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Basic Study
Bariatric surgery on glucose and lipid metabolism, and liver and kidney functions in food-derived obese diabetic rats

Long H et al. Food-derived obese diabetic rats
Abstract

BACKGROUND
Obesity usually causes diabetes and is a serious danger to human health. Type 2 diabetes mostly occurs along with obesity. Foodborne obesity diabetes was caused by an excessive long-term diet and surplus energy. Bariatric surgery can improve the symptoms of type 2 diabetes in some obese patients. But different types of bariatric surgery may have different effects.

AIM
To investigate the effect of bariatric surgery on glucose and lipid metabolism, and liver and kidney functions in rats.

METHODS
Male Sprague-Dawley rats aged 6-8 wk underwent Roux-en-Y gastric bypass surgery (RYGB), sleeve gastrectomy (SG), gastric banding (GB). Glucose and insulin tolerance tests, analyses of biochemical parameters, histological examination, western blotting, and quantitative real-time polymerase chain reaction were conducted.

RESULTS
In comparison to the sham operation group, Roux-en-Y gastric bypass surgery, SG, and GB groups had decreased body weight and food intake, reduced glucose intolerance and insulin insensitivity, downregulated biochemical parameters, alleviation of morphological changes in the liver and kidneys, and decreased levels of protein kinase C β/P66shc. The effect in the RYGB group was better than in the SG and GB groups.

CONCLUSION
These results suggest that RYGB, SG and GB may be helpful for the treatment of foodborne obesity diabetes.
Key Words: Diabetes; Obesity; Bariatric surgeries; Liver and kidney functions


Core Tip: Bariatric surgery can improve the symptoms of type 2 diabetes in some obese patients. But different types of bariatric surgery may have different effects. In the current study, in comparison to the sham operation group, Roux-en-Y gastric bypass surgery (RYGB), sleeve gastrectomy (SG), and gastric banding (GB) groups had decreased body weight and food intake, reduced glucose intolerance and insulin insensitivity, downregulated biochemical parameters, alleviation of morphological changes in the liver and kidneys, and decreased levels of protein kinase C β/P66shc. The effect in the RYGB group was better than in the SG and GB groups. Here, we introduced bariatric surgeries as a helpful tool for the treatment of food-derived obese diabetes.

INTRODUCTION
Diabetes mellitus (DM), a well-known chronic metabolic disease, is a substantial global problem characterized by a common outcome of hyperglycemia. Type 2 DM (T2DM), accounting for > 90% of all cases of DM, is considered the chief cause of diabetic complications, including diabetic nephropathy, diabetic neuropathy, cardiovascular disease, and diabetic retinopathy[1].

It is widely acknowledged that obesity, increasingly influenced by lifestyle factors, is an essential risk for the development of T2DM[2,3]. Obesity is involved in the upregulated circulating free fatty acids that play a distinct role in progressive dysfunction of pancreatic beta cells, damaging their ability to compensate for insulin resistance (IR), and thus contributing to T2DM pathogenesis[4]. The growing prevalence
of T2DM has resulted in various approaches focusing on discovering novel therapeutic targets for treating hyperglycemia.

Higher blood glucose serves as an absolute risk factor for all-cause mortality, and bariatric surgery can increase survival rates of patients with obesity\cite{5,6}. Bariatric surgery can promote sustained weight loss and is more efficacious than traditional medical strategies for long-acting control of T2D\cite{7}. Bariatric surgery quickly diminishes IR and blood glucose before any fathomable weight loss\cite{8,9}. Bariatric surgery can ameliorate glycemic control and glucose homeostasis, including in patients with T2DM\cite{10}. However, the underlying mechanism remains to be investigated.

Protein kinase C (PKC), a family member of serine/threonine protein kinases consisting of \textit{>} 12 members, exerts a pivotal role in intracellular crosstalk and signal transduction\cite{11}. It has been reported that pharmacological blockade or gene deletion of PKC\(\beta\) can decrease infarct size, protect ischemic myocardium, and promote ventricular functional recovery\cite{12}. The Shc adaptor protein family contains P46shc, P52shc and P66shc, but only P66shc plays an essential role as a redox enzyme associated with the generation of mitochondrial reactive oxygen species (ROS) and the transformation of oxidative signals into apoptosis\cite{13}. Hyperglycemic and oxidative stress can activate the PKC\(\beta\)2 isoform to stimulate phosphorylation of P66shc at ser36 to transfer phosphorylated (p)-P66shc from the cytosol to the inner mitochondrial membrane, where p-P66shc can increase oxidative stress and catalyze the generation of ROS via cytochrome oxidation\cite{14,15}. Therefore, we hypothesize that there may be a PKC\(\beta\)/P66shc signaling pathway in the pathogenesis of DM.

In the current study, we investigated the effects of bariatric surgery, including Roux-en-Y Gastric bypass (RYGB), sleeve gastrectomy (SG), and gastric banding (GB), on the foodborne obesity diabetes in rats and possible mechanisms related to the PKC\(\beta\)/P66shc signaling pathway.

**MATERIALS AND METHODS**

**Animals**
Male Sprague-Dawley rats aged 6-8 wk with body weight of 200-220 g were purchased from the Guangdong Medical Laboratory Animal Center and adapted to the environment for 7 d before the experiments. All rats were housed in specific pathogen-free cages with a 12-h light/dark cycle and had free access to drinking water and food.

**Bariatric surgery and groups**

All rats were randomly divided into four groups of 10: RYGB, SG, GB and sham operation, with 10 rats in each group. The rats were anesthetized with 0.5% pentobarbital (45 mg/kg) by intraperitoneal injection before the operation.

In the RYGB group, rats were routinely fasted with water 12 h before the operation. After weighing the rats, anesthesia was induced by intraperitoneal injection of 3% pentobarbital (0.15 mL/100 g). When the limbs were soft, we used an animal shaver to prepare the operation area, dipped a sterile cotton swab in iodophor, and disinfected the skin around the incision area three times. At the sterile area about 3 cm below the sternum of the rat, a longitudinal incision was made, separated layer by layer into the abdominal cavity, and an electrocoagulator was used to stop bleeding as needed. After entering the abdominal cavity, we confirmed the position of the distal ligament of Treitz. At a distance of 10-15 cm from the distal end of the ligament, we freed a segment of small intestine to avoid damaging the mesenteric vessels and intestinal serosa, separated and cut off the jejunum, wiped off the digestive fluid with a cotton swab, and disinfected the distal and proximal intestinal tubes with iodophor. End-to-side full-thickness anastomosis was performed at the proximal jejunum 10 cm below the distal jejunum to keep the anastomosis unobstructed. We gently exposed the stomach body, esophagus and cardia with a sterile cotton swab, and used the electrocoagulator to dissociate the stomach towards the lower part of the cardia. The stomach was severed at 10 cm at the distal end of the cardia, and < 20% of the gastric sacs were retained. The proximal gastric sacs were anastomosed with the distal jejunum laterally. After confirming the patency of the anastomosis, the distal remnant stomach was closed by suture. Both gastrojejunostomy and jejunoojejunostomy were sutured with 6-0
absorbable sutures. Before closing the abdominal cavity, we flushed the abdominal cavity with normal saline solution containing gentamicin in three times. Intermittent suture was used for abdominal closure.

In the SG group, preoperative treatment was the same in the RYGB group. We cut the skin and subcutaneous tissue layer by layer, exposed the esophagus, stomach, duodenum and other organs, freed the stomach from the abdominal cavity, and ligated the vessels of the greater curvature according to the scope of resection. And 75%-80% of the whole stomach volume was cut off, including the fundus and most of the stomach body tissue. After resection, we cleaned the contents of the remnant stomach with a cotton swab. The stump stomach was sutured and closed, and the abdominal cavity was washed with physiological saline. After dipping the physiological saline with cotton ball, the stump stomach was covered with omentum, and the abdominal cavity was closed layer by layer.

In the GB group, preoperative treatment was the same as in the RYGB group. The midline incision of the upper abdomen was 3 cm long and entered the abdomen. The inner diameter of the lower part of the upper gastric cardia was bound and fixed to one third of the original position with a buckle type silicon tape at 1 cm below the cardia. The proximal end formed a 20% small gastric sac. Routine abdominal closure was performed.

In the sham operation group, rats were fasted with water 12 h before the operation, and the jejunum and gastric body were cut at the same position as in the RYGB group, and then the broken end was cut and anastomosed. The cross section of the gastrointestinal tract was performed at the site where RYGB underwent gastrotomy, and the anastomosis was performed at the original cutting site. The operation time should be the same as that of RYGB, and normal saline containing gentamicin was used to wash the abdomen. The physiological flow of food through the gastrointestinal tract remained intact. After surgery, the rats were placed into a single cage, and wound care was applied.
Glucose and insulin tolerance tests

At 4 wk, the oral glucose tolerance test (OGTT) was performed as previously described\textsuperscript{[16]} by glucose gavage (5 g D-glucose/kg) following an overnight fast. After the tail of rats was pierced using a needle, a drop of venous blood was collected to determine blood glucose concentration, and 300 μL of tail vein blood was gathered and centrifuged for 15 min at 2000 rpm to collect the serum for the measurement of insulin. Blood glucose was determined using a glucometer (Roche Diagnostics, Switzerland) following glucose gavage for 0 h, 1 h, and 2 h, and insulin was monitored at the same timepoints. The insulin tolerance test (ITT) was performed as previously described\textsuperscript{[17]}. After fasting for 8 h, the rats were intraperitoneally injected with insulin (0.5 U/kg). The area under the curve (AUC) of OGTT and ITT was also calculated.

Analysis of biochemical parameters

At 4 wk, blood samples gathered from the abdominal aorta of rats under anesthesia were centrifuged for 15 min at 3000 rpm to determine the serum concentrations of triglyceride (TG), total bile acids (TBA), alanine aminotransferase (ALT), and aspartate aminotransaminase (AST) by a microplate (Reitman-Frankel colorimetric assay) (Nanjing Jiancheng Corp., Nanjing, China).

Histological examination

Histological examination was performed as previously described\textsuperscript{[17]}. Liver and kidney tissues were fixed in 4% paraformaldehyde solution, following embedding in paraffin blocks. Tissue sections were obtained using a microtome, and after staining with hematoxylin and eosin (HE), they were examined under a microscope (Olympus, Japan).

Western blotting

Western blotting was performed as previously described\textsuperscript{[18]}. Generally, protein samples were separated using 10% SDS-PAGE at 90 V for nearly 1.5 h, followed by
electroblotting onto polyvinylidene difluoridemembranes for 2 h at 300 mA. The membranes were blocked with 5% bovine serum albumin (#9048-46-8, Beijing Solarbio Science & Technology Co., Ltd., City, China) diluted in Tris-HCl buffer supplemented with 0.1% Tween-20 (TBST; pH = 7.4), following incubation with the primary antibodies: rabbit anti-PKCβ (1:1000, #ab32026, Abcam Cambridge, United Kingdom) and rabbit anti-P66shc (1:1000, #ab33770, Abcam), overnight at 4 °C. Membranes were washed three times with TBST for 5 min each. The membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000, #ab288151, Abcam) diluted in TBST for 1 h at room temperature. Membranes were washed with TBST three times for 5 min each. An enhanced chemiluminescence solution (Bio-Rad, Hercules, CA, United States) was used to visualize the immunoreactive signals. Band intensity was quantified using ImageJ 5.0.

**Quantitative real-time polymerase chain reaction**

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as previously described[27]. After blood collection, the rats were killed, the liver and kidney tissues were quickly removed, and 15-20 mg of pancreatic tail tissue was separated on the ice bed for fluorescent qPCR detection. Liver and kidney tissues were treated with TRIzol reagent, cut, and homogenized on ice until complete lysis. After centrifugation at 1200 g, absorb the supernatant, add 200 μL of isopropanol, mix it upside down, centrifuge at 1200 g, and discard the supernatant. We added 1 mL 75% ethanol, centrifuged at 1200 g, and discarded the supernatant. RNA was dried at room temperature for 30 min and stored at -80 °C. We measured the absorbance at 260 and 280 nm with a UV spectrophotometer, and calculated the ratio, which was controlled to 1.8-2.0. RNA content was calculated according to OD_{260} value. RT reaction: Add total RNA into EP pipe 8 μL, 10 μmol/L Oligo (dT) 1 μL. DEPC water 3 μL. Mix well and centrifuge, and immediately dissolve in water for 3 min. Add 5 * M-MLV buffer 4.0 μL into the above pipes, 10 mmol/L dNTP 2.0 μL, RNAser 1 μL, M-MLV 1 μL. After mixing, jog centrifugation, and complete the following operations on the PCR
instrument: 42 °C for 60 min, 70 °C for 5 min. Take out the above reaction solution to obtain cDNA and store it at -20 °C. Fluorescent quantitative PCR reaction: Complete the detection of gene expression under RT method, and the reaction system is as follows: 2 * qRT-PCR, the total volume of PCR reaction is 10 μL. Including forward primer (10 μmol/L) 0.4 μL. Reverse primer (10 μmol/L) 0.4 μL, 50 * ROXII0.4 μL, cDNA 2.0 μL. Double distilled water 6.8 μL. The relative mRNA level of each gene was analyzed by the 2^-ΔΔCt method. The sequences of each primer is shown in Table 1.

**Statistical analyses**

Data were shown as mean ± SEM and analyzed using GraphPad Prism v7.03 with one-way analysis of variance with Dunnett’s post-hoc test. Results were considered statistically significant at P < 0.05.

**RESULTS**

*Serum biochemical analysis and physical conditions*

The levels of serum total cholesterol (TC), TG, IR and TBA were significantly upregulated in all four groups after building the foodborne obesity diabetes rat models at week 8 compared with before (week 1).

After 8 wk, body weight, blood glucose, and serum levels of TC, TG, IR and TBA were not significantly different among the four groups (P > 0.05) (Figures 1A-F). One rat in the RYGB group died of massive hemorrhage during surgery, and one died on postoperative day 4. Anatomical analysis showed that the cause of death might be anastomotic leakage (Figures 1G and H), and one died of wound infection (Figure 1I). Seven survived until the end of the experiment. Two rats in the SG group died of wound infection 1 wk after the operation (Figure 1J). Eight survived until the end of the experiment. All rats in the GB and sham groups survived.

*Effect of bariatric surgery on body weight and food intake in rats*
In comparison to the sham group, the body weight in the RYGB, SG and GB groups was significantly decreased. Among the three groups, body weight was lowest in the RYGB group and highest in the GB group ($P < 0.05$) (Figure 2A). Each group differed significantly compared with other groups. In comparison to the sham group, food intake in the RYGB, SG and GB groups was significantly decreased. Among the three groups, food intake as lowest in the RYGB group and highest in the GB group ($P < 0.05$) (Figure 2B). Each group differed significantly compared with other groups.

**Effect of bariatric surgery on glucose intolerance and insulin insensitivity in rats**

In comparison to the sham group, fasting blood glucose in the RYGB, SG and GB groups was significantly decreased ($P < 0.05$). Among the three groups, fasting blood glucose was lowest in the RYGB group and highest in the GB group (Figure 2C). Each group differed significantly compared with other groups.

In comparison to the sham group, fasting blood IR level in the RYGB, SG and GB groups was significantly decreased ($P < 0.05$). Among the three groups, fasting blood IR level in RYGB group is bigger than those in the GB and SG groups, with no significant difference between the RYGB and SG groups (Figure 2D). In comparison to the sham group, blood glucose level in the OGTT in the RYGB, SG and GB groups was significantly decreased ($P < 0.05$). Among the three groups, blood glucose level in the OGTT was lowest in the RYGB group and highest in the GB group. Each group differed significantly compared with other groups (Figure 2E). A similar pattern of AUC of OGTT was observed (Figure 2F).

In comparison to the sham group, the blood glucose level in the ITT in the RYGB, SG and GB groups was significantly decreased ($P < 0.05$). Among the three groups, blood glucose level in the ITT was lowest in the RYGB group and highest in the GB group (Figure 2G). Each group differed significantly compared with other groups. A similar pattern of AUC of ITT was observed (Figure 2H).

**Effect of bariatric surgery on biochemical parameters in rats**
In comparison to the sham group, the serum levels of TG, TC, TBA, ALT and AST were significantly decreased ($P < 0.05$). Among the three groups, the serum levels of TG, TC, TBA, ALT and AST were lowest in the RYGB group and highest in the GB group (Figure 3). Each group differed significantly compared with other groups.

**Effect of bariatric surgery on morphological changes in the liver and kidneys in rats**

At the time of dissection, naked eye observation revealed that the liver volume of rats in the sham group increased, with a milky white appearance, the edge became blunt, the texture was soft; in the RYGB group, the liver was small, bright red, with sharp edges. HE staining showed a large number of fatty vacuoles in the liver of rats in the sham group, hepatic cord disorder, and narrowing of hepatic sinuses, indicating the presence of severe fatty liver. Compared with the sham group, the fatty vacuoles in the SG and GB groups were significantly reduced, the hepatic cord was unclear, and the shape was curved, indicating mild fatty liver. In the RYGB group, the liver lobules were normal, the hepatic cords were orderly arranged, the hepatic sinuses were normal, and no obvious degeneration of hepatocytes was observed (Figures 4A-D).

In the sham group, renal tubular lesions were more obvious, epithelial cells showed vacuolar lesions, glomerular mesangial injury, and shedding of epithelial cells; the lesions in the SG and GB groups were significantly alleviated; and there was no obvious kidney disease in the RYGB group (Figures 4E-H).

**Effect of bariatric surgery on PKCβ/P66shc pathway in rats**

In comparison to the sham group, the levels of P66shc and PKCβ were significantly decreased in the RYGB, SG and GB groups ($P < 0.05$). Among the three groups, the levels of P66shc and PKCβ were lowest in the RYGB group and highest in the GB group (Figures 5A-C). Each group differed significantly compared with other groups.

In comparison to the sham group, the mRNA levels of P66shc and PKCβ were significantly decreased in the RYGB, SG and GB groups ($P < 0.05$). Among the three groups, the mRNA levels of P66shc and PKCβ were lowest in the RYGB group and
highest in the GB group (Figures 5D and E). Each group differed significantly compared with other groups.

DISCUSSION

In previous studies, bariatric surgery promoted sustained weight loss, ameliorated glycemic control and glucose homeostasis in patients with T2DM\textsuperscript{[7,10]}. In the current study, we revealed that bariatric surgery, including RYGB, SG and GB, can decrease body weight and food intake, reduce glucose intolerance and insulin insensitivity, downregulate biochemical parameters, alleviate morphological changes in the liver and kidneys, and diminish the levels of PKCβ/P66shc pathway, laying the foundation for bariatric surgery to be a novel treatment for foodborne obesity diabetes.

It is well-known that obesity predicts progression to T2DM, characterized by increased blood glucose, glucose intolerance and IR\textsuperscript{[19,20]}. Here, we observed that bariatric surgery decreased body weight and food intake, and reduced glucose intolerance and insulin insensitivity.

In complex diseases, including T2DM, there are several genes involved, affecting biological function in groups rather than alone. Therefore, to understand the signaling pathways involved in the pathological mechanisms and identify which of these pathways are affected in each patient may provide more understanding of T2DM and could lead to new strategies for diagnosing, treating and preventing disease. It has been reported that acupuncture induced improvement of oxidative stress by regulating PKCβ/P66shc signaling in obese diabetic rats\textsuperscript{[21]}. Here, we observed that bariatric surgery decreased the levels of PKCβ/P66shc pathway.

Although these results look promisingly, the bariatric surgery, including RYGB, SG and GB, has its own advantages and disadvantages: For RYGB, advantages: It has a small wound size, low risk, good prognosis, and is generally less prone to recurrence. The way of food flow after surgery can also promote insulin secretion, effectively reduce the apoptosis of islet cells, restore the function of islets, and thus effectively treat diabetes. Disadvantages: Some rats will have abdominal discomfort, local inflammation
of the anastomosis, high blood sugar, which is easy to lead to incomplete healing of the surgical incision, infection, intestinal adhesion and other complications. Some rats may also experience symptoms such as gastric paresis, gastrointestinal dysfunction, abdominal distension, and inability to eat, mainly related to postoperative reduction of gastric volume.

For SG, it can effectively control type 2 diabetes and obesity related complications. By reducing the volume of stomach, surgery can reduce weight, improve type 2 diabetes and reduce the risk of obesity related cardiovascular and cerebrovascular complications. Disadvantages: Surgery that completely removes the fundus of the stomach may increase the risk of developing gastroesophageal reflux disease.

For GB, advantages: Like SG, it is a surgical method of reducing weight by reducing food intake. It reduces the entry passage for food by installing binding straps. The surgical damage is minimal, and there is no need to modify the digestive tract, resulting in faster postoperative recovery. Disadvantages: The restraining strap is prone to displacement and expansion, and the surgical effect is not very good, resulting in limited weight loss.

CONCLUSION
Bariatric surgery may be a novel treatment for foodborne obesity diabetes.

ARTICLE HIGHLIGHTS
Research background
Obesity usually causes diabetes and endangers human health seriously, and type 2 diabetes mellitus (T2DM) usually occurs along with obesity. Foodborne obesity diabetes was caused by the excessive long-term diet and surplus energy.

Research motivation
Bariatric surgery can improve the symptoms of T2DM in some obese patients, but different types of bariatric surgery may have different effects.
Research objectives
To investigate the effect of different types of bariatric surgery on glucose and lipid metabolism, and liver and kidney functions in rats, and the underlying mechanisms.

Research methods
Male Sprague-Dawley rats aged 6-8 wk underwent Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (SG), gastric banding (GB). Glucose and insulin tolerance tests, analysis of biochemical parameters, histological examination, western blotting, and quantitative real-time polymerase chain reaction were conducted.

Research results
In comparison to the sham group, RYGB, SG and GB groups had decreased body weight and food intake, reduced glucose intolerance and insulin insensitivity, downregulated biochemical parameters, alleviated morphological changes in the liver and kidneys, and decreased levels of protein kinase C (PKC) β/P66shc pathway. Among the three groups, the effect in the RYGB group was better than in the SG and GB groups.

Research conclusions
Bariatric surgeries, including RYGB, SG, and GB operation can modulate the glucose and lipid metabolism, and liver and kidney functions in food-derived obese diabetic rats via mediating the PKCβ/P66shc pathway.

Research perspectives
Bariatric surgery may be helpful for the treatment of foodborne obesity diabetes.
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