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Original article

The effect of selective gut stimulation on glucose metabolism after gastric bypass in the Zucker diabetic fatty rat model

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Abstract

Background: Potential mechanisms underlying the antidiabetic effects of Roux-en-Y gastric bypass (RYGB) include altered nutrient exposure in the gut. The aim of this study was to evaluate the effects of selective gut stimulation on glucose metabolism in an obese diabetic rat model.

Methods: Sixteen male Zucker diabetic fatty rats were randomly assigned to 1 of 2 groups: RYGB with gastrostomy tube (GT) insertion into the excluded stomach or a control group with GT insertion into the stomach. An insulin tolerance test (ITT), oral glucose tolerance test (OGTT), and mixed meal tolerance test (MMTT) were performed before and 14–28 days after surgery. A glucose tolerance test via GT (GTT-GT) and MMTT via GT were performed postoperatively.

Results: Postoperatively, the RYGB group had significant decreases in weight and food intake. Both the ITT and OGTT tests revealed significantly improved glucose tolerance after RYGB. The GTT-GT showed a reversal of the improved glucose tolerance in the RYGB group. In response to meal stimulation, postoperatively, the RYGB group increased glucagon-like peptide 1 (GLP-1) secretion via the oral route and peptide YY secretion by both oral and GT routes.

Conclusion: When foregut exposure to nutrients was reversed after RYGB, the improvement in glucose metabolism was abrogated. This model can be extended to identify the role of gut in glucose homeostasis in type 2 diabetes. (Surg Obes Relat Dis 2013;▮:00-00.)

Keywords : Type 2 diabetes; Obesity; Insulin resistance; Roux-en-Y gastric bypass; Gastrointestinal hormones

There is a growing consensus that bariatric surgery is an effective treatment for type 2 diabetes mellitus (T2DM) in obese patients [1,2]. All forms of weight loss surgery lead to caloric restriction, weight loss, and a decrease in fat mass, which are associated with improvements in glucose metabolism [3]. The effect of purely restrictive procedures on improving glucose control is directly proportional to the degree of weight loss [4]. In contrast, T2DM improves or resolves within a few days to weeks after bypass procedures such as Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion, before significant weight loss has occurred [1,2]. An additional weight-independent antidiabetic mechanism is thought to contribute to this dramatic effect of bypass surgery. Currently, there are 2 leading hypotheses to explain the rapid surgery-induced improvement in glucose metabolism in T2DM patients, namely, the hindgut hypothesis and the foregut hypothesis [5,6]. The former proposes that surgical rerouting of nutrients to the distal gut results in increased secretion and concomitant glucose-lowering effects that are produced by glucagon-like peptide 1 (GLP-1). The latter suggests that surgical bypass of the foregut prevents the

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release of an unidentified nutrient-induced diabetogenic signal in susceptible individuals [7]. However, the relative contribution of each is still unknown.

We have previously established a rodent model to investigate the physiologic effects of RYGB on glucose metabolism [8]. We have also developed a selective gut stimulation model that provides access to the bypassed foregut in the Sprague-Dawley diet-induced obesity model [9]. The aim of this study was to extend this work to an obese type 2 diabetes model, assess the feasibility of testing the gut hormone hypothesis, and gain further understanding of the role of the proximal and distal bowel in T2DM remission after gastric bypass surgery.

Methods

Animal care and surgery

Sixteen male Zucker Diabetic Fatty (ZDF) rats (fa/fa) aged 8–10 weeks were purchased from Charles River Laboratories (Wilmington, MA). Preoperatively, the rats had free access to tap water and were fed an ad libitum diabetogenic diet (Purina 5008, 56.4% carbohydrate) for 6–8 weeks to induce T2DM. Diabetes was confirmed from baseline fasting hyperglycemia. All rats were housed individually in a temperature- and humidity-controlled facility on a 12-hour light/dark cycle. The experimental protocols were approved by, and conducted in compliance with, the Cleveland Clinic Institutional Animal Care and Use Committee.

Before surgery, the animals were randomly assigned to 1 of 2 groups: RYGB with gastrostomy tube (GT) inserted into the excluded stomach (n = 8) or sham-operated controls with GT inserted into an intact stomach (n = 8). Preoperatively, the rats were fasted overnight. An isoflurane gas chamber was used for anesthesia induction, which was switched to nosecone flow for maintenance of anesthesia during the procedure. Ceftriaxone 75 mg/kg was administered intramuscularly for antimicrobial prophylaxis, and 25 mL/kg of saline was given subcutaneously for hydration before and immediately after surgery.

Surgical procedures were performed as previously described [9]. In brief, the RYGB group had a 20% proximal gastric pouch created, with a 30-cm biliopancreatic limb and a 10-cm alimentary limb. A GT (1.9-mm polyethylene tube) was then inserted into the gastric remnant. The GT was secured in the gastric lumen by means of 2 purse-string sutures placed circumferentially around the gastrostomy site. The GT was then tunneled through the abdominal muscles and subcutaneous fat until it emerged through the skin of the dorsolateral torso. The tube was fixed in place on the skin. The control group underwent GT insertion in a similar fashion without bypassing the stomach.

After surgery, all rats were kept on a heating pad and observed for recovery from anesthesia. Buprenorphine .1 mg/kg was administered subcutaneously for analgesia postoperatively and then daily for postoperative days 1–2. The animals were not allowed to eat until 24 hours after surgery but were hydrated with 50 mL/kg saline injected subcutaneously. Approximately 24 hours after surgery, the animals were started on an ad libitum liquid diet with Boost (Nestle, Buffalo Grove, IL) and tap water, and this regimen was maintained up to postoperative day (POD) 5. Thereafter, they were again fed an ad libitum Purina 5008 diet until POD 35. Food intake and weight were measured daily for the duration of the study. The GT was flushed daily with 1 mL of water to maintain patency throughout the study period.

Insulin tolerance test (ITT)

An ITT was performed preoperatively and repeated on POD 14 after an overnight fast. Blood glucose was measured in conscious rats by tail vein puncture before and 10, 20, 30, 60, 90, and 120 minutes after intraperitoneal injection of .5 IU/kg human insulin (Novo Nordisk Inc., Princeton, NJ), using a standard glucometer (Alpha TRAK, Abbott Laboratories, Abbott Park, IL).

Glucose tolerance test

A fasting oral glucose tolerance test (OGTT) was performed in conscious rats preoperatively and repeated on POD 21, by measuring glucose levels via tail vein puncture before and 10, 20, 30, 60, 90, 120, 150, and 180 minutes after oral gavage with a 3.0 mg/kg glucose solution. A fasting glucose tolerance test via the GT (GTT-GT) was performed on POD 23, with an identical glucose load delivered to the gastric remnant in RYGB rats or to the intact stomach in control rats. AUCs were determined by the trapezoidal method, using 9 time points when blood samples were taken.

Mixed meal tolerance test (MMTT)

A fasting MMTT via the oral route was performed preoperatively and repeated on POD 28. A baseline blood sample was collected from the femoral vein, which was exposed using a cut-down technique after rats were anesthetized using isoflurane gas. After the animal emerged from anesthesia, a 2.4-mL/kg liquid meal, containing 45 g carbohydrate, 14 g fat, and 14 g protein of Boost (360 kcal, 237 mL), was given by oral gavage. Postgavage blood samples, .5 mL at each time point, were obtained in the same manner at 30 and 60 minutes. MMTT via the GT was performed on POD 30, with an identical meal load delivered to the gastric remnant in RYGB rats or the intact stomach in control rats. Blood samples were collected in tubes containing 50 mmol/L ethylene-diaminetetraacetic