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Effects of duodenal-jejunal exclusion on beta cell function and hormonal regulation in Goto-Kakizaki rats

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Bariatric surgery;
Beta cell;
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Rat model

Abstract

BACKGROUND: The aim of our work was to investigate the hormones that control glycemic status and in vitro β-cell function in diabetes mellitus after a duodenal-jejunal exclusion in Goto-Kakizaki rats (Taconic, Denmark).

METHODS: Twenty-three rats (age, 12–14 wk) were randomized as follows: group 1 (n = 14), no intervention (control); or group 2 (n = 9), duodenal-jejunal exclusion.

RESULTS: In group 2, levels of glucagon and leptin were lower than in group 1 at 1 week and at 8 weeks. Glucagon-like peptide 1 levels had a significant increase at 8 weeks from basal value in group 2 and this value was higher than in group 1. The insulin secretion at 60 minutes in group 2 was higher than in group 1 (group 1, 12.9 ± 12.0 μg/L vs group 2, 41.9 ± 36.3 μg/L; $P < .05$). Messenger RNA (mRNA) expression of insulin at 2 months was higher in the rat pancreas of the experimental group than in the control group (group 1, .99 ± .48 mRNA amount vs group 2, 1.66 ± .33 mRNA amount; $P < .05$).

CONCLUSIONS: Gastrojejunal bypass in this model improves glucose ratios, with a significant increase of glucagon-like peptide 1 and decrease of homeostasis model assessment, glucagon, and leptin levels after surgery. This type of surgery improves mRNA insulin expression in pancreatic islets and insulin secretion as well.

Type 2 diabetes mellitus is a chronic metabolic disease characterized by insulin resistance and progressive deterioration of islet functions.[1] The resolution of type 2 diabetes mellitus has been detected as an outcome of surgical treatment of obesity.[2] Two procedures, biliopancreatic diversion and Roux-en-Y gastric bypass (RYGBP), are more effective treatments for diabetes than other procedures[3] and control or improve diabetes in 90% to 100% of morbidly obese patients with diabetes mellitus.[4–6]

The antidiabetic effect of bariatric surgery has been interpreted as a result of the surgically induced weight loss.[7] However, glycemic control often occurs within days or hours, before weight loss has been reached.[8] Efforts have been made to understand the mechanism of bariatric surgery on diabetes mellitus, however, the exact mechanism still remains elusive.

Both techniques, biliopancreatic diversion and RYGBP, include bypassing the proximal jejunum and duodenum. The potential mechanisms responsible for this reversal of

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diabetes mellitus type 2 must include changes on several hormones in this part of the bowel, which are involved in the β-cell function.[9–11] It also has been reported that after RYGB surgery, some patients developed nesidioblastosis, with hypertrophy and hyperplasia pancreatic islets. These data suggest that RYGB may be associated with the regeneration and neogenesis of the pancreatic islets.[12] However, the enteroinsular hormone that modulates this response remained unknown and the influence of these potential changes on pancreatic islet function (messenger RNA [mRNA] expression, and production and secretion of insulin) remained unclear as well.

We observed, in a previous study,[13] an improvement in glucose control, with statistical changes in leptin and glucagon levels in a short protocol of 4 weeks (Goto-Kakizaki rats), without evaluation of β-cell function. Taking into account our previous study,[13] the aim of our present study was to investigate in vitro β-cell function and enteroinsular hormone levels, which control diabetes mellitus, after duodenal-jejunal exclusion in Goto-Kakizaki rats in an 8-week study.

Materials and Methods

Animals and diet

This study was approved by the Investigation Committee of the Hospital Universitary Rio Hortega and 23 male Goto-Kakizaki rats, 10 to 12 weeks of age, were purchased from Taconic M&B A/S (Lille Skensved, Denmark). Animals had ad libitum access to tap water and were fed 20 to 25 g/d of 5% fat rat chow diet (Altromin 04, Lage, Germany) during the protocol.

Experimental protocol

After the rats were acclimated for 3 weeks, weight and fasting glycemia were measured. Insulin, glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), glucagon, and leptin levels were measured after 20 minutes of glucose overload (3 g/kg). An oral glucose tolerance test (OGTT) also was performed. Then, in a first step of experiments, 12- to 14-week-old rats randomly underwent one of the following procedures: group 1 (n = 14), no intervention (controls), or group 2 (n = 9), gastrojejunal bypass (study group). Both groups were fed with the same type of diet. Rats undergoing duodenal-jejunal exclusion were fasted overnight and anesthetized with ketamine 150 mg/kg, atropine .3 mg/kg, and diazepam 1 mg/kg. The proximal jejunum was bypassed and gastric volume was maintained intact. At baseline (preoperative) and after intervention (1 week and 8 weeks), weight and fasting glycemia were measured. Hormone levels (insulin, GLP-1, GIP, glucagon, and leptin) were measured after 20 minutes of glucose overload (3 g/kg) (at 1 week and 8 weeks). An OGTT also was performed. At the end of the protocol (2 months), pancreatic islets were cultivated from both groups. These pancreatic islets were used to assess insulin secretion at baseline (5 mmol/L glucose) and after stimulation (16.7 mmol/L glucose) conditions, insulin production in a sonicated sample of islets, and insulin expression with reverse-transcription polymerase chain reaction.

Biochemical measurements

For plasma hormone measurements, blood from the jugular vein was collected. After centrifugation at 3,000 rpm for 10 minutes, plasma immediately was separated and stored at -80°C until analyzed. Enzyme-linked immunosorbent assay (ELISA) kits were used for measurements of insulin (DRG, Marburg, Germany), glucagon (WAKO Chemicals, Dallas, TX), leptin (DRG), GLP-1 (Phoenix, Belmont, CA), and GIP (Phoenix). The homeostasis model assessment (HOMA) was used to measure insulin resistance. The HOMA was calculated as follows: glucose * insulin/22.5.

For a fasting glycemia evaluation, after a fasting period of 12 hours, blood was collected from the tail. For the oral glucose tolerance test, after 12 to 14 hours of fasting, blood glucose level was measured in conscious rats before (baseline) and then 10 and 120 minutes after the administration of 3 g/kg glucose by oral gavage. Plasma glucose levels, fasting, and postoverload were determined by using a Glucometer (One Touch Lifescan; Johnson and Johnson, Buckinghamshire, UK). Weight was measured at baseline and 1 week and 8 weeks after the intervention in both groups.

Islets measurements. On the day the rats were killed (after a 2-month study period), each rat first was injected intraperitoneally with 5-bromo-2-deoxyuridine (Sigma, St. Louis, MO) at 50 mg/kg, and 2 hours later the rats were killed with an overdose of anesthesia. Pancreases were inflated with collagenase solution (2 U/mL) in Hank's balanced salt solution supplemented with 1% fetal bovine serum. After digestion period, islets were the hand-picked twice under stereomicroscopic control to exclude extra-islets debris.

Insulin secretion and insulin amount. The islets from control and experimental rats were cultured for 2 days in 5 mL of Ham's F-10 medium (Gibco, Tokyo, Japan) containing bovine serum albumin (10 mg/mL), penicillin (.075 mg/mL), streptomycin (.1 mg/mL), and glutamine (2 mmol/L) in a CO_2 incubator (37°C, 95% air/5% CO_2). The media were exchanged after 1 day of culture. The control medium condition contained 5 mmol/L glucose and the experimental condition had 16.7 mmol/L glucose. During each condition, media were collected and aliquots of the medium of 7 intact islets were analyzed at baseline, at 30