Ileal interposition improves glucose tolerance and insulin sensitivity in the obese Zucker rat

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Submitted 28 December 2009; accepted in final form 13 July 2010

Ileal interposition improves glucose tolerance and insulin sensitivity in the obese Zucker rat. Am J Physiol Gastrointest Liver Physiol 299: G751–G760, 2010. First published July 15, 2010; doi:10.1152/ajpgi.00525.2009.—The hindgut hypothesis posits improvements in Type 2 diabetes after gastric bypass surgery are due to enhanced delivery of undigested nutrients to the ileum, which increase incretin production and insulin sensitivity. The present study investigates the effect of ileal interposition (IT), surgically relocating a segment of distal ileum to the proximal jejunum, on glucose tolerance, insulin sensitivity, and glucose transport in the obese Zucker rat. Two groups of obese Zucker rats were studied: IT and sham surgery ad libitum fed (controls). Changes in food intake, body weight and composition, glucose tolerance, insulin sensitivity and tissue glucose uptake, and insulin signaling as well as plasma concentrations of glucagon-like peptide-1 and glucose-dependent insulinoorphic peptide were measured. The IT procedure did not significantly alter food intake, body weight, or composition. Obese Zucker rats demonstrated improved glucose tolerance 3 wk after IT compared with the control group (P < 0.05). Euglycemic, hyperinsulinemic clamp and 1-[14C]-2-deoxyglucose tracer studies indicate that IT improves whole body glucose disposal, insulin-stimulated glucose uptake, and the ratio of phospho- to total Akt (P < 0.01 vs. control) in striated muscle. After oral glucose, the plasma concentration of glucagon-like peptide-1 was increased, whereas GIP was decreased following IT. Enhanced nutrient delivery to the ileum after IT improves glucose tolerance, insulin sensitivity and muscle glucose uptake without altering food intake, body weight, or composition. These findings support the concept that anatomic and endocrine alterations in gut function play a role in the improvements in glucose homeostasis after the IT procedure.

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tion of glucagon-like peptide (GLP)-1, decreased insulin resistance, and improved glucose tolerance. However, the relative importance of foregut bypass vs. enhanced delivery of undigested nutrients to the ileum (the so-called “hindgut hypothesis”) in post-RYGB glycemic control remains controversial.

The present study is the first to examine the effects of ileal interposition (IT) on glucose tolerance and insulin sensitivity using the obese ZR model. The IT procedure interposes a 10-cm segment of neurovasculally intact terminal ileum into the proximal jejunum on a mesenteric pedicle (30). In contrast to the RYGB procedure there is no mechanical restriction of meal size, no loss of absorptive surface, and no bypass of the foregut (5). Consequently, the IT procedure specifically examines the effects of enhanced nutrient delivery to the ileum on glucose homeostasis.

Obesity in the ZR is an autosomal recessive trait (fa/fa) caused by defective leptin receptors. Heterozygous lean ZRs are normal, whereas the homozygous obese ZR develops progressive insulin resistance, glucose intolerance, hyperlipidemia, and hypertension (15). The obese ZR has been used extensively to study obesity-related insulin resistance and is therefore an excellent model for investigating how IT improves glucose homeostasis (4). In the ZR model peripheral insulin resistance is characterized by moderately elevated circulating glucose levels, hyperinsulinemia, abnormal glucose tolerance, and increased pancreatic β-cell mass (15). Insulin resistance in this model is due to defective insulin signaling, minor reductions in basal insulin-sensitive glucose transporter (GLUT-4) expression, and defective insulin-stimulated GLUT-4 membrane translocation (3, 27).

Alterations in the secretion or activity of enteric hormones have been implicated in the resolution of T2DM after RYGB surgery (1, 11). Incretins are peptides secreted by the gut that augment insulin secretion and glycemic control in response to oral (vs. intravenous) glucose, protein, and fat intake (1). Glucose-dependent insulinoorphic peptide (GIP) is secreted by K cells in the duodenum and jejunum whereas glucagon-like peptide-1 (GLP-1) is secreted by L cells in the distal small bowel and colon (1). Both GIP and GLP-1 bind specific receptors on pancreatic β-cells to increase islet cell mass and stimulate insulin secretion (1). Extrapancreatic effects of GLP-1 include the stimulation of glucose metabolism in liver and muscle (23, 33). GIP levels are not altered in T2DM, but reductions in β-cell GIP receptors and postreceptor defects in GIP signaling have been identified (17). Impaired GLP-1 release and action have also been reported in T2DM (12). Thus alterations in incretin synthesis or activity represent a potential
mechanism for improved insulin sensitivity following bariatric surgery.

The present study examines the effects of IT on body weight, body composition, glucose tolerance, insulin sensitivity, insulin signaling, incretin production, free fatty acid levels, and plasma bile acid levels. Our data indicate that IT-induced changes in GI anatomy improve glucose tolerance and insulin action without significantly altering food intake, body weight, or composition. Potential mechanisms for these beneficial actions include increased postprandial GLP-1 secretion and circulating bile acids, both of which may increase insulin sensitivity. Collectively, these data support the “hindgut and foregut hypotheses” of glycemic control after gastrointestinal surgery.

MATERIALS AND METHODS

Animal care and surgery. Two groups of obese male ZRs, 12 wk of age (Charles River Breeding Laboratories, Wilmington, MA) were studied: IT and sham surgery fed ad libitum (AL). Except for pretest overnight fasting and the immediate postoperative period, animals had free access to water and chow (Harlan Teklad 2018). The experimental protocols were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University, College of Medicine.

The IT procedure was performed by using a modification of the technique described by Strader et al. (30). Following an overnight fast and randomization, rats were weighed, then anesthetized with isoflurane (1.5–3%). In the IT group the ileum was divided 5 cm from the cecum and again at 15 cm, isolating a 10-cm segment of neurovascularily intact ileum on a mesenteric pedicle. The ileum was then anastomosed with interrupted 5-0 silk. The jejunum was then divided 5 cm from the ligament of Treitz and the ileal segment was anastomosed with interrupted 5-0 silk. The jejunum was then divided and randomization, rats were weighed, then anesthetized with isoflurane (1.5–3%). In the IT group the ileum was divided 5 cm from the cecum and again at 15 cm, isolating a 10-cm segment of neurovascularily intact ileum on a mesenteric pedicle. The ileum was then anastomosed with interrupted 5-0 silk. The jejunum was then divided 5 cm from the ligament of Treitz and the ileal segment was anastomosed in an isoperistaltic direction with 5-0 interrupted silk sutures. The abdominal closure was done with 5-0 silk in the fascia and 4-0 nylon for the skin. Surgical incisions were injected with 0.5 ml of 0.25% bupivacaine to minimize postoperative discomfort. The sham surgical procedures included six enterotomies that were closed primarily to correspond with the IT group. The remainder of the ZRs in the AL group underwent intestinal manipulation followed by abdominal closure as a sham surgical procedure. There were no differences between these two sham procedures in terms of weight, food consumption, or glucose tolerance as well as plasma insulin and incretin concentrations (Supplemental Figs. S1–S7; the online version of this article contains supplemental data), so data were combined into a single sham group. All rats received postoperative care as previously described (19).

Body composition. Nuclear magnetic resonance (1H-NMR) was performed after an overnight fast on postoperative week 8 to determine the effect of IT on body composition. To detect a change in body composition, conscious rats were placed the NMR analyzer (Bruker LF90 proton-NMR Minispec; Bruker Optics; The Woodlands, TX) for rapid determination of body fluid and lean and adipose tissue masses and was then returned to their respective cages.

OGTTs, hyperinsulinemic clamp, and glucose uptake studies. Oral glucose tolerance tests (OGTTs) were performed on postoperative weeks 3 and 5 after an overnight fast. Blood was collected by tail snip before (t0), and 30, 60, 90, and 120 min after oral gavage with 1.25 g/kg 25% dextrose in tubes containing 50 mmol/l EDTA, 12 IU/ml apropinin, and 100 μmol/ml dipeptidyl peptidase-4 (DPP-4) inhibitor. Glucose was measured by glucometer (Contour, Beyer, Tarrytown, NY). Insulin, total GLP-1, and glucagon were measured by multiplexed ELISA (Mesoscale Discoveries, Gaithersburg, MD). Changes in glucose tolerance were compared by analyzing area under the curve (AUC) by the trapezoidal rule. AUC was calculated by using the t0 starting and t1,20 ending points for each animal with baseline set as the lowest t0 value in the control group.

On postoperative week 8, hyperinsulinemic clamp studies were performed as previously described (19). During the steady-state phase of the clamp (140 min), a tracer amount of 1-[14C]-2-deoxyglucose (8 μCi; MP Biochemicals, Irvine, CA) was given as an intravenous bolus for the determination of tissue-specific glucose uptake. Serial blood samples were collected (t = 142, 145, 150, 160, 170, 180) to determine the AUC of plasma 2-deoxyglucose during the last 40 min of the experiment. At the end of the clamp (t = 180 min), animals were euthanized. Tissues were rapidly excised and snap frozen between liquid nitrogen cooled clamps for later processing. Intestinal mucosa from the jejunum and ileum were isolated by scraping (36). Tissues were subsequently weighed and homogenized in ice-cold 0.5 N perchloric acid (0.4 ml/100 mg tissue), then centrifuged at 3,000 g for 15 min. The supernatant was collected and neutralized with an equal molar amount of potassium hydroxide and then assayed for total 14C radioactivity by using a dual-channel liquid scintillation counter (Beckman Coulter). The 14C radioactivity in these samples represents the total counts from both the 2-deoxyglucose and the phosphorylated 2-deoxyglucose present in the tissue sample. An aliquot of the neutralized extract was subjected to Somogyi reaction, which removes the nonphosphorylated 2-deoxyglucose, and counted. Thus phosphorylated 2-deoxyglucose was calculated as the total counts minus the counts remaining after Somogyi extraction. The basal glucose rates of appearance and disappearance, glucose turnover rate, and hepatic glucose output were calculated as previously described (19). The glucose metabolic rate (μg·g tissue−1·min−1) in each tissue was determined by dividing the phosphorylated 2-deoxyglucose and by the integrated area under the 14C-2-deoxyglucose curve during the last 40 min of the clamp.

Measurement of insulin signaling. Samples of gastrocnemius and heart from the clamp study were homogenized in a 1:4 ratio of ice-cold homogenization buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM NaF, 100 mM KCl, 0.2 mM EDTA, 50 mM β-glycerophosphate, 1 mM DTT, 0.1 mM PMSF, 1 mM benzamidine, and 0.5 mM sodium vanadate) with a Polytron homogenizer and centrifuged at 10,000 rpm for 10 min. The supernatant was aliquoted into microcentrifuge tubes, and 2× sample buffer (2 ml of 1 M Tris, pH 6.8, 4 ml of glycerol, 4 ml of 10% SDS, 0.4 ml of β-mercaptoethanol, 0.32 g of bromophenol blue, and 5.6 ml of water to a final volume of 16 ml) was added in a 1:1 ratio. Western blots were performed as previously described using antibodies to total Akt or phospho-specific Akt Ser473, and total insulin receptor substrate-1 (IRS-1) or phospho-IRS-1-Ser307 (Cell Signaling Technology, Beverly, MA). After development, the film was scanned (Microtek ScanMaker IV) and analyzed by use of NIH Image 1.6 software.

Measurement of insulin, GLP-1, GIP, free fatty acids, and total bile acids. Insulin and total-GLP-1 was measured by multiplexed ELISA (Mesoscale Discoveries) according to the manufacturer’s guidelines. Plasma levels of GIP were measured on serial collected plasma samples before and after oral gavage with 1.25 g/kg 25% dextrose collected during the OGTT described above, using a commercially available ELISA for rat GIP (Linco Research, St. Charles, MO). Nonesterified fatty acids (NEFAs) were measured on the fasting plasma samples by use of a colorimetric kit (Wako Diagnostics, Richmond, VA). Total bile acid levels were measured on plasma samples collected as part of the OGTT by use of an enzyme cycling-based total bile acid assay kit from diazime (San Diego, CA).

Statistical analysis. Data are presented as means ± SE. The number of animals in each experimental group is specified in the figure legends. Analysis of the data was performed by ANOVA for multiple measures followed by the Tukey-Kramer or Student-Newman-Keuls posttest using InStat GraphPad 5.02 (San Diego, CA). AUC measurements were performed by using the Prism 5 software with the trapezoidal integration model with the baseline assigned at the lowest basal value. Statistical analysis of the hyperinsulinemic-euglycemic clamp study was done with a Student’s t-test. Differences among groups were considered significant at P < 0.05.
RESULTS

The mean body weights of the IT (432 ± 9 g) and AL (444 ± 8 g) groups prior to surgical intervention were similar. The change in body weight over time for the two experimental groups is shown in Fig. 1A. Both groups gained weight throughout the 8-wk study period. There was a trend toward decreased weight gain in the IT rats that did not reach statistical significance when analyzed by ANOVA for repeat measures. Although many of the later time points for weight are significant when analyzed by Student’s t-test, they did not achieve significance by ANOVA. However, there were no significant differences in body weight or food intake (Fig. 1B) between the groups at any point. When body composition was assessed by NMR, there were no significant differences in body fat, lean mass, or fluid mass between the groups (Fig. 1C).

OGTTs were performed to assess the effect of IT on glucose tolerance. Improvements in OGTT were noted as early as week 3 (Supplemental Fig. S3) after the IT procedure. By postoperative week 5 (Fig. 2A), the mean fasting plasma glucose levels were elevated in the AL group (157 ± 10) compared with the IT group (114 ± 5). Glucose levels were elevated to a greater extent in the AL rats 30 min postgavage and remained significantly higher throughout the 120-min postgavage period. Glucose tolerance in the IT animals was significantly improved as indicated by the 60% reduction in glucose AUC (Fig. 2B). As shown in Fig. 2, C and D, pregavage insulin levels were significantly decreased in the IT group (P < 0.05 vs. AL). Both groups peaked at 30 min and then returned to the basal levels for each respective group. Because of the initial elevation in insulin, the plasma insulin level determined at each time point after the OGTT was higher in the AL compared with IT group (Fig. 2C). Although baseline values were different, the insulin AUC was similar in the AL and IT groups.

The hyperinsulinemic-euglycemic clamp is the “gold standard” for assessing in vivo insulin action. With this technique, insulin is infused to maintain a maximally stimulating insulin concentration while the glucose infusion is titrated to maintain euglycemia. Basal fasting glucose concentrations were similar between the IT and AL groups (Fig. 3A). Steady-state glucose concentrations achieved during the clamp were not different between the groups (Fig. 3C). Plasma insulin concentrations were not determined because previous studies demonstrated that the insulin infusion rate in the present study achieves circulating levels of insulin that maximally stimulate glucose uptake by peripheral tissue (e.g., skeletal muscle) and completely suppress hepatic gluconeogenesis (19). The basal glucose turnover rate (Fig. 3B) was not different between the groups. However, insulin-stimulated glucose disposal was 3.5-fold greater in the IT group compared with the AL group (Fig. 3D).

IT animals showed increased tissue-specific 14C-glucose uptake in all three types of striated muscle examined: gastrocnemius (fast-twitch), soleus (slow-twitch) and heart compared with AL control (Fig. 4A). However, there were no significant changes in glucose uptake observed for the three different adipose depots: epididymal, retroperitoneal, or subcutaneous (Fig. 4B). Although glucose uptake was not increased in the jejunal mucosa, a twofold increase in glucose uptake was observed when the interposed ileal mucosa from the IT group was compared with the same segment of ileum (left in situ) in the AL group (Fig. 4C). There was no change in glucose uptake in the internal control tissues of liver, kidney, or brain (Fig. 4D).

After identifying muscle as a tissue of interest with increased insulin-stimulated glucose uptake, we sought to characterize
the molecular differences in insulin signaling. Measurement of Ser473 phosphorylated-Akt demonstrated increased phosphorylation in the IT animals vs. the AL for the gastrocnemius and heart, and this change was independent of a difference in the total Akt (Fig. 5). Increased serine-307 phosphorylation of IRS-1 correlates with insulin resistance in muscle (35). However, the relative abundance of phosphorylated IRS-1-Ser307 was not significantly different between the groups for gastrocnemius or heart (data not shown). The improvement in glucose tolerance and insulin sensitivity observed after the IT procedure prompted additional investigation for potential mechanisms. Fasting plasma levels of total GLP-1 were similar in the AL and IT groups (Fig. 6A). However, the fourfold increase in plasma GLP-1 observed 30 min after dextrose gavage in the IT group (*P < 0.001 vs. AL) demonstrates an enhanced incretin response of enteric L cells following the IT procedure. We also examined fasting and postgavage plasma levels of total GIP in the IT and AL groups (Fig. 6B). Fasting GIP levels were not different between the groups. Moreover, the increase in plasma GIP observed 30–60 min after glucose gavage was significantly diminished in the IT group (P < 0.05 vs. AL). There were no differences in the plasma concentration of either free fatty acids or glucagon between the IT and AL groups (data not shown). However, as shown in Fig. 7, both fasting and postgavage plasma total bile acid levels were increased two- to threefold in the IT group (P < 0.05 vs. AL).

DISCUSSION

The present study is the first to perform the IT procedure in the obese ZR model and characterize glucose tolerance, insulin sensitivity, and tissue glucose uptake. Changes in glucose homeostasis after bariatric surgery have been attributed to decreased food intake, body weight, or postsurgical alterations in body composition. However, none of these parameters was significantly altered by the IT procedure in the present study. The results of previously reported studies are difficult to compare due to differences in experimental models. Chen et al. (5) examined the effects of IT on food preference and body weight in 52-wk-old female, obese ZRs. In this study, the IT rats demonstrated decreased preference for fat calories after surgery and consequently gained less body weight than controls (5). In a high-fat diet model of obesity, Strader et al. (30)
noted a 7% reduction in body weight 6 wk after IT which appeared due to reduced intake of the high fat diet vs. malabsorption of ingested fat. More recent studies in the lean Goto-Kakizaki (GK) and low-dose streptozotocin (STZ)-induced diabetes models suggest that IT did not significantly affect either food intake or body weight (21, 29, 34). The minimal effects of IT on body weight or composition in the present study are likely due to the use of a standard chow diet after surgery since IT seems to preferentially impact consumption of dietary fat. In that regard, changes in glucose tolerance and insulin sensitivity in the present study are due to alterations in GI anatomy vs. postsurgical changes in body weight or composition.

Improvements in glucose tolerance and insulin action after IT are somewhat variable depending on the experimental model and timing of OGTT. Glucose tolerance was unchanged 3 wk after IT in a high-fat diet model of obesity, whereas insulin tolerance measured 6 wk after IT demonstrated improved insulin sensitivity (30). This may be the result of a lower 0.75 g/kg glucose challenge used in these studies, compared with the 1.25 g/kg challenge used in the present investigation. In the GK rat model, a 30% reduction in glucose AUC was observed 6 wk after IT, and improvements in insulin sensitivity index required 20 wk to develop (21). More recently, Wang et al. (34) reported improvements in glucose and insulin tolerance 10 wk after IT in the GK model. Serial OGTTs demonstrating a 15% reduction in glucose AUC at 4 wk and a 25% reduction in glucose AUC with similar insulin values at 12 wk in the STZ model suggest glucose tolerance improves over time after the IT procedure (29).

The 60% reduction in glucose AUC observed in the present study is among the most dramatic improvements in glucose tolerance reported after IT. Interestingly, the almost threefold reduction in glucose AUC after IT was greater than the 20% reduction in glucose AUC observed 3 wk after RYGB in the ZR model, although we cannot exclude the possibility that this difference may be due to the measurements occurring at different time points (19). The severity and duration of T2DM, as well as the magnitude of postoperative weight loss, appear to be predictive of T2DM resolution after RYGB in morbidly obese patients (26). Therefore, differences in experimental models and postoperative timing of OGTT studies in the literature most likely explain the variability in glucose tolerance after IT.

The associated reduction in plasma insulin during the OGTT suggests that insulin sensitivity was also improved by the IT procedure. The present study is the first to characterize the effects of IT on insulin action using the euglycemic, hyperinsulinemic clamp. There were no differences in basal glucose or basal hepatic glucose output, which indicates that improvements in glucose homeostasis after IT are not the result of reductions in hepatic glucose output. In contrast, the 3.5-fold
increase in whole body glucose disposal demonstrates that the IT procedure dramatically improves peripheral insulin responsiveness and glucose uptake. Similar improvements in insulin action and glucose disposal were noted after RYGB in the obese ZR model (19). Improvements in insulin action are commonly observed in patients after RYGB (7). However, the relative contributions of decreased food intake, reductions in body weight and changes in intestinal anatomy after RYGB are difficult to ascertain. Our data clearly indicate the IT procedure improves the ability of insulin to increase peripheral glucose uptake. Furthermore, the improvement in insulin responsiveness seen after the IT procedure cannot be attributed to postsurgical reduction in food intake, body weight, or adiposity.

To further characterize the effects of IT on glucose homeostasis we injected 1-[14C]-2-deoxyglucose to quantify tissue-specific glucose uptake. GLUT-4 is the predominant insulin-responsive glucose transporter that is located predominantly in striated muscle and adipose tissue. Reductions in insulin-stimulated glucose uptake in skeletal muscle are caused by insulin resistance and represent an important cause of hyperglycemia in patients with T2DM. The relative abundance of total GLUT-4 in muscle or adipose tissue is not dramatically altered in patients with T2DM or the obese ZR model (27).

Instead, defects in insulin-stimulated GLUT-4 translocation to the cell membrane in myocytes and adipocytes represent the primary defect in cellular glucose transport during T2DM (38). Consistent with this observation, insulin-stimulated glucose uptake by striated muscle was increased after the IT procedure. In contrast, glucose uptake did not differ for any of the fat depots or visceral tissues measured, except for the mucosa of the translocated ileal segment. The differences in mucosal glucose uptake in the translocated ileal segment may be the result of increased glucose transporter expression or activity due to increased luminal glucose exposure in the proximal GI tract. This was not further investigated because it is beyond the scope of this report. Post-RYGB alterations in intestinal glucose transport and metabolism have been recently described (28, 31, 36). However, because muscle represents the principal site of insulin-stimulated glucose transport in vivo, the observed increase in muscle glucose uptake presumably represents the predominant mechanism for improved glucose homeostasis after the IT procedure (14, 27).

The inflammatory effects of obesity are posited to inhibit insulin signaling by a mechanism involving serine phosphorylation of IRS-1 (10). Muscle tissue from the clamp experiment was assayed for phosphorylated-Akt to determine

Fig. 4. Tissue-specific glucose uptake. Tissue-specific insulin-stimulated glucose uptake was measured at the conclusion of the clamp procedure, during steady state, using a bolus tracer dose of 1-[14C]-2-deoxyglucose as described in the MATERIALS AND METHODS. A: glucose uptake in 3 types of striated muscle. Gastroc, gastrocnemius. B: glucose uptake in 3 adipose depots. Epi, epididymal fat pad; Retro, retroperitoneal fat; Sub-Q, subcutaneous fat. C: glucose uptake from plasma in the mucosa of the ileum and jejunum. D: glucose uptake in control tissues of liver, kidney, and brain. Data are mean glucose uptake (µg·g tissue⁻¹·min⁻¹, ± SE) for IT (n = 9) and AL (n = 8). *P < 0.05 IT vs. AL for all panels.
whether insulin signaling was influenced by IT. The ratio of phosphorylated to total Akt was increased in muscle following the IT procedure, suggesting an improvement in insulin signaling. In the ZR rat, obesity-related defects in muscle insulin signaling are reversed by anti-inflammatory therapies including salicylates and disruption of the IκB kinase pathway (37). Our findings are consistent with reports of improved muscle glucose transport and insulin action after RYGB in morbidly obese patients (2). However, we were unable to identify a reduction in serine phosphorylation of IRS-1 in the present study, potentially because of differences in experimental de-

![Graph of muscle insulin signaling](image1)

Fig. 5. Muscle insulin signaling. At the completion of the hyperinsulinemic-euglycemic clamp procedure, muscle was frozen, stored at −70°C, then homogenized, and a Western blot analysis was performed to measure Ser473 phosphorylated (p)-Akt and total Akt. A: relative densitometry of p-Akt/Total Akt for IT (n = 8) and AL (n = 8). Data are mean relative densitometry units (RDU) ± SE for gastrocnemius and heart tissue, *P < 0.001 IT vs. AL. B: representative Western blots.

![Graph of incretin secretion after OGTT](image2)

Fig. 6. IT alters incretin secretion after OGTT. Fasting (t0) and postgavage plasma samples were collected at 30, 60, 90, and 120 min after gavage on postoperative week 7 as described in MATERIALS AND METHODS. Data are means ± SE, *P < 0.001 IT vs. AL for all panels. A: total GLP-1 is in pg/ml. B: total GIP is reported in ng/ml. IT (●; n = 15), and AL (○; n = 10).
sign or the effects of the hyperglycemic clamp on insulin signaling.

Enhanced exposure of the ileum to undigested nutrients after RYGB may improve glucose homeostasis by resulting in enhanced secretion of GLP-1 and other gut peptides by enteroadipose L-cells. An increased postprandial secretion of GLP-1 has been demonstrated after RYGB in patients and in the obese ZR model (16, 19). In addition, increased postprandial levels of plasma GLP-1 are uniformly reported in all of the experimental IT models. Consistent with this observation, we noted a significant increase in postgavage GLP-1 in the IT rats compared with AL controls. In contrast, postgavage GIP levels were dramatically reduced after the IT procedure.

GLP-1 improves glucose homeostasis by several mechanisms including enhanced insulin secretion, improved insulin sensitivity, extrapancreatic effects on insulin-independent glucose disposal/metabolism in liver and muscle, enhanced β-cell proliferation, and inhibition of β-cell apoptosis (1, 11, 20, 25). The postgavage increases in plasma GLP-1 and insulin concentrations observed in the IT group suggest that the insulinotropic effect of GLP-1 on pancreatic β-cells contributes to glycemic control. However, gene therapy with GLP-1 has also been shown to improve insulin sensitivity and GLP-1-mediated, insulin-independent improvement in glycemic control (23, 33). Interestingly, basal and glucose-stimulated insulin levels were lower in the IT animals, suggesting that the insulin-independent effects on insulin sensitivity play a more predominant role than the insulinotropic effects of GLP-1. The reduction in GIP after IT was unexpected as GIP secretion has been reported to be unaltered by IT (30). This may be explained by the anatomic impact of interrupting jejunal continuity with the IT procedure. However, resistance to the insulinotropic effects of GIP has been reported in the Zucker model of T2DM as a result of reductions in β-cell GIP receptors and postreceptor defects in β-cell GIP signaling (17, 19). Consequently, evaluating the effects of IT on GIP bioactivity will require a more detailed analysis of the relative abundance of GIP receptors and postreceptor signaling events in pancreatic β-cells.

The release of NEFAs from adipose tissue can also produce insulin resistance during T2DM (14). Hence, we measured NEFAs in fasting plasma samples because reductions in plasma NEFAs after IT could contribute to improvements in insulin action. However, we did not identify any changes in plasma NEFA levels after the IT procedure. Our results are consistent with those of Tsuchiya et al. (32), who noted IT had no effect on triglyceride absorption. Wang et al. (34) identified a decrease in plasma fatty acids in the GK model after IT in the fasting, but not the nonfasting state, and attributed the differences to the hormonal milieu. However, our data suggest that a change in plasma free fatty acid concentration was not responsible for the improvement in insulin action after IT in the obese ZR model.

Finally, we measured the effects of IT on total plasma bile acids. Increased GI absorption of bile acids after IT were initially described in 1996 (32). More recently, Strader et al. (29) noted a threefold increase in fasting total bile acids after IT in the dietary fat obesity model. Duodenjejunal diversion of bile is associated with improved glucose tolerance (18). Furthermore, IT has been shown to increase plasma bile acids and improve glucose tolerance in the STZ model of diabetes (29). Increased plasma bile acids were recently described after RYGB in morbidly obese patients (22). Consistent with these observations, a threefold increase in plasma bile acids after IT was detected in the present study. The endocrine effects of bile acids on intracellular signaling, iodothyronine deiodinase activity, and energy metabolism were recently reviewed and represent a potentially important mechanism for the metabolic sequelae of the IT procedure (13). However, additional studies will be necessary to further understand the relationship between changes in circulating bile acids after IT and improvements in glucose tolerance.

DePaula et al. (8, 9) have published two reports detailing their experience in humans of laparoscopic IT in conjunction with sleeve gastrectomy. They find a similar improvement in diabetes and enhanced GLP-1 secretion to what we report in our rat model; 86% of their patients had resolution of their diabetes and all patients had significant improvements. Interestingly, they do report decreases in body mass index (BMI) and NEFAs, which were not seen in our model. This difference may be explained by our model not having a sleeve gastrectomy and hence lacking a restrictive component. These reports support the clinical relevance of IT in surgically correcting diabetes, particularly in patients with BMI below 35 who would not normally be candidates for bariatric surgery.

In conclusion, IT produces improvements in glucose tolerance, muscle insulin responsiveness, and muscle insulin signaling in the obese ZR model that are independent of weight loss, body composition, or food intake. This observation provides evidence that postsurgical changes in intestinal anatomy...
and function, especially enhanced exposure of the ileum to nutrients, contribute to improved glucose homeostasis after GI surgery. Alternatives in GIP secretion suggest a humoral effect altering foregut hormonal response and suggest an uncharacterized interplay between the hindgut and foregut taking place in the setting of bariatric surgery.

ACKNOWLEDGMENTS

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GRANTS

This work was supported in part by National Institute of Health Grants GM-55639 (to R. N. Cooney), GM-38032 (to C. H. Lang), and DK-062880 (C. Lynch). This project is funded, in part, under grants with the Pennsylvania Department of Health using Tobacco Settlement Funds (to R. N. Cooney) and Penn State Institute for Diabetes and Obesity (to C. Lynch). The Department specifically disclaims responsibility for any analysis, interpretations, or conclusions.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

