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The role of obestatin in Roux-en-Y gastric bypass surgery in the obese, type 2 diabetes Zucker rat

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ABSTRACT

Aims: Roux-en-Y gastric bypass (RYGB) is a novel therapy for diabetes and the exact mechanisms of this procedure remain unclear. Obestatin is an important gut hormone. We aimed to explore the role of obestatin in the therapeutic mechanism of RYGB.

Methods: Twenty obese Zucker rats and twenty Wistar rats were randomly assigned to two groups: RYGB and sham surgery. We evaluated plasma obestatin and insulin levels pre- and post-RYGB. Additionally, obestatin expression levels in the gastrointestinal tract were assessed using immunohistochemical staining.

Results: In Zucker rats, plasma obestatin and insulin levels gradually increased after RYGB. At post-operation week 7, plasma levels of obestatin were higher in the RYGB group than the sham operation group, and fasting plasma insulin levels were significantly increased the in RYGB group compared with the sham operation group. Furthermore, we observed a positive relationship between obestatin and insulin plasma levels. Among 10 zucker rats, high expression of obestatin was only seen in the jejunum of 2 rats before the operation; however, high expression of obestatin was seen in the Roux limb of 8 rats and in the ileum of 7 rats after RYGB. The expression of obestatin was significantly higher in the intestine in the RYGB group than the sham operation group postoperatively.

Conclusions: We propose that obestatin maybe a potential mediator to improve glucose homeostasis after RYGB. The increase of obestatin secretion may be an important mechanism through which RYGB alleviates obesity and type 2 diabetes mellitus.

1. Introduction

Obesity and type 2 diabetes mellitus (T2DM) have currently become two of the most common chronic diseases globally [1]. Traditional treatment modalities for diabetes do not satisfactorily control the disease or its complications [2], and current emerging treatments remain in the exploratory or extension phase. Recently, accumulating evidence has

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demonstrated that bariatric surgery is a more effective way to produce greater and sustained body weight reduction compared with nonsurgical treatments [3,4]. The Roux-en-Y gastric bypass (RYGB), a novel approach among the available bariatric surgery procedures [5], has proved effective in curing diabetes with reported remission rates of T2DM up to 80% [6], but the therapeutic mechanisms have remained unclear, and various hypotheses have been debated. Suggested possible explanations for the dramatic effect of bariatric surgery on T2DM include surgically induced decrease of caloric intake, weight loss, carbohydrate and fat malabsorption, and especially alterations in gut hormone release [7–11].

Obestatin, a recently discovered gut hormone, was proposed as a ligand of GPR39, an orphan receptor belonging to the ghrelin receptor family [12]. The biological activity of obestatin depends on the amidation at its carboxyl terminus. Obestatin has been reported to reduce food intake, body weight gain, gastric emptying, and jejunal motility [12–14]. Some recent studies in humans show that fasting obestatin plasma levels are reduced in obese compared with normal weight individuals [15–17], therefore supporting a role for obestatin in the regulation of body weight and energy homeostasis. Moreover, there is a significant correlation between insulin and obestatin concentrations in the postnatal pancreas [18] raising the possibility that obestatin could also play a role in the regulation of energy and glucose metabolism. However, Vicennati et al. [19] showed that in the presence of obesity, women had higher circulating levels of obestatin compared with normal subjects. The role of obestatin in the regulation of metabolism is still under debate. Expression patterns of obestatin and their correlation with the outcome of patients who have undergone RYBG have not been extensively studied.

The purpose of this study was to investigate the mechanisms of RYGB by testing gut hormone hypotheses in Zucker rats (ZRs), an obese animal model of T2DM. To shed light on the putative role of obestatin in RYGB, we measured the circulating blood levels of obestatin before and after RYGB, and its relationship with weight and the glucose–insulin system.

2. Materials and methods

2.1. Animals and chow diet

Two groups of male 10-week-old ZRs (Charles River Laboratories, Bei Jing, China) were studied: RYGB operation group (ZRR, $n = 10$) and sham operation group (ZRS, $n = 10$). ZRs are a widely used animal model of obesity and T2DM. They are characterized by leptin resistance, insulin resistance, and hyperphagia [20]. Normal control animals, age-matched Wistar rats (WRs), were studied: RYGB operation group (WRR, $n = 10$) and sham operation group (WRS, $n = 10$). Both obese animals and normal controls were housed under constant ambient temperature and humidity and in a 12-h light, 12-h dark cycle. All animals had free access to tap water and chow, ZRs were fed ad libitum with Formulab Diet 5008 (LabDiet, USA). The study was approved by the China Ethics Committee and performed in accordance with the ethical standards.

2.2. Interventions

ZRs and WRs underwent RYGB and sham operations. Prior to surgery, animals were randomized to RYGB or sham operation. Rats were fasted overnight prior to surgery. Following randomization, rats were weighed then anesthetized with isoflurane (3% for induction, 1.5% for maintenance). Cefuroxim 100 mg/kg i.m. (Esseti Farmaceutici s.r.l.) was given as a prophylactic antibiotic. Under sterile conditions, a midline laparotomy was performed. The sham operation group underwent intestinal manipulation followed by abdominal closure as a sham surgical procedure.

In the RYGB groups, the stomach was divided to create a 20% gastric pouch; the remnant of stomach was closed with interrupted 5–0 silk. A length of 20 cm from the ligament of Treitz was measured to choose the site for the gastrojejunal anastomosis. Samples of stomachs and jejunum were removed for further processing. The continuity of biliopancreatic secretions was reconstructed by anastomosing the biliary limb to the alimentary limb of small bowel 15 cm distal to the gastrojejunal anastomosis in a Roux-en-Y fashion. The gastrojejunal and jejunojejunostomies were performed by using interrupted 6–0 silk sutures, followed by abdominal closure using 3–0 silk. Surgical incisions were injected with 0.5 ml of 0.25% bupivacaine to minimize postoperative discomfort.

All rats were injected subcutaneously with normal saline (50 ml/kg, prior to the start of surgery, immediately after surgery). To allow the surgical anastomoses to heal, animals were not allowed to eat or drink until 24 h after surgery. Approximately 24 h after surgery, animals were given access to water and 5% glucose sodium chloride solution ad libitum. Regular chow was started on postoperative day 3, to ensure adequate healing of the stomach and bowel anastomoses.

At 7 weeks after operation, rats were euthanized with CO_2 before decapitation. A 3 ml trunk blood sample was obtained. All animals were processed for morphology. The entire gastrointestinal tract was removed and immersion fixed and stored in 4% phosphate buffered formaldehyde until further processing.

2.3. Body weight, blood glucose, oral glucose tolerance test (OGTT)

Weight was measured daily for the duration of the study. After 12–14 h of fasting, blood glucose was measured in conscious rats before (baseline) and at 30, 60, 90, and 120 min after administration of 4 g/kg glucose by oral gavage. Blood was obtained from the tail and analyzed using a glucometer (One Touch Ultra, Lifescan, Johnson & Johnson, Milpitas, CA). Changes in glucose tolerance were compared by analyzing area under the curve (AUC). AUC was calculated using the area under the start and end points, t_0 and t_{120}, for each experimental group.

2.4. Measurement of obestatin, insulin, and insulin sensitivity

From the tail of conscious rats, blood was collected in tubes containing 50 mmol/L ethylenediaminetetraacetic acid,