Effects of sleeve gastrectomy and ileal transposition, alone and in combination, on food intake, body weight, gut hormones, and glucose metabolism in rats

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Nausheen S, Shah III, Pezeshki A, Sigalet DL, Chelikani PK. Effects of sleeve gastrectomy and ileal transposition, alone and in combination, on food intake, body weight, gut hormones, and glucose metabolism in rats. Am J Physiol Endocrinol Metab 305: E507–E518, 2013. First published June 25, 2013; doi:10.1152/ajpendo.00130.2013.—Bariatric surgeries are hypothesized to produce weight loss and improve diabetes control by multiple mechanisms including gastric restriction and lower gut stimulation; the relative importance of these mechanisms remains poorly understood. We compared the effects of a typical foregut procedure, sleeve gastrectomy, (SG) with a primarily hindgut surgery, ileal transposition (IT), alone and together (SGIT), or sham manipulations, on food intake, body weight, gut hormones, glucose tolerance, and key markers of glucose homeostasis in peripheral tissues of adult male Sprague-Dawley rats (450–550 g, n = 7–9/group). SG, IT, and SGIT surgeries produced transient reduction in food intake and weight gain; the effects of SG and IT on intake and body weight were nonadditive. SG, IT, and SGIT surgeries resulted in increased tissue expression and plasma concentrations of the lower gut hormones glucagon-like peptide-1 and peptide YY and decreased plasma glucose-dependent insulinoctopic peptide, insulin, and leptin concentrations. Despite transient effects on intake and weight gain, the SG, IT, and SGIT surgeries produced a significant improvement in glucose tolerance. In support of glycemic improvements, the protein abundance of key markers of glucose metabolism (e.g., GLUT4, PKA, IRS-1) in muscle and adipose tissue were increased, whereas the expression of key glucomoeogenic enzyme in liver (G-6-Pase) were decreased following the surgeries. Therefore, our data suggest that enhanced lower gut stimulation following SG, IT, and SGIT surgeries leads to transient reduction in food intake and weight gain together with enhanced secretion of lower gut hormones and improved glucose clearance by peripheral tissues.

sleeve gastrectomy; ileal transposition; food intake; body weight; gut hormones; glucose metabolism

Bariatric surgeries are some of the most effective antiobesity interventions producing durable weight loss in obese subjects. The surgeries often lead to dramatic improvements in diabetes and other comorbidities of obesity beyond the benefits accrued from weight loss alone (13, 19, 25, 36, 49). A recent position statement from the International Diabetes Federation recommends bariatric surgery as a treatment option for obese subjects (BMI $\geq$35 kg/m²), and as an alternative treatment option for moderately obese subjects (BMI 30–35 kg/m²) with poorly controlled diabetes (22). Despite such recommendations and benefits, the underlying mechanisms by which bariatric procedures improve control of diabetes remain largely unknown.

Bariatric surgeries typically involve manipulations of the foregut and/or hindgut. The foregut surgeries include sleeve gastrectomy (SG) and gastric banding, whereas jejunoileal bypass, biliopancreatic diversion, and ileal transposition (IT) are hindgut surgeries (49). Roux-en Y gastric bypass (RYGB) surgery involves manipulation of both the foregut and hindgut and is considered a reference standard for bariatric procedures. The weight loss and metabolic effects of RYGB are hypothesized to be due to multiple mechanisms including exclusion of foregut and/or enhanced stimulation of the hindgut (25); the relative importance of these mechanisms to improvements in glucose homeostasis remains to be determined. In contrast to RYGB, SG is primarily a restrictive surgery in which the stomach volume is decreased by resection along the greater curvature of the stomach without altering the length of intestines. In recent human clinical trials, SG was found to be comparable to RYGB in producing weight loss with significant remission of diabetes (30, 51); similar weight-independent effects of SG and RYGB were also observed in rodent models (12). In contrast to SG, in IT a segment of ileum is transposed to the upper jejunum without altering the anatomy of the stomach or the length of the intestines. Thus, IT permits the assessment of the exclusive role of ileal activation as a mediator of surgically induced weight loss and metabolic improvements. IT surgeries were found to produce significant improvements in glycemic control independent of weight loss in rat models (16, 18, 32, 34, 54, 55). Recently, a combination of SG and IT (SGIT) surgeries was found to be as effective as RYGB in preventing weight gain and improving glucose tolerance in obese rats (5). SGIT was also shown to produce significant remission of diabetes with improvements in other comorbidities of obesity in humans (20, 21, 62); the underlying mechanisms for these benefits remain to be characterized.

The benefits of bariatric surgeries are often attributed to altered secretion of gut hormones (3, 36). Enhanced lower gut stimulation with consequent secretion of the hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are believed to play a key role in the reduction of food intake, weight loss, and improvements in glucose homeostasis following these surgeries (3, 36, 49). Antagonism of the GLP-1 receptor has been shown to attenuate the improvements in glucose homeostasis following SG (12) and IT (24) surgeries, supporting a direct role for GLP-1 in glyemic improvements. GLP-1 and...
glucose-dependent insulinotropic peptide (GIP) are incretins with strong insulinotropic properties; however, less is known of GIP secretion following bariatric surgeries (3, 36). Although the surgeries apparently improve glucose clearance by peripheral tissues, the underlying mechanisms remain elusive. SG was reported to inhibit hepatic glucose output (12), and IT was found to increase muscle glucose uptake (16) as well as increase the abundance of the insulin-sensitive glucose transporter (GLUT4) and insulin receptor substrate-1 (IRS-1) in muscle (64) of rats. Insulin signaling in vitro is enhanced, in part, through a reciprocal upregulation of Ser636-phosphorylated IRS-1 and a downregulation of Ser630-phosphorylated IRS-1 (IRS-1) (IRS-1 P636/630) (41). However, little is known of the intracellular signaling pathways in muscle, adipose tissue and liver that mediate the improvements in glucose homeostasis following bariatric surgeries. Therefore, to gain insights into the mechanisms of weight loss and glycemic improvements of bariatric surgeries, for the first time, we compared the effects of a foregut procedure (SG) with a primarily hindgut procedure (IT), alone and together (SGIT), on multiple parameters, including food intake, body weight, glucose tolerance, lower gut hormones, and key markers of glucose metabolism in skeletal muscle, adipose tissue, and liver in rats.

**MATERIALS AND METHODS**

**Animals.** All experiments were performed in accordance with the Canadian Council for Animal Care Guidelines under protocols approved by the University of Calgary Animal Care Committee. Male Sprague-Dawley rats (Charles River, Montreal, QC, Canada) weighing 350–400 g were housed individually in wire mesh cages in a room with controlled temperature (20 ± 2°C) and a 12:12-light-dark cycle (lights off at 1000). The animals were provided pelleted rat chow (Labdiet PROLAB RMH2500 Rodent diet; PMI Nutrition International) and water ad libitum and allowed to adapt for a minimum of 2 wk to the environment.

**Surgical procedures.** The rats (450–550 g) were randomly allotted to either SG (n = 9), IT (n = 9), SGIT (n = 9), or sham surgeries (n = 7; control, C). Prior to surgeries, the animals were fasted for ~12 h but had ad libitum access to water. Surgeries were performed under isoflurane anesthesia (2–4%; 1 l/min O₂ flow) with administration of an antibiotic (Baytril Bayer HealthCare, Toronto, ON, Canada), analgesic (Buprenorphine), and saline (Baxter, Mississauga ON, Canada) during the preoperative period and at ~12-h intervals for 48 h postoperatively. SG was performed following the procedure of Pietrakos et al. (45) with some modifications. Briefly, following a 4.5-cm middle abdominal incision, stitches were placed on the stomach with 6-0 silk (Ethicon, Markham, ON, Canada) to on the stomach with 6-0 silk (Ethicon, Markham, ON, Canada) to 5 cm from the ligament of Treitz. It is likely that a minor section of the jejunum may have been included in the proximal part of the transposed ileal segment. The SGIT rats were subjected to both SG and IT surgeries. The sham operations involved sham firing of the stapler over the stomach and intestinal incisions as in IT with reanastomosis. Another group of rats (n = 8), age-matched to the control group, were housed in shoebox cages with free access to chow and water for the duration of the study. These chow animals were intended primarily as age-matched controls for the Ensure-fed control rats in the intraperitoneal glucose tolerance test (IPGTT) described below. The Chow-fed animals were housed in pairs, but they were not subjected to any surgical manipulations and their daily intakes and body weights were not recorded.

**Food intake and body weight.** At onset of the dark period (1000), the animals were provided daily a fresh, complete, mixed-nutrient liquid diet Ensure Plus (57, 15, and 28% calories from carbohydrate, protein, and fat, respectively, 1.5 kcal/ml; Abbott Laboratories, Saint-Laurent, QC, Canada). Daily food intake (23 h) and body weight were recorded between 0900 and 1000. At 7 wk postsurgery, under ad libitum conditions, the amount of Ensure Plus consumed was recorded from all rats at 30, 60, and 120 min after dark onset for 3 consecutive days. Mixed-nutrient liquid diets were originally used to characterize the effects of IT surgeries on intake and gut hormones in rat models (35). When given access to a high-fat solid diet and Ensure Plus liquid diet, rats consume 80% of their total calories from Ensure Plus (14), and we previously used this mixed nutrient liquid diet to compare the effects of IT and RYGB surgeries on food intake and body weight (15).

We estimated that the Ensure Plus liquid diet would either meet or exceed the requirements for minerals and vitamins, as well as the total caloric contributions from carbohydrates, fats, and protein for rats in the current study. Therefore, the Ensure Plus liquid diet was used in the current study because it permits comparisons with previous studies, is highly palatable for rats, allows for more rapid passage of food across the sites of anastomosis with minimal blockage, and liquid diets permit more precise recording of intakes with relatively less spillage. Although the rats were nonobese at study onset, we demonstrate below that with Ensure Plus feeding the control rats rapidly gained weight and were glucose intolerant compared with age-matched Chow-fed animals. Furthermore, in support of our animal model, numerous studies had characterized the anorexic, weight loss, and other metabolic effects of bariatric surgeries using relatively older, nonobese, Chow-fed Wistar or Sprague-Dawley rats (1, 2, 7–9, 37, 38, 43), or maintained obese Long-Evans rats (12) or normal Wistar rats (35) completely on a mixed-nutrient liquid diet following surgical interventions.

**Intraperitoneal glucose tolerance test.** Three days after completion of the dark period, food intake measurements mentioned above, all animals were fasted overnight and subjected to an injection of 50% dextrose at 2 g/kg body wt ip. Tail vein blood glucose concentrations were determined using a hand-held glucose meter (Accu-Chek glucose meter; Roche Diagnostics, QC, Canada) at 0 (baseline), 30, 60, and 120 min after dextrose injection.

**Blood sampling and tissue harvesting.** At 3–4 days after IPGTT, the C, SG, IT, and SGIT rats were subjected to blood sampling from the tail as we described previously (15). Briefly, the fasted animals were allowed to consume ~8 ml of Ensure Plus over a 15-min period, and blood samples were collected at 0, 30, 60, and 120 min after consumption. Blood samples were collected on ice in 1.5-ml Eppendorf tubes containing EDTA (1 mg/ml blood; Sigma, St. Louis, MO), Dipeptidyl peptidase-4 inhibitor (10 μl/ml blood; Millipore) and protease inhibitor cocktail (10 μl/ml blood; Sigma-Alrhich), and centrifuged at 1,000 g for 10 min at 4°C within 30 min of collection. Plasma was immediately separated, aliquoted, and stored at ~8°C until analysis. The animals were euthanized with Euthanyl (Euthanyl Bimed-MTC, ON, Canada), and representative samples of the epididymal fat, leg muscle, and liver were collected, rinsed in sterile saline, immediately snap-frozen in liquid nitrogen, and stored at −80°C. Furthermore, a segment of the transposed ileum in IT and SGIT rats, and a comparable ileal segment in C and SG rats, were collected, rinsed in sterile saline, and bisected with one fragment fixed in 10% formalin (EMD Chemical, NJ) and the remainder was snap-frozen in liquid nitrogen and stored at −80°C.

**RNA isolation and semiquantitative PCR.** RNA isolation and realtime qPCR were performed as we described previously (48). Briefly,
total RNA was isolated from tissues using an RNeasy Mini Kit (Qiagen, Toronto, ON, Canada) and cDNA synthesized using reagents from Invitrogen (Burlington, ON, Canada). The qPCR was performed in duplicate using SYBR Green master mix (Applied Biosystems) on a Mastercycler ep realplex thermocycler (Eppendorf, Ontario, Canada) with gene-specific primers (Table 1). The relative expression level of target genes was determined using the 2^−ΔΔCt method (39, 52).

### Histomorphometry and immunohistochemistry

For histomorphometry, segments (~0.5 cm) of formalin-fixed gut tissues were embedded in paraffin, soaked overnight in cold water, and sectioned (6 µm) with a microtome (Finesse 325; Shandon Science, Cheshire, UK). The tissue sections were mounted on silane-coated slides, deparaffinized in xylene (EMD Chemicals), and then rehydrated through serial dilutions of ethanol (100, 95, 90, and 70%) followed by staining with hematoxylin and eosin (Sigma-Aldrich, Oakville, ON, Canada) solutions. For each sample slide, measurement of villus height, villus width, crypt depth, and muscular thickness (circular and longitudinal) were recorded from 10 well-oriented villi under ×20 objective of an Olympus BX51 microscope (Tokyo, Japan).

For immunohistochemistry, immunohistochemical staining for Ki-67 (mouse anti-rat Ki-67 antibody, MIB-5; Cedarlane Labs, Burlington, ON, Canada), a marker of gut epithelial proliferation, was performed as described (29), and the number of Ki-67-stained cells per crypt was determined. The mucosal surface area of the gut segments was estimated by histological surface magnification ratio, which was calculated using measurements of villus width, villus length, and crypt width (33). For PYY and GLP-1 immunostaining, sections were prepared as described above and incubated at 4°C overnight with the primary antibodies anti-GLP-1 (1:500 dilution, HYB 147–06, sc-57166; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-PYY (1:1,000 dilution, ab22663; Abcam, Cambridge, MA). The slides were washed for a minimum of 3 × 8 min in fresh phosphate-buffered saline solution (PBS). Next, the slides were incubated with 1:500 dilution of biotinylated donkey anti-mouse IgG or goat anti-rabbit secondary antibodies (715–065–150, 111–065–003, Cedarlane Labs) for 30 min at 37°C. The slides were then washed, incubated with horseradish peroxidase (HRP)-conjugated streptavidin (Cedarlane Labs) for 30 min at 37°C, and the sections were developed with diaminobenzidine (Vector Laboratories, Burlington, ON, Canada) for 1–2 min. Next, slides were counterstained with hematoxylin for 15 s and mounted with aquamount (Thermo Scientific, Santa Cruz, CA) solutions. For each sample slide, measurement of villus height, villus width, crypt depth, and muscular thickness (circular and longitudinal) were recorded from 10 well-oriented villi under ×20 objective of an Olympus BX51 microscope (Tokyo, Japan).

### Protein extraction and western blotting

Segments of the gut (ileum), muscle, liver, and fat samples (100–200 mg) were homogenized in 660 µl of a mix of NP40 buffer (Invitrogen, Burlington, ON, Canada), 10× protease inhibitor cocktail, and 0.3 M phenylmethylsulfonyl fluoride (Sigma-Aldrich, Oakville, ON, Canada). After brief sonication (10 s), the cellular debris was removed by centrifuging the homogenates at 2,500 g for 15 min (4°C). Cell lysate was carefully decanted, and protein concentrations were determined using the Bradford assay (Bio-Rad Lab, Mississauga, ON, Canada). The primary antibodies and their dilutions used in the study were: anti-AMP-activated protein kinase-α (AMPKα, 1:1,000, 2793S; Cell signaling Technology, MA), anti-GLP-1 receptor (GLP-1R, 1:1,000, sc-66911; Santa Cruz Biotechnology), anti-GLUT4 (1:1,000, ab6545; Abcam), anti-IRS-1 (1:500, ab52167; Abcam), anti-IRS-1 pS636 (1:1,000, ab47764; Abcam), and protein kinase A (PKA, 1:2,000, ab32390; Abcam). Equal amounts of the sample (2 mg/ml) were resuspended in 2× Laemmli sample buffer (Sigma-Aldrich, St. Louis, MO) heated at 95°C for 3 min (except for GLUT4 and GLP-1R) and run at 130 V for 90 min in 8–10% SDS-PAGE depending on the size of protein of interest. The proteins were transferred from gel to a nitrocellulose membrane by running at 100 V for 1 h (Sigma-Aldrich, Oakville, ON, Canada). Blots were incubated with primary antibody overnight at 4°C and then blocked in 5% skimmed milk for 1 h. Blots were washed with PBST (PBS with 1% Tween 20) solution (3 × 10 min), and then probed with HRP monoclonal anti-rabbit/anti-mouse secondary antibodies (Cedarlane Labs) by incubating at room temperature for 1 h. Immunoreactivity was detected by chemiluminescence (ECL solution; Invitrogen), and images were captured with a GelDoc system (Chemidoc MP System; Bio-Rad, Mississauga, ON, Canada). To normalize the protein of interest, the blots were reprobed with β-actin (sc-7778; Santa Cruz Biotechnology). The images were scanned, and band densitometry was assessed using image lab software (Chemidoc MP System, Bio-Rad). For each protein of interest, antibody dilutions were optimized, the linearity of signal was confirmed between 5 and 40 µg of a pooled protein sample, and for negative controls primary antibodies were omitted from the immunoblotting protocol.

### Hormone assays

Plasma PYY and GLP-1 concentrations were quantified by ELISA (S-1359, S-1274; Bachem Americas, Torrance, CA) following the instructions of the manufacturer. The PYY assay kit (S-1274) cross-reacts with both PYY-(1–36) and PYY-(3–36), and the intra- and interassay CVs were 7.19 and 4.02%, respectively. The GLP-1 assay cross-reacts with GLP-1-(7–36) and GLP-1-(7–37), and the intraassay CVs were 7.19 and 4.02%, respectively. The intra- and interassay CVs were 3.76 and 18.06%, respectively. Plasma concentrations of insulin, leptin, and GIP were measured in a single assay using the Milliplex Rat gut hormone panel (RGT 88K; Millipore Luminex, Austin, TX) on a Luminex platform (Bio-Plex 200). The intra-assay CVs for insulin, leptin, and GIP were 1.07, 2.02, and 1.81%, respectively.

### Statistical analyses

Data are represented as means ± SE. Daily food intake, cumulative food intake during first 2 h of dark period, and weight gain are represented as means ± SE. Statistical analysis was performed with a two-way analysis of variance (ANOVA; SigmaStat, Jandel Scientific, San Rafael, CA). Differences were considered statistically significant at P < 0.05.

<table>
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<th>Genes Sequence (5'→3')</th>
<th>Location on Template (bp)</th>
<th>Amplicon size (bp)</th>
<th>GenBank Acc. No.</th>
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<td>Peptide YY</td>
<td>F: CGGCTATGGAACAGAAGACTC</td>
<td>255–276</td>
<td>110</td>
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<td></td>
<td>R: ACACCGTGTCGACACCTTCG</td>
<td>344–364</td>
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<td>Preproglucagon</td>
<td>F: GAGGCGGCAGAACAGAAAGG</td>
<td>397–416</td>
<td>103</td>
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<tr>
<td></td>
<td>R: TCTGGGCGCAAGCCCTGACG</td>
<td>479–499</td>
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<tr>
<td>Phosphoenolpyruvate carboxykinase</td>
<td>F: GGCAAGCATAAAGGGCAAGGT</td>
<td>1545–1564</td>
<td>74</td>
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<tr>
<td></td>
<td>R: TGGCGGAGTTAGGCAAAA</td>
<td>1599–1618</td>
<td></td>
</tr>
<tr>
<td>Phosphofructokinase (liver)</td>
<td>F: CTCACGGCTGAGCTGAGGAAAGTG</td>
<td>71–93</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>R: CTGCAAGGACGACCATGATCGT</td>
<td>148–171</td>
<td></td>
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<tr>
<td>Glucokinase</td>
<td>F: AGGACTCGGGGACCCGAAGA</td>
<td>1003–1022</td>
<td>128</td>
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<tr>
<td></td>
<td>R: CAGATATGGGCGGCAGGATT</td>
<td>1111–1130</td>
<td></td>
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<tr>
<td>Glucose-6-phosphatase</td>
<td>F: TCCACCTTGACACATACACCC</td>
<td>818–837</td>
<td>250</td>
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<td></td>
<td>R: GGCGCGTCTGACCTC</td>
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<td>β-Actin</td>
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<td></td>
<td>R: AAGACGGCTGAAGTACAGGGCG</td>
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Table 1. Primer sequences forward (F) and reverse (R), locations on template (bp), amplicon sizes, and GenBank accession numbers for genes used for quantitative PCR in the current study.
body weight, glucose tolerance, and hormonal changes over the course of the experiment were analyzed by repeated-measures ANOVA followed by Dunnett’s test. To test for additive inhibitory effects of SG and IT surgeries on intake and weight, the differences in intakes and weights of controls from SG or IT rats were summed, and these predicted responses were then compared with the reduction of intakes and weights of SGIT rats relative to controls by repeated-measures ANOVA followed by an unpaired t-test. Gut histological parameters, immunohistochemistry data, protein levels by Western blotting, and qPCR data were analyzed using one-way ANOVA followed by post hoc Dunnett’s test. Statistical significance was declared at $P < 0.05$.

**RESULTS**

**Food intake and body weight.** The SG, IT, and SGIT surgeries produced a significant reduction in daily food intake (Fig. 1A) and body weight (Fig. 1C) compared with controls. The mean daily food intake prior to surgeries did not differ among treatments. Compared with C, the intakes of SG rats decreased by 26–35% for the first two postoperative weeks, those of IT rats decreased by 30–53% for 3 wk, and the intakes of SGIT rats were decreased by 30–57% for 5 wk after surgery, respectively (Fig. 1A). After 5 wk, the intakes did not differ among treatments. The inhibition of daily food intake of SGIT rats was less than that predicted from a summation of the inhibition of the intakes of SG alone and IT alone relative to control rats (Fig. 1A). At 8 wk, when the cumulative food intake during the first 2 h of dark period was considered (Fig. 1B), relative to C rats the intakes of SG, IT, and SGIT rats were decreased by 24–25% during 1 h, and the intakes of SGIT rats were decreased by 17% by 2 h.

Mean preoperative body weight did not differ among treatments (C 508 ± 13, SG 483 ± 30, IT 469 ± 14, SGIT 463 ± 14, Chow 466 ± 16 g). At study termination, the weights of the SG and C rats did not differ, whereas the weights of the IT, SGIT, and chow-fed rats were lower than those of C rats (C 635 ± 23, SG 600 ± 28, IT 560 ± 20, SGIT 550 ± 27, Chow 547 ± 10 g). Relative to the C rats, the weights of SG rats were decreased by 10–17% for the first 7 wk following surgery, while the weights of the IT and SGIT rats were decreased by 16–26 and 19–28%, respectively, throughout the study (Fig. 1C). Compared with their own presurgical body weights, weights of SG rats were decreased by 6% for the first week, and the weights of IT and SGIT rats were decreased by ∼9% for 2–3 wk postsurgery. Similar to intakes, the relative reduction of body weight of SGIT rats was less than the decrease of weight predicted from a summation of the reduction of weights.
of SG alone and IT alone (Fig. 1C). However, when the rate of change in weight was compared across treatments, relative to weight gain of the C rats the SG rats had a transient reduction in weight gain for 1 wk, whereas the reduction in weight gain lasted for 3 wk in IT and SGIT rats (Fig. 1D). Together, these results demonstrate that enhanced gut stimulation through IT or SGIT surgeries produce comparable effects on food intake and body weight, and these effects were prolonged vs. those of SG surgeries. Interestingly, SG and IT surgeries do not have additive effects in decreasing food intake or weight gain in our rat model.

**IPGTT.** There was a significant improvement in glucose tolerance in all surgical treatment groups compared with C rats (Fig. 2A). Relative to C, blood glucose concentrations were decreased by 28–41% for SG, 28–39% for IT, and by 27–39% for SGIT. Compared with C rats, the total glucose area under the curve (AUC) was decreased by ~22% in all surgical groups (Fig. 2B). Interestingly, the chow animals had 39% lower blood glucose AUC than C, and the glucose concentrations of the chow rats were similar to those of SG, IT, and SGIT groups.

**Gut histomorphology.** At termination, histomorphometry of transposed or comparable ileal segments revealed a significant increase in villus height in SG, IT, and SGIT and an increase in villus width in SG compared with C rats (Table 2 and Fig. 3A). The increase in Ki-67 immunoreactivity in IT and SGIT treatments is indicative of enhanced crypt cell proliferation in these groups; crypt width and depth did not differ among treatments. The thicknesses of the circular and longitudinal muscle layers were increased by 145 and 43% in IT and by 122 and 61% in SGIT, respectively, compared with C rats. The histological surface index increased by 31% in SG, 43% in IT, and 84% in SGIT groups compared with C.

**Gut hormones.** Compared with C, the mRNA abundance of preproglucagon in gut segments showed a numerical increase in SG and IT (\(P = 0.17\) and \(P = 0.29\), respectively; Fig. 4A), and the numbers of GLP-1-immunopositive cells in transposed or comparable ileal segments were increased in SG, IT, and SGIT surgeries (Figs. 3B and 4B). The mRNA abundance of PYY increased significantly in IT (Fig. 4D), and the numbers of PYY-immunopositive cells were increased in IT and SGIT compared with C rats (Figs. 3C and 4E).

Meal-induced changes in plasma concentrations of GLP-1, PYY, insulin, leptin, and GIP were measured at the end of study (Figs. 4 and 5). Relative to C rats, following a meal, plasma GLP-1 concentrations were increased by 28–39% in IT, and by 27–39% in SGIT (Fig. 2B). Interestingly, the chow animals had 39% lower blood glucose AUC than C, and the glucose concentrations of the chow rats were similar to those of SG, IT, and SGIT groups.

**Table 2. Effects of sleeve gastrectomy (SG) and ileal transposition (IT) surgeries on gut adaptation and hepatic gene expression**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>SG</th>
<th>IT</th>
<th>SGIT</th>
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<tbody>
<tr>
<td>Vector (µm)</td>
<td>348 ± 45</td>
<td>431 ± 15†</td>
<td>459 ± 12†</td>
<td>510 ± 19‡</td>
</tr>
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<td>Villus width (µm)</td>
<td>154 ± 15</td>
<td>90 ± 7†</td>
<td>139 ± 12</td>
<td>113 ± 4</td>
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<tr>
<td>Crypt depth (µm)</td>
<td>145 ± 26</td>
<td>127 ± 9</td>
<td>188 ± 12</td>
<td>193 ± 17</td>
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<tr>
<td>Crypt width (µm)</td>
<td>52 ± 2</td>
<td>55 ± 6</td>
<td>58 ± 9</td>
<td>44 ± 3</td>
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<tr>
<td>Ki-67-positive cells/µm</td>
<td>2.75 ± 0.31</td>
<td>3.10 ± 0.33†</td>
<td>4.54 ± 0.37†</td>
<td>5.18 ± 0.42‡</td>
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<tr>
<td>Muscle thickness (µm)</td>
<td>119 ± 29</td>
<td>115 ± 11</td>
<td>253 ± 22‡</td>
<td>261 ± 10†</td>
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<tr>
<td>Glucose-6-phosphatase/β-actin</td>
<td>0.125 ± 0.04</td>
<td>0.88 ± 0.18</td>
<td>0.38 ± 0.18</td>
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<td>Phosphoenolpyruvate carboxyl kinase/β-actin</td>
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<td>0.59 ± 0.08</td>
<td>0.73 ± 0.14</td>
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Data are presented as means ± SE. Rats (\(n = 6–8\)/group) were subjected to SG, IT, combination (SGIT), or sham control (C) surgeries. At study termination, samples of liver and segments of transposed ileum from IT and SGIT and comparable ileal segments from SG and C rats were collected. *\(P < 0.05\), †\(P < 0.01\), ‡\(P < 0.001\) vs. control (C).
decreased by 49–51% in SG, by 48–68% in IT, and by 66–75% in SGIT rats (Fig. 5A). Plasma GIP concentrations were decreased by 63% in SG rats, by 62–84% in IT, by 24–66% in SGIT rats (Fig. 5B). Plasma leptin concentrations decreased by 57% in SG, by 59–72% in IT, and by 45–72% in SGIT rats (Fig. 5C).

**Markers of glucose metabolism in skeletal muscle.** To explore the mechanisms of improved glucose tolerance following SG, IT, and SGIT surgeries, we determined the relative protein abundance of key markers of glucose metabolism in skeletal muscle and adipose tissue. In muscle, relative to C, GLUT4 protein abundance was increased by 101, 113, and 170% in SG, IT, and SGIT rats, respectively (Fig. 6A). The relative protein abundance of GLP-1R protein did not differ among treatments (Fig. 6B). The relative protein abundance of PKA was increased by 130 and 173% in SG and IT (Fig. 6C). The relative protein abundance of IRS-1 protein (Fig. 6D) increased by 243%, whereas that of IRS-1 pS636 (Fig. 6E) was decreased by 36% in IT rats compared with C; the other treatments did not differ. The IRS-1 pS636/IRS-1 ratio was decreased with IT but not with other treatments (C 12.5 ± 4.3, SG 4.1 ± 0.8, IT 2.9 ± 0.8, SGIT 9.5 ± 2.1). The AMPKα protein abundance was increased by 78% in IT rats (Fig. 6F).

**Markers of glucose metabolism in adipose tissue.** Similar to muscle, in adipose tissue, compared with C, GLUT4 protein abundance was increased by 128, 147, and 121% in SG, IT, and SGIT rats, respectively (Fig. 7A). GLP-1R protein abundance did not differ among treatments (Fig. 7B). The relative protein abundance of PKA was increased in SG and IT by 141 and 222%, respectively (Fig. 7C). The relative protein abundance of IRS-1 (Fig. 7D) increased by 415% in IT rats; the relative abundance IRS-1 pS636 (Fig. 7E) did not differ among treatments in adipose tissue. The IRS-1 pS636/IRS-1 ratio was decreased with SG and IT but not with SGIT (C 13.1 ± 4.3, 6.7 ± 0.8, IT 2.5 ± 0.1, SGIT 8.5 ± 3.1). The AMPKα protein abundance was increased by 311 and 213% in the adipose tissue of IT and SGIT rats, respectively (Fig. 7F).

**GLYCOLYSIS AND GLOUCONEOGENESIS MARKERS IN LIVER.** The mRNA abundance of glucokinase and phosphofructokinase, which are rate-limiting glycolytic enzymes, did not differ among treatments in liver. We also quantified the mRNA abundance of the key rate-limiting enzymes of gluconeogenesis, G-6-Pase and phosphoenolpyruvate carboxykinase (PEPCK). The mRNA abundance of G-6-Pase decreased following IT surgery compared with C animals, and the abundance of PEPCK showed a tendency (P = 0.17) to decrease in the liver of SG rats (Table 2).

**DISCUSSION**

There is substantial evidence supporting that bariatric surgeries produce improvements in diabetes, and other comorbidities of obesity, even before there is significant weight loss. However, the underlying mechanisms by which bariatric surgeries produce such metabolic benefits remain largely unknown. In the present study, for the first time, we compared the effects of a typical foregut bariatric procedure—sleeve gastrectomy (SG), with a hindgut surgical procedure, ileal transposition (IT), either alone or in combination (SGIT), on weight gain, glucose tolerance, lower gut hormones, and key markers of glucose metabolism in peripheral tissues in rats. Our data reveal several important findings. First, SG, IT, and SGIT surgeries produced transient reductions in food intake and weight gain, with the effects of IT and SGIT being more prolonged than those of SG surgeries. Interestingly, the inhibitory effects of SGIT surgeries on food intake and body weight were nonadditive to the inhibitory effects of SG and IT sur-
geries. Second, SG, IT, and SGIT surgeries resulted in increased tissue expression and plasma concentrations of the lower gut hormones GLP-1 and PYY while decreasing plasma GIP, insulin, and leptin concentrations. Third, despite producing relatively transient effects on food intake and body weight, both foregut and hindgut surgeries resulted in significant improvements in glucose tolerance. The glycemic improvements were further supported by findings of increased protein abundance of key markers of glucose metabolism in muscle and adipose tissue together with a downregulation in the expression of key gluconeogenic enzymes in liver.

The transient reduction in food intake and weight gain following SG surgeries in our study is consistent with other studies in obese and nonobese rodents, where SG surgery was shown to decrease weight gain and cumulative food intake for 2–6 wk (10, 12, 27, 40, 42, 45, 53, 59). Although the daily intakes of SG did not differ from controls after 2 wk, the cumulative intake during the first hour of dark were still lower.

![Fig. 4. Effects of SG and IT surgeries on GLP-1 and PYY expression and secretion. Preproglucagon mRNA abundance (A), relative numbers of GLP-1 immunopositive cells (B), plasma GLP-1 concentrations (C), PYY mRNA abundance (D), relative numbers of PYY immunopositive cells (E), and plasma PYY concentrations (F) are shown. mRNA abundance and immunoreactivity to GLP-1 and PYY were determined in the transposed ileum of rats subjected to IT (○), SGIT (●), or a comparable ileal segment of rats subjected to SG (△) or sham-control (△, C) manipulations. At ~8 wk after surgeries, plasma GLP-1 and PYY concentrations were determined at baseline (0 min) and 30, 60, and 120 min after consumption of a mixed-nutrient liquid meal (Ensure Plus). Values are means ± SE. *P < 0.05 vs. controls.](image-url)
at study termination. The reduction in gastric volume following SG may have contributed to the reduction in food intake during the early part of the dark period. However, after this initial reduction, the animals may have compensated so that the daily intakes of the SG rats did not differ from the controls at the end of the study. In the current study, IT surgery decreased food intake for 4 wk and weight gain for 3 wk. In agreement with our findings, transposition of a 20-cm ileal segment decreased intake and weight gain (15, 35), whereas transposition of shorter, 10-cm lengths of ileum either had no significant impact (16, 18, 46, 47, 54) or decreased food intake and weight gain in rats (35). The temporal profile of body weight changes of IT animals in the current study is similar to the weight change of IT animals that we previously reported (15), attesting to the reproducibility of our animal model. In our study, SGIT surgeries reduced food intake for 5 wk and weight gain for 3 wk. Similar to our findings, in obese Zucker rats, SGIT was shown to produce a transient ~10% weight loss for 3–4 wk (5). In another recent report, SG performed 7 wk after IT apparently produced a greater reduction in food intake and body weight than SG alone in chow-fed Sprague-Dawley rats (31). However, it is unclear from this study whether the effects of IT alone were greater than those of SG, whether the effects of IT were comparable to SGIT, and whether the surgical interventions produced a greater inhibition of intake and weight loss compared with control rats for the duration of the study (31).

As SG is primarily a foregut restrictive procedure, the degree of hindgut stimulation and consequent reduction of food intake and body weight is expected to be considerably less than with IT, which is a purely hindgut procedure with no gastric restriction. Therefore, by design, a combination of SG and IT in the SGIT animals would include both gastric restriction and hindgut stimulation and was predicted to produce additive effects on food intake and body weight. However, as the effects of SGIT surgeries on intake and weight were nonadditive compared with SG and IT surgeries, our data suggest that these effects of SGIT are beyond a simple combination of either surgery alone; the underlying mechanisms mediating such interactions remain to be determined.

Despite the foregut and hindgut surgeries (SG, IT, and SGIT) showing differential effects on intake and weight gain, interestingly, all treatments demonstrated marked improvement in glucose tolerance at study termination. The attenuation of glycemic excursions following the surgeries were similar to those of age-matched chow-fed animals, indicating that the surgeries prevented the worsening of glucose tolerance associated with chronic Ensure feeding. Previous studies had shown that following SG surgery blood glucose homeostasis was improved often in association with weight loss (12, 17, 42, 56). Although the daily intakes and body weights of the SG rats were similar to those of controls by 8 wk after surgery in the current study, we could still detect a remarkable improvement in glucose clearance, suggesting that SG improves glucose homeostasis likely through weight-independent effects in our rat model. Consistent with previous reports of IT-induced improvements in glucose metabolism in obese and diabetic rats (16, 17, 24, 32, 34, 54, 55) and SGIT-induced increase in glucose clearance in obese rats (5), we also observed significant improvement in glucose tolerance following IT and SGIT surgeries. Interestingly, these glycemic improvements occurred despite normalization of daily food intake to control rats. Though the early transient reduction in weight in the surgical groups may have contributed to later improvements in glucose tolerance, it remains to be determined whether such glycemic improvements are completely independent of weight changes in our animal model.

The reduction in food intake and weight gain, and improvements in glycemic control following the surgeries (SG, IT, SGIT), could likely be due to increased lower gut stimulation with resultant changes in lower gut hormones and/or other

Fig. 5. Effects of SG and IT surgeries on postprandial plasma concentrations of insulin (A), glucose-dependent insulino-metotropic peptide (GIP; B), and leptin (C). Plasma hormone concentrations were determined at baseline (0 min) and 30, 60, and 120 min after consumption of Ensure Plus in rats subjected to SG (△), IT (○), combination of sleeve gastrectomy and ileal transposition (●, SGIT), or sham control (⊗, C) surgeries. Values are mean ± SEM. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with controls.
metabolic adaptations. Our gut histomorphology data indicate that the foregut and hindgut surgeries resulted in increased villus height and width and increased mitogenic capacity, as well as increased muscular thickness, demonstrating adaptive hypertrophic and hyperplastic changes in the ileal segments. These adaptive changes were associated with increased GLP-1 and PYY immunoreactivity in the gut as well as meal-induced increases in plasma GLP-1 and PYY concentrations. Rapid transit of ingested nutrients to the lower gut following SG and early exposure of lower gut to nutrients following IT surgeries may contribute to the enhanced secretion of GLP-1 and PYY and is consistent with previous reports in rodents (12, 16, 17, 24, 32, 34, 54, 55). Interestingly, with SGIT the early exposure of the transposed ileum to nutrients might have been negated by the SG-mediated rapid flow of partially digested food with a consequent attenuation of nutrient stimulation of GLP-1 and PYY secretion. Although recent studies indicate that SGIT leads to increased GLP-1 expression in ileum (31) and enhanced plasma PYY concentrations in rats (5), this is the first study to compare the expression and secretion of GLP-1 and PYY among SG, IT, and SGIT surgeries. Importantly, the meal-induced elevations in plasma GLP-1 and PYY concentrations across the surgical treatments were temporally associated with a reduction in food intake during the first 2 h of the dark period, suggesting that these hormonal signals may play a role in the early anorexia following these surgeries. However, a cause-effect relationship between the anorexia and the elevations in lower gut hormones following the surgeries remains to be determined.

An important finding from the current study is that, together with improvement in glucose tolerance, the surgeries also resulted in elevation in circulating concentrations of GLP-1 and PYY, and a decrease in plasma GIP, insulin, and leptin concentrations. Blockade of the GLP-1R been shown to worsen glycemic control following IT (24), duodenojejunal exclusion (32), and SG (12) surgeries in rats. Thus, the SG, IT, and SGIT-induced elevations
in GLP-1 concentrations could likely contribute to the improvements in glucose tolerance in the current study. Though exogenous PYY is reported to be protective against β-cell loss and improve glucose tolerance in the presence of insulin (50, 60, 61), it remains to be determined whether PYY blockade attenuates the improvements in glycemic control following bariatric surgeries. The reduction in plasma GIP concentrations following the surgeries is consistent with some studies (16, 35) but not others (18, 54, 63). In our study, rapid transit of the liquid meal through the K cell-rich duodenum and jejunum may have attenuated the postprandial increases in GIP following SG surgeries, and in IT the anatomic interruption of the K cell continuity in the duodenum and jejunum by the transposed ileum might have decreased nutrient stimulation of K cells with consequent reduction in GIP secretion. Immunoneutralization of circulating GIP and antagonism of GIP receptors improves glucose tolerance and insulin sensitivity in animal models of obesity and diabetes (28). It is likely that low plasma GIP concentrations might play a permissive role in the improvement in glycemic control, in part, by decreasing plasma insulin concentrations across the surgical treatments.

Despite a robust increase in plasma GLP-1 concentrations, GLP-1R protein abundance in muscle and fat did not differ among treatments. It is speculated that a GLP-1-like receptor exists in peripheral tissues such as muscle, fat, and liver that may differ from the authentic GLP-1R (6, 23); the identity of such a putative receptor remains to be established. Regardless of the identity of the receptor, SG and IT surgeries resulted in a significant increase in protein abundance of the GLP-1R signaling intermediaries PKA and AMPKα in muscle and fat. Consistent with other studies on GLP-1-induced glucose uptake in muscle (4, 11, 26), our findings suggest that GLP-1 may signal through PKA and AMPKα to increase glucose uptake via the insulin-sensitive GLUT4 in muscle and fat. As exogenous PYY enhances glucose uptake in muscle and adipose tissue of mice (60), it is likely that elevated PYY concentrations may also signal to increase GLUT4 abundance in peripheral tissues and improve glucose tolerance following the surgeries. In IT animals, the protein abundance of IRS-1 was increased, whereas the abundance of IRS-1 pS636, a negative modulator of IRS-1, and the IRS-1 pS636/IRS-1 ratio was
decreased in muscle and fat. A similar reduction in IRS-1 pS636/IRS-1 ratios in fat was also observed in SG animals. These changes are suggestive of an upregulation of insulin sensitivity and insulin signaling in peripheral tissues following IT and SG surgeries. Furthermore, for the first time, we demonstrate that the mRNA abundance of the rate-limiting gluconeogenic enzyme G-6-Pase was decreased following IT, and PEPCk tended to decrease following SG, suggesting that both surgeries may downregulate hepatic gluconeogenesis. In support of our findings, both RYGB and SG surgeries decreased hepatic glucose output in obese rats (12). The marginal improvements in markers of glucose metabolism in peripheral tissues of SGIT might be a consequence of an attenuated GLP-1 and PYY secretion and/or other metabolic adaptations that are distinct from the effects of SG and IT surgeries. Taken together, our data suggest that SG- and IT-induced elevations in systemic GLP-1 concentrations, with consequent upregulation of GLP-1 signaling, may interact cooperatively with insulin signaling to improve insulin sensitivity and glucose clearance by muscle and adipose tissue while concurrently decreasing hepatic glucose production.

In conclusion, this is the first study to compare the effects of a typical foregut surgery, SG, with a prototypical hindgut surgery, IT, alone and in combination, on multiple parameters, including food intake, body weight, gut hormones, and glucose homeostasis. We demonstrate that the surgeries produced transient reductions of food intake and body weight together with enhanced secretion of GLP-1 and PYY. Importantly, the dramatic improvements in systemic glucose clearance following the surgeries are supported by significant improvements in multiple markers of glucose metabolism in muscle, adipose tissue, and liver. An understanding of the molecular mechanisms mediating the improvement in glucose homeostasis following the foregut and hindgut bariatric surgeries may lead to the development of less invasive procedures for treating obesity and diabetes.

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DISCLOSURES
No conflicts of interests are reported by the author(s).

AUTHOR CONTRIBUTIONS
S.N., I.H.S., P.K.C., study concept and design; S.N., I.H.S., animal work; S.N., A.P., P.K.C., tissue analyses; S.N., A.P., D.L.S., P.K.C., analysis and interpretation of data; P.K.C., obtained funding; S.N., A.P., drafting of the manuscript; S.N., A.P., D.L.S., P.K.C., critical revision of the manuscript for important intellectual content.

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