Research Paper

Esophagus-duodenum Gastric Bypass Surgery Improves Glucose and Lipid Metabolism in Mice

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ABSTRACT

Background: Despite of its significant therapeutic effects on obesity and metabolic diseases, Roux-en-Y gastric bypass (RYGB) has limited clinical application because of considerable impacts on the gastrointestinal structure and postoperative complications. This study aims to develop a simplified surgical approach with less damage and complication but efficient metabolic benefit.

Method: The effects of Esophagus-Duodenum gastric bypass (EDGB) on body weight, food intake, glucose and lipid metabolism were compared to RYGB in mice.

Findings: EDGB is simple, has higher survival rate and less complication. Relative to RYGB, EDGB demonstrated modest body weight control, identical improvement of glucose and lipid metabolism in obese mice. Blood glucose increased significantly 15 and 30 min after oral glucose administration, then markedly decreased in both EDGB and RYGB groups relative to the sham surgery, indicating a quicker absorption of oral glucose and improvement in glucose uptake by insulin targeted tissues. Insulin sensitivity was identically improved. EDGB significantly decreased plasma and hepatic triglyceride levels, while increased browning in visceral and subcutaneous white adipose tissue to the extent identical to RYGB. Levels of ghrelin and nesfatin-1 increased significantly after EDGB and RYGB.

Interpretation: EDGB is a valuable model to study the metabolic benefit of bariatric surgery in mice.

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1. Introduction

Obesity and its associated morbidities such as Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver diseases (NAFLD) impose a significant health burden worldwide. Conventional treatment of obesity and its associated chronic metabolic dysfunction is limited and often inefficient. Effective and long term management of obesity and T2DM remains a major challenge.

Bariatric surgery has demonstrated a significant efficacy in the treatment of obesity and T2DM. Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG) are the most commonly performed bariatric surgeries (Debs et al., 2016; Haruta et al., 2017). RYGB is the most effective for durable treatment of T2DM imparting both weight loss dependent and independent improvement in glycemic control in 83.8% of morbidly obese patients (Buchwald et al., 2004). However, considerable alteration in structure and physiology of the gastrointestinal tract and adverse complications have significantly limited its application (Breznikar and Dinevski, 2009). Although infrequent, serious long-term complications may require reversal procedures. Bariatric surgery traditionally has been recommended for severely obese patients with a body mass index (BMI) of ≥40 kg/m2 or for severely obese patients with BMI of ≥35 kg/m2 with at least 1 co-morbid condition. More recently, the indications have been expanded to include obese patients with a BMI of 30–40 kg/m2 and a metabolic condition, such as type 2 diabetes (Ponce et al., 2015; Brethauer, 2013).

Improvement in glycemic control after bariatric surgery can be either weight loss dependent and independent. RYGB often produces a massive weight reduction and a large extent in the improvement of glycemic control, but the improvement of glucose occurs before weight reduction. Weight loss after RYGB surgery, in particular in rodent model, occurs rapidly, with 20–30% weight lost within two weeks after surgery. This rapid weight loss makes it difficult to investigate the weight-loss-independent mechanisms for improvement of glucose homeostasis (Hao et al., 2013). Further, Asian patients are rarely “severely obese” because of differences in genetic background and diets. Interest in the mechanism independent of weight loss after bariatric surgery for patients with moderate obesity has thus been growing (Ezzati, 2016).
alternative bariatric surgery to replace RYGB is needed. VSG is rapidly gaining popularity with a trend to replace RYGB surgery because of its less damage and fewer complications. However, studies in mice have found that VSG is less effective for lasting reduction in body weight and improvement on glycemic control relative to RYGB, the gold standard surgery, in mice (Hao et al., 2017). Further, removal of majority of the stomach renders it impossible to reverse the procedure in case complications such as anemia and severe bone loss occur (Angrisani et al., 2015; Buchwald et al., 2004). These observations indicate that it is premature to replace RYG with VSG surgery (Nguyen et al., 2016).

For the gastroduodenal anastomosis (Billroth I) surgery, the antrum and pylorus are removed and the stomach is attached to the duodenum along its greater curvature. This bariatric surgery is performed much less often because its postoperative complications such as reflux gastritis and esophagitis are significantly higher than RYGB surgery (Zong and Chen, 2011). In this study, we presented a simplified bariatric surgery: Esophagus-Duodenum Gastric Bypass Surgery (EDGB) in mice. In this procedure, the 5% small gastric pouch is end-to-side connected to the duodenum, rendering food bypassing the stomach only. We compared the changes in food intake, body weight, glycemic control and lipid metabolism between the EDGB and RYGB surgeries in obese mice induced by high fat diet (HFD).

2. Materials and Methods

2.1. Animals

Four weeks-old C57BL/6 J mice weighted 16 ± 3 g were purchased and fed with a high-fat diet (45% fat, D12451; Research Diets, USA) for 12 weeks. At the time of surgery, obese mice were weight-matched and divided into Sham, EDGB and RYGB groups. Mice were fed with high-fat diet for 8 weeks after surgery until the end of experiments. All experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Health Science Center of Peking University.

2.2. Surgical Procedures

Animals were fasted 4 to 6 h before operation and anesthetized with pentobarbital sodium (60 mg/kg body weight). Standard aseptic procedures were used throughout.

2.2.1. Roux-en-Y Gastric Bypass Surgery

We used the mouse RYGB model in which a pouch size of about 5% of the total stomach volume was created similar to typical RYGB surgery in humans. Briefly, mice were anesthetized and stomach exposed. Perigastric ligaments were ligated and cut to release the stomach. The left gastric vessel was separated bluntly from the cardia to make room for pouch operation without impairing the gastric blood supply. A titanium clip was applied to generate a small pouch size of about 5% of the total stomach volume. The stomach was transected right above the esophagus were traced, and the branch entering to the stomach was identified and cut, the branch to liver remained intact. The abdominal cavity was closed with suture as described above.

2.2.2. Esophagus-Duodenum Gastric Bypass Surgery

After anesthesia, perigastric ligaments were ligated and cut to release the stomach and the small pouch size of about 5% of the total stomach volume was created using a titanium clip as described above in the RYGB procedure. The small gastric pouch was then sutured end-to-side to the duodenum bulb using 11-0 nylon suture in an uninterrupted fashion. Then the stomach and duodenum were put back in position. The abdominal cavity was closed with interrupted suture using 5-0 nylon suture.

2.2.3. Sham Surgery

For the sham operation, the stomach was released and the left gastric vessel separated from the cardia as described in RYGB and EDGB procedures. The stomach, esophagus, and small intestine were exposed and the abdominal cavity closed with interrupted suture.

2.2.4. Vagotomy

After anesthesia, perigastric ligaments were ligated and cut to release the stomach. The vagal nerves descending along the surface of the esophagus were traced, and the branch entering to the stomach was identified and cut, the branch to liver remained intact. The abdominal cavity was closed with suture as described above.

2.2.5. Postoperative Care

For the first 12 h after the operation, mice were placed on a heating pad set at 37 °C. Immediately after the surgery, mice were administrated subcutaneously with 0.7 ml of 5% dextrose and carprofen (5 mg/kg) for analgesia. Water and high-fat diet was made available after recovery from anesthesia. One day after surgery, mice were free to drink and fed with high-fat diet until euthanasia.

2.3. Measurement of Body Weight and Food Intake

Body weight was measured every week before and after surgery. Food intake was measured every 3 days after surgery. Spillage was weighted and subtracted.

2.4. Glucose Metabolism

Basal levels of glucose were measured using blood drawn from the tail vein 1 week before surgery and 8 weeks after surgery. For oral glucose tolerance tests (OGTT), mice were fasted for 16 h before gastric administration of glucose (3 g/kg body weight) by gavage. Blood was drawn from a cut at the tip of the tail at 0, 15, 30, 60, 90 and 120 min after glucose administration, and glucose concentrations were detected immediately. Area under the glucose curve (AUC) was calculated.

Intrapertoneal glucose tolerance tests (IPGTT) was also performed after intraperitoneal injection of glucose at a dose of 1.5 g/kg body weight.

For insulin tolerance tests (ITT), mice were fasted for 6 h, followed by intraperitoneal injection of insulin at a dose of 1 IU/kg body weight. Blood was drawn from a cut at the tip of the tail at 0, 15, 30, 60, 90 and 120 min for determination of glucose concentration.

2.5. Plasma Ghrelin and Nesfatin-1

Blood samples from mice were transcardially collected after anesthesia, immediately transferred to chilled polypropylene tubes containing EDTA-2Na (12.5 mg/ml) and aprotinin (1000 units/ml), and centrifuged at 1500g for 10 min at 4 °C. Plasma was separated and stored at − 80 °C before use. Blood levels of total ghrelin were measured using an enzyme linked immunosorbent assay (ELISA). Acylated ghrelin was measured using radio immunoassay kit (Linco Bioscience Institute, St. Charles, MO) according to the manufacturer’s instructions. Aprotinin was purchased from Amersham Biosciences (Pittsburgh, PA). Nesfatin-1 was purchased from Amersham Biosciences (Pittsburgh, PA).
was measured using ELISA kits from RayBio (Nesfatin Enzyme Immunoassay Kit, Mouse Nesfatin ELACODE: EIA-M-NES).

2.6. Measurements of Plasma and Hepatic Triglyceride

Plasma and hepatic triglyceride was measured as described before (Li et al., 2014; Yin et al., 2015). Briefly, blood samples were transcardially collected. The plasma was separated and stored at −80 °C before use. Plasma triglyceride was measured by Triglyceride Colorimetric Assay Kit from Cayman Chemical Company according to the manufacturer’s instructions. Liver tissues were homogenized according to manufacturer’s instructions, and the supernatant was used for triglyceride detection. Values were normalized to protein concentrations determined by using the Pierce BCA protein quantitative assay kit (Thermo-Fisher Scientific).

2.7. Histological Examination

Pancreas, liver and adipose tissues were harvested and fixed with neutral buffered formalin containing 4% formaldehyde for 6 h. Tissue sections were cut at 6 μm, adhered to charged slides, air dried for 5 min, and rehydrated with 0.01 M PBS. The sections were stained with hematoxylin and eosin for H&E staining. Liver frozen sections were rinsed in PBS three times, and then fixed with 4% paraformaldehyde for 10 min. After washing, slices were incubated in 0.3% oil-red staining solution for 1 h at room temperature. Samples were then counterstained with hematoxylin for 30 s, followed by wash in running water for 80 min. All slides were mounted with 90% glycerol and stored at 4 °C before observation. The signal intensity was analyzed by software ImageJ.

2.8. Western Blotting

Protein was measured with western blotting as described before (Li et al., 2014; Yin et al., 2015). Liver and skeletal muscle tissues extracts were immunoblotted with AKT, phospho-AKT, IRS-1, phospho-IRS-1, Glu4, nesfatin-1, β-actin. Specific reaction was detected using IRDye-conjugated second antibody and visualized using the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE). The antibodies are listed in Table 1.

2.9. Gene Expression Analysis

For gene expression analyses, RNA was isolated from mouse liver or hepatic primary cells by using TRIzol (Invitrogen) and reverse-transcribed into cDNAs using the First-Strand Synthesis System for RT-PCR kit (Invitrogen). SYBR Green-based quantitative RT-PCR was performed by using the Mx3000 multiplex quantitative PCR system (Stratagene). Triplicate samples were collected for each experimental condition to demonstrate no significant difference among all groups, suggesting that the experimental design was adequate.

2.10. Statistical Analysis

Data were presented as mean ± SEM and compared among groups using one-way analysis of variance (ANOVA). GraphPad Prism 6.0 software was used for statistical analysis. P < 0.05 denotes statistical significance.

3. Results

3.1. EDGB Surgery Is Easier and Safer

The surgery for sham, EDGB and RYGB was outlined in Fig. 1A. In the sham group, food pass was normal from esophagus into stomach. In the EDGB group, food passed the esophagus and 5% small gastric pouch, then entered into duodenum, bypassing 95% stomach. In the RYGB group, food also passed through the esophagus and the small gastric pouch, but entered into distal jejunum, bypassing 95% stomach, duodenum and about 2 cm jejunum. Shown in Fig. 1B was the experimental design. Fig. 1C showed the survival rates of sham, EDGB and RYGB surgeries performed by the same surgeon in different periods representing distinct experience levels. The survival rate of EDGB surgery in different periods was similar to sham group. On the other hand, it took 4 years for an experienced surgeon to increase the RYGB survival rate from 18.6% ± 6.9% to 77.7 ± 7.3%. Further, post-operative complications were rare in EDGB group, whereas RYGB demonstrated a significantly higher rate of complications such as gastroesophageal reflux, nutrition deficiency, anastomotic leak (Fig. 1D). Because of its complexity in jejunum end-to-side and gastro-jejunum end-to-end anastomosis, the RYGB procedure typically took 1 h. EDGB was finished in 30–40 min because only one gastro-duodenum end-to-side anastomosis is performed.

3.2. EDGB Produces Moderate Weight Loss Independent of Food Intake

Average body weight before surgery was similar between Sham, EDGB and RYGB groups (Fig. 2A upper panel left and bottom panel left). In the first week after surgery, all animals lost weight with the RYGB group demonstrating the most significant weight reduction. For the sham group, body weight became stable in the second post-operative week, increased starting from the third week. For EDGB group, body weight reduction lasted for two weeks, and then became steady until the end of experiments. For RYGB group, weight loss continued for 4 weeks, and then remained at a stable level until the end of experiments. Relative to RYGB group, animals with EDGB surgery demonstrated a significantly less reduction in body weight. At 8 weeks after operation, mice with RYGB and EDGB lost 37.3 ± 3% and 20.2 ± 2% body weight respectively (Fig. 2A upper panel right and bottom panel middle). Representative animals were shown in Fig. 2A bottom panel right. The decline of body weight in EDGB mice was more steady and rational than those in RYGB mice.

Interestingly, cumulative food intake and total food intake (Fig. 2B) demonstrated no significant difference between all groups, suggesting...
that weight loss and metabolic benefits after surgery are independent of food intake.

3.3. EDGB Improves Glucose Metabolism to an Extent Comparable to RYGB

As shown in Fig. 3A, glucose tolerance was significantly impaired in obese mice induced by HFD. EDGB significantly improved the oral glucose tolerance (OGTT) assessed at 8 weeks after surgery (Fig. 3B left). Similarly, insulin sensitivity (ITT) was also significantly improved (Fig. 3B middle). A slight but non-significant greater improvement in OGTT and ITT was observed for RYGB relative to EDGB. Unexpectedly, intraperitoneal glucose tolerance remained unchanged by either EDGB or RYGB (Fig. 3B right).

Examination of the pancreas revealed a significant increase in the percentage of pancreas weight over body weight in both EDGB and RYGB groups (Fig. 3C left). Consistently, islets areas demonstrated a significant increase in both EDGB and RYGB groups relative to the sham animals (Fig. 3C middle and right). EDGB surgery decreased plasma insulin to the levels comparable to those in RYGB group (Fig. 3D).

We next examined the effects of EDGB on insulin signaling in the liver and skeletal muscle. EDGB significantly increased insulin signaling to an extent comparable to RYGB.

3.4. EDGB Improves Lipid Metabolism to an Extent Comparable to RYGB

Plasma levels of triglyceride were significantly reduced by EDGB to an extent comparable to RYGB group. Levels of cholesterol remained unchanged in both EDGB and RYGB groups (Fig. 4A).

To further determine the effects of EDGB on lipid metabolism, we first examined the change in visceral and subcutaneous white adipose tissues. Adipose tissue weight and adipocyte size in visceral (Fig. 4B) and subcutaneous white adipose tissue (Fig. 4C) were significantly reduced in EDGB groups. Levels of brown adipose marker genes such as

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Table 2

Sequences of primers.

| Gene       | Upstream primer (5′-3′)          | Downstream primer (5′-3′)          | Gene accession number |
|------------|----------------------------------|-----------------------------------|----------------------|
| Mouse UCP1 | GGCACGACCCCTAATCCTAATG           | CATTAGATTAGGGTGTCGCC              | NM_009463.3          |
| Mouse UCP3 | ATCCGGATTCGGCCGGCC               | GCTGGCCAGATCTGTT                 | NM_009464.3          |
| Mouse prdm16| ATGACCAACCCACTCAGGAC            | AAGGGCAAGCGCCTGGCCCTA            | NM_008904.2          |
| Mouse ghrelin| CCATCTGCAATTTCTCTGTCTA       | GCAGTTAGCTGGTGGCTCCCTC           | NM_001001131.1       |
| Mouse nesfatin-1| GCA AGA CTG CCG ATG CTC AT     | ACT CTC TCC GCT GTT CC           | NM_001130479.2       |
| Mouse β-actin | ATCTGGCACCACACCTTC         | AGCCAGGTCAGACGGCA                 | NM_007393            |

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Fig. 1. EDGB surgery. a. Schematic illustration of sham (left), EDGB (middle) and RYGB (Right) surgery. b. Experimental protocol. Four weeks-old C57BL/6J mice were fed with a high-fat diet (45% fat, D12451; Research Diets, USA) for 12 weeks. Obese mice were weight-matched, group and performed for Sham, EDGB and RYGB surgery. n = 15, 14, 13 for sham, EDGB and RYGB respectively. c. Survival rate of Sham, EDGB and RYGB surgery performed by the same surgeon at different periods. Statistical differences were analyzed by log-rank (mantel-cox) test. d. Complications of sham, EDGB and RYGB surgery.
uncoupling protein 1 (UCP1), UCP3 and PR domain containing 16 (PRDM16) were significantly increased (Fig. 4B, C). These alterations were comparable to those observed in RYGB groups.

We next examined the alteration of hepatic lipid contents. EDGB significantly decreased the volume of liver, while increased hepatic weight. Hepatic steatosis was significantly improved as evidenced by the decrease in hepatocyte ballooning, Oil red staining and triglyceride contents. Hepatic cholesterol was unchanged (Fig. 4D). Again, the improvement in hepatic steatosis was identical between EDGB and RYGB.

3.5. Identical Effects on Gastric Hormones by EDGB and RYGB

Ghrelin and nesfatin-1 are two counteracting hormones derived from the gastric X/A like endocrine cells. We thus examined the effects of EDGB

Fig. 2. EDGB produces modest weight loss independent of food intake a. Body weight. b. Food intake. Values are the mean ± SEM. n = 15, 14, 13 for sham, EDGB and RYGB respectively. Statistical differences were analyzed by one-way ANOVA followed by the Student’s t-test. *P < 0.05 vs sham.

Fig. 3. EDGB improves glucose metabolism to an extent comparable to RYGB a. Pre-operation OGTT. b. Post-operation OGTT, ITT and IPGTT. c. Pancreas weight, histology and average islet area. Islet area was measured in serial sections of pancreas, calculated and expressed as mean ± SEM. d. Plasma levels of insulin expressed as mean ± SEM. e. Levels of AKT and IRS-1 phosphorylation in liver. f. Levels of AKT and IRS-1 phosphorylation, and Glut 4 protein in skeletal muscle. *P < 0.05 vs sham. n = 15, 14, 13 for sham, EDGB and RYGB respectively. NCD: normal calorie diet.
on these two hormones. As shown in Fig. 5A, EDGB significantly increased the mRNA levels of ghrelin in the gastric mucosa and circulating levels of total ghrelin and acyl-ghrelin. Gastric and circulating levels of nesfatin-1 were also markedly increased in EDGB group (Fig. 5B). The extent of change in ghrelin and nesfatin-1 was comparable between EDGB and RYGB.

3.6. Vagotomy Stimulates Food Intake but Demonstrates No Effect on Body Weight, Glucose Metabolism and Hepatic Steatosis

During the EDGB and RYGB surgery, vagal nerve cannot be spared and the vagotomy was performed. To determine whether vagal reflex contributes to the beneficial effects of EDGB on metabolic profiles, we...
performed the vagotomy in obese mice and assessed its effects on food intake, body weight, glucose metabolism and hepatic lipid contents. Unlike EDGB and RYGB, vagotomy markedly increased food intake (Fig. 6A). However, body weight (Fig. 6A), visceral adipose tissue weight and subcutaneous adipose tissue weight (Fig. 6C), pancreas weight, OGTT and ITT (Fig. 6D), liver weight and liver lipid drops (Fig. 6E) remained unchanged in vagotomy group.

4. Discussion

Using the diet-induced-obese (DIO) mouse model, our present studies demonstrate that EDGB surgery can produce a metabolic benefit including modest weight reduction, sustained improvement in glucose metabolism and hepatic steatosis. This metabolic benefit is comparable to the RYGB which is the gold standard of bariatric surgeries. Relative to RYGB, EDGB is safer and easier to perform in mice.

Rodent models have been increasingly used to study the mechanisms underlying the metabolic benefit of bariatric surgery (Bueter et al., 2010; Chandarana et al., 2011; Nestoridi et al., 2012). Mouse models are particularly important as genetic manipulation allow direct testing of specific hormone or signaling molecule (Ding et al., 2016; McGavigan et al., 2017; Mokadem et al., 2014). Among bariatric surgeries, Roux-en-Y gastric bypass (RYGB) is the most effective and commonly performed in the treatment of obesity and its associated metabolic dysfunctions such as type-2 diabetes and NAFLD (Haruta et al., 2017; Beaulac and Sandre, 2017). Existing mouse models vary greatly in the surgical detail and do not closely mimic typical RYGB surgery in humans (Troy et al., 2008; Yin et al., 2011; Hatoum et al., 2012). In the present study, a mouse RYGB model based on a pouch size of about 5% of the total stomach volume, which is similar to typical RYGB surgery in humans, was used as a gold standard for the evaluation of metabolic efficacy.

We did not detect any anemia in all mice with EDGB. As a result, survival rate for EDGB was significantly higher than RYGB. EDGB is thus easier and safer than RYGB. Weight regain and type-2 diabetes relapse has been reported in a significant proportion of VSG both in patients and in mice (Felsenreich et al., 2016). Further, VSG is less effective to lastingly suppress body weight and improve glycemic control compared with RYGB in mice (Hao et al., 2017). Together with our observation that EDGB provides a metabolic benefit identical to RYGB, these findings suggest that EDGB may be a better approach for weight reduction and glycemic control relative to VSG, although the long-term effects remain to be investigated.

While weight loss was rapid and profound in RYGB group (37.3 ± 3%), EDGB mice demonstrated a modest weight loss (20.2 ± 2%) which sustained for the entire period (8 weeks) of experiments. Despite of this modest weight reduction, EDGB produced a metabolic benefit similar to RYGB. EDGB improves glucose and lipid metabolism in obese mice to the extent comparable to RYGB. This observation indicates that weight loss independent mechanism is critically involved in the improvement of glucose and lipid metabolism induced by bariatric surgery. The significance of these findings is two-folds: (1) EDGB may serve as an alternative model to study the weight-loss-independent mechanisms for improvement of glucose homeostasis; (2) In the clinical setting, EDGB may be a preferred procedure for intervention of T2DM with no or mild obesity.

Mechanisms by which bariatric surgery improves obesity and metabolic dysfunction remain disputed. Reduction in food intake has been proposed as a driving force for long-term body weight loss and metabolic improvement. Our studies indicate a mechanism independent of food intake. Neither EDGB nor RYGB demonstrated a significant effect on body weight in obese mice. Similarly, studies by Hao et al. (2017) have reported that total food intake is similar after VSG, RYGB, and sham surgery. All these observations suggest the involvement of food independent mechanism for the metabolic benefit of bariatric surgery. It is worth noting that reduction in nutrient absorption sufficiency which is not detected in our experiments may contribute to the phenotypic

![Fig. 5.](image-url) Identical effects on gastric hormones by EDGB and RYGB. a. Increase in ghrelin. Shown are the mRNA levels of ghrelin in the gastric mucosa (left) and circulating levels of total ghrelin (middle) and acyl-ghrelin (right). b. Increase in nesfatin-1. Shown are mRNA and protein levels of nesfatin-1 in the gastric mucosa and the circulating nesfatin-1. *P < 0.05 vs sham. n = 9 mice per group.
characterization after RYGB surgery. Indeed, several studies (Mokadem et al., 2014; Liou et al., 2013; Hao et al., 2017) have reported that fecal energy intake may reduce 1–3 kcal/day after RYGB surgery. Further studies should investigate whether EDGB affects the nutrition absorption sufficiency and energy expenditure in a manner distinct from the RYGB and VSG.

The “foregut” hypothesis represents an alternative mechanism for improved glucose control after bariatric surgery. This hypothesis is built on the premise that exclusion of the foregut, in particular duodenum, is critical for normalization of glycemic control (Rubino et al., 2010; Wang et al., 2008). Nutrients entering the duodenum stimulate the release of an unknown hormone, an anti-incretin that counteracts the action of glucagon like peptide 1 (GLP-1) (Buteau et al., 2004). Bypassing this segment of the gut inhibits the release of this anti-incretin, thus allowing normalization of glycemic control (Mosinski and Kirwan, 2016). Again, this concept is under debate. Supporting for this concept are the observations that the metabolic benefit of RYGB surgery was abrogated when obese rats or patients were fed through a gastric tube placed directly into the bypassed segment of the gut. In our EDGB model, food bypasses the stomach and enters directly into the duodenum. However, EDGB still produces significant improvement in glycemic control to the extent similar to RYGB. This finding indicates that food bypassing the duodenum is not required for the glycemic control after bariatric surgery. Recent studies also challenge the central role of the incretin in the metabolic benefit of bariatric surgery. Although animal and clinical studies support a significant role for GLP-1 in postprandial β-cell function and glycemic control, other studies demonstrate that RYGB is still able to produce metabolic benefit in GLP-1-deficient mice (Mokadem et al., 2014; Ye et al., 2014). These findings indicate the complex mechanisms underlying the beneficial effects of bariatric surgery.
surgery. To explore the alternative mechanism, we first analyzed the levels of ghrelin and nesfatin-1: two counteracting hormones derived from the gastric X/A like endocrine cells. Unexpectedly, both gastric ghrelin mRNA and circulating total and acyl-ghrelin increased significantly after EDGB and RYGB. This finding contradicts previous reports showing a decrease in ghrelin after bariatric surgery. The biggest decline in ghrelin levels was observed after VSG in which majority of the stomach is removed (Alamuddin et al., 2017). Since over 80% of circulating ghrelin derives from the stomach, it is reasonable that removal of the stomach results in the significant decrease in ghrelin. Further, secretion of ghrelin has been demonstrated to be inhibited by the mechanical and chemical signals initiated from the food ingestion (Cummings et al., 2001; Xu et al., 2009). Further studies should thus examine whether release of the inhibitory signals derived from food ingestion in the stomach contributes to the increase of ghrelin after EDGB and RYGB. We also observed a significant increase in gastric and circulating nesfatin-1 (Li et al., 2012; Ogiso et al., 2011), our findings suggest that nesfatin-1 may partially contribute to the metabolic benefit after EDGB and RYGB.

The current mechanism by which there is no difference in IPGTT between EDGB, RYGB and sham surgery remains unclear. The size and weight of the pancreas is increased, and we also found the area of islets is significantly increased to the same degree between EDGB and RYGB, which might contribute a small proportion of the overall area of the organ, but could not rule out the increase in pancreatic exocrine weight after the gastric bypass surgery. The increase in islet area would theoretically predict an alteration in glucose tolerance. The observation of no difference in IPGTT between sham, EDGB and RYGB suggests that mechanism other than islet function may contribute to the improvement in glycemic metabolism. These alternative mechanisms includes reprogramming of intestinal glucose distribution, gastrointestinal endocrine homeostasis and vagon function. A recent study demonstrating that reprogramming of intestinal glucose metabolism contributes to the glycemic control after RYGB (Saedi et al., 2013). Together with our previous studies (Xu et al., 2009; Xu et al., 2012; Li et al., 2013), these findings suggest that intestinal fuel sensing is critical for glucose disposal in intestine and the subsequent improvement in glycemic control.

Vagal innervation has been also proposed as an alternative mechanism for the metabolic benefit of bariatric surgery. Studies by Hao et al. indicate that signals carried by vagal afferents from the mid and lower intestines contribute to the early RYGB-induced body weight loss and reduction of food intake. Further, vago-vagal reflex contributes to the up-regulation of GLP-1 and improvement of glycemic control after RYGB (Hao et al., 2013). In contrast to these findings, our studies indicate that vagotomy does not significantly alter body weight, glucose tolerance, insulin sensitivity and hepatic steatosis in obese mice. These observations suggest that vagal innervation may not be indispensable for the metabolic benefit after bariatric surgery.

The size and weight of the pancreas is increased. It is unlikely that increase in islet mass contributes to the gain of pancreas weight after EDGB and RYGB because islet mass only consists of a small proportion of the overall pancreas. Whether increase in pancreatic exocrine weight accounts for the weight gain of this organ after the gastric bypass surgery remains to be explored.

In conclusion, our studies demonstrate EDGB as an easier and safer bariatric surgery with beneficial effects of glucose and lipid control identical to RYGB in mice. EDGB may provide an alternative approach for the intervention of obesity, and its associated metabolic dysfunctions such as T2DM and NAFLD.

Conflict of Interest

The authors have nothing to disclose.
Mcgavigan, A.K., Garibay, D., Henseler, Z.M., Chen, J., Bettaieb, A., Haj, F.G., Ley, R.E., Chouinard, M.L., Cummings, B.P., 2017. TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. Gut 66, 226–234.

Mokadem, M., Zechner, J.F., Margolskee, R.F., Drucker, D.J., Aguirre, V., 2014. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. Mol. Metab. 3, 191–201.

Mosinski, J.D., Kirwan, J.P., 2016. Longer-term physiological and metabolic effects of gastric bypass surgery. Curr. Diab. Rep. 16, 50.

Nestoridi, E., Kvas, S., Kucharczyk, J., Stylopoulos, N., 2012. Resting energy expenditure and energetic cost of feeding are augmented after Roux-en-Y gastric bypass in obese mice. Endocrinology 153, 2234–2244.

Nguyen, N.T., Vu, S., Kim, E., Bodunova, N., Phelan, M.J., 2016. Trends in utilization of bariatric surgery, 2009–2012. Surg. Endosc. 30, 2723–2727.

Ogiso, K., Asakawa, A., Amitani, H., Nakahara, T., Ushikai, M., Haruta, I., Koyama, K., Amitani, M., Harada, T., Yasuhara, D., Inui, A., 2011. Plasma nesfatin-1 concentrations in restricting-type anorexia nervosa. Peptides 32, 150–153.

Ponce, J., Nguyen, N.T., Hutter, M., Sultan, R., Morton, J.M., 2015. American Society for Metabolic and Bariatric Surgery estimation of bariatric surgery procedures in the United States, 2011–2014. Surg. Obes. Relat. Dis. 11, 1199–1200.

Ye, J., Hao, Z., Humphrey, M.B., Townsend, R.L., Patterson, L.M., Stylopoulos, N., Munzberg, H., Morrison, C.D., Drucker, D.J., Berthoud, H.R., 2014. GLP-1 receptor signaling is not required for reduced body weight after RYGB in rodents. Am. J. Phys. Regul. Integr. Comp. Phys. 306, F352–F62.

Zong, L., Chen, P., 2011. Billroth I vs Billroth II vs Roux-en-Y following distal gastrectomy: a meta-analysis based on 15 studies. Hepato-Gastroenterology 58, 1413–1424.