Gastric mucosal devitalization reduces adiposity and improves lipid and glucose metabolism in obese rats

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Background and Aims: The gastric mucosa is an endocrine organ that regulates satiation pathways by expression of orexigenic and anorexigenic hormones. Vertical sleeve gastrectomy (VSG) excludes gastric mucosa and reduces gastric volume. Our study aimed to investigate the independent effects of altering gastric mucosa on obesity and its related comorbidities.

Methods: Gastric mucosa devitalization (GMD) of 70% of the stomach was achieved by argon plasma coagulation in a high-fat diet rat model and was compared with VSG and sham surgery. In an 8-week follow-up study, we quantified body weight, visceral adiposity, insulin resistance index, cholesterol profiles, and free fatty acid profiles by enzyme-linked immunosorbent assay (ELISA). Following a 2-hour oral glucose tolerance test, the kinetics of ghrelin, glucagon-like peptide-1, peptide YY, and serum and liver bile acid levels were measured. Liver lipid content was quantified by ELISA.

Results: GMD resulted in significant reductions in body weight, visceral and subcutaneous adipose tissue, and hepatic steatosis as well as an improvement in lipid metabolism. GMD resulted in significant reductions in food intake and intestinal malabsorption of free fatty acids, both contributing to improved body composition and metabolic profile. Mechanistically, GMD resulted in a significant reduction in serum palmitate levels as well as an increase in serum and liver bile acid levels, known to alter glucose and lipid metabolism. Similar changes were noted when VSG rats were compared with sham surgery rats.

Conclusions: Devitalization of gastric mucosa, independent of altering gastric volume, was able to reduce obesity-related comorbidities. The gastric mucosa may be a potential target for treating obesity and its associated comorbidities.

(footnotes appear on last page of article)

Roux-en Y gastric bypass and vertical sleeve gastrectomy (VSG), despite being anatomically different, result in similar improvements in metabolic profile.[1-3] One element common to both surgeries is the exclusion or excision of the gastric mucosa.[3] The gastric mucosa secretes orexigenic (ghrelin) and anorexigenic (leptin, obestatin, and nesfatin-1), making it a complex regulator of food intake as well as glucose and lipid metabolism.[4-6] Increased insulin secretion and improved insulin sensitivity are observed in diabetic obese patients immediately after VSG, before any weight loss.[7-9] Additionally, VSG results in improved lipid metabolism, particularly reduction in secretion of triglycerides in a weight-independent manner.[10,11] This supports the hypothesis that the benefits of VSG are a result of changes of gastric origin and are neither meal related nor weight change related.

To investigate the hypothesis that gastric mucosa is an independent regulator of obesity-related comorbidities, we used a high-fat diet (HFD) rat model and designed a method of gastric mucosal devitalization (GMD) to selectively obliterate the gastric mucosa, without altering gastric volume or intestinal anatomy. To assess the independent effects of gastric mucosa we included a rat model of VSG, which combines excision of gastric mucosa with the addition of a reduction in gastric volume.

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MATERIALS AND METHODS

Animals

All animal procedures followed the international guidelines for the prevention of animal cruelty[12] and were approved by the Landesdirektion Leipzig, the local authority for animal care.[13] We used 4-week-old male Sprague-Dawley rats (n = 110; MEZ, Medical Experimental Center, University of Leipzig, Leipzig, Germany; 100-150 g). Rats were fed either an HFD (Research Diets, energy from fat 45%, carbohydrate 35%, and protein 20%; ssniff-Spezialdiaeten; Soest, Germany) or a standard chow diet (CD) (Research Diets, energy from fat 11%, carbohydrate 66%, protein 23%; ssniff-Spezialdiaeten). Rats were housed under controlled conditions (12:12-hour light-darkness cycle, 50%-60% humidity, 25°C, free access to water and food except where noted). At the end of each study, animals were killed by placement in a CO_2 chamber for 10 minutes. The allocation of animals is described in Supplemental Figure 1, available online at www.giejournal.org.

First, we validated a previously developed rat model of obesity characterized by increased body weight, visceral and subcutaneous adiposity, hepatic steatosis, dyslipidemia, and disturbed glucose metabolism.[14,15] Twenty rats were randomized into 2 groups receiving either CD (n = 10) or HFD (n = 10) with free access to food and water. Weight gain and food intake were monitored twice a week over 11 weeks. Second, we stratified rats that had been fed the HFD for 11 weeks (n = 90) into 3 intervention groups (GMD, n = 30; VSG, n = 30; sham surgery, n = 30) in order to assess various outcomes (Supplemental Fig. 1). From each cohort, 10 rats were randomized to be killed at 2, 4, or 8 weeks after intervention. HFD was continued in all intervention groups until death. The day before death, fecal samples were collected over 24 hours.

Interventions

An identical anesthesia protocol was used (intraperitoneal injection of ketamine 100 mg/kg, xylazine 10 mg/kg, and atropine 0.1 mg/kg) for all interventions. Following completion of the designated intervention, the stomach was reintegrated into the abdominal cavity, and the abdominal wall was closed.

VSG

A laparotomy incision was performed and the stomach mobilized outside the abdominal cavity. Loose gastric connections to the spleen and liver were released along the greater curvature, and the suspensory ligament supporting the upper fundus was severed. The lateral 70% of the stomach was excised by using a TX30B 30 mm staple gun (Johnson & Johnson Medical GmbH, Norderstedt, Germany), leaving a tubular gastric remnant in continuity with the esophagus and duodenum.

GMD

Argon plasma coagulation (APC) was used to perform GMD. The anesthetized animals were fixed on a metal plate, which was coupled with a neutral electrode. After a laparotomy incision and mobilization of the stomach outside the abdominal cavity, a small gastric incision in the fundus was followed by insertion of a 2-mm rigid endoscope (STORZ 1232AA Hopkins II, Storz, Germany) and a 1.5-mm APC-probe (VIO 300D/APC2-HF-generator; ERBE Elektromedizin, Tubingen, Germany). The activated probe was then fired (pulsed APC, effect 2 at 25 W with an argon flow rate of 0.2 L/minute) by a non-contact technique for a duration of 30 seconds. We decided to ablate 70% of the surface area of the stomach along the greater curvature aspect to match the amount of mucosa removed at VSG. The area treated could be visualized by the operator with the use of the endoscope and from outside because of the plasma beam of the activated probe (Supplemental Fig. 2, available online at www.giejournal.org).

A single operator performed all GMD procedures and only after the operator was able to achieve proficiency in the technique (homogeneous ablation of 70% of the gastric mucosa in ex vivo and in vivo models) was the formal study commenced.

Sham surgery

A laparotomy incision was made and the stomach mobilized outside the abdominal cavity. A gastric incision was performed to allow the entry of a 8F catheter, and the stomach was lavaged with 20 mL of sterile water at 37°C.

Postoperative care. Postoperative care included daily subcutaneous injection of antibiotics (0.1 mL/100 g ceftriaxon-ratiopharm 1.0; ratiopharm GmbH, Ulm, Germany) for 5 days and daily admixing of analgesic (0.5 mL metamizol [ratiopharm GmbH] + 30 mL 20% glucose + 70 mL water) to the water for 3 days. HFD was resumed ad libitum on post-intervention day 2. Weight, food, and water intake were assessed daily for 2 weeks and then twice weekly. An oral glucose tolerance stimulation test (OGTT) was performed after a 10-hour fast in the last postoperative week, with samples being drawn every 30 minutes for 2 hours. Animals were killed at the 2, 4, and 8 week timepoints.

Phenotypical characterization, methods of blood plasma analysis, the details of protein and lipid analysis by using enzyme-linked immunosorent assay, and activity assays as well as detailed methods of tissue histology are shown in Supplementary Methods 1 (available online at www.giejournal.org).

Statistical analysis

Data analysis was performed by using Prism version 5.0 (GraphPad Software; La Jolla, Calif). Statistical differences were calculated by an independent *t* test if comparing CD and HFD after 11 weeks and analysis of variance with