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ORIGINAL CONTRIBUTIONS

The Effect of Gastric Bypass with a Distal Gastric Pouch on Glucose Tolerance and Diabetes Remission in Type 2 Diabetes Sprague-Dawley Rat Model

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Abstract

Background Gastric bypass with a proximal gastric pouch (Roux-en-Y gastric bypass) induces early diabetes remission. The effect of gastric bypass with a distal gastric pouch remains unknown.

Objective To observe the effect on glucose tolerance and diabetes remission of gastric bypass with a distal gastric pouch.

Method A type 2 diabetes (T2D) model was created in 44 Sprague-Dawley (SD) rats that randomly underwent Roux-en-Y gastric bypass (RYGB, $n = 8$); gastric bypass with duodenal-jejunal transit (GB-DJT, $n = 8$); distal-pouch gastric bypass with duodenal-jejunal transit (DPGB-DJT, $n = 8$); distal-pouch gastric bypass with duodenal-jejunal bypass (DPGB-DJB, $n = 8$); sham ($n = 6$); and Roux-en-Y gastric bypass with esophageal re-anastomosis (RYGB-Er, $n = 6$) surgery. In the DPGB-DJT and the DPGB-DJB groups, the gastric pouch was created in the distal stomach. In the RYGB and the GB-DJT groups, the gastric pouch was created in the proximal stomach. An oral glucose tolerance test (OGTT), insulin tolerance test (ITT) and mixed-meal tolerance test (MMTT) conducted preoperatively were repeated postoperatively.

Results GLP-1 AUC recorded preoperatively was significantly increased 8 weeks postoperatively in the RYGB, GB-DJT, and DPGB-DJB groups. Increased GLP-1 AUC in the DPGB-DJT did not reach statistical significance. Improved glucose tolerance in the RYGB and GB-DJT groups was significantly higher than DPGB-DJT group. DPGB-DJB did not improve glucose tolerance significantly. Gastrin level was increased significantly in the DPGB-DJT and DPGB-DJB groups.

Conclusion In gastric bypass, creating the gastric pouch in the distal region of the stomach significantly impairs the glucose tolerance and diabetes remission in spite of the increased GLP-1 and insulin responses in T2D SD rat model, suggesting that bypassing the distal stomach may be the key mediator of early diabetes remission after RYGB.

Keywords Gastric bypass · Type 2 diabetes · Foregut · Duodenal-jejunal bypass · GLP-1 · Gastrin · Distal stomach, antrum

Introduction

Diabetes ranks as the ninth major cause of death globally. It is estimated that 1 in 11 adults globally suffer from diabetes, and most (> 90%) of them have T2D [1].

Over the years, a number of measures have been used to prevent, control, or reverse the progression of diabetes and the associated comorbidities.

Lately, bariatric surgery, particularly Roux-en-Y gastric bypass (RYGB) has emerged as an effective treatment for T2D. Even though the long-term results are still being investigated, RYGB has proving to be superior to conventional treatment for T2D [2, 3]. However, RYGB is invasive and prone to severe complication and malnutrition.

Therefore, unraveling the underlying mechanism of diabetes remission following gastric bypass has attracted significant interest in the hope of developing a noninvasive alternative of similar efficacy to surgery.

Of particular interest lately, is the weight independent or early diabetes remission following RYGB [4].

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One of the leading and widely accepted theories of the mechanisms of early diabetes remission following RYGB is the foregut theory. Proponents of the foregut theory believe that excluding the duodenum and the proximal jejunum in gastric bypass, eliminates pro-diabetic factor(s) produce in the duodenum, which leads to the diabetes remission [5, 6].

The foregut theory is principally based on observations of a significant improvement in glucose tolerance with procedures that involve duodenal-jejunal bypass (DJB) compare to non-DJB procedures [7]. However, recent studies suggest that DJB alone without gastric exclusion in human does not achieve diabetes remission [8, 9].

Proponents of the foregut theory also point to the reversal in glucose tolerance that occurs following the stimulation of the bypassed gastroduodenal-jejunal limb (foregut) in gastric bypass [10] or following the occurrence of gastro-gastric fistula in RYGB [11] as evidence in support of the foregut theory. They believe that the reversal in glucose tolerance with foregut stimulation is a result of the duodenal-jejunal stimulation and therefore, excluding the duodenal-jejunal limb in RYGB leads to the improved glucose tolerance and diabetes remission. Unfortunately, the effect of stimulating the gastric component (gastric remnant) of the foregut on glucose tolerance in the foregut theory has largely remained unexplored.

Therefore, it is not clear whether the early antidiabetic effect of RYGB is due to the bypass of the duodenal-jejunal limb or the remnant stomach. Similarly, it is also unclear whether the reversal in glucose tolerance with foregut stimulation (or following the occurrence of gastro-gastric fistula) after RYGB is due to the gastric or duodenal stimulation.

We recently demonstrated that preserving duodenal-jejunal stimulation in gastric bypass rat model does not impede the glucose tolerance and diabetes remission [12].

In this study, we aim to observe the effects of stimulating the distal (remnant) stomach on the glucose tolerance and diabetes remission in gastric bypass.

To achieve our aim, we created gastric bypass models in rat with the gastric pouch in the distal stomach with DJB and without DJB respectively. Rats were followed for 12 weeks. Glucose tolerance and incretin response were assessed.

Materials and Methods

Fifty male Sprague-Dawley rats, 8–12 weeks old were purchased from the Xuzhou Medical University laboratory Animal Department. Rats were housed individually in shoe box cages under constant ambient temperature and humidity on a 12-h day/night cycles. Care and protocol were in compliance with the national guidelines for the care of animal of the People's Republic of China.

T2D Model

In order to create a T2D model, rats were fed high-fat diet and administered intraperitoneal injection of low-dose streptozotocin (35 mg/kg). Rats presenting with 16 mmol/L or greater random blood glucose on three consecutive tests were selected [13].

Surgical Groups and Technique

Forty-four rats were selected and randomly assigned to the Roux-en-Y gastric bypass (RYGB, $n = 8$); gastric bypass with duodenal-jejunal transit (GB-DJT $n = 8$); distal pouch gastric bypass with duodenal-jejunal transit (DPGB-DJT, $n = 8$); distal pouch gastric bypass with duodenal-jejunal bypass (DPGB-DJB $n = 8$); sham surgery ($n = 6$); and Roux-en-Y gastric bypass with esophageal re-anastomosis (RYGB-Er, $n = 6$) groups.

After an overnight fast, rats were sedated with an intraperitoneal injection of 5% chloral hydrate (0.5 mL/100 g). Under strict sterile condition, the rat was placed on the operating table and the incision site (mid-abdomen) cleaned with 5% povidine iodine without hair removal.

RYGB Surgery and GB-DJT Surgery RYGB surgery (Fig. 1a) and GB-DJT surgery (Fig. 1b) were performed in a similar fashion as described in our previous study [12]. Briefly, after the creation of a 20% gastric pouch around the cardia, the rest of the procedure involved (1) transection of the jejunum 30 cm distal to the Trietz ligament,; (2) anastomosing the distal end of the transected jejunum to the gastric pouch; (3) anastomosing the proximal end of the transected jejunum 10 and 40 cm distal to the gastrointestinal anastomosis in the RYGB and GB-DJT groups respectively; and finally (4) in the GB-DJT group, the construction of a side-to-side anastomosis between the duodenum and jejunum at a point 10 and 2 cm distally to the gastrojejunal anastomosis and pylorus respectively and then followed by closure of the jejunum 2 cm below the duodenal-jejunal anastomosis to guarantee duodenal transit.

DPGB-DJT Surgery and DPGB-DJB Surgery DPGB-DJT surgery (Fig. 1c) and DPGB-DJB surgery (Fig. 1d) involved (1) the creation of a 20% gastric pouch around the pylorus; (2) transecting the esophagus (with care to avoid damage to the vagal nerves); (3) anastomosing the esophagus to the distal gastric pouch; (4) transecting the jejunum 30 cm distal to the Trietz ligament; (5) anastomosing the distal end of the transected jejunum to the proximal gastric remnant; (6) anastomosing the proximal end of the transected jejunum 10 and 30 cm distal to the gastrojejunal anastomosis in the DPGB-DJB and the DPGB-DJT group respectively, and finally; (7) in the DPGB-DJB group, an additional anastomosis was created between the jejunum and the distal gastric pouch at 5 cm distal to the gastric (remnant) jejunal anastomosis, followed by the closure of the duodenum 1 cm below the pylorus to guarantee DJB.

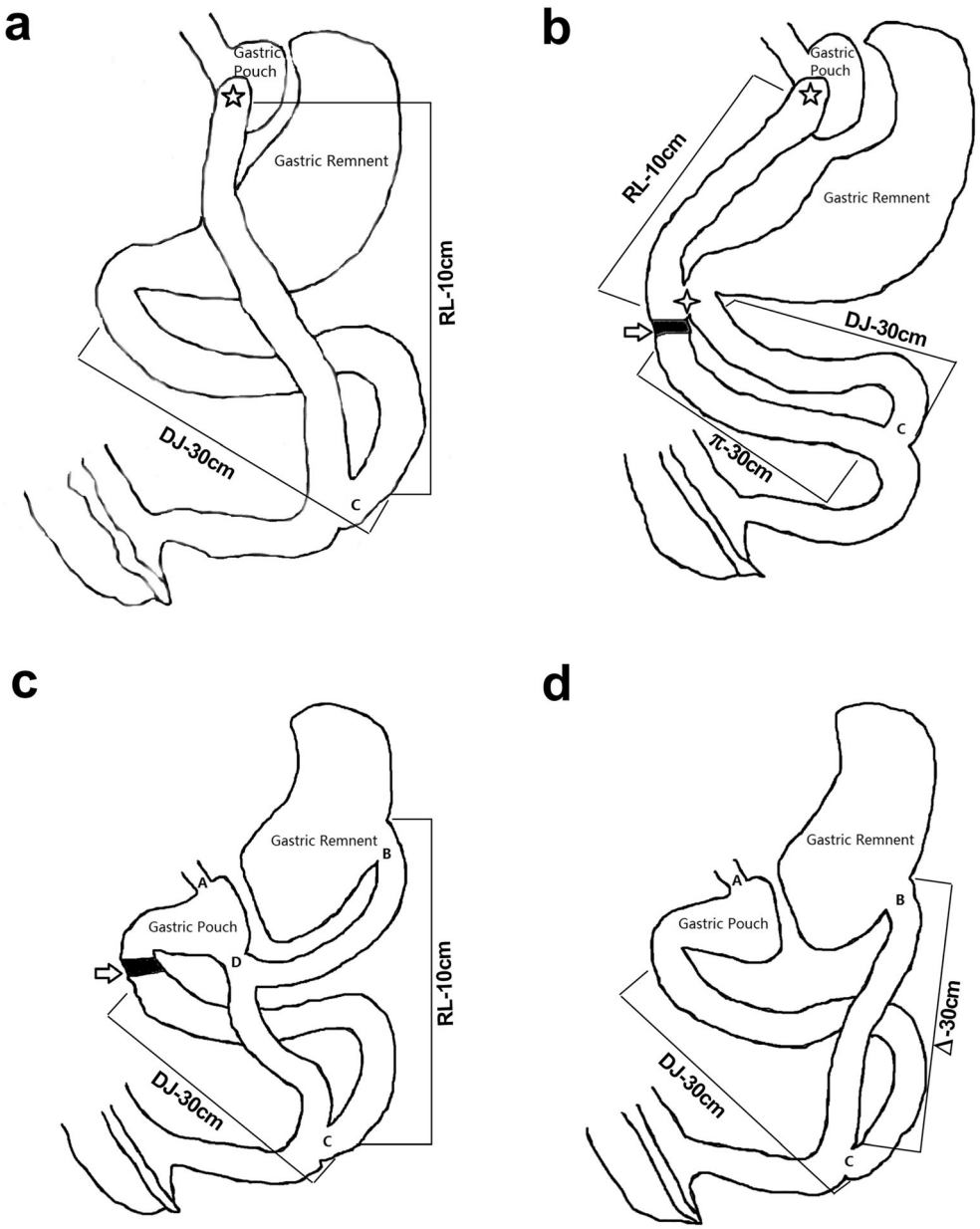

Fig. 1 Illustrates **a** Roux-en-Y gastric bypass (RYGB), **b** gastric bypass with duodenal-jejunal transit (GB-DJT), **c** distal pouch gastric bypass with duodenal-jejunal bypass (DPGB-DJB), and **d** distal pouch gastric bypass with duodenal-jejunal transit (DPGB-DJT). Five-point star, gastrojejunal anastomosis; four-point star, duodenal-jejunal anastomosis; arrowhead, closure of the duodenum; A, gastroesophageal anastomosis; B, gastro (remnant) jejunal anastomosis; C, jejuno-jejunal anastomosis; D, gastro (pouch) jejunal anastomosis; RL, roux limb; DJ, duodenal-jejunal limb; CL, common limb; π, excluded limb to mimic the DJB; Δ, extended pseudo roux limb to mimic the DJB

Sham Surgery Sham surgery involved the transection and direct anastomosis of the bowel 30 cm distal to the Treitz ligament and then the transection and direct re-anastomosis of the esophagus to mimic the bowel and esophageal manipulations respectively. A small incision was made on the stomach and closed immediately to mimic gastric trauma in the surgery groups.

RYGB-Er Surgery Roux-en-Y gastric bypass was performed in similar fashion to the RYGB group, plus esophageal transection (similar to the distal pouch models) and direct re-anastomosis to the proximal pouch as a control for the esophageal manipulation in the distal pouch models.

All anastomoses were constructed with 7-0 silk in a continuous fashion. Ceftriaxone (75 mg/kg) was injected intraperitoneally as antimicrobial prophylaxis before closing the abdomen. The abdomen was closed in layers using a 3-0 non-absorbable suture. Operation time was 60 ± 15 min in the bypass groups and 20 ± 5 min in the sham group.

The study was approved by the ethics committee of Xuzhou Medical University Research Animal Center.

Biochemical Test

Biochemical analyses were made on blood collected from the retro-orbital vein of rats in conscious state. Glucose levels

were ascertained using a hand-held glucose meter in conscious rats. Blood for hormones analysis was collected in chilled ethylenediamine tetraacetic acid (EDTA) tubes treated with protease (DPP-4) inhibitor. After an immediate centrifuge at 3000 rpm for 10 min, serum was collected and stored at − 80 °C awaiting hormone analysis. Assay was performed using an enzyme-linked immunosorbent assay (ELISA) kit according the to manufacturer's instruction (Cloud-Clone Corp, 1304 Langham Creek Dr, Suite 226, Houston, TX 77084 USA). The homeostasis of insulin resistance (HOMA-IR) index [(Fasting glucose × fasting insulin)/22.5] was used to calculated insulin resistance.

A fasting oral glucose tolerance test (OGTT) and an insulin tolerance test (ITT) with oral gavage of 50% glucose solution (3.0 mg/kg) and ITT with an intraperitoneal injection of 0.5 IU/kg of human insulin were performed preoperatively and repeated on separate days, 3 and 10 weeks postoperatively in all the groups. Blood glucose was measured before and then again at 15, 30, 60, 90, and 120 min from the tail vein after intervention using a hand-held gluco-meter in conscious rats.

Glucagon-like peptide-1 (GLP-1), gastric-inhibitory polypeptide (GIP), insulin, gastrin, ghrelin, and glucagon levels were measured on blood samples obtained before and then again at 30 and 60 min after gavage (TPF, 7.68 ml/kg, Nutrison liquid) during a repeat of mixed-meal tolerance test (MMTT) at week 8 postoperatively.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Differences between groups were assessed by one-way analysis of variance (ANOVA, LSD post-test). A Student *t* test was used to assess differences within group. Statistics were performed using SPSS, version 18.0, statistical software (SPSS Inc., Chicago, IL). The trapezoidal integration was used to calculate areas under curve (AUC). All test were two-tailed (statistically significant with $p < 0.05$).

Results

Body Weight, Food Intake and Fasting Blood Glucose

The change in body weight, food intake, and fasting blood glucose are illustrated in Figs. 2 a, b, and c respectively.

Body weight decrease significantly ($p < 0.05$) following operation in all the surgical groups. The decreases in mean body weight 12 weeks after operation were 11.7 ± 1.5%, 12.7 ± 1.9%, 14.0 ± 4.1%, and 12.0 ± 1.3% in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. The amount of decrease in body weight did not differ significantly among the gastric bypass groups.

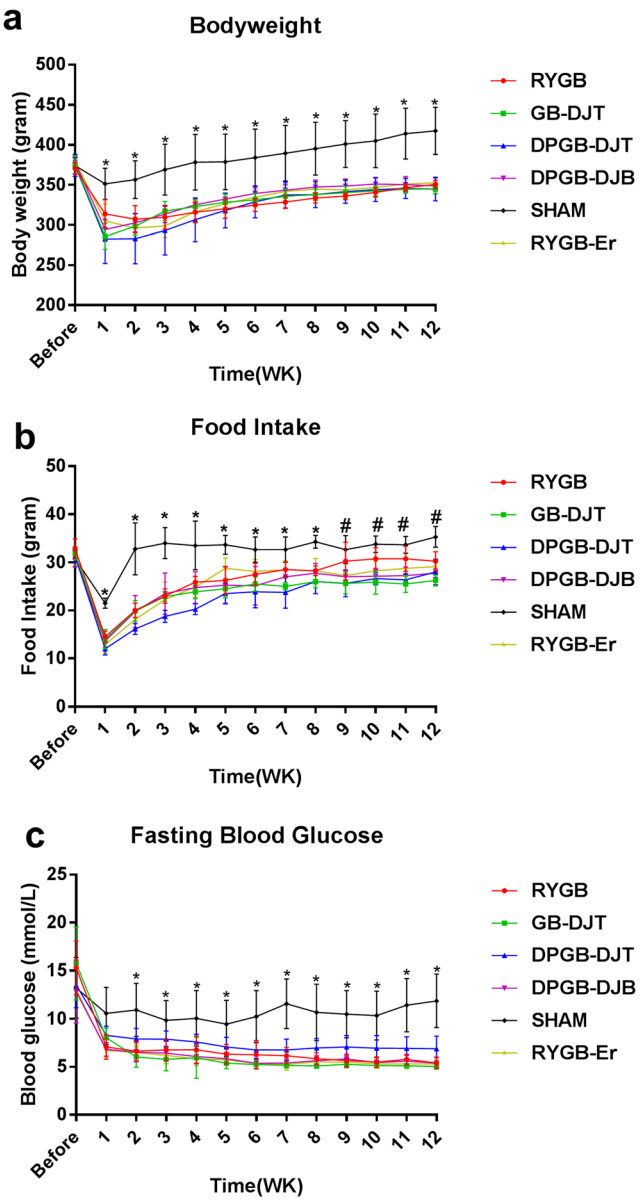

Fig. 2 Illustrates the change in body weight (**a**), food intake (**b**), and fasting blood glucose (**c**). RYGB, Roux-en-Y gastric bypass; GB-DJT, gastric bypass with duodenal-jejunal transit; DPGB-DJB, distal pouch gastric bypass with duodenal-jejunal bypass; DPGB-DJT, distal pouch gastric bypass with duodenal-jejunal transit; RYGB-Er, Roux-en-Y gastric bypass with esophageal re-anastomosis; SHAM, sham surgery; *, significant compare with SHAM ($p < 0.05$); #, significant GB-DJT, DPGB-DJB, and DPGB-DJT compare with SHAM ($p < 0.05$)

Food intake decrease significantly ($p < 0.05$) following operation in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups. The decreased in mean food intake 12 weeks after operation were 19.5 ± 7.9%, 25.4 ± 3.8%, 27.9 ± 4.7%, and 20.7 ± 4.7% in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. The amount of decrease in food intake in the DPGB-DJT group was significantly higher than the RYGB and the DPGB-DJB groups ($p < 0.01$).

Fasting blood glucose decrease significantly ($p < 0.05$), following operation in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups. The decreases in the mean fasting blood glucose 12 weeks after operation were 58.5 ± 8.6%, 62.8 ± 8.2%, 45.2 ± 10.8%, and 51.5 ± 13.2% in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. The mean decrease in fasting glucose in the GB-DJT was significantly higher than the DPGB-DJT ($p < 0.01$) and the DPGB-DJB ($p < 0.05$) groups.

Glucose and Insulin Test

Figure 3 illustrates OGTT curve at week 3 (a) and 10 (b), and ITT curve at week 3 (c) and 10 (d). Glucose AUC and HOMA-IR are illustrated in Figs. 3 e and f respectively.

RYGB, GB-DJT, and DPGB-DJT decreased glucose levels significantly ($p < 0.05$) from preoperative level during OGTT at weeks 3 and 10 after surgery. The amount of decrease in blood glucose levels during OGTT at weeks 3 and 10 in the RYGB and GB-DJT groups were significantly greater than the amount of decrease in DPGB-DJT group ($p < 0.05$). The amount of decrease in glucose level at 3 and 10 weeks after surgery in the DPGB-DJB did not reach statistical significance ($p > 0.05$).

The mean peak glucose recorded during OGTT 3 weeks after surgery were 59 ± 11%, 51 ± 11%, 35 ± 8%, and 24 ± 6% lower than preoperative level in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. On a repeat of OGTT at week 10, the mean peak glucose in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups were 60 ± 12%, 58 ± 9%, 39 ± 8% and 25 ± 10% lower than preoperative level respectively.

During ITT at week 3 and 10 postoperatively, the lowest blood glucose was recorded 90 min after insulin injection which were lower than preoperative levels. Intraperitoneal injection of 0.5 IU/kg of human insulin 3 weeks after surgery decreased blood glucose significantly from the baseline by 42.6 ± 10.8%, 35.1 ± 8.8%, 41.5 ± 9.9%, and 45.3 ± 11.8% in the RYGB, GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. On a repeat at week 10 after surgery, blood glucose decreased from the baseline by 39.5 ± 6.9%, 33.6 ± 12.4% 45.7 ± 6.9%, and 45.4 ± 4.2% in the RYGB GB-DJT, DPGB-DJT, and DPGB-DJB groups respectively. The amount of decrease in blood glucose at week 3 and 10 were significantly greater than the amount of decrease recorded preoperatively in the RYGB, GB-DJT, and DPGB-DJT groups (24.3 ± 4.0%, 19.8 ± 7.1%, and 24.8 ± 8.1%, respectively, $p < 0.05$). In the DPGB-DJB group, the difference was only significant at week 10 (postoperation, 45.4 ± 4.2% vs. preoperation, 24.8 ± 8.1%).

GLP-1

Figures 4 a, b illustrate mean GLP-1 response and AUC respectively in all groups. Postoperative GLP-1 responses to enteral stimulation during MMTT conducted 8 weeks after surgery in the RYGB, GB-DJT, and DPGB-DJB groups were significantly higher ($p < 0.05$) than preoperative response. The increased in GLP-1 response in the DPGB-DJT groups postoperatively did not reach statistical significance ($p = 0.07$).

GIP

Figures 4 c and d illustrate GIP response and AUC respectively in all groups. RYGB, GB-DJT, DPGB-DJB, and DPGB-DJT did not change GIP response significantly from preoperative level.

Insulin

Figures 4 e and f illustrate insulin response and AUC respectively in all the groups. Fasting insulin levels were decreased in the RYGB and GB-DJT groups after surgery. Insulin AUC was significantly increased in the DPGB-DJB group ($p < 0.05$). The increased insulin AUC in the RYGB, GB-DJT, and DPGB-DJT did not reach statistical significance.

Gastrin

Figures 5 a and b illustrate gastrin response and AUC respectively in all groups. Compared to preoperative response, gastrin response to oral gavage in the DPGB-DJT and DPGB-DJB groups after surgery were significantly increased from preoperative level. RYGB and GB-DJT did not change gastrin response significantly from preoperative level.

Ghrelin

Figures 5 c and d illustrate ghrelin response and AUC respectively in all groups. Unlike the RYGB group, fasting ghrelin level decreased following oral gavage in the GB-DJT, DPGB-DJT, and the DPGB-DJB groups postoperatively. Postoperative ghrelin AUC was decreased significantly in the DPGB-DJT group. The decreased postoperative ghrelin AUC in the DPGB-DJB group did not reach statistical significance. Ghrelin AUC in the RYGB group was significantly increased from preoperative level. Postoperative ghrelin AUC in the GB-DJT group did not differed from preoperative level.

Glucagon

Figures 5 e and f illustrate glucagon response and AUC respectively in all groups. Fasting glucagon level and AUC were increased significantly in the GB-DJT and DPGB-DJT groups after surgery ($p < 0.05$). Postoperative glucagon AUC was decreased significantly ($p < 0.05$) in the RYGB group. The decreased glucagon level in the DPGB-DJB group did not reach statistical significance. Unlike the DPGB-DJT and the DPGB-

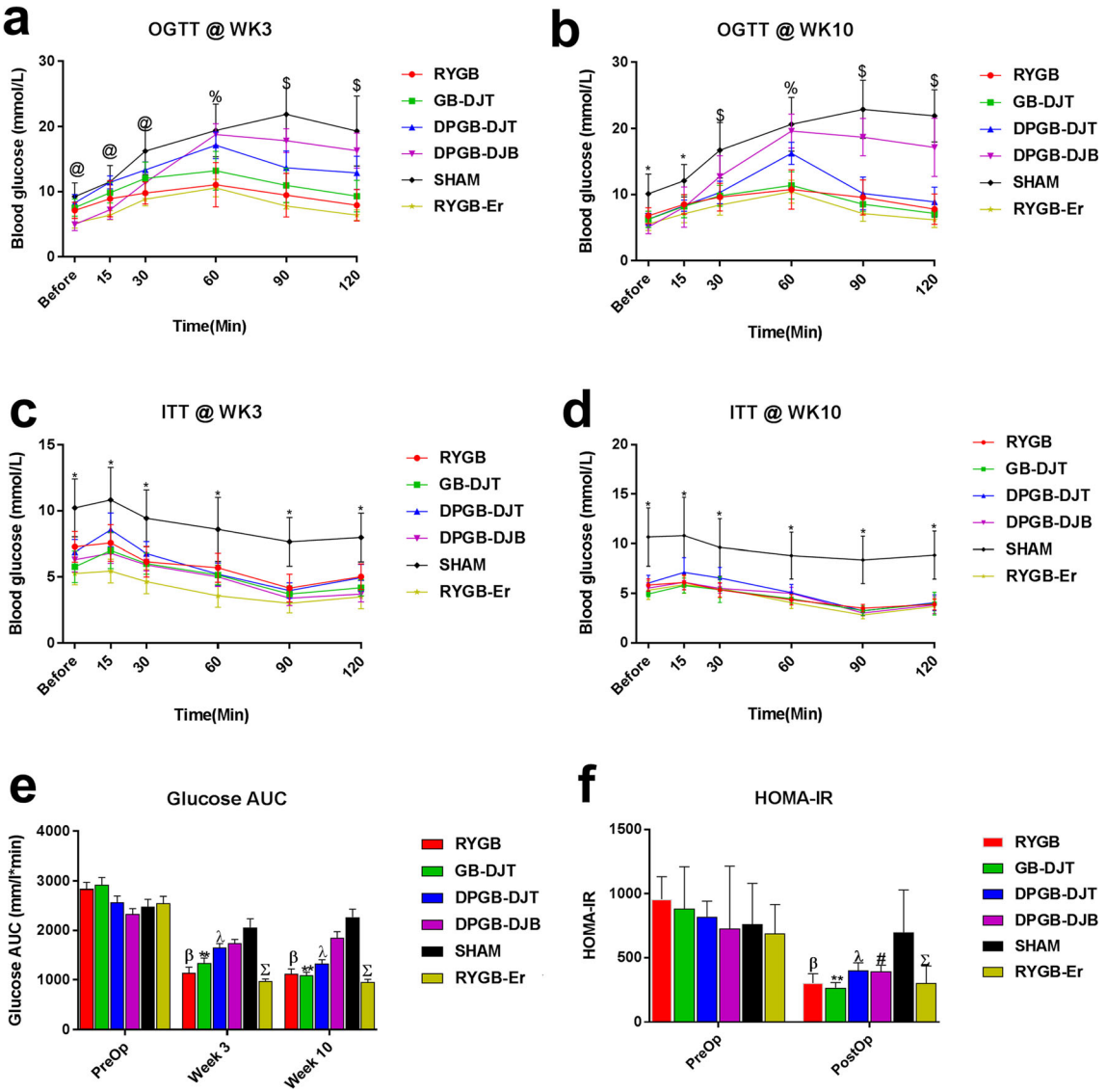

Fig. 3 Illustrates the oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). OGTT at weeks 3 and 10 (**a**, **b**); ITT at weeks 3 and 10 (**c**, **d**); glucose AUC and HOMA-IR (**e**, **f**). RYGB, Roux-en-Y gastric bypass; GB-DJT, gastric bypass with duodenal-jejunal transit; DPGB-DJB, distal pouch gastric bypass with duodenal-jejunal bypass; DPGB-DJT, distal pouch gastric bypass with duodenal-jejunal transit; SHAM, sham operation; RYGB-Er, Roux-en-Y gastric bypass with esophageal re-anastomosis; Before, before gavage; Preop, preoperation; Postop, postoperation; $^{@}$, significant RYGB, GB-DJT, and DPGB-DJB compared with the SHAM group ($p < 0.05$); $^{\%}$, significant RYGB and GB-DJT compared with the SHAM group ($p < 0.05$); $^{\$}$, significant RYGB, GB-DJT, and DPGB-DJT compared with the SHAM group ($p < 0.05$); *, significant compared with preoperation ($p < 0.05$); β, significant RYGB compared with preoperation ($p < 0.05$); **, significant GB-DJT compared with preoperation ($p < 0.05$); λ, significant DPGB-DJT compared with preoperation ($p < 0.05$); #, significant DPGB-DJB compared with preoperation ($p < 0.05$); Σ, significant RYGB-Er compared with preoperation ($p < 0.05$)

DJB groups, the fasting glucagon level in the GB-DJT and the RYGB groups decreased following oral gavage postoperatively.

The esophageal re-anastomosis in the RYGB-Er group did not affect the glucose tolerance and the incretin response significantly; as the amount of postoperative improvements in glucose tolerance and incretin response in the RYGB-Er group did not differ from the RYGB group. Sham operation did not change body weight, glucose tolerance, and incretin response significantly from the preoperative level.

Discussion

Results from our experiment showed that, in gastric bypass, creating the gastric pouch in the distal region of the stomach significantly impairs the glucose tolerance and diabetes remission in spite of the increased GLP-1 and insulin responses. We also found that the DJB in the model with the distal pouch was ineffective in alleviating the poor glucose control, which argues against the proposed antidiabetic effects of DJB suggested by the foregut theory. Therefore, we believe that in

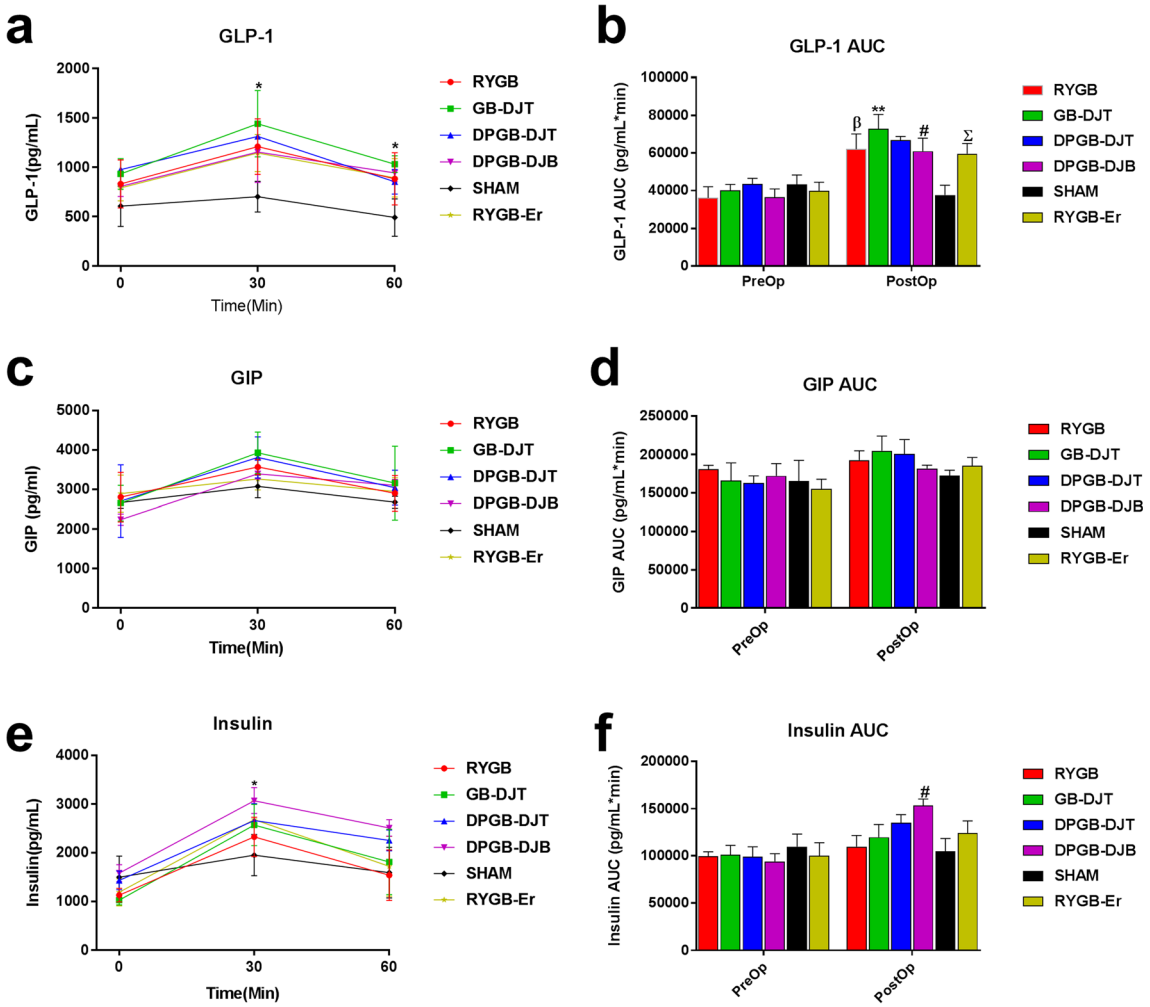

Fig. 4 Illustrates mixed-meal tolerance test (MMTT). GLP-1 response and AUC (**a**, **b**); GIP response and AUC (**c**, **d**); insulin response and AUC (**e**, **f**). RYGB, Roux-en-Y gastric bypass; GB-DJT, gastric bypass with duodenal-jejunal transit; DPGB-DJB, distal pouch gastric bypass with duodenal-jejunal bypass; DPGB-DJT, distal pouch gastric bypass with duodenal-jejunal transit; SAH, sham operation; RYGB-Er, Roux-en-Y gastric bypass with esophageal re-anastomosis; Before, before gavage; Preop, preoperation; Postop, postoperation; *, significant compared with sham ($p < 0.05$); &, significant RYGB and GB-DJT compared with SHAM ($p < 0.05$); β, significant RYGB compared with preoperation ($p < 0.05$); **, significant GB-DJT compared with preoperation ($p < 0.05$); #, significant DPGB-DJB compared with preoperation ($p < 0.05$); Σ, significant RYGB-Er compared with preoperation ($p < 0.05$)

RYGB, excluding the gastric remnant may be the principle determinant of the early improvement in glucose tolerance and diabetes remission after gastric bypass surgery.

Glucose Control

Bypassing the remnant stomach in RYGB is not known to be the key mediator of the improved glucose control or the early diabetes remission that follows the surgery. It is believed that dividing the stomach into a small gastric pouch and a gastric remnant in RYGB surgery principally plays a restrictive role [14, 15].

Early diabetes remission after RYGB is believed to be associated with the increase in incretin response (hindgut theory) and the eradication of putative factor with DJB (foregut theory). It is therefore unclear whether excluding meal stimulus from the remnant stomach in RYGB plays any role(s) in the early diabetes remission that follows RYGB.

In this experiment, in spite of the similar amount of decrease in food intake and bodyweight, the amount of improvement in glucose tolerance differed significantly between the models with the proximal pouch (RYGB and GB-DJT) and the models with the distal pouch (DPGB-DJT and DPGB-DJB) throughout the study. The glucose tolerance in the distal pouch models after surgery was barely improved from the preoperation level. This finding disputes a purely restrictive role of dividing the stomach into a gastric pouch and a gastric remnant in RYGB.

It was earlier demonstrated in a study by Shimizu H, et al. [10] that in RYGB, stimulating the bypassed foregut significantly reverses the glucose tolerance effects of RYGB in rats. In their study, even though they delivered the nutrients into the

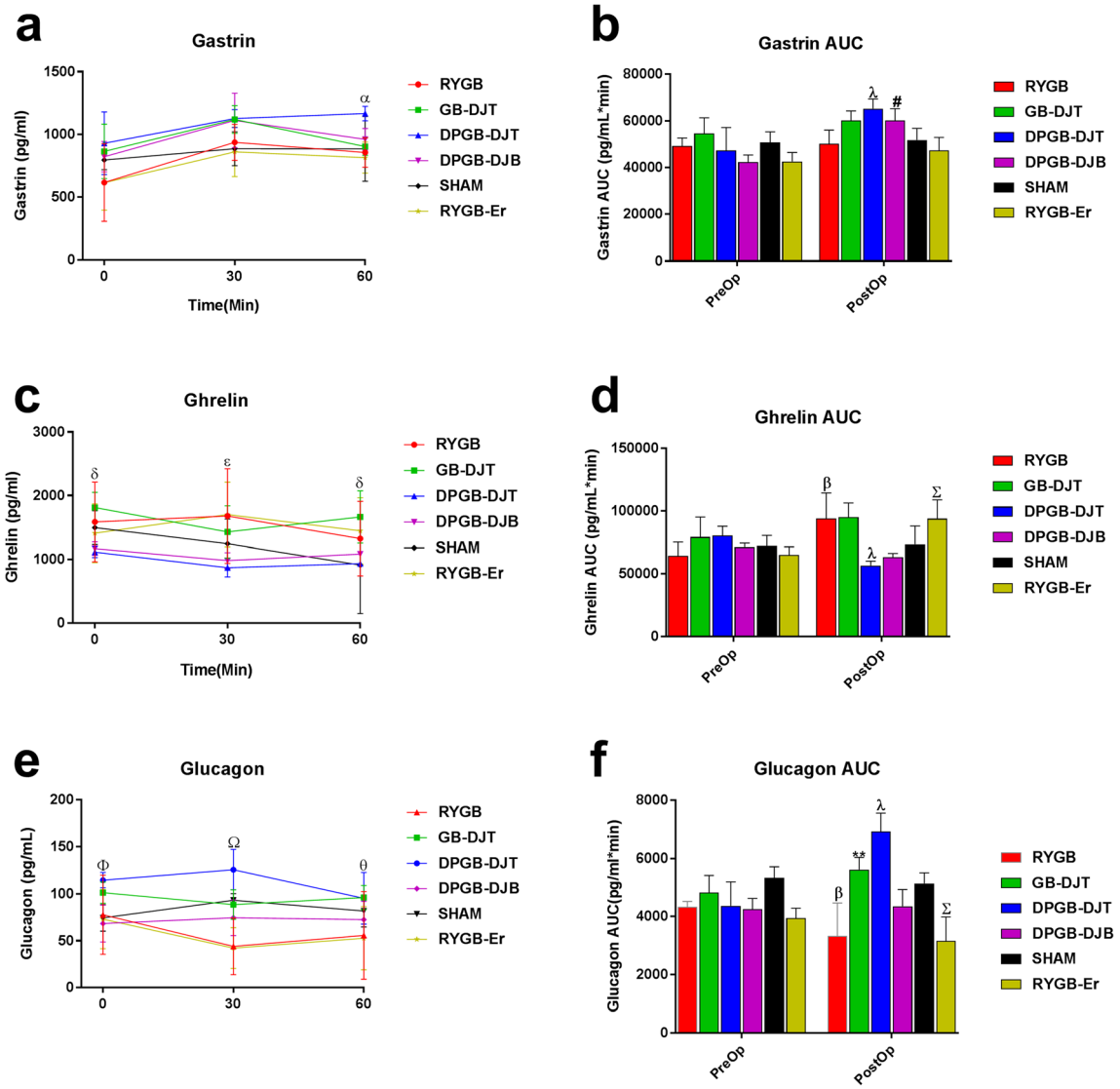

Fig. 5 Illustrates mixed-meal tolerance test (MMTT). Gastrin response and AUC (**a**, **b**); ghrelin response and AUC (**c**, **d**). Glucagon response and AUC (**e**, **f**). RYGB, Roux-en-Y gastric bypass; GB-DJT, gastric bypass with duodenal-jejunal transit; DPGB-DJB, distal pouch gastric bypass with duodenal-jejunal bypass; DPGB-DJT, distal pouch gastric bypass with duodenal-jejunal transit; SHAM, sham operation; RYGB-Er, Roux-en-Y gastric bypass with esophageal re-anastomosis; Preop, preoperation; Postop, postoperation; β, significant RYGB compared with preoperation ($p < 0.05$); #, significant DPGB-DJB compared with preoperation ($p < 0.05$); λ, significant DPGB-DJT compared with preoperation ($p < 0.05$); δ, significant DPGB-DJT and SHAM compared with DPGB-DJB ($p < 0.05$); $\mathcal{E}$, significant DPGB-DJT compared with DPGB-DJB ($p < 0.05$); ϕ, significant RYGB, DPGB-DJB, and SHAM compared with DPGB-DJT ($p < 0.05$); Ω, significant compared with DPGB-DJT ($p < 0.05$); , significant RYGB compared with DPGB-DJT and GB-DJT ($p < 0.05$); Σ, significant RYGB-Er compared with preoperation ($p < 0.05$)

gastric remnant, they attributed the reversal in glucose tolerance to the duodenal-jejunal stimulation (as opposed to the gastric stimulation) in support of the foregut theory. We wonder why the reversal in glucose tolerance could not be associated with the gastric stimulation.

The results of our previous study in rat challenged the foregut theory by finding that duodenal-jejunal transit (DJT) in gastric bypass does not impair the glucose tolerance [12]. We therefore believe that stimulation of the gastric remnant, not the duodenal-jejunal limb was responsible for the impaired glucose tolerance recorded in the study by Shimizu H et al. [10].

In our current study, findings with the DPGB-DJB model further challenged the foregut theory in that, DJB in the model was ineffective in alleviating the poor glucose control.

If the foregut hypothesis (based on DJB) was true, one would expect a similar degree of glucose control between the DPGB-DJB and the RYGB (or the RYGB-Er) groups, given that the surgical anatomies in both the DPGB-DJB and RYGB models included the creation of a 20% gastric pouch plus DJB. Remarkably, glucose tolerance with DPGB-DJB surgery barely differed from the sham surgery. Given that the key difference between the surgical anatomy in the DPGB-DJB model and

the surgical anatomy RYGB model was the position of the gastric pouch (proximal in RYGB and distal in DPGB-DJB), we believe that the impaired glucose control in the DPGB-DJB model was a result of the distal gastric stimulation. We therefore believe that bypassing the distal region of the stomach may be key to the improved glucose tolerance in RYGB surgery.

We also recorded poor glucose control in the DPGB-DJT group (also with a distal gastric pouch but without DJB) compared to RYGB and GB-DJT groups (proximal pouch). Even though inferior to the RYGB and GB-DJT groups, surprisingly, the glucose control in the DPGB-DJT group (with DJT) was superior to the glucose control in the DPGB-DJB group (with DJB). Could the DJT in the DPGB-DJT model somehow account for the slightly better glucose control? Suggesting positive effects of DJT on glucose tolerance may be provoking, but the DJT in this study at least did not appear to impede the glucose excursion. This is rather similar to the transit bipartition surgery which has been reported to significantly improve diabetes without duodenal exclusion [16]. More studies will be required to assess the effect of DJT on glucose tolerance.

GLP-1 and Insulin

The surge in GLP-1 after RYGB is widely accepted to be the key mediator of the improved glucose tolerance after RYGB by stimulating insulin release [17].

In this study, we were surprised to find that the postoperative GLP-1 response was also significantly increased in the DPGB-DJB and the DPGB-DJT groups given that the glucose control in the models was barely improved from the preoperation level.

In our opinion, this finding raises questions about the theory that the increased in GLP-1 response after RYGB is the principle mediator of the improved glucose tolerance after RYGB.

Previous authors have also raised similar questions [18–20]. Patel et al. [20] suggested that the improvement in glucose tolerance after bypass surgery is independent of the changes in GLP-1 and insulin levels. Results in our study seem to partly agree with such suggestion, but does not seem to agree that the improvements in glucose tolerance is entirely independent of the changes in GLP-1 response. The minimum improvement in glucose tolerance in the presence of a significantly increased GLP-1 response in the distal pouch models in this study seems to suggest that the incretin response may only be one factor in the mechanism of improved postprandial glucose control. This appears to agree with the suggestion by the anti-incretin theory about the existence of a counter-regulatory mechanism, possibly a physiological factor(s) in the foregut that counteract the hypoglycemic effects of postprandial incretin response [20, 21]. If the anti-incretin theory is true, our results suggest that such factor may exist in the gastric component of the foregut. It will therefore imply that, bypassing the distal stomach in RYGB would eliminate or reduce the release of such factor, thereby shifting the balance in favor of the incretins. In this case, the unopposed incretin effects (not simply the incretin response), will most probably be the mechanism of the improved glucose tolerance after RYGB. Unfortunately, since the anti-incretin theory was proposed, that exact mediator and mechanism remains a mystery. More studies may be warranted in this regard.

Gastrin

In order to explore possible factor(s) in the distal stomach that may have affected the glucose tolerance in the models with the distal pouch, we assessed changes in gastrin level. Gastrin is one of the most studied peptide release by the distal stomach that affects glucose homeostasis, which level generally declines after gastric bypass [22]. Gastrin is believed to be released from G cells mainly in the pyloric antrum and functions predominantly to stimulate acid release from the enterochromaffin-like cell (ECL cells) [22].

In this study, not surprisingly, preserving the gastric antrum in the DPGB-DJB and DPGB-DJT groups significantly increased gastrin level compared to the RYGB and the GB-DJT groups. It is therefore tempting to speculate that the hypergastrenemia in the DPGB-DJB and the DPGB-DJT models may have somehow contributed to the poor glucose clearance in the groups, and by contrast, the lower level in the RYGB and the GB-DJT models may have contributed to the superior glucose control in the groups. There is some evidence to support this speculation. A study by Stenstrom et al. [23] reported that reversing hypogastrinemia with gastrin infusion following gastric bypass reactivates enterochromaffin-like cell function and reverses the weight loss in rats. Unfortunately, the author did not access the impact on glucose tolerance. We are eager to see the effect on glucose tolerance of reversing the hypogastrenemia in RYGB (probably by gastrin infusion).

Ghrelin

Ghrelin is another key gastric hormone believed to be release from the gastric fundus in response to food ingestion. Even though recorded levels of ghrelin following gastric bypass have proven inconsistent, a decline is believed to facilitate the reduction in food intake further enhancing outcomes [24].

In this study, the postoperative ghrelin level was significantly increased in the RYGB group, which will explain the slightly higher food intake in the RYGB group. As expected, the postoperative ghrelin level was decreased in the DPGB-DJT and the DPGB-DJB groups, which may as well explain the slightly lower food intake compared to the RYGB group. Other than the slight differences in food intake, the postoperative changes in ghrelin level did not appear to affect the glucose tolerance. The long-term effects of higher ghrelin level on body weight and glucose homeostasis remains to be seeing.

GIP

GIP is a foregut hormone thought to be released principally in the proximal intestine [25]. GIP response was increased in our models with the duodenal stimulation (DPGB-DJT and GB-DJT), which further confirms the foregut association. GIP is also believed to stimulate insulin and glucagon release, which may explain the higher levels of insulin and glucagon in the DPGB-DJT and GB-DJT models.

Glucagon

Glucagon released from the pancreatic alpha cells acts principally to stimulate hepatic glucose production. It is believed that impaired glucagon secretion is one hallmark of T2D. The well-known glucose suppression of glucagon release is thought to be impaired in T2D leading to postprandial hyperglucagonaemia [26, 27].

In this study, glucagon level was decreased significantly in RYGB group after surgery, possibly a result of the improved beta cell function. Interestingly, the postoperative glucagon level in the GB-DJT group was increased significantly even though the postoperative insulin response in the GB-DJT group did not differ from the postoperative insulin response in the RYGB group. Similarly, in the groups with the distal pouch, the glucagon level in the DPGB-DJT group (with duodenal stimulation) was significantly increased compared to the DPGB-DJB group. We suspect that factors other than the improved insulin response may have contributed to the different changes in the postoperative glucagon level.

Glucagon release is thought to be principally regulated by blood glucose level. Surprisingly, assessment of glucose tolerance postoperatively showed no specific relationship between postoperative glucagon level and glucose homeostasis in this study. The differences in glucagon level did not correlate with the degree of improve glucose tolerance**.** Generally, compared to the groups with the DJT (GB-DJT and DPGB-DJT), postoperative glucagon level was significantly lower in the groups with the DJB (RYGB and DPGB-DJB).

Ramracheya et al. [26] reported that the decrease in glucagon level following gastric bypass in rats is a result of the increased PYY level. PYY is thought to inhibit glucagon release from the pancreatic alpha cells. It is therefore possible that an increase in PYY level with intact food reaching the ileum in the RYGB and DPGB-DJB group (with DJB) may explain the decrease in the glucagon level. Unfortunately, we did not measure PYY level in this study.

Alternatively, the possibility of an extra-pancreatic glucagon release has also previously been indicated. It has been suggested that an aberrant proglucagon in L cells leads to the co-secretion of GLP-1 and glucagon in response to the meal stimulus [28]. If true, our results suggest that such cells may predominately exist within the proximal intestine, in that, the higher glucagon release was recorded in the groups with the DJT (GB-DJT and DPGB-DJT group). In this case, the DJB may explain the decreased glucagon level in the RYGB group in the first place. This was also seeing in our DPGB-DJB group.

Summary

Consistently, in this study, stimulating the distal stomach was associated with poor glucose clearance, irrespective of the surge in GLP-1 or the presence of DJB. Our study appears to indicate that factor(s) uniquely associated with distal gastric stimulation may be relevant to postprandial glucose homeostasis. Notably, glucose tolerance in the distal pouch models only deteriorated following enteral stimulation but not in fasting state. This occurrence was not observed in the models with the proximal pouch.

Whether this phenomenon in the distal pouch models was uniquely a result of the distal pouch stimulation or secondary to other associated factors such as the increased gastrin level, it will require more rigorous studies. The anti-incretin theory may also be of relevance.

Finally, we do not dispute that DJB may hold potential antidiabetic effects; however, results in our study (in this study as well as our previous study) indicate that duodenal-jejunal stimulation at least does not impair glucose tolerance. The results in this study suggest the distal stomach as the likely culprit for the impaired glucose tolerance with foregut stimulation. The increased gastrin level with distal gastric stimulation appears to be a putative factor in this study.

These results, therefore, lay the basis for further rigorous studies into the mechanism of impaired glucose tolerance with foregut stimulation or the reversal in diabetes remission following the occurrence of gastro-gastric fistula in RYGB [11], which could provide potentially novel therapeutic target in the treatment of T2D.

Meanwhile, if the findings in this study of impairments to glucose control following stimulation of the distal gastric mucosa (or improvement to glucose control without stimulating the distal gastric mucosa) in T2D rat can be extrapolated in human, then endoscopically manipulating the distal gastric mucosa in human might be clinically relevant in the treatment of diabetes.

Lastly, we cannot rule out the effects of changes in the gut microbiota and bile acid metabolism that may be associated with the different gastrointestinal reconstruction (e.g., proximal vs. distal pouch, separation vs. no separation of food and bile flow into the common channel) on the glucose tolerance in this study.

Limitations

Unfortunately, we could not identify in this study the exact mechanism by which the distal gastric stimulation impaired the glucose clearance in spite of the increased GLP-1 and insulin

responses. The possible unintended effects of esophageal manipulation in the distal pouch models remain an important limitation to this study. However, similar manipulation in the RYGB-Er group did not appear to impede the glucose control.

Conclusion

This study does not dispute the incretin effect of GLP-1 or possible improvements to glucose tolerance with DJB. Our experiment indicates that bypassing the distal stomach is required to achieve glucose tolerance and diabetes remission (early and late) in gastric bypass rodent model. Therefore, excluding the gastric remnant in RYGB surgery may be key to the improved glucose tolerance and diabetes remission after surgery.

Author Contributions Ponnie Robertlee Dolo (ponnie85@yahoo.com) and Xiaocheng Zhu (zhuxccf@163.com) designed the experiment. Ponnie Robertlee Dolo performed the experiment and drafted the Manuscript. Chao Li (18068718716@189.cn) and Yong Shao (shaoyutong1975@163.com) supervised the experiment and offered technical support. Libin Yao (yaolibin_123@163.com) and Xiaocheng Zhu, revised the manuscript. Hui Wang (15996960499@139.com) Provided Material and logistical support.

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Compliance with Ethical Standards

The study was approved by the ethics committee of Xuzhou Medical University Research Animal Center.

Conflict of Interest The authors declare that they have no conflict of interest.

Statement of Animal Rights All applicable institutional and national guidelines of the People's Republic of China for the care and use of animals were followed.

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