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Impact of bariatric surgery on glucose and lipid metabolism and liver and kidney function in food-induced obese diabetic rats

Hong Long, Lei Zhao, Zhong-Sheng Xiao, Shu-Xiang Li, Qiu-Lin Huang, Shuai Xiao, Liang-Liang Wu

BACKGROUND
Obesity usually causes diabetes mellitus (DM) and is a serious danger to human health. Type 2 DM (T2DM) mostly occurs along with obesity. Foodborne obesity-induced DM is caused by an excessive long-term diet and surplus energy. Bariatric surgery can improve the symptoms of T2DM 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 function in rats.

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

RESULTS
In comparison to the sham operation group, the 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 β/P66shc. The effect in the RYGB group was better than that in the SG and GB groups.

CONCLUSION
These results suggest that RYGB, SG and GB may be helpful for the treatment of...
The rats were anesthetized with 0.5% pentobarbital (45 mg/kg) by intraperitoneal injection before the operation. All the rats were randomly divided into four groups: RYGB, SG, GB, and sham operation (SO), with 10 rats in each group.

Animal groups and bariatric surgery

Male Sprague-Dawley rats aged 6-8 wk with a body weight of 200-220 g were purchased from the Guangdong Medical Animals 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.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats aged 6-8 wk with a 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.

Animal groups and bariatric surgery

All the rats were randomly divided into four groups: RYGB, SG, GB, and sham operation (SO), 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, the abdominal cavity was opened layer by layer, 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 the 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 three times. Intermittent suture was used for abdominal closure.

In the SG group, preoperative treatment was the same as that 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 a cotton ball, the stump stomach was covered with part of the omentum, and the abdominal cavity was closed layer by layer.

In the GB group, preoperative treatment was the same as that in the RYGB group. The midline incision of the upper abdomen was 3 cm long. 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 SO group, rats were fasted 12 h before the operation, and the jejunum and gastric body were cut at the same position as that 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 gastrotomy was performed in RYGB, 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 [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 time points. The insulin tolerance test (ITT) was performed as previously described [17]. After fasting for 8 h, the rats were intra-peritoneally injected with insulin (0.5 U/kg). The area under the curve (AUC) of the 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) with a microplate reader (Reitman-Frankel colorimetric assay) (Nanjing Jiancheng Corp., Nanjing, China).

**Histological examination**

Histological examination was performed as previously described [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 blot analysis**

Western blot was performed as previously described [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., Beijing, China) diluted in Tris-HCl buffer supplemented with 0.1% Tween-20 (TBST; pH = 7.4), followed by incubation with the primary antibodies rabbit anti-PKCβ1 (1:1000, #ab33770, Abcam), rabbit anti-P66shc (1:1000, #ab33770, Abcam), overnight at 4 °C. The membranes were washed three times with TBST for 5 min each and then incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000, #ab289151, Abcam) diluted in TBST for 1 h at room temperature. After the 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[17]. 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. The complementary DNA (cDNA) was generated based on the RNA templates by using a Transcriptor Reverse Transcriptase kit (Roche Applied Science, IN, United States). The mRNA levels of P66shc and PKCβ were determined by qPCR using a KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Inc., MA, United States) and the Miniopticon™ Real-Time PCR Detection System (Bio-Rad, CA, United States). β-actin was used as the internal control. 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 analysis**

Data are shown as the mean ± SEM and were analyzed using GraphPad Prism v7.03 by one-way analysis of variance with the 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 the induction of foodborne obesity-induced diabetes 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 1H), 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 SO groups survived.

**Effect of bariatric surgery on body weight and food intake in rats**

In comparison to the SO group, the body weight in the RYGB, SG, and GB groups was significantly decreased. Among the latter three groups, body weight was lowest in the RYGB group and highest in the GB group \( (P < 0.05) \) (Figure 2A). There was a significant difference in body weight between any two of the groups. In comparison to the SO group, food intake in the RYGB, SG, and GB groups was significantly decreased. Among the latter three groups, food intake was lowest in the RYGB group and highest in the GB group \( (P < 0.05) \) (Figure 2B). There was a significant difference in food intake between any two of the groups.

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

In comparison to the SO group, fasting blood glucose in the RYGB, SG, and GB groups was significantly decreased \( (P < 0.05) \). Among the latter three groups, fasting blood glucose was lowest in the RYGB group and highest in the GB group (Figure 2C). There was a significant difference in fasting blood glucose between any two of the groups.

In comparison to the SO group, fasting blood IR level in the RYGB, SG, and GB groups was significantly decreased \( (P < 0.05) \). Among the latter three groups, fasting blood IR level in RYGB group was significantly higher than those in the GB and SG groups, but with no significant difference between the RYGB and SG groups (Figure 2D). In comparison to the SO group, blood glucose level in the OGTT in the RYGB, SG, and GB groups was significantly decreased \( (P < 0.05) \). Among the latter three groups, blood glucose level in the OGTT was lowest in the RYGB group and highest in the GB group. There was a significant difference in blood glucose level in the OGTT between any two of the groups (Figure 2E). A similar pattern of AUC of the OGTT was observed (Figure 2F).

In comparison to the SO group, the blood glucose level in the ITT in the RYGB, SG, and GB groups was significantly decreased \( (P < 0.05) \). Among the latter three groups, blood glucose level in the ITT was lowest in the RYGB group and highest in the GB group (Figure 2G). There was a significant difference in blood glucose level in the ITT between any two of the groups. A similar pattern of AUC of the ITT was observed (Figure 2H).

**Effect of bariatric surgery on biochemical parameters in rats**

In comparison to the SO group, the serum levels of TG, TBA, ALT, and AST were significantly decreased in the RYGB, SG, and GB groups \( (P < 0.05) \). Among the latter 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). There was a significant difference in the serum levels of TG, TC, TBA, ALT, and AST between any two of the groups.

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

Grossly, the liver volume of rats in the SO group increased, with a milky white appearance, the edge became blunt, and the texture was soft; in the RYGB group, the liver was small and bright red, with sharp edges. HE staining showed a large number of fatty vacuoles in the liver of rats in the SO group, hepatic cord disorder, and narrowing of hepatic sinuses,
Table 1 Primers used for quantitative real-time polymerase chain reaction

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<th>Gene</th>
<th>5’ to 3’</th>
<th>Product length (bp)</th>
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<tr>
<td>P66 shc</td>
<td>Forward: TGCCCCCTCCCTCCAGGACAT</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>Reverse: CGCAACCCCATGTCACCGAAC</td>
<td></td>
</tr>
<tr>
<td>PKCβ</td>
<td>Forward: GACTTCATTGGGGCTCAGGG</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Reverse: TTGCTCGTGGGTCACAGA</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward: GTTCGACATTCCAGGAGAC</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Reverse: ACCAACACACACAGTA</td>
<td></td>
</tr>
</tbody>
</table>

PKC: Protein kinase C.

Figure 1 Serum biochemical analysis and physical conditions. A-F: Different changes in body weight (A), blood glucose (B), insulin (C), triglyceride (D), total cholesterol (E) and total bile acids (F) in foodborne obese diabetic rats before and after modeling (n = 10); G: Representative images of body shape between obese diabetic and normal rats; H: One rat in the Roux-en-Y gastric bypass surgery (RYGB) group died of anastomotic fistula; I: One rat in the RYGB group died of wound infection; J: One rat in the SG group died of wound infection. *P < 0.05. TG: Triglyceride; TC: Total cholesterol; TBA: Total bile acid; RYGB: Roux-en-Y gastric bypass surgery; SG: Sleeve gastrectomy; GB: Gastric banding; SO: Sham operation.

indicating the presence of severe fatty liver. Compared with the SO group, the number of fatty vacuoles in the SG and GB groups was 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 SO group, renal tubular lesions were more obvious, and epithelial cells showed vacuolar lesions, glomerular mesangial injury, and shedding. 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 SO group, the protein expression levels of P66shc and PKCβ were significantly decreased in the RYGB, SG, and GB groups ($P < 0.05$). Among the latter three groups, the protein expression levels of P66shc and PKCβ were lowest in the RYGB group and highest in the GB group (Figures 5A-C). There was a significant difference in the protein expression levels of P66shc and PKCβ between any two of the groups.

In comparison to the SO group, the mRNA levels of P66shc and PKCβ were significantly decreased in the RYGB, SG, and GB groups ($P < 0.05$). Among the latter 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). There was a significant difference in the mRNA expression levels of P66shc and PKCβ between any two of the groups.

DISCUSSION

In previous studies, bariatric surgery promoted sustained weight loss, and achieved glycemic control and glucose homeostasis in patients with T2DM[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 expression levels of PKCβ and P66shc, suggesting that bariatric surgery may be a novel treatment for foodborne obesity-induced DM.

It is well-known that obesity predicts progression to T2DM, characterized by increased blood glucose, glucose intolerance, and IR[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 multiple 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 a better understanding of T2DM and could lead to new
Figure 3 Changes in biochemical indexes. A-E: Different changes in triglyceride (A), total cholesterol (B), total bile acids (C), alanine aminotransferase (D), and aspartate aminotransaminase (E) in foodborne obese diabetic rats after bariatric surgery \((n = 10)\). *\(P < 0.05\). TC: Total cholesterol; TBA: Total bile acids; ALT: Alanine aminotransferase; AST: Aspartate aminotransaminase; RYGB: Roux-en-Y gastric bypass surgery; SG: Sleeve gastrectomy; GB: Gastric banding; SO: Sham operation.

Figure 4 Histomorphological changes in the liver and kidneys. A-H: Representative images of liver tissue in sham operation (SO) group (A), sleeve gastrectomy (SG) group (B), gastric banding (GB) group (C), and Roux-en-Y gastric bypass surgery (RYGB) group (D) in foodborne obese diabetic rats after bariatric surgery, as well as representative images of kidney tissue in SO group (E), SG group (F), GB group (G), and RYGB group (H) in foodborne obese diabetic rats after bariatric surgery are shown. Scale bars, 20 μm.

strategies for diagnosing, treating, and preventing this disease. It has been reported that acupuncture induced improvement of oxidative stress by regulating PKCβ/P66shc signaling in obese diabetic rats\(^{[21]}\). Here, we observed that bariatric surgery decreased the expression levels of PKCβ and P66shc.

Although these results look promising, each bariatric surgery, including RYGB, SG, and GB, has its own advantages and disadvantages. For RYGB, it has a small wound size, low risk, and 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. However, some rats undergoing RYGB will have abdominal discomfort, local inflammation of the anastomosis, and 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 the postoperative reduction of gastric volume.

For SG, it can effectively control T2DM and obesity related complications. By reducing the volume of the stomach, this surgery can reduce weight, improve T2DM, and reduce the risk of obesity related cardiovascular and cerebrovascular complications. However, SG completely removes the fundus of the stomach and may increase the risk of developing gastroesophageal reflux disease.
CONCLUSION

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

ARTICLE HIGHLIGHTS

Research background
 Obesity usually causes diabetes mellitus (DM) and endangers human health seriously, and type 2 DM (T2DM) usually occurs along with obesity. Foodborne obesity-induced DM is 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 function in rats, and to explore the underlying mechanisms.

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

Research results
In comparison to the sham operation group, the 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. Among the three groups, the effect in the RYGB group was better than that in the SG and GB groups.
Research conclusions
Bariatric surgeries, including RYGB, SG, and GB, can modulate the glucose and lipid metabolism, and liver and kidney function 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-induced DM.

FOOTNOTES

Author contributions: Long H, Zhao L, Li SX, and Wu LL designed the research; Long H and Wu LL performed the research and wrote the paper; Zhao L, Huang QL, and Xiao S supervised the report; Li SX contributed to the data analysis; Xiao ZS provided clinical advice.

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