SG, and the effects of oligofructose on calorie intake, insulin levels, GLP-1 levels, LPS levels and the gut microbiota may

contribute to this protective function.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus is one of the important complications of obesity. Bariatric surgery can treat obesity and diabetes effectively, but the recurrence of diabetes after surgery is still one of the problems to be solved. However, whether oligofructose has an effect on metabolism after bariatric surgery remains to be further studied.

Research motivation

Prebiotics promote the weight loss and improve the metabolism of glucose and lipid. The mechanism of these benefits may be due to reduced energy intake, regulation of gut microbiota, improvement of low-grade inflammation and increase of intestinal hormones, such as glucagon like peptide-1 (GLP-1) and peptide YY (PYY), which prompted us to explore whether prebiotics can reduce or delay the recurrence of diabetes after surgery.

Research objectives

The present study aimed to find the effect and mechanism of oligofructose on diabetic remission in diabetic rats after sleeve gastrectomy.

Research methods

SG and SHAM operation were performed on diabetes rats induced by HFD, nicotinamide and low-dose streptozotocin (STZ). Then the rats in SHAM and SG groups were continuously provided with HFD, and the rats in sleeve gastrectomy- oligofructose (SG-OF) group were provided with a specific HFD containing 10% oligofructose. Body weight, calorie intake, OGTT, HOMA-IR, lipid profile, serum insulin, glucagon-like peptide 1 (GLP-1), total bile acids (TBA), lipopolysaccharide (LPS) and colonic microbiota levels were determined and compared at the designated time points. All statistical analysis

was performed using SPSS version 19.0 (IBM, USA), and the statistically significant difference was considered at $P < 0.05$.

Research results

Oligofructose treatment reduced body weight, energy intake and LPS levels, increased GLP-1 levels, improved insulin resistant and lipid profiles. Oligofructose also altered the gut microbiota in the SG-OF group.

Research conclusions

Oligofructose partially prevents HFD-induced metabolic damage of glucose and lipid after SG in rats, and the effects of oligofructose on calorie intake, insulin, GLP-1, LPS and gut microbiota may contribute to protective function for damaged metabolism.

Research perspectives

The results of this study provide evidence that oligofructose could be a promising agent for the treatment in diabetic rats after SG. Further studies that assess the effect of oligofructose on the mechanism of preventing HFD-induced metabolic damage of glucose and lipid after SG may substantiate our findings and pave the path for clinical translation of the therapeutic effects of oligofructose.

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Footnotes

Institutional review board statement: This study protocol was reviewed and approved by the First Affiliated Hospital of Shandong First Medical University (Approval No.2020S396).

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care Committee of the First Affiliated Hospital of Shandong First Medical University, Jinan, China (Protocol No.2018-006).

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Figure Legends

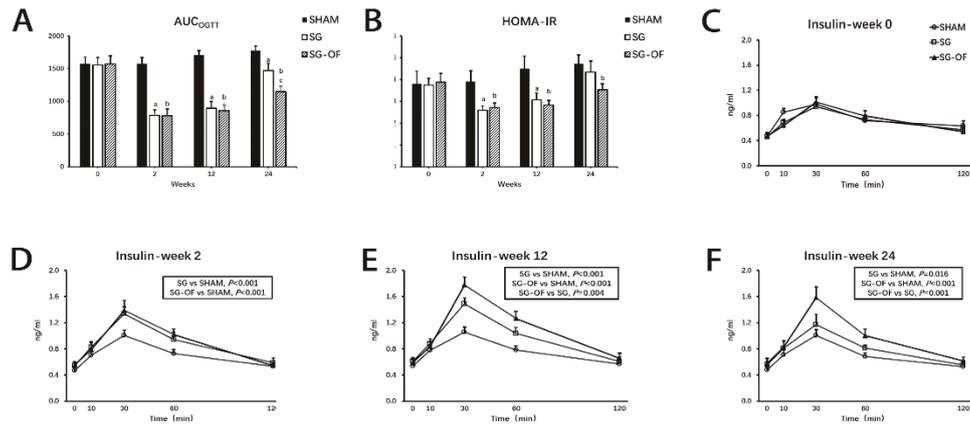


Figure 1 AUC_{OGTT} , HOMA-IR and serum insulin levels. AUC_{OGTT} (A), HOMA-IR (B) and serum insulin levels were measured during the OGTT at baseline (C) and 2 (D), 12 (E) and 24 (F) weeks after surgery (**a** $P < 0.001$, SHAM vs SG; **b** $P < 0.001$, SHAM vs SG_OF; **c** $P < 0.001$, SG vs SG_OF). The concentration of insulin during the OGTT was analyzed using a mixed model ANOVA followed by Bonferroni post hoc comparison. The P value is shown in the rectangular frame. A statistically significant difference was considered at $P < 0.05$.

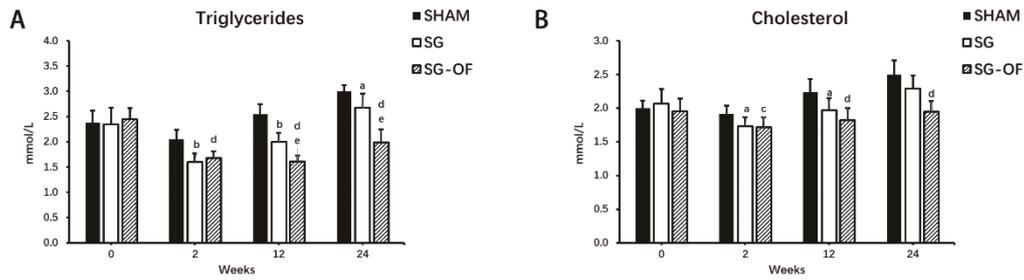


Figure 2 Fasting serum triglyceride and cholesterol levels. The fasting serum triglyceride (A) and cholesterol (B) levels were determined (^aP < 0.05, ^bP < 0.001, SHAM vs SG; ^cP < 0.05, ^dP < 0.001, SHAM vs SG_OF; ^eP < 0.001, SG vs SG_OF). A statistically significant difference was considered at P < 0.05.

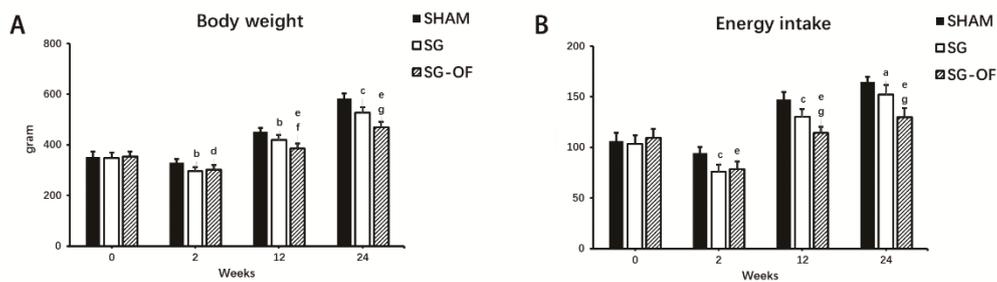


Figure 3 Body weight and calorie intake. The body weight (A) and calorie intake (B) were measured at baseline and 2, 12 and 24 weeks after surgery (^aP < 0.05, ^bP < 0.01, ^cP < 0.001, SHAM vs SG; ^dP < 0.01, ^eP < 0.001, SHAM vs SG_OF; ^fP < 0.01, ^gP < 0.001, SG vs SG_OF). A statistically significant difference was considered at P < 0.05.

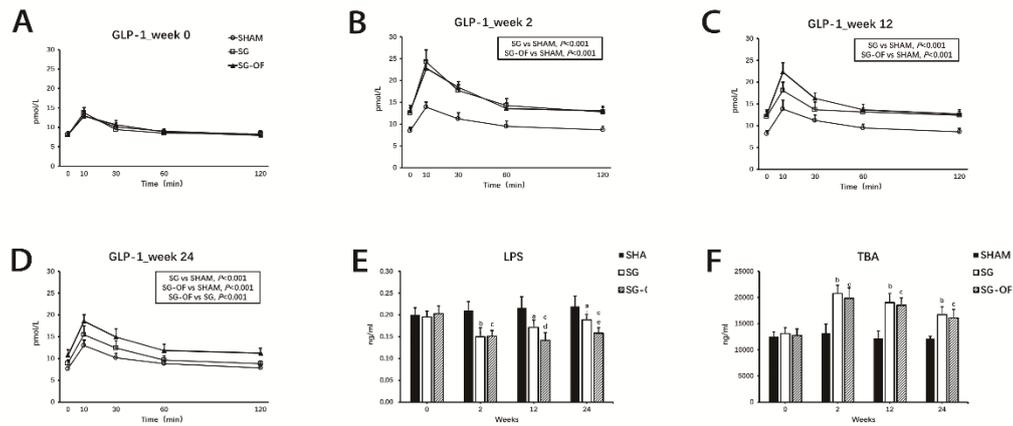


Figure 4 GLP-1, fasting serum LPS and TBA levels. The fasting serum GLP-1 levels were measured at baseline (A) and 2 (B), 12 (C) and 24 (D) weeks after surgery, and the P value is shown in a rectangular frame. The fasting serum LPS (E) and TBA (F) levels were also determined (^aP < 0.01, ^bP < 0.001, SHAM vs SG; ^cP < 0.001, SHAM vs SG_OF; ^dP < 0.05, ^eP < 0.01, SG vs SG_OF). A statistically significant difference was considered at P < 0.05.

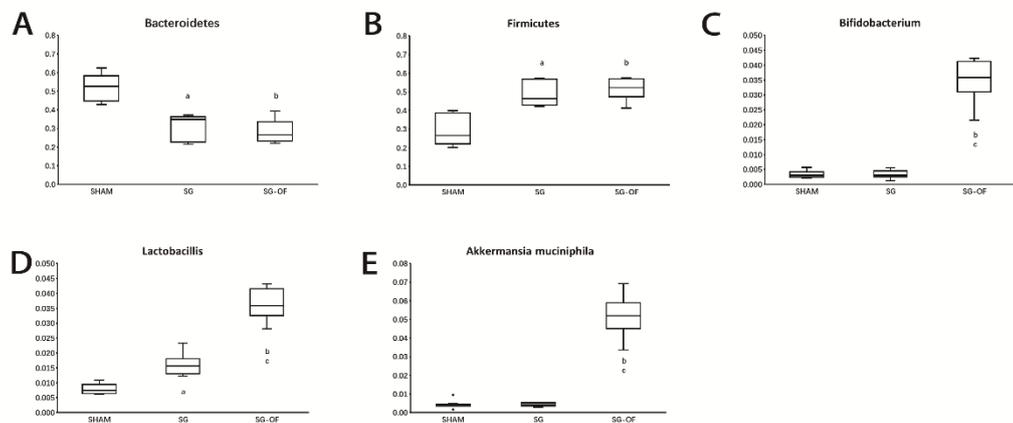


Figure 5 Gut microbiota. The relative abundances of *Bacteroidetes* (A), *Firmicutes* (B), *Bifidobacterium* (C), *Lactobacillus* (D) and *Akkermansia_muciniphila* (E) between groups were analyzed using the ANOVA and a statistically significant difference was considered at P < 0.05. (^aP < 0.001, SHAM vs SG; ^bP < 0.001, SHAM vs SG_OF; ^cP < 0.001, SG vs SG_OF).

