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2009 SSAT PLENARY PRESENTATION

Duodenal-Jejunal Exclusion Improves Glucose Tolerance in the Diabetic, Goto-Kakizaki Rat by a GLP-1 Receptor-Mediated Mechanism

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Abstract
Background Gastric bypass results in the rapid resolution of type 2 diabetes. No causal evidence exists to link specific gut hormone changes with improvements in glucose homeostasis post-operatively. We hypothesized that surgical augmentation of the glucoregulatory factor GLP-1 would improve glucose tolerance in diabetic GK rats. We compared two procedures that increase distal small bowel stimulation, ileal interposition (IT), and duodenal-jejunal exclusion (DJE).
Methods DJE, IT, DJE Sham, or IT Sham were performed in GK rats. Glucose tolerance was tested at 4 and 6 weeks, the latter with and without Exendin-[9-39], a GLP-1 receptor antagonist. Small bowel segments were harvested for GLP-1 protein content 2 weeks after DJE or Sham surgery.
Results Despite similar weight profiles, a significant improvement in the OGTT was noted at 4 weeks after DJE and IT. Plasma GLP-1 levels were significantly elevated after DJE and IT. Intestinal GLP-1 was increased in the mid-jejunum and ileum after DJE. Exendin-[9-39] abolished the improvement in glucose tolerance after DJE.
Conclusions DJE increased GLP-1 secretion and improved glucose tolerance, an effect that was reversed by GLP-1 receptor antagonism. This study provides direct evidence that improvement of glucose tolerance following a gastric bypass-like surgery is mediated by enhanced GLP-1 action.

Keywords Gastric bypass · Glucagon-like peptide-1 · Ileal interposition · Incretin

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Abbreviations

RYGB	Roux-en-Y gastric bypass
GK	Goto-Kakizaki
DJE	Duodenal-jejunal exclusion
IT	Ileal interposition
OGTT	Oral glucose tolerance test
GLP-1	Glucagon-like peptide-1
LOT	Ligament of Treitz
AUC	Area under the curve

Introduction

Roux-en-Y gastric bypass (RYGB), the most commonly performed bariatric surgery in the United States, results in the rapid improvement of type 2 diabetes for morbidly obese patients.[1] The reported rate of resolution of diabetes after RYGB is approximately 80%.[2–7] Mechanisms beyond weight loss and calorie restriction are quite probable given the rapid and sustained improvement in type 2 diabetes

found in post-RYGB patients. Common explanations for this response are based on changes in gastrointestinal hormone release that occur due to alterations in gastrointestinal anatomy.[8–14] However, there is as yet no direct evidence from animal or human studies that changes in gastrointestinal hormone secretion cause the improvement of glucose tolerance seen after gastric bypass surgery.

The distal jejunum and ileum contain the majority of enteroendocrine L cells, which secrete the incretin hormone, glucagon-like peptide-1 (GLP-1). The incretin hormones GLP-1 and gastric inhibitory polypeptide are responsible for up to 70% of post-prandial insulin secretion.[15,16] GLP-1 is a 30 amino acid peptide secreted by intestinal L cells in response to enteral carbohydrates and fats.[16] GLP-1 also decreases glucagon secretion, suppresses endogenous glucose production, and enhances peripheral glucose uptake.[17–19] In addition, GLP-1 functions as an "ileal brake" by slowing gastric emptying, inhibiting food intake, and prolonging intestinal transit.[20–22] The administration of GLP1R agonists or DPPIV inhibitors, which retard the degradation of endogenous GLP-1, improve HgbA1c levels, and fasting and postprandial glucose concentrations of type 2 diabetic patients.[23–25] Post-prandial plasma GLP-1 levels are almost universally increased after RYGB, as early as 2 days after surgery, and this is likely due to increased delivery of nutrients to distal small bowel L cells.[11,26–28]

Duodenal-jejunal exclusion (DJE) is an experimental, metabolic surgery similar to RYGB, including duodenal and proximal jejunal exclusion to nutrients, a jejunal Roux-en-Y reconstruction, and early nutrient delivery to the distal small bowel. Several authors have shown dramatic, early improvements in glucose homeostasis in rodents following DJE surgery.[9,12,29] Ileal interposition (IT) is another experimental, metabolic, gastrointestinal surgery originally described in rats by Koopmans.[30] In an IT surgery, a distal segment of ileum is moved more proximally in the small bowel resulting in increased secretion of the ileal gut hormones, including GLP-1 and peptide YY.[31–33] Previously, a study comparing DJE and IT surgeries in lean, diabetic Goto-Kakizaki (GK) rats found that both surgeries resulted in the same improvement in glucose homeostasis, leading the authors to postulate that the distal small bowel was the responsible factor.[34] However, rats in this study had a significant weight loss after DJE and IT surgeries compared to sham controls, rendering definitive differentiation between the effect of weight loss and the surgical procedure itself difficult.

We hypothesized that DJE and IT surgeries would improve glucose tolerance in GK rats through early stimulation of the distal small bowel by nutrients resulting in increased secretion of GLP-1. We therefore directly compared the effects of DJE and IT on glucose tolerance and GLP-1 secretion in GK rats without a difference in post-surgical weight profiles. To further test if GLP-1 was the responsible hormone released from the distal small bowel, we acutely administered the GLP1R antagonist, Exendin-[9-39] (Ex-9), during an oral glucose tolerance test (OGTT) performed 6 weeks after surgery in DJE and DJE Sham rats.

Methodology

Animals and Experimental Design At the time of study initiation, 12- to 14-week old, male, GK rats (Taconic, Germantown, NY), or age-matched Wistar rats (Charles River Laboratories, Wilmington, MA) were housed individually. GK rats are an inbred, lean model of type 2 diabetes derived from Wistar rats. Rats were allowed to acclimate to their environment for 1 week prior to the beginning of the study. All animal procedures and protocols were approved by the University of Cincinnati's Internal Animal Care and Use Committee.

The first experiment involved rats in five different study groups (n=9 per group). These groups included: (1) GK DJE, (2) GK DJE Sham, (3) GK IT, (4) GK IT Sham, and (5) Wistar IT Sham. A Wistar IT Sham group allowed for a comparison to non-diabetic animals. Food intake and body weight were followed for 30 days post-operatively. An OGTT was performed pre-operatively and at 2 and 4 weeks post-operatively. An insulin tolerance test (ITT) was performed at 3 weeks post-operatively. At 5 weeks post-operatively, a mixed meal test was performed following the insertion of a jugular cannula for the measurement of systemic incretin hormones.

The second experiment included GK rats in two different study groups, DJE (n=7) and DJE Sham (n=6 Sham). At 2 weeks after surgery, intestinal segments from the duodenum, mid-jejunum, and ileum were harvested for GLP-1 protein content. We chose this time point because we had seen from Experiment #1 an improvement in glucose tolerance in DJE rats compared to Sham rats during an OGTT as early as 2 weeks after surgery.

The third experiment again had two different groups of GK rats, DJE (n=8) and DJE Sham (n=6). Animals were followed for 6 weeks after surgery. After 6 weeks, the animals were acutely challenged with Exendin-9 during the administration of an OGTT to test the involvement of GLP1R signaling in the improvement in glucose homeostasis after duodenal-jejunal exclusion.

Surgical Procedures

(1) Duodenal-jejunal exclusion. Animals were fasted for 18 h pre-operatively. Under isoflurane anesthesia, the peritoneum was entered through a midline incision. Similar to the duodenal exclusion described by Rubino

et al.[29] the most proximal portion of the duodenum and the jejunum 10 cm distal to the Ligament of Treitz (LOT) were divided (Fig. 1). The proximal segment of duodenum was anastamosed to the distal segment of divided jejunum in end-to-end fashion. The distal stump of duodenum was sewn closed. A partial enterotomy was made 15 cm distal to the duodeno-jejunostomy and a jeju-jejunostomy was made with the proximal segment of divided jejunum in end-to-side fashion. The abdomen was irrigated and closed in two layers. Rats had free access to water for the first 24 h post-operatively. Twenty-four hours after surgery, the rats were started on an ad libitum liquid diet (Regular Ensure, Abbott Laboratories, Columbus, OH). After 24 h of a liquid diet (post-operative day 2), the rats were transitioned back to their pre-operative standard chow diet (Harlan Teklad diet 7012).

(2) Ileal interposition. An ileal interposition was performed similar to the procedure previously described by Strader et al.[31] Rats were also fasted for 18 h pre-operatively. The abdomen was entered under isoflurane anesthesia. The cecum was identified and the ileum was divided at 5 and 15 cm proximal to the cecum (Fig. 1). After division of the jejunum 10 cm distal to the LOT, the isolated segment of ileum was interposed into the divided segment of proximal jejunum. The divided segment of proximal and distal ileum were then re-anastamosed in end-to-end fashion. The abdomen was irrigated and closed in two layers. The post-operative care was the same as that described for DJE above.

(3) Sham surgeries. All Sham rats received the same pre- and post-operative care as the DJE and IT rats. For the DJE Sham surgery, a full enterotomy with division of the mesentery and re-anastamosis in end-to-end fashion was made at the proximal duodenum, 10 cm distal to the LOT and 25 cm distal to the LOT. The IT Sham surgery included an enterotomy, mesenteric division, and re-anastamosis at 10 cm distal to the LOT, 5 cm proximal to the cecum, and 15 cm proximal to the cecum.

(4) Jugular cannulation and gastric tube insertion. Animals were fasted overnight. Under isoflurane anesthesia, the right internal jugular vein was identified and isolated. A catheter (0.014 ID/0.033 OD, Braintree Scientific, Braintree, MA) was inserted in the jugular vein and advanced to the level of the right atrium. The distal catheter was tunneled subcutaneously and exteriorized at the posterior aspect of the neck. Under the same anesthetic period, the abdomen was entered through the previous midline incision. The stomach was mobilized and a small enterotomy was made along the anterior aspect of the greater curvature. A catheter (0.04 ID/0.085 OD, VWR International, West Chester, PA) was inserted into the stomach and secured with a purse-string stitch. The gastric catheter was exteriorized through the right flank, and the abdomen was closed in two layers. Animals were kept in restraint cages post-operatively. The mixed meal study for experiment #1 was started after 2 h of anesthetic recovery.

Insulin Tolerance Test An ITT was performed at 3 weeks post-operatively in experiment #1. Insulin, 0.5 U/kg, was administered subcutaneously followed by blood sample collection from the tail vein at 15, 30, 45, and 60 min post-injection. Blood samples were immediately assayed in duplicate for glucose concentration using a handheld glucometer. Due to unacceptable hypoglycemia in Wistar rats, a 0.5 U/kg dose of insulin could not be used and subsequently the Wistar IT group was not used for comparison of insulin sensitivity.

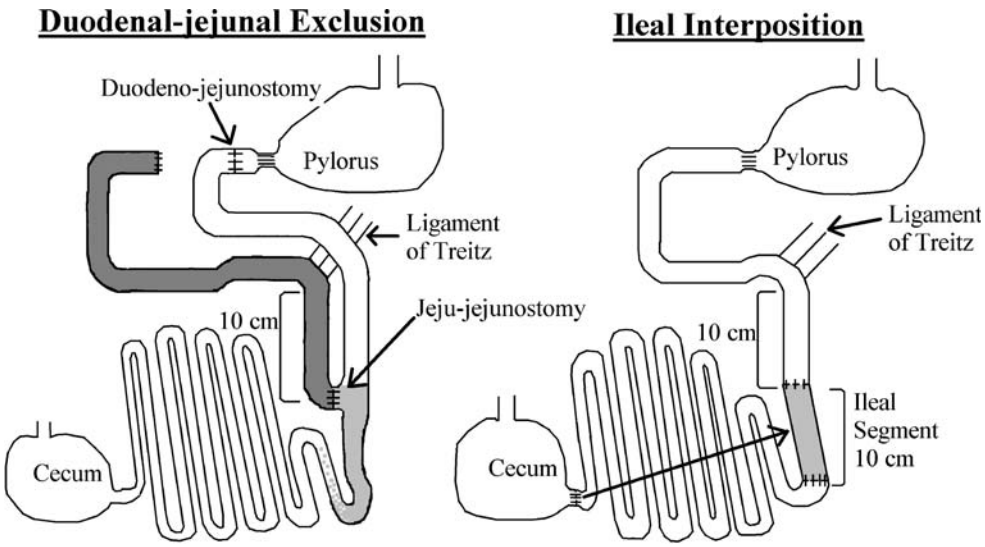

Figure 1 Duodenal-jejunal exclusion (DJE) and ileal interposition (IT) are two experimental, metabolic surgeries used for the investigation and treatment of type 2 diabetes mellitus. As diagramed on the *left*, DJE bypasses the entire duodenum and 10 cm of proximal jejunum (*dark grey color*). IT (*right panel*) leaves anatomically normal nutrient flow to the proximal small bowel. Both surgeries offer early nutrient delivery to the distal small bowel (*light grey color*).

Oral Glucose Tolerance Test For experiment #1, a 2 g/kg D-glucose OGTT was performed pre-operatively and at 2 and 4 weeks post-operatively. Blood samples were collected from the tail vein at 0, 10, 30, 60, and 120 min after the glucose gavage and immediately assayed in duplicate for glucose concentration using a handheld glucometer. Blood samples from the 4 week OGTT were also collected in EDTA coated collecting tubes. Samples were spun at 4,000×*g* for 10 min at 4°C, and the plasma was stored at −20°C until assayed for insulin concentration using a commercially available ELISA kit (Millipore, St Charles, MO). For experiment #3, an OGTT was performed on two separate days at 6 weeks post-operatively with the co-administration of either subcutaneous saline or the GLP-1R antagonist Ex-9 as described below. To better characterize the glucose response, blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after the glucose challenge.

Mixed Meal Test We have previously shown that GK rats have a more robust secretion of GLP-1 to a mixed meal bolus over a solitary nutrient, such as glucose.[35] We therefore used a mixed meal test in experiment #1 to maximize GLP-1 secretion and plasma measurement. A mixed meal of Regular Ensure (7.68 ml/kg) was given intragastric to all rats in Experiment #1 at 5 weeks post-operatively. Blood samples were collected from the jugular catheter at 0 and 30 min after the mixed meal bolus. Blood samples were collected into EDTA-coated collecting tubes with the addition of a 1% DPPIV inhibitor (Millipore, St Charles, MO) and spun at 4000×*g* for 10 min at 4°C. Plasma was stored at −20°C until assayed for GLP-1 concentration. GLP-1 samples were assayed using an active GLP-1 ELISA kit (Millipore, St Charles, MO).

Small Bowel GLP-1 Protein Content For experiment #2, 2 cm intestinal segments were isolated from three separate sections of small intestine under anesthesia. These sections included (1) the second segment of the duodenum, (2) 25 cm distal to the LOT (Sham animals) or just distal to the jeju-jejunostomy (DJE animals), and (3) the distal ileum. Tissues were weighed and frozen at −20°C. Frozen segments were homogenized in 2 M glacial acetic acid (5 ml/g tissue weight). Samples were incubated at 95°C for 10 min followed by a 10-min incubation on ice. After centrifugation at 4,000×*g* for 10 min at 4°C, the supernatant was removed, frozen, and lyophilized. Once lyophilized, the segments were resuspended in dH_2O, diluted, and assayed the same day for total protein concentration and GLP-1 concentration using an active GLP-1 ELISA kit (Millipore, St Charles, MO).

GLP1R blockade with Ex-9 In experiment #3, GK rats at 6 weeks after surgery were given either a subcutaneous dose of 200 μl of saline or 25 nM of Ex-9 (Bachem, Torrence, CA). This was followed 10 min later by a 2 g/kg D-glucose OGTT as described above.

Statistical Analysis Area under the curve (AUC) was calculated using the trapezoidal rule. Comparisons between surgical groups were made using a one-way analysis of variance (ANOVA) or a two-way ANOVA for the Exendin-9 study to account for separate treatments and surgeries. Comparisons between surgical groups over time were performed using a two-way repeated-measures ANOVA. A student's *t*-test was used to compare GLP-1 content of the intestinal segments. All values are presented as the mean±standard error. Values were determined as statistically significant if $p<0.05$.

Results

DJE and IT do not Affect Body Weight or Food Intake in GK Rats

In experiment #1, Wistar IT rats weighed significantly more than all of the GK surgical groups for every time point of the study (Fig. 2a). There was no difference in body weight between any of the GK surgical groups for each day measured post-operatively. As shown in Fig. 2b, Wistar IT rats also ate significantly more food per day compared to all GK rat groups (excluding post-operative days 0–2 when rats were fasted or on a liquid diet). GK DJE rats ate the same amount of daily chow as GK DJE Sham rats except for post-operative day 28 (GK DJE 26.9 g±2.20 vs. GK DJE Sham 22.1±2.48, $p<0.05$). Similarly, GK IT rats ate the same amount of daily chow as GK IT Sham rats except for post-operative day 12 (GK IT 22.5±0.98 versus GK IT Sham 26.8 g±2.25, $p<0.05$). In experiment #2 and 3, there was no difference in post-operative body weights for any day measured between GK DJE and GK DJE Sham rats (data not shown).

DJE and IT Significantly Improves Glucose Tolerance by 4 weeks after Surgery in GK Rats Without Changing Plasma Insulin Concentrations

An OGTT was performed at 0, 2, and 4 weeks post-operatively in experiment #1. There was no difference in pre-operative glucose tolerance AUC among the 4 GK groups (Fig. 3), and pre-operative Wistar IT Sham rats had a significantly lower glucose concentration throughout the OGTT compared to the GK groups (data not shown). As shown in Fig. 3, by 4 weeks after surgery, both GK DJE and GK IT rats had a significantly lower late-phase glucose AUC (60–120 min) compared to GK DJE Sham and GK IT Sham

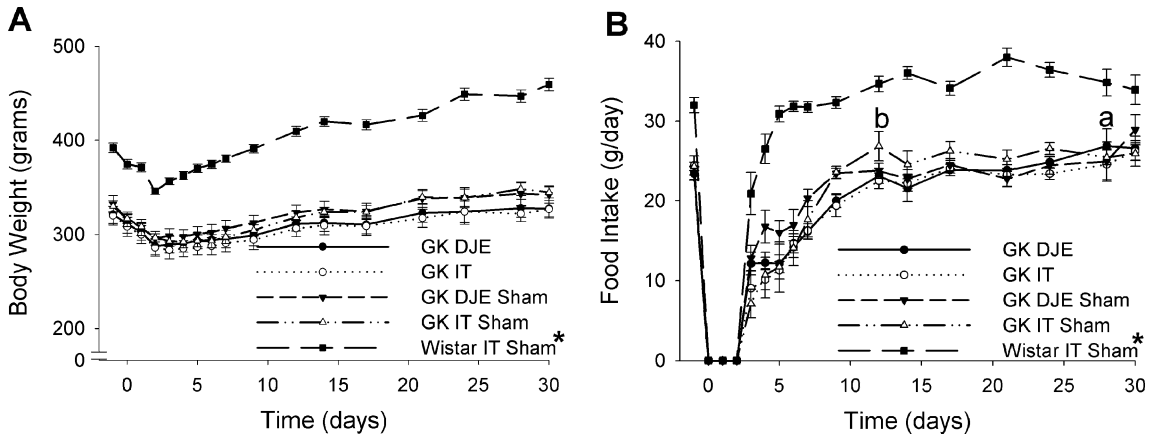

Figure 2 Body weight and food intake after gastrointestinal surgery in GK and Wistar rats. Body weights (**a**) and food intake (**b**) were assessed daily pre-operatively and for 30 days post-operatively. *Statistically different for all days when comparing Wistar IT sham to all GK surgical groups when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

rats (GK DJE 13,267 (mg/dl)min±457 vs. GK DJE Sham 15,696 (mg/dl)min±663, $p<0.05$; GK IT 13,327 (mg/dl)min±936 vs. GK IT Sham 15,769 (mg/dl)min±360, $p<0.05$).

At 2 weeks after surgery, both DJE and IT rats had a lower glucose concentration at 120 min compared to their respective sham groups during an OGTT (GK DJE 189.3 mg/dl±8.5 vs. GK DJE Sham 237.1 mg/dl±15.3, $p<0.05$; GK IT 197.4 mg/dl±13.3 vs. GK IT Sham 238.0 (mg/dl)min±17.6, p=NS; data not shown). As reflected in Fig. 4a, the glucose concentration over time during an OGTT at 4 weeks was significantly lower in GK DJE compared to GK DJE Sham rats at 60 min (244 mg/dl±7.7 vs. 282 mg/dl±15.7, respectively, $p<0.05$) and 120 min (198 mg/dl±10.6 vs. 241 mg/dl±15.0, respectively, $p<0.05$). Similar to GK DJE rats, GK IT rats had a significantly lower glucose concentration compared to GK IT sham rats at 120 min (GK IT 192 mg/dl±17.4 vs. GK IT Sham 242 mg/dl±8.2, $p<0.05$). Surprisingly, there was no difference in insulin secretion profiles (Fig. 4b) during the 4 week OGTT between the GK experimental and their respective GK sham group at any time point. GK rats lacked a rapid increase and peak in insulin secretion seen at 30 min in Wistar IT Sham rats (30 min insulin concentration, 2.1 ng/ml±0.16, $p<0.05$ compared to all GK groups).

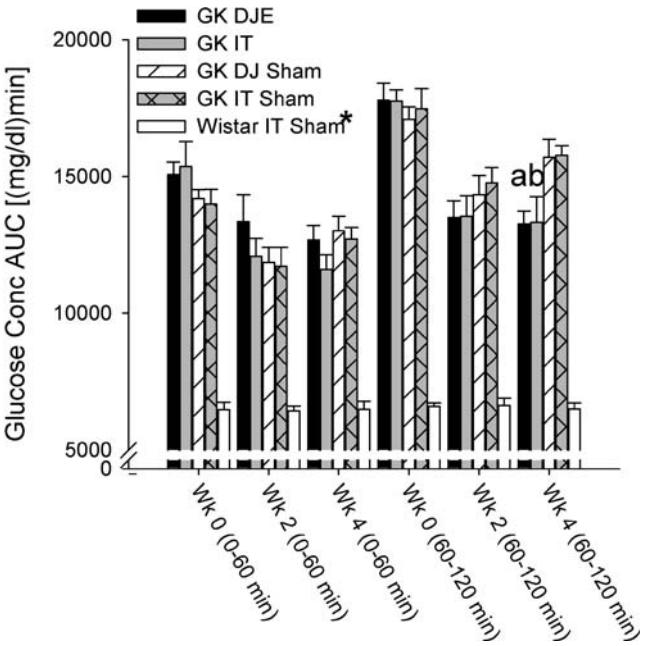

Figure 3 Oral glucose tolerance test AUC was determined by measuring glucose concentrations before and after (10, 30, 60, and 120 min) the administration of an oral glucose load (2 g/kg D-glucose). AUC was determined using the trapezoidal rule. Glucose tolerance tests were performed pre-operatively and at 2 and 4 weeks post-operatively. *Statistically different AUC when comparing Wistar IT sham to all GK surgical groups when $p<0.05$ (intra-week comparisons only). Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$ (intra-week comparisons only). Data are presented as mean±SE.

DJE and IT do not Affect Insulin Sensitivity in GK Rats

Plasma glucose concentrations were determined after the administration of 0.5 U/kg of insulin subcutaneously to all GK surgical groups (Fig. 5) at 3 weeks post-operatively. There was no statistical difference in glucose concentrations at any time point between any of the GK surgical groups, suggesting that neither DJE nor IT surgery acutely affects insulin sensitivity in GK rats after surgery.

DJE and IT Increase Post-prandial Plasma GLP-1 Concentrations

Plasma GLP-1 levels were measured from the jugular vein after administration of a mixed meal tolerance test at

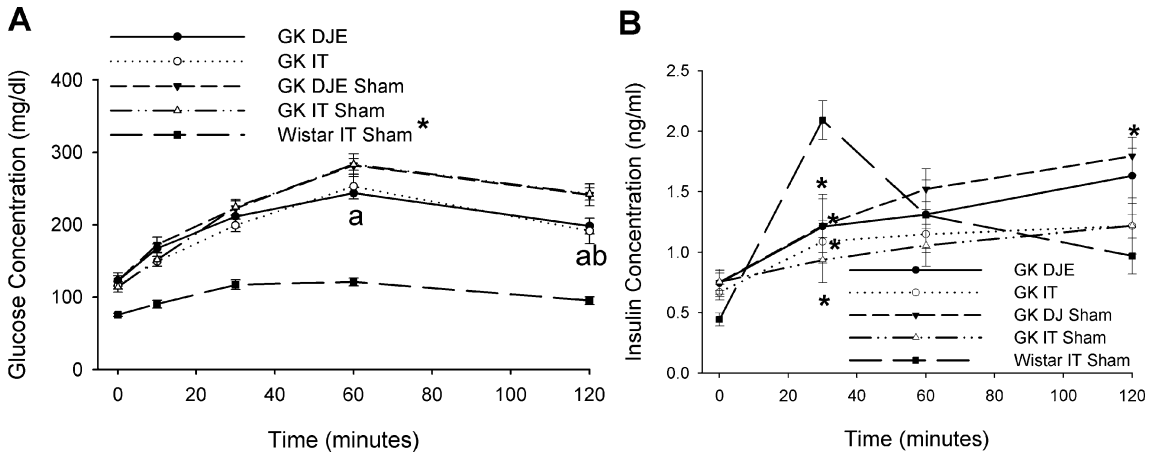

Figure 4 Plasma glucose (**a**) and insulin (**b**) concentrations were measured before and after (10, 30, 60, and 120 min) the administration of an oral glucose tolerance test (2 g/kg D-glucose) at 4 weeks post-operatively. *Statistically different for all time points (**a**) or designated time points and groups (**b**) when comparing Wistar IT sham to all GK surgical groups when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

5 weeks after surgery (Fig. 6). There was a statistically significant increase in fasting GLP-1 levels of GK DJE rats, 3.5 pM±0.20, compared to Wistar IT Sham rats, 2.3 pM±0.19, ($p<0.05$). Both GK DJE and IT surgical groups had significantly higher plasma GLP-1 concentrations at 30 min post-prandial compared to their respective GK sham groups (GK DJE 4.5 pM±0.36 versus GK DJE Sham 2.7 pM±0.22, $p<0.05$; GK IT 4.4 pM±0.52 versus GK IT Sham 3.1 pM±0.25, $p<0.05$). Both GK DJE and IT groups also had a significantly higher GLP-1 concentration at 30 min compared to the Wistar IT Sham group, 2.5 pM±0.11.

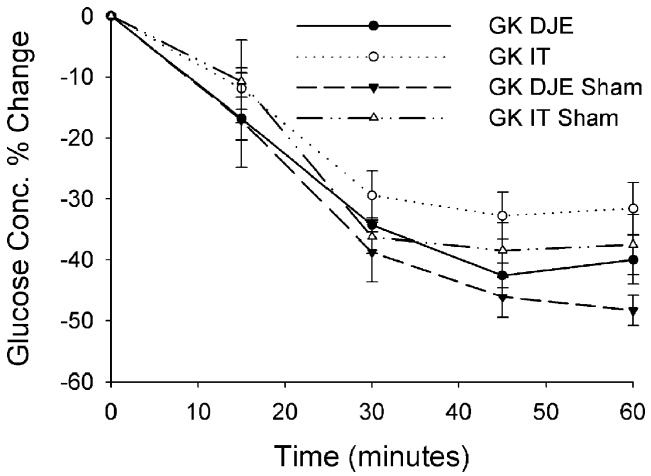

Figure 5 An insulin tolerance test was performed at 3 weeks post-operatively. Plasma glucose concentrations were measured before and after (15, 30, 45, and 60 min) the administration of insulin (Humalin 0.5 U/kg for GK rats). Values are presented for each surgical group as a percent glucose concentration change compared to each groups respective fasting values. There were no differences in glucose concentrations between any of the GK surgical groups at any time point after insulin administration with significance determined as $p<0.05$.

DJE Increases Distal Small Bowel GLP-1 Protein Content

By 2 weeks after surgery, there was a significant increase in the GLP-1 content of the distal small intestine (Fig. 7). As expected, DJE did not significantly alter the duodenal GLP-1 concentration compared to sham animals (0.33×10^{-6}%±0.067 vs. 0.263×10^{-6}%±0.039, respectively, $p=0.43$). DJE compared to Sham surgery significantly increased both mid-jejunal GLP-1 content (2.34×10^{-6}%±0.29 vs. 1.44×10^{-6}%±0.22, respectively, $p=0.03$) and ileal GLP-1 content compared to sham rats (5.19×10^{-6}%±0.42 vs. 2.88×10^{-6}%±0.24 respectively, $p<0.001$).

Ex-9 Administration Ablates the Significant Improvement in Glucose Tolerance at 6 weeks after DJE in GK Rats

Similar to experiment #1 as seen 4 weeks after surgery, there was a statistically significant late-phase improvement in glucose concentrations in DJE rats compared to DJE Sham rats at both 60, 90, and 120 min after an oral glucose load performed at 6 weeks after surgery (Fig. 8a). DJE rats at 60 min had an average glucose concentration of 285.0 mg/dl±5.9 compared to 316.9 mg/dl±4.1 for Sham rats, $p=0.007$. At 120 min, the average glucose concentration for DJE rats was 211.1 mg/dl±10.3 compared to 255.7 mg/dl±13.5 for Sham rats, $p<0.001$. As shown in Fig. 8c, there was a significant improvement in glucose concentration AUC for DJE rats (28,786 (mg/dl)min±571) compared to Sham rats (32,113 (mg/dl)min±593, $p=0.035$). The administration of Exendin (9-39) to DJE and DJE Sham rats resulted in similar glucose concentration curves, with the loss of the statistically significant late-phase improvement for the DJE group over time (Fig. 8b). As shown in Fig. 8c, there was no difference ($p=0.439$) in

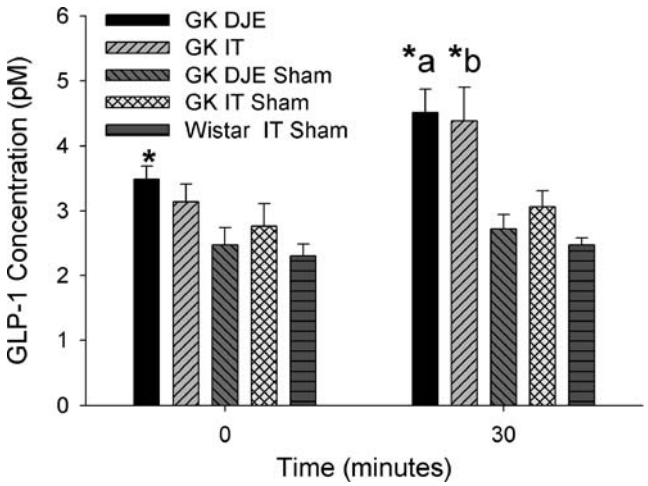

Figure 6 GLP-1 concentrations were measured from jugular plasma samples before and 30 min after a mixed meal bolus of Ensure (7.68 ml/kg) via an intragastric catheter. *Statistically different when compared to Wistar IT Sham rats when $p<0.05$. Represents statistically significant comparisons between GK DJE and GK DJE Sham (*a*) and GK IT and GK IT Sham (*b*) when $p<0.05$. Data are presented as mean±SE.

OGTT AUC observed between the two groups after the administration of Ex-9.

Discussion

In this study, we found that independent of weight loss, both DJE and IT in GK rats result in a statistically significant improvement in glucose tolerance by 4 weeks

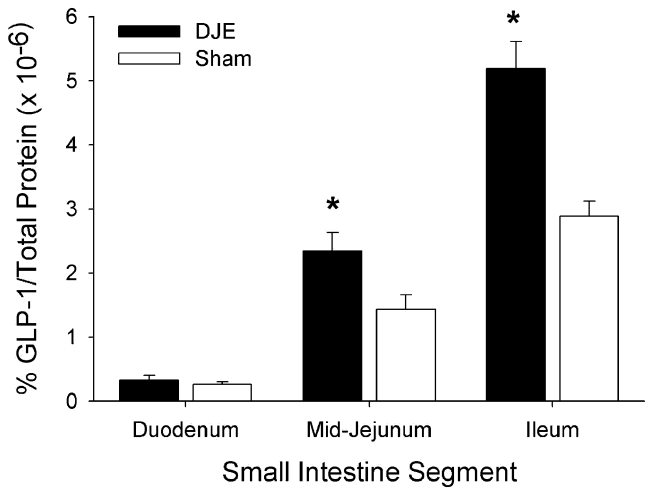

Figure 7 Percentage intestinal GLP-1 protein content was determined at 2 weeks after DJE ($n=7$) or DJE Sham ($n=6$) surgery in GK rats. Intestinal segments were taken from the second segment of the duodenum, mid-jejunum (distal to the jeju-jejunostomy in DJE rats or 25 cm distal to the ligament of Treitz in Sham rats), and distal ileum. *Statistically different for the tested segment of small bowel between DJE and Sham rats when $p<0.05$. Data are presented as mean±SE.

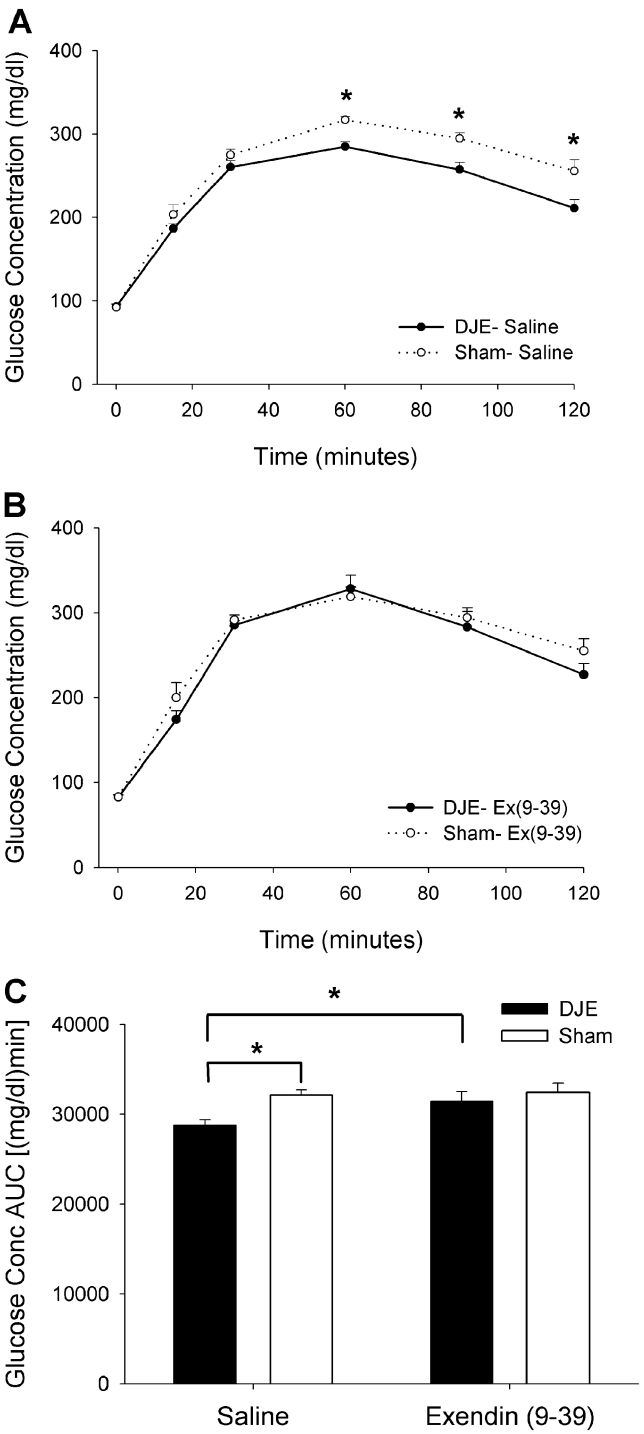

Figure 8 An OGTT was performed in male, GK rats 6 weeks after DJE ($n=8$) or DJE Sham ($n=6$) surgery. Plasma glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min after a 2 g/kg D-glucose oral gavage with the co-administration of 200 µl of saline (**a**) or 200 µl of 25 nM of the GLP1R antagonist Ex-9 (**b**) subcutaneously. (**c**) depicts glucose concentration AUC for the 6-week OGTT. *Statistically different for the designated time points (**a** and **b**) or between groups (**c**) when $p<0.05$. Data are presented as mean±SE.

after surgery. Both metabolic surgeries did not acutely change plasma insulin concentrations or insulin sensitivity. Supporting a mechanism mediated by enhanced nutrient delivery to the distal small bowel, the common feature of DJE and IT, we found a similar magnitude of elevation of post-prandial plasma GLP-1. Furthermore, intestinal GLP-1 protein levels were significantly increased by 2 weeks after surgery in not only the ileum (the major focus of L cells in the non-operated gut) but also the mid-jejunum at the new post-surgical site of primary nutrient absorption. The administration of the GLP1R antagonist, Ex-9, ablated the significant improvement in glucose tolerance seen after DJE surgery at 6 weeks. Thus, the improvement in glucose tolerance noted after DJE in this model is mediated by GLP1R signaling.

RYGB results in the early and sustained improvement in glucose homeostasis for the majority of morbidly obese, type 2 diabetic patients. Multiple mechanisms stemming from the rearrangement of small bowel anatomy may be involved beyond weight loss and calorie restriction. For this reason, we compared two different experimental, metabolic surgeries, DJE and IT, to determine if one surgery offered an advantage over the other regarding glucose tolerance in a lean, rodent model of diabetes. Both surgeries increase distal small bowel exposure to nutrients but only DJE, like RYGB, bypasses the duodenum and proximal jejunum. It has been proposed that exclusion of the duodenum from nutrient stimulation is a predominant mechanism responsible for the improvement in glucose homeostasis after RYGB.[8,9] Results of this study indicate that increased GLP-1 secretion and GLP1R stimulation, and not duodenal exclusion, is the predominate mechanism involved in the early improvement in glucose tolerance after DJE surgery in GK rats.

The GLP1R is a specific G-protein coupled receptor located on the lung, brain, kidney, pancreatic islets and gastrointestinal tract.[36–38] We were unable to specifically identify which action of GLP1R signaling was responsible for the improvement in glucose tolerance. Although we did not detect an absolute increase in plasma insulin levels following DJE, this does not exclude the possibility that the surgery enhances insulin secretion via increased GLP1R stimulation. Because both DJE and IT result in reduced glucose concentrations without a change in insulin sensitivity, it is possible that a relatively greater secretion of insulin for the given glucose concentration accounts for some of the effect of surgery. This relative increase in insulin secretion could be the dominate GLP1R mechanism in this model, as some clinical studies have found an increase in post-prandial insulin secretion after RYGB.[39,40]

Ayala et al. have shown that GLP1R −/− mice have an impaired suppression of hepatic glucose production independent of insulin secretion.[19] Activation of GLP1R signaling suppresses glucagon secretion and could possibly mediate the suppression of hepatic glucose production. Le Roux et al. administered octreotide as a non-specific blocker of GLP-1 and PYY to post-RYGB and gastric banding patients and found an increase in meal size and decrease in satiety unique to the RYGB group; however, the effect on glucose tolerance, insulin, and glucagon secretion was not assessed.[28] There is a lack of consensus regarding the changes in glucagon secretion after RYGB, including a decrease, no change, or increase in glucagon secretion.[41–44] Because we did not measure plasma, or more specifically, portal vein glucagon concentrations, we cannot exclude the possibility that the effects of DJE surgery are mediated by the suppression of glucagon secretion via a GLP1R mechanism.

The administration of Ex-9 in vivo completely abolishes the stimulatory effect of endogenous GLP-1 on insulin secretion, with no effect on co-stimulators of insulin such as gastric inhibitory polypeptide and vasoactive intestinal polypeptide.[45,46] While Ex-9 is specific for the GLP1R, there are cross-reactive hormones of the GLP1R besides GLP-1, including the intestinal proglucagon alternative splice product oxyntomodulin. Oxyntomodulin as yet does not have an identified separate receptor and has been found to mediate glucoregulatory actions including stimulation of insulin secretion through a functional GLP1R.[47,48] We did not measure oxyntomodulin concentrations in this study and are unaware of any published reports regarding the effect of RYGB on oxyntomodulin secretion. However, oxyntomodulin acts only partially via the GLP1R. Because we found a full reversal of the improvement in glucose tolerance after DJE with use of the GLP1R antagonist, we expect that the hormone involved is mediated only by GLP1R signaling, making GLP-1 the likely candidate. The increase in GLP1R signaling could be from a physiologically relevant increase in GLP-1 or due to increased sensitivity and enhanced incretin effect of GLP-1 on the GLP1R, regardless of the quantitative changes in GLP-1 secretion.

We found no change in insulin sensitivity assessed by a subcutaneous ITT. While the euglycemic-hyperinsulinemic clamp offers greater precision compared to an ITT in assessing peripheral insulin sensitivity, we were not surprised to find that insulin sensitivity was not acutely affected by DJE or IT. Some studies have suggested unique changes in insulin sensitivity after RYGB.[49] However, when RYGB patients are compared to patients with similar degrees of weight loss (gastric banding patients), the improvement in insulin sensitivity correlates to the magnitude of post-surgical weight loss,[50,51] and thus would not be expected in our surgical model.

Our study does not find the robust improvement in glucose tolerance as previously reported by some investigators after DJE in GK rats.[12,29,45] Differences in surgical technique and post-operative care are possible reasons for

this difference. Also, there are differences in phenotypic severity between different colonies of GK rats.[52] The GK rat is a lean, inbred model of type 2 diabetes derived from Wistar rats. These rats have reduced β-cell mass, decreased pancreatic insulin reserves, and a defective secretion of insulin to a glucose stimulus.[53,54] With age, GK rat islets have a decreased number of β-cells, reduced islet insulin content, and exhibit abnormal islet morphology.[55,56] It is possible that in a rat strain dominated by pancreatic insulin insufficiency, there is a point of "no return" in reversing pancreatic failure and a sub-maximal amount of recovery that can be obtained with DJE surgery. Recent data has shown that the rate of resolution of diabetes after RYGB is highest for patients who have had a short duration of disease (less than 4 or 5 years) or mild disease (diet-controlled).[4,57] The lack of a consentient improvement in glucose tolerance after DJE surgery points to the need for further research to determine what factors (duration of diabetes, type of diabetes, insulin requirements, beta cell reserve, etc.) enable or prevent a maximum surgical response.

While this study did not produce dramatic improvements in glucose tolerance by 4 to 6 weeks after DJE or IT, our results parallel the findings of recently published results with IT surgery. IT performed in streptozocin-induced diabetic rats had a similarly significant although small improvement in glucose homeostasis by 4 weeks after surgery without a change in insulin secretion.[32] By 11 weeks, IT surgery in these rats resulted in a more dramatic improvement in glucose concentrations after a glucose tolerance test. We suspect that with a longer observation period, improvements in glucose tolerance would have been more pronounced for both surgeries due to β-cell recovery as seen by other investigators after IT or with exogenous GLP-1 treatment.[58,59]

Conclusion

To our knowledge, this study offers the first direct evidence documenting a causal relationship between a change in GLP-1 signaling induced by bypass surgery and the subsequent improvement in post-operative glucose tolerance. It is possible that in other animal models, specifically in a diet-induced obesity model, DJE may cause other positive hormonal changes beyond GLP1R signaling that affect glucose tolerance. Clinically, it is yet unknown if the combination of effects that bypass surgery can achieve induced by weight loss, calorie restriction, and augmented hormone signaling are superior to pharmacologic intervention in a population of type 2 diabetic patients with a BMI< 35 (especially when considering cost effectiveness, morbidity, and mortality). However, evidence, as shown in this study, that RYGB-like surgeries, independent of weight loss and calorie restriction, can benefit type 2 diabetes mellitus in animal models by enhancing incretin signaling, supports the further careful and cautious investigation of RYGB for the use as a treatment for type 2 diabetic patients without morbid obesity.

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