Weight loss through ileal transposition is accompanied by increased ileal hormone secretion and synthesis in rats

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Strader, April D., Torsten P. Vahl, Ronald J. Jandacek, Stephen C. Woods, David A. D’Alessio, and Randy J. Seeley. Weight loss through ileal transposition is accompanied by increased ileal hormone secretion and synthesis in rats. Am J Physiol Endocrinol Metab 288: E447–E453, 2005. First published September 28, 2004; doi:10.1152/ajpendo.00153.2004.—Bariatric surgeries, such as gastric bypass, result in dramatic and sustained weight loss that is usually attributed to a combination of gastric volume restriction and intestinal malabsorption. However, studies parceling out the contribution of enhanced intestinal stimulation in the absence of these two mechanisms have received little attention. Previous studies have demonstrated that patients who received intestinal bypass or Roux-en-Y surgery have increased release of gastrointestinal hormones. One possible mechanism for this increase is the rapid transit of nutrients into the intestine after eating. To determine whether there is increased secretion of anorectic peptides produced in the distal small intestine when this portion of the gut is given greater exposure to nutrients, we performed ileal transpositions (IT) in rats. In this procedure, an isolated segment of ileum is transposed to the jejunum, resulting in an intestinal tract of normal length but an alteration in the normal distribution of endocrine cells along the gut. Rats with IT lost more weight ($P < 0.05$) and consumed less food ($P < 0.05$) than control rats with intestinal transections and reanastomosis without transposition. Weight loss in the IT rats was not due to malabsorption of nutrients. However, transposition of distal gut to a proximal location caused increased synthesis and release of the anorectic ileal hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY; $P < 0.01$). The association of weight loss with increased release of GLP-1 and PYY suggests that procedures that promote gastrointestinal endocrine function can reduce energy intake. These findings support the importance of evaluating the contribution of gastrointestinal hormones to the weight loss seen with bariatric surgery.

glucagon-like peptide-1; peptide YY; obesity surgery; ileum

Obesity has reached epidemic proportions in the Western world (43). Despite dramatically increased research efforts aimed at developing effective treatments for this rising health issue, there are few treatments that reliably cause significant and long-lasting weight loss. Bariatric surgery remains the most efficacious treatment option presently available, with published weight loss of 30–60% of excess weight persisting for up to 5 yr (9). In response to the increased prevalence of obesity, bariatric surgery is becoming much more common. It is estimated that the number of people treated surgically for weight loss has increased from fewer than 20,000 surgeries in 1992 to 47,000 in 2001 (35). Between 2001 and 2002, the number of surgeries performed increased 70% to 80,000, according to a report by the contracting firm Frost & Sullivan; this number is projected to reach 250,000 by 2005.

Historically, the surgical strategies for weight loss have relied on volume restriction, malabsorption, or both (31). As one example, gastroplasty has been an effective weight loss measure that works primarily by restricting the amount of food that can be ingested by severely limiting the gastric volume (31). Alternatively, the practice of jejunooileal bypass resulted in decreased food intake and malabsorption (8). As a consequence, jejunooileal bypass was unfortunately associated with significant malnutrition and was discontinued (38, 48). The current gold standard for bariatric surgery is the Roux-en-Y gastric bypass (see Refs. 4 and 28), which combines gastric restriction and significant bypass of the stomach and proximal intestine. In this procedure, a small stomach pouch (~30 ml) is created, the jejunum is transected, and the distal portion of the small intestine (midjejunum and ileum) is connected directly to the gastric reservoir so that meal contents bypass the lower stomach and upper small bowel. The duodenal-upper jejunal segment is anastomosed at a distal site in the jejunum so that the biliary and exocrine pancreatic drainage contacts luminal nutrients only in the latter half of their passage through the gut. Roux-en-Y gastric bypass is primarily a restrictive procedure and does not typically involve substantial malabsorption unless significant lengths of intestine are bypassed, as seen with the long duodenojejunal limb gastric bypass (10). One structural feature that the gastric bypass shares with other types of bariatric procedures (32), and more generally with gastric surgery, is accelerated delivery of partially digested nutrients to the distal small bowel early in the postigestive period. Recently, there has been an increase in the number of investigations into the gastrointestinal endocrine changes associated with gastric bypass surgery. Most notable is the finding that the circulating levels of the stomach-derived orexigenic hormone ghrelin are significantly decreased (13, 24, 25, 46) in many cases after gastric bypass surgery and that this decrease potentially contributes to the reduction in hunger after surgery. In addition to changes in plasma ghrelin, gastric bypass surgery has been associated with greatly enhanced release of certain intestinal hormones, most prominently the products of intestinal proglucagon (19, 29, 39, 40, 42). Several of these intestinal hormones, such as glucagon-like peptide-1 (GLP-1) and peptide tyrosine-tyrosine [PYY(3–36)], have anorectic effects (5, 6, 16, 45, 47). Therefore, higher circulating levels of satiety

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E447
peptides because of accentuated stimulation of the distal intestine by rapid passage of nutrients in the gut may contribute to sustained weight loss with bariatric surgery (32).

To test the possibility that accelerated stimulation of the ileum and consequent release of GLP-1 and/or PYY-(3–36) contribute to the effects of some bariatric surgery, we used the ileal transposition (IT) model in rats. In this procedure, a 10-cm portion of distal intestine (which includes the ileum) is transected and transposed in the original peristaltic direction into the proximal intestine. First described by Koopmans and Scalfani (21) in 1981, IT was found to elicit weight loss and reduced food intake in diet-induced obese and ventromedial hypothalamic-lesioned rats (7, 11). This procedure has actually been proposed as being potentially useful in treating glucose intolerance related to obesity because of the potential for increasing GLP-1 secretion (27). As an experimental model, IT is well suited for specifically studying ileal hormones, since it allows enhanced nutrient stimulation of the distal gut during a meal potentially without the malabsorption or restriction in more common bariatric procedures. We therefore ascertained the behavioral, endocrine, and metabolic effects of premature distal gut stimulation using the IT model.

METHODS

Animals

Adult male Long-Evans rats (~350–375 g) were placed on a high-fat (HF; 41% butterfat by kcal/g; Dyets, Bethlehem, PA; see Ref. 50) diet for 6–8 wk before surgery. Rats had ad libitum access to HF diet and water unless otherwise specified in the experimental protocol. Rats were housed individually and maintained on a 12:12-h light-dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. The following studies included IT and sham-operated groups.

Surgery

IT surgery was performed as described by Koopmans and Scalfani (21). Animals were anesthetized using isoflurane followed by an intraperitoneal injection of a ketamine (60 mg/ml)-xylazine (8 mg/ml) mixture that was administered per kilogram body weight. Next, a midline abdominal incision was made, and the cecum was located and removed from the abdomen on saline-soaked sterile gauze. A 10-cm segment of ileum located 5–15 cm proximal to the ileocecal valve was isolated and transected. The segment was carefully placed on saline-soaked gauze. An anastomosis was then made with the two open ends of the ileum, using eight stitches with 7-0 silk suture (Ethicon, Cincinnati, OH). The remaining small intestine was then transected 5–10 cm distal to the ligament of Treitz. The isolated ileal segment with full neural innervation and intact vascular supply to the transposed segment was then inserted in the original peristaltic direction by making two additional end-to-end anastomoses (see Fig. 1; modified from Ref. 49).

Sham IT surgeries were performed by externalizing the cecum and making transections through the ileum in the same locations as occurred in the IT group (+5 and +15 cm proximal to the ileocecal valve). After transection, the intestines were immediately attached by anastomosis. Next, the small intestines were externalized, and a third transection was made distal to the ligament of Treitz (where the ileal segment would be inserted in an IT animal) and reattached. Both the sham and the IT surgeries took ~50 min/animal.

Nutrient-stimulated GLP-1 and PYY Secretion

To compare nutrient-driven ileal hormone secretion, rats were fasted for 16 h and orally gavaged with d-glucose solution (20%; 1 g/kg) for measurement of GLP-1 or an Intralipid solution (1.5 g/kg; 20%) for measurement of PYY (2). Blood was collected from the tail vein before and after the gavage.

Absorption Assessment

We used the fat balance methodology facilitated by the inclusion of a nonabsorbable marker to determine the percentage of dietary fat that was absorbed (18). Absorption of dietary fat by normal rats is typically 95–99%, as measured by this method. Animals from both the IT and sham-operated groups received an ad libitum diet that contained 38% lipid during the fat balance measurement. A HF diet with slightly lower fat content was used on the basis of the requirements for the absorption method (18). Rats consumed the food for 3 days, after which feces were collected for the measurement of fat absorption.

Glucose Tolerance Test

To assess the effects of IT surgery on glucose tolerance, fasted rats were given an oral gavage of glucose (20% d-glucose; 1 g/kg) on day 21 after surgery. Plasma glucose and insulin concentrations were measured at 0, 5, 15, 30, and 45 min. The 0-, 5-, and 15-min samples from the glucose tolerance test were also used to measure glucose-stimulated GLP-1 (as described below).

Insulin Tolerance Test

To determine whether IT surgery causes changes in insulin sensitivity, rats were given an insulin tolerance test on day 42 after surgery. During the insulin tolerance test, fasted rats were given an intraperitoneal injection of insulin (250 mU/kg), and blood samples were taken from the tail vein at 0, 15, 30, 45, and 60 min for measurement of plasma glucose. Plasma concentrations are expressed as a percentage of baseline glucose values, and the glucose disappearance rate was calculated for each group as the slope of the natural logarithm of the glucose values at every time point.

Gene Expression for Preproglucagon and PYY

RNA isolation and cDNA synthesis. At the termination of the study, rats were fasted overnight and killed by CO2 asphyxiation. For the IT rats, a 1-cm segment of ileum was rapidly removed from the center of the transposed piece of intestine, and for the sham-operated rats a 1-cm segment was collected from the center of the transected ileum that was returned to its natural location. Ileal segments were placed in RNA Later (Ambion, Austin, TX) and stored at 4°C for 1 wk and then moved to −80°C until use for RNA isolation. RNA was subsequently isolated from the samples with TRI-Reagent (Molecular Research Center, Cincinnati, OH). All RNA samples were DNase treated using DNase free (Ambion). The Superscript III first-strand synthesis system (Invitrogen, Carlsbad, CA) was used to synthesize cDNA from 3 μg of total RNA.

Semiquantitative expression of preproglucagon and PYY mRNA (real-time PCR). The following primers were designed to amplify a portion of the rat preproglucagon and PYY gene. Primer sequences from 5′ to 3′ were forward TCTGGGAAACTGGGAATTG and reverse TCTGCGATGTGGTGGGTTAATG and forward GAGCTGAGCCTGGCTGTTTA and reverse TCCGCGT- GTGCCTCTGAGGGTT for PYY. The constitutively expressed ribosomal protein L-32 was used to obtain relative quantification of each primer. The gene sequences for L-32 were forward CATTCTGTA-GAAAGAGCAAGCAG and reverse GCCACACAGCCTCATCTATC. The identity of the amplification products was confirmed for all three primer pairs by gel electrophoresis and melt curve analysis.

All PCR reactions were performed using the Bio-Rad iCycler (Bio-Rad, Hercules, CA) at a final volume of 25 μl. cDNA (2 μl) was amplified using iQ SYBR Green Supermix (Bio-Rad) with a final primer concentration of 400 nM. After an initial denaturation step (95°C for 3 min), 40 cycles of PCR were performed with denaturation
at 95°C for 10 s and a combined annealing/extension step at 58°C for 30 s. A standard curve for all genes was performed using a dilution series spanning seven orders of magnitude (dilution factor of 5), and samples were measured within the linear part of the standard curve. The PCR efficiencies for L-32, preproglucagon, and PYY were all between 95 and 100%. Correlation coefficients for all standard curves were between 0.997 and 1.00. The PCR efficiency was calculated from the slope of the plot threshold cycle ($C_T$) vs. log starting concentration. Samples were run in triplicate and normalized to the constitutively expressed ribosomal protein L-32. Data are expressed as percent mean ± SE of preproglucagon and PYY relative to L-32 using the $\Delta C_T$ subtraction method (Applied Biosystems).

**Assays**

Blood samples were placed in tubes containing heparin for determination of glucose and insulin and 50 mM EDTA plus 500 kallikrein inhibitor units/ml aprotinin for assay of GLP-1 and PYY. Blood samples were immediately placed on ice and centrifuged. Plasma was removed and stored at −20°C until assay. Plasma glucose was measured using a glucose oxidase method. Insulin concentrations were determined by RIA using a guinea pig anti-insulin serum, iodinated insulin, and a double-antibody separation method (14). Total GLP-1 immunoreactivity was measured by RIA using antisera 89390 (kindly provided by Dr. Jens Holst, Panum Institute, Copenhagen, Denmark) from ethanol extracts of plasma, as previously described (33). This antibody recognizes both the intact hormone GLP-1-(7–36)-NH$_2$ and the metabolite GLP-1-(9–36)-NH$_2$ and is a reliable index of secreted hormone. The intra-assay variability is <7% and the interassay variability is <11%. PYY immunoreactivity was measured using the Phoenix Pharmaceuticals RIA kit for PYY (Belmont, CA). This assay recognizes PYY-(1–36) and PYY-(3–36). The intra-assay variability is <8.42% and interassay variability <14.52%.

**Statistical Analysis**

Body weight changes and food intake over the course of the experiment were analyzed by repeated-measures ANOVA. Cumulative food intake was compared with Student’s $t$-test. Plasma changes in PYY, GLP-1, glucose, and insulin were analyzed using repeated-measures ANOVA. Gene expression data for preproglucagon and PYY were compared using Student’s $t$-test. Absorption percentages and glucose disappearance rates were also compared using Student’s $t$-test. Plasma concentrations of leptin and adiponectin were compared with Student’s $t$-test. Data are presented as means ± SE.

**RESULTS**

**Effects of IT on Body Weight**

IT surgery (as shown in Fig. 1) resulted in significant weight loss compared with sham-operated rats ($P < 0.05$; Fig. 2). After surgery, both groups lost a similar amount of weight until approximately the 11th postoperative day, after which the sham-operated group started regaining lost body weight whereas the rats with IT lost additional weight before regaining weight. The rats with IT surgery never attained a weight similar to the sham-operated group throughout the remaining study period (6 wk). Contributing to the lost body weight was a significant decrease in food intake by the rats with IT (Fig. 3; $P < 0.05$). In contrast, there was no evidence of impaired fat absorption to contribute to the reduced body weight in the rats with IT (not significant; Fig. 4).

**Effects of IT on Ileal Hormone Secretion and Synthesis**

IT resulted in hypersecretion of both GLP-1 and PYY. Glucose-stimulated GLP-1 in rats with IT was nearly three times that of sham-operated rats (Fig. 5A; $P < 0.05$). Consistent with an increase in GLP-1 secretion, synthesis of GLP-1 as measured by preproglucagon gene expression was also highly elevated in rats with IT (Fig. 5B; $P < 0.05$). Transposition of the ileum also resulted in increased Intralipid-stimulated PYY secretion. PYY secretion in response to an oral gavage of Intralipid increased earlier and remained higher in rats with IT compared with sham-operated rats (Fig. 6A; $P < 0.05$). Fasting levels of PYY mRNA were also significantly greater in rats with IT surgery compared with sham-operated rats (Fig. 6B; $P < 0.05$).

**Effects of IT on Glucose Tolerance and Insulin Sensitivity**

Glucose tolerance tests for rats with IT revealed no significant difference compared with sham-operated controls (Fig. 7A). Plasma insulin concentrations during the glucose tolerance tests were similar between sham-operated and IT rats.
In contrast, rats with IT surgery were more insulin sensitive compared with sham-operated controls (Fig. 8). Rats with IT surgery had a greater glucose disappearance rate compared with sham-operated rats \((P < 0.05)\). Despite the increased insulin sensitivity seen after IT, rats with IT surgery did not have significantly higher adiponectin levels (sham-operated rats \(3,558.8 \pm 248.2\) ng/ml vs. IT rats \(3,037.9 \pm 475.3\) ng/ml). However, plasma leptin was significantly lower in the rats with IT surgery (sham-operated rats \(3.8 \pm 0.51\) ng/ml vs. IT rats \(2.0 \pm 0.55\) ng/ml; Student’s \(t\)-test; \(P < 0.05)\).

**DISCUSSION**

The present experiments sought to look at the effect of exposing a distal portion of the ileum to increased direct nutrient stimulation. To do so, we used the IT model (21) to move a portion of distal ileum to the upper gut where it is exposed to meal contents soon after food intake. Similar to previous observations (7, 11, 21), rats with the IT did not regain weight after surgery as fast as rats with a carefully constructed sham surgical procedure (involving transections and anastomoses). Rats with IT surgery had a greater glucose disappearance rate compared with sham-operated rats \((P < 0.05)\). Despite the
that had IT surgery. The weight difference between the groups is at least partially attributable to reduced calorie intake after the IT surgery. The size of the effect in the current data is not as large as in previous reports, despite the surgery having been performed in rats of similar age, weight, and diet exposure. However, because the present experiments used Long-Evans rats while previous work used Wistar and Sprague-Dawley rats, there is a possibility that strain differences contribute to the size of the effect observed after IT.

The results presented herein corroborate and extend previous work with IT in several ways. First, the present data support previous documented findings indicating that IT is not malabsorptive (7, 11). Using a different method (18), we also demonstrate that intestinal fat absorption in rats with IT surgery is normal and comparable to that in the sham-operated rats. This is an important point, because it indicates that the relative weight difference between IT and sham-operated rats is not a function of surgically compromised absorption of nutrients. Second, the current studies demonstrate that IT causes an increase in the synthesis and secretion of important gastrointestinal regulatory peptides. Previous studies have shown that ileal segments transposed into the duodenojejunal region become extremely hypertrophied after transposition and secrete significantly more enteroglucagon compared with sham-operated rats (12, 22). Although enteroglucagon has been used in the past as a measure of intestinal peptides derived from proglucagon, we have now shown that IT surgery is associated with increased plasma GLP-1. In these studies, glucose-stimulated increases in GLP-1 levels are greatly en-

Fig. 6. A: mean ± SE plasma levels of peptide YY (PYY) after Intralipid administration after IT or sham surgery. B: mean ± SE mRNA levels of fasting PYY in the transposed ileal segment after IT or sham surgery (t-test; *P < 0.05).

Fig. 7. Mean ± SE plasma glucose (A) and insulin (B) after an oral glucose gavage (20% D-glucose; 1 g/kg) in sham-operated and IT rats.

Fig. 8. Mean plasma glucose concentrations (expressed as a percentage of baseline values ± SE) after ip administration of insulin (250 mU/kg; 2-way repeated-measures ANOVA; *P < 0.05). The glucose disappearance rates were 0.73 ± 0.18 and 0.27 ± 0.08 10^-7/min^-1 for IT and sham-operated groups, respectively (*P < 0.05).
lected in rats with IT compared with sham-operated controls. This might be because glucose tolerance was impaired in our sham-operated rats, whereas one can prove glucose tolerance similar to that of sham-operated rats. After all, when animals are failing to extract sufficient calories from their consumed food, the typical response is to increase food intake (17, 20), not to decrease food intake, such as is observed both in the IT and Roux-en-y procedures (51). These findings point to additional and more complicated mechanisms by which bariatric surgical procedures produce their effects. Furthermore, these findings make the IT more interesting, since it is a model of gastrointestinal-induced weight loss that is clearly not associated with malabsorption.

In addition to rapid weight loss after bariatric surgery, patients with impaired glucose homeostasis experience dramatic improvements (36). Reductions in plasma glucose and insulin after bariatric surgery are likely a consequence of weight loss; however, some studies suggest these improvements have occurred before substantial weight loss. This finding implicates other factors for the changes in glucose homeostasis (37). To assess whether this effect of bariatric surgery is apparent in the absence of alterations of the stomach or malabsorption, it was important to assess parameters of glucose homeostasis in the IT model. Interestingly, rats with IT surgery demonstrated glucose tolerance similar to that of sham-operated controls. This might be because glucose tolerance was not impaired in our sham-operated rats, whereas one can speculate that IT may have improved glucose tolerance in diabetic rats. It has been well documented that mice or rats with elevated GLP-1 with deletions or mutations in the GLP-1 receptor have decreased food intake and weight loss that are partly the result of a reduction in food intake; yet, compared with sham-operated controls, these effects are relatively modest. Strikingly, in the absence of either restriction or malabsorption, large changes in intestinal hormone secretions and synthesis are evident. Many of the physiological roles for gut hormones, such as PYY and GLP-1, have only recently been determined, leaving the IT model in which these hormones are greatly increased as an obviously useful tool for examining them. Satiation is just one of many physiological effects mediated by GLP-1 and PYY (3–36). The important role of GLP-1 in improving glucose homeostasis and pancreatic function has made it an important target for the treatment of type 2 diabetes. Therefore, the IT model may be useful not only for understanding the mechanisms underlying bariatric surgical methods but also for understanding the improvement in comorbid conditions, such as diabetes (27).

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