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Metabolic responses to xenin-25 are altered in humans with Roux-en-Y gastric bypass surgery

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Abstract

Xenin-25 (Xen) is a neurotensin-related peptide secreted by a subset of enteroendocrine cells located in the proximal small intestine. Many effects of Xen are mediated by neurotensin receptor-1 on neurons. In healthy humans with normal glucose tolerance (NGT), Xen administration causes diarrhea and inhibits postprandial glucagon-like peptide-1 (GLP-1) release but not insulin secretion. This study determines i) if Xen has similar effects in humans with Roux-en-Y gastric bypass (RYGB) and ii) whether neural pathways potentially mediate effects of Xen on glucose homeostasis.

Eight females with RYGB and no history of type 2 diabetes received infusions with 0, 4 or 12 pmoles Xen/kg/min with liquid meals on separate occasions. Plasma glucose and gastrointestinal hormone levels were measured and insulin secretion rates calculated. Pancreatic polypeptide and neuropeptide Y levels were surrogate markers for parasympathetic input to islets and sympathetic tone, respectively. Responses were compared to those in well-matched non-surgical participants with NGT from our earlier study.

Xen similarly increased pancreatic polypeptide and neuropeptide Y responses in patients with and without RYGB. In contrast, the ability of Xen to inhibit GLP-1 release and cause diarrhea was severely blunted in patients with RYGB. With RYGB, Xen had no statistically significant effect on glucose, insulin secretory, GLP-1, glucose-dependent insulinotropic peptide, and glucagon responses. However, insulin and glucose-dependent insulinotropic peptide secretion preceded

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GLP-1 release suggesting circulating GLP-1 does not mediate exaggerated insulin release after RYGB. Thus, Xen has unmasked neural circuits to the distal gut that inhibit GLP-1 secretion, cause diarrhea, and are altered by RYGB.

Keywords

Xenin; GIP; GLP-1; Gastric Bypass; Insulin Secretion

INTRODUCTION

Xenin-25 (Xen) is a 25-amino acid neurotensin-related peptide reportedly produced by a subset of glucose-dependent insulinotropic peptide (GIP)-producing enteroendocrine cells that reside in the proximal small intestine [1,2]. Like GIP, it has been reported that Xen release is stimulated by ingestion of food. However, recent studies from our laboratory suggest that Xen is produced by a subset of serotonin-producing enterochromaffin cells, not K cells, that reside in the proximal gut (Submitted). Intriguingly, serotonin containing granules are localized at the apical tip as well as at the basal portion of enterochromaffin cells in the rat duodenum [3] raising the possibility that Xen, like serotonin, can be secreted bidirectionally. Lumenally released Xen could potentially elicit novel responses. Both central and peripheral administration of Xen can exert biological effects, many of which have been shown to be mediated by Xen activation of neurotensin receptor-1 on neurons [4–9]. In addition, Xen acts as antagonist to neurotensin receptor-2 and can activate neurotensin receptor-3 with very low affinity [10]. In animals, Xen administration delays gastric emptying [4], reduces food intake [5,6,11], augments gut motility [12], increases gall bladder contractions [7], stimulates exocrine pancreas secretion [13], and amplifies effects of GIP on insulin secretion [14,15]. Mouse studies with isolated islets, insulin producing cell lines, and the in situ perfused pancreas showed that Xen does not act directly on beta cells to amplify the effects of GIP on insulin secretion. Rather, this response is mediated by a cholinergic relay to islets [16]. In human studies, our laboratory has shown that intravenous administration of Xen amplified the effects of GIP on insulin, glucagon, and pancreatic polypeptide release during graded glucose infusions [17,18]. During liquid mixed meal tolerance tests, Xen infusion delayed gastric emptying, reduced postprandial glycemia, and inhibited glucagon-like peptide-1 (GLP-1) release and insulin secretion [19]. Importantly, the reduced insulin secretion was a consequence of lower glucose levels and not an inhibition of insulin release per se. Diarrhea was the only side effect of Xen administration in the human studies. Neurotensin receptor-1 is present on neurons, but not endocrine cells, in the human pancreas [18] and stomach [19] suggesting that effects of Xen on islet and intestinal physiology are mediated by neurons in humans as well as in animals. Thus, Xen represents a unique peptide to probe neural regulation of glucose homeostasis in humans.

Roux-en-Y gastric bypass (RYGB) is one of the most common types of bariatric procedures performed to treat obesity. A small pouch is created by dividing the upper end of the stomach and is attached to the distal jejunum. This allows nutrients to bypass the lower stomach, duodenum and the first portion of the jejunum [20,21]. In patients with pre-surgical normal glucose tolerance (NGT), intrinsic beta cell function remains normal after RYGB

[21]. In those with prior type 2 diabetes mellitus (T2DM), RYGB is associated with a profound improvement in glucose homeostasis [20–25] although normal beta cell function may not be fully restored [25]. Immediate and long-term improvements in insulin sensitivity result from caloric restriction and weight loss, respectively [26,27]. In addition, early postprandial GLP-1 release is greatly exaggerated after surgery because the re-positioned jejunum contains the GLP-1-producing cells [28,29] that can in theory directly sense nutrients immediately after eating. It is commonly thought that the elevated GLP-1 circulates and then acts directly on beta cells to increase insulin secretion [21].

In addition to GLP-1 and GIP, neurotransmitters and peptides released from nerves that innervate the islets can regulate insulin and glucagon release [30,31]. Acetylcholine, vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide, and gastrin releasing peptide secreted from parasympathetic neurons increase insulin secretion. In contrast, noradrenaline, neuropeptide Y (NPY) and galanin released from sympathetic neurons inhibit insulin release. However, it is not known if RYGB alters neural pathways that could potentially mediate some of the beneficial effects of this procedure. In the present study, liquid mixed meal tolerance tests with and without infusion of Xen were administered to humans with prior RYGB to unmask neural and other pathways altered by RYGB that could potentially explain mechanisms mediating beneficial effects of this surgical procedure.

MATERIALS AND METHODS

Human Subjects with prior RYGB

All protocols were approved by Washington University's Human Research Protection Office and the FDA (IND#103,374). The study was registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00949663) (NCT00949663) and performed in the Clinical Research Unit of the Institute of Clinical and Translational Sciences of Washington University after obtaining written informed consent. Males and females aged 18 to 65 and with prior RYGB were eligible for enrollment. However, nearly all RYGB patients in our institution's Research Participant Registry are female and thus, only females were studied. Women of childbearing potential were required to use birth control. Because oral glucose tolerance tests (OGTT) are not well-tolerated after RYGB, glucose tolerance was assessed by determining HOMA-IR during the screening visit. Subjects were required to have a HOMA-IR <3 and no previous history of T2DM or treatment with hypoglycemic agents. These selection criteria were designed to exclude subjects with impaired pre- or post-surgery beta cell function. All patients were medically and surgically stable (at least 1 year post surgery). Subjects with history or risk for pancreatitis, history of any cancer other than skin cancer and history of liver, gastrointestinal or renal disorders were excluded. Group characteristics are shown in Table 1.

Study Design

This is a crossover study in which each patient received all treatments. Subjects were blinded to treatments. Studies were performed after a 12 hour overnight fast. One intravenous catheter was placed into a hand vein. This hand was kept in a thermostatically controlled box (50–55°C) to sample arterialized venous blood [32,33]. A second intravenous line was inserted in the opposite hand for administration of Xen or Albumin alone (Alb). All

subjects were infused with Xen at a dose of 0 (Alb), 4 (Lo-Xen), or 12 (Hi-Xen) pmol/kg/min and each dose was administered on a separate visit. Study visits were typically separated by at least 2 weeks. Hemoglobin was checked before each visit and anyone with a value <11.2 g/dL had that visit rescheduled. In our earlier study, Xen infusions were initiated with meal ingestion. Because Xen infusion rapidly inhibited GLP-1 release in this prior study [19], Alb or Xen infusions were started 15 min before the meal to ensure any inhibitory effects of Xen were operative at the start of the meal. Importantly, without meal ingestion, infusion of Xen alone had no effect on plasma glucose or glucagon levels and ISRs [16].

Meal Tolerance Tests

Boost Plus (Nestle Health Science, Florham Park, New Jersey) is a liquid mixed meal (360 calories), containing 14 gram (g) of fat, 45 g of carbohydrates, and 14 g of protein. To prevent carbohydrate-induced nausea and vomiting in bariatric surgery patients, the liquid meal was ingested over a 30 minute period [7 equal volumes (~36 mL each) every 5 minutes from 0 to 30 minutes. Acetaminophen (1.5g) was added to the liquid meal because it was used to estimate the rate of gastric emptying in our other studies. Blood was sampled for glucose, insulin, C-peptide, glucagon, total GIP, intact GLP-1, PP, and NPY at minutes: -30, -20, -15, -3, -1, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and every 30 min thereafter until 300 minutes. For Lo-Xen, infusion rates from 0-3, 3-7, 7-10, and 10-300 minutes were 10.8, 7.7, 5.6, and 4.0 pmol/kg/min. For Hi-Xen, the Xen concentration in the infusate was tripled but administered at the same flow rates as with Lo-Xen. Postprandial hypoglycemia is common in a subset of patients after RYGB [34]. Studies were aborted if a subject experienced symptomatic hypoglycemia (plasma glucose <60 mg/dL) or if blood glucose dropped below 50 mg/dL regardless of symptoms. Participants experiencing hypoglycemia were given 50 mL of 10% dextrose by intravenous infusion. Plasma glucose was monitored every 15 minutes until blood glucose normalized. Subjects were blinded to treatments. Because potential responses to Xen infusion in subjects with RYGB were unknown, the Alb and Lo-Xen infusions were administered in random order and the Hi-Xen was administered as long as the Lo-Xen infusion was well-tolerated.

Xenin-25

Xen was synthesized under GMP conditions, analyzed, and prepared for infusions as previously described [17,19].

Measurements

Glucose, insulin, C-peptide, Xen, GIP, GLP-1, complete metabolic profiles and hemoglobin A1c were measured as previously described [17,19]. Of note, the GIP ELISA (Millipore, St. Charles, MO) recognizes both the intact (GIP₁₋₄₂) as well as N-terminally cleaved, inactive form (GIP₃₋₄₂) and the GLP-1 ELISA (MesoScale Discovery, Rockville, MD) recognizes only the active form of the peptide [GLP-1 (7-36) amide]. Glucagon was measured using an ELISA specific for mature glucagon that does not cross react with other proglucagon-derived peptides (Mercodia, Uppsala, Sweden). PP was measured as previously described and involves an extraction step to remove compounds that interfere with the assay [18]. NPY was measured by ELISA (EMD Millipore, Saint Charles, MO). The number and severity of

diarrheal episodes were determined by surveys taken during the infusions and post infusion telephone follow-ups.

Human Subjects without prior RYGB

The study protocol was approved by Washington University's Human Research Protection Office and the FDA (IND#103,374). This study was registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00949663) (NCT00949663). Study procedures and an initial set of results have previously been published [19]. No new subjects were enrolled or studied for the present report and only archived plasma samples from subjects with NGT from the prior study were analyzed (Table 1; n=10). Patients had given written consent for future analyses of archived samples. Liquid meal tolerance tests were administered as described for the RYGB patients except the primed-constant intravenous infusion of Alb or Xen (4 or 12 pmol/kg/min) was initiated along with Boost Plus ingestion at time zero and the entire Boost Plus meal was ingested within 3 minutes.

Calculations and Data Analysis

Insulin secretion rates (ISRs) were derived by stochastic deconvolution of the peripheral C-peptide concentrations as in earlier studies [17,19] using population-based estimates of C-peptide clearance kinetics [35]. Areas under the curves (AUCs) were calculated using the trapezoid method over time periods indicated and incremental areas under the curves (iAUCs) relative to baseline were then calculated. In order to compare statistical significances of ISR, GLP-1, and GIP temporal responses during each infusion within the RYGB group shown in figure 4, respective values for each individual were converted to the percent of maximal response during infusion of Alb alone. For example, the difference between the minimum and maximum values for each outcome for each person during infusion of Alb alone was given a value of 100%. The values during infusion with Lo-Xen and Hi-Xen were then normalized to the appropriate 100% value. AUCs and iAUCs were analyzed using the mixed effects model with subject as a random effect and peptide as a fixed effect using SAS 9.4 Version 20. Within each group, pairwise comparisons were limited to evaluating the effects of Hi-Xen versus Alb and Lo-Xen versus Alb. Repeated measures two-way ANOVAs were used to determine if time and Xen interactions were statistically significant for comparisons of individual outcomes compared to placebo as well as for comparisons of different outcomes with the same infusate within each group using GraphPad Prism Version 5.04. Multiple comparison procedure (Bonferroni method) was used to adjust raw *p* values in order to control for the inflated type-I error in the two-way ANOVAs. Two-tailed tests were used for all analyses and *p*<0.05 was considered significant. Differences in baseline characteristics and glucose, ISR, GIP, and GLP-1 AUCs and iAUCs between groups were evaluated with Student's t-tests using GraphPad Prism Version 5.04. In one subject, both Xen infusions were terminated at 75 minutes due to hypoglycemia. Data for this subject was omitted from all 300-minute AUCs and iAUCs and included in other analyses as indicated in figure legends. Group responses $\pm$ SEMs for all 3 outcomes were then compared during administration of each infusate using repeated measures two-way ANOVAs.

RESULTS

Subject characteristics

For the RYGB study, 17 potential participants were screened. Twelve patients met the inclusion criteria and were enrolled. One participant was excluded before the first study visit due to anemia and a second one after receiving the Lo-Xen infusion due to ongoing anemia. One subject withdrew from the study after receiving only the Lo-Xen infusion due to unpleasant response to the Boost Plus. One subject was removed after the Alb infusion due to hypoglycemia. The remaining 8 patients received all 3 infusions. However, both Xen infusions were terminated at 75 minutes in one subject due to hypoglycemia. RYGB subjects were studied on average 73 months after surgery. Baseline characteristics for the 8 RYGB participants as well as for the 10 previously studied non-surgical subjects with NGT are shown in Table 1. Although lower in the RYGB group, fasting glucose levels were normal in both groups. Fasting insulin, hemoglobin A1c, HOMA-IR and body mass index were not different between groups. Thus, recruitment based on HOMA-IR (RYGB group) versus OGTT (non-surgical group) yielded groups with similar characteristics.

Metabolic responses to a liquid mixed meal are different in humans with and without RYGB

Glucose, insulin secretory, GIP, GLP-1 and glucagon responses to a liquid mixed meal were determined in patients with RYGB and compared to those in the non-surgical subjects with NGT from our previous study (Fig 1). In agreement with other reports [20,21], postprandial glucose, insulin secretory, GLP-1 and glucagon peak responses are much higher in subjects with prior RYGB. However, the 300-minute iAUCs for glucose and ISR are not different between groups whereas the iAUC for GLP-1 is nearly 5-fold higher after RYGB. In contrast to GLP-1, postprandial peak GIP responses and 300-minute iAUCs are not different between groups.

Infusion of Xen increases plasma levels of immunoreactive Xen (IR-Xen)

We previously showed that endogenously released IR-Xen is not detectable in plasma before or after meal ingestion [16,17,19]. As shown in figure 2A, IR-Xen was not detectable in plasma from subjects in either group during infusion of Alb alone (i.e. no Xen). In the RYGB group, intravenous infusion of Xen at rates of 4 and 12 pmol/kg/min increased steady state plasma IR-Xen concentrations to 105 and 486 pmol/mL, respectively. These levels were not statistically different from those obtained in healthy humans with an intact bowel in our earlier study.

Xen-induced diarrhea is reduced in humans with RYGB

In our earlier studies, diarrhea has been the only side effect associated with Xen infusion [17,19]. When administered during a liquid mixed tolerance test, 58% and 77% of the non-surgical subjects experienced diarrhea with Lo-Xen and Hi-Xen, respectively (Fig 2B). In sharp contrast, 0% and 17% of the RYGB patients infused with Lo-Xen and Hi-Xen respectively, experienced diarrhea. As in our earlier studies, Xen did not have a statistically significant effect on mean arterial pressure, resting heart rate or body temperature when

compared to infusion with Alb (Not Shown). Further, based on qualitative surveys taken before, during, and after each study visit, Xen infusion, regardless of dose, was not associated with nausea, vomiting, chest pains, dizziness, heart palpitations, shortness of breath, fever, chills, blurred vision, or changes in salivation, sweating, or frequency of urination.

Xen inhibition of GLP-1 release is blunted in humans with RYGB

We previously showed in healthy humans that Xen infusion at a dose of 12 pmol/kg/min delayed gastric emptying, reduced postprandial glucose and GLP-1 levels, but did not decrease ISRs and GIP levels. As shown in figure 3, infusion of Xen had no significant effect on plasma glucose, GIP, GLP-1, insulin, and C-peptide levels or ISRs in the RYGB group. However, ISRs and GIP levels appeared to increase earlier and more rapidly than GLP-1 concentrations.

Secretion of GIP and insulin precedes GLP-1 release after RYGB

To further explore temporal differences in the GLP-1, GIP and insulin secretory responses, data for each infusate were re-plotted on single graphs (Fig 4). During infusion of Alb, GIP levels and ISRs rapidly increased and were superimposable from 0–50 minutes whereas the GLP-1 response was delayed relative to ISRs and GIP (Fig 4A). This delay in GLP-1 release is further exacerbated during infusion of Lo-Xen (Fig 4B) and Hi-Xen (Fig 4C) in a dose-dependent manner.

Autonomic signaling remains intact after RYGB

Xen rapidly increased pre- and postprandial PP (Fig 5A) and NPY (Fig 5G) levels in a dose-dependent fashion in patients with RYGB. Elevated peptide levels persisted for the duration of the infusion ($p<0.0001$ for Xen-time interaction). Xen also increased the postprandial PP and NPY responses in the non-surgical group (Fig 5B,H). Respective PP and NPY responses during Alb versus Lo-Xen and Alb versus Hi-Xen were statistically different within each group but not different between groups (Fig 5C–F and I–L). Note that the increased PP and NPY levels in the RYGB group at time zero are due to Xen infusions starting 15 minutes before meal ingestion compared to 0 minutes in the non-surgical group.

DISCUSSION

There is a marked improvement in glucose homeostasis in patients after RYGB [22–24]. The mechanism(s) for this beneficial outcome is not known but has been proposed to result from increased GLP-1 release, improved insulin sensitivity, and/or weight loss itself. In the current study, liquid mixed meal tolerance tests, with and without infusions of Xen, were used to increase our understanding of the mechanism (s) by which RYGB improves glucose homeostasis.

Peripheral GLP-1 levels increase profoundly after meal ingestion in humans with RYGB [36]. It is commonly thought that the elevated GLP-1 circulates and then acts directly on beta cells to increase insulin secretion [21]. However, in the RYGB subjects, the postprandial increase in ISRs preceded that of GLP-1. In contrast, GIP levels increased in

tandem with ISR for the first 40 minutes after initiating meal ingestion. These results suggest that GIP, rather than GLP-1, could have an important role in increasing postprandial insulin secretion after RYGB. Normally, GIP is produced by enteroendocrine K cells located in the proximal small intestine and secretion is stimulated by nutrients present in the gut lumen, but not by those in the blood [37,38]. After RYGB surgery, the proximal small intestine is bypassed and thus, the postprandial GIP response would be expected to decrease. Paradoxically, postprandial GIP responses were maintained in subjects with RYGB. Thus, there could be an adaptive increase in the number of K cells in the distal small intestine after bypass surgery. Alternatively, it is possible that GIP release is only indirectly regulated by nutrients present in the lumen of the gut. In support of this concept, others have shown that beta-adrenergic receptors and hypercalcemia can stimulate GIP release in humans [28]. Moreover, we have shown that glucose stimulates GIP secretion in vivo [17] but not from GIP-producing cell lines [39]. Finally, the ability of fat to stimulate GIP release is dependent upon chylomicron formation by enterocytes [40]. Thus, luminal nutrients may indirectly stimulate GIP release from K cells.

In our previous study [19], infusion of Hi-Xen in subjects with NGT reduced postprandial GLP-1 secretion (6-fold) without affecting ISRs (when normalized to glucose levels). In the RYGB subjects, infusion of Hi-Xen caused only a small decrease in the GLP-1 response (15% reduction in the iAUC) compared to that observed in the non-surgical group. Thus, the ability of Xen to inhibit GLP-1 release is severely blunted after RYGB. This result also indicates that Xen does not act directly on intestinal L cells to regulate GLP-1 release. Because effects of Xen are mediated by neurotensin receptor-1 on neurons [8,41–43], we hypothesize that a Xen-sensitive pathway, mediated by enteric neurons, that inhibits GLP-1 secretion is severed during the RYGB procedure itself. The loss of this inhibitory pathway could play an important role in the exaggerated postprandial GLP-1 release after RYGB. Intriguingly, guanylin peptide is produced in the same region of the bowel as GLP-1, causes diarrhea, and is secreted in response to neural input [44–47]. Thus, altered neural signaling to distal bowel could also explain the reduction in diarrhea in the RYGB group.

It must be noted that infusion of exendin-9-39, a GLP-1 receptor antagonist, modestly reduces postprandial insulin release after RYGB [48–51] suggesting GLP-1 does play a role in regulating insulin secretion after RYGB. However, these studies do not define the role of gut-derived, circulating GLP-1 for regulating insulin release. For examples: i) Exendin-9-39 blocks all peripheral GLP-1 receptors including those on enteric neurons contacting L cells and also central GLP-1 receptors [52]. This latter point is important because GLP-1 is also produced in the brain [53] and centrally administered exendin-9-39 decreases the insulin response to oral glucose [54]; ii) Intestinal peptides in addition to GLP-1 activate GLP-1 receptors to enhance beta cell function [55]; and iii) Exendin-9-39 has been reported to antagonize GIP as well as GLP-1 receptors [56]. Thus, locally released rather than circulating GLP-1 may be important for regulating insulin secretion. Consistent with our results in humans, rodent studies have shown that beta cell GLP-1 receptors are not required to maintain normal oral glucose tolerance and GLP-1 receptor signaling is not required for the reduction of body weight after RYGB [57–59].

Several limitations to our study should be addressed. *First*, our study did not compare the same patients before and after RYGB surgery. However, the extremely blunted effects of Xen on GLP-1 release and diarrhea in the RYGB group were unexpected and thus, plasma samples collected from the non-surgical group from our previous study were further analyzed. Importantly, baseline characteristics for the two groups were remarkably well-matched which allowed us to determine the effects of the RYGB procedure itself on outcomes. This would not have been possible if the same subjects were studied before and after RYGB because baseline characteristics in patients before and after surgery are fundamentally different with respect to body mass index, insulin resistance, plasma levels of incretin hormones, and numerous other metabolic parameters which would have confounded interpretation of results. Importantly, the mean time after RYGB was 7 years in our study and thus, metabolic differences between the surgery and control groups could not be attributed to the rapid weight loss that occurs immediately after surgery. Hence, differences between the two groups can be attributed to the surgical rearrangement of the gut rather than to alterations in body weight, beta cell function and insulin resistance. Thus, comparing these two groups should be considered a strength of our study. *Second*, in the RYGB group, the liquid meal was consumed over 30 minutes rather than within 3 minutes as in our earlier study with healthy subjects in order to prevent nausea and vomiting associated with the surgical procedure. Further, because Xen inhibited postprandial GLP-1 release in our earlier study, the Xen infusion was started at minus 15 minutes in the RYGB study to ensure that GLP-1 release would be fully inhibited before meal ingestion. We have repeated the RYGB protocol in a single patient with vertical sleeve gastrectomy and similar to our non-surgical controls, Xen infusion inhibited GLP-1 release (10-fold at 30 min) without affecting plasma glucose levels or ISRs. Thus, the timing of meal and Xen administration is unlikely to explain the different responses to Xen in patients with RYGB versus non-surgical controls. *Third*, Xen infusion increased plasma PP and, to a lesser degree, NPY levels before initiating meal ingestion in the RYGB group. However, these preprandial increases were not associated with concomitant changes in ISRs and plasma levels of glucose, GIP, and GLP-1. *Finally*, the RYGB study included only women because this surgery is prevalent in this patient population.

In spite of the above limitations, our results have unmasked several important neural and metabolic changes associated with RYGB in humans. Because the RYGB and non-surgical groups were matched for BMI and HOMA-IR, our data suggest that surgical rearrangement of the gut alters metabolically important neural circuits that directly or indirectly connect the proximal to distal gut. These circuits may be mediated by enteric rather than autonomic neurons because PP and NPY responses to Xen were not different in groups with and without RYGB. Coupled with the observation that postprandial increases in GIP levels and ISRs precede those for GLP-1 release, a host of neural and metabolic changes in addition to increased GLP-1 levels could potentially contribute to the beneficial effects of RYGB. However, it is unclear if these changes are causally or casually related to the improvements in glucose homeostasis after RYGB. Nevertheless, mechanisms in addition to elevated GLP-1 levels, weight loss and caloric restriction might play an important role(s) in the improvement in glucose homeostasis after RYGB.

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Highlights

- Many effects of xenin-25 are mediated by neurotensin receptor-1 on neurons
- Xenin increased autonomic responses in humans with and without RYGB
- Ability of xenin to inhibit GLP-1 release and cause diarrhea was blunted after RYGB
- Insulin and GIP secretion preceded GLP-1 release after RYGB
- Neural circuits that regulate glucose homeostasis are altered by RYGB

Figure 1. Postprandial plasma glucose and hormone profiles are different in subjects with and without RYGB

Panels A–E: Outcomes were determined at the indicated times in patients with and without RYGB. Panels F–J: iAUCs were calculated for the 0 to 300 minute time window. All subjects received the Alb infusion until 300-min and were included in the analysis. *P* values for iAUCs were determined by Student's *t*-test. Patients with and without RYGB ingested the meal from 0–3 and 0–30 minutes, respectively.

Figure 2. Xen-induced diarrhea is greatly reduced after RYGB

Panel A: Xen was measured in plasma samples collected at the 300-minute time point. The 75-minute time point was used for the RYGB subject with the aborted Lo-Xen and Hi-Xen infusions. Note that Xen levels are similarly increased in a dose-dependent fashion in subjects with and without RYGB. **Panel B:** The number of subjects with and without prior RYGB experiencing diarrhea during or after infusion of Xen at the indicated dose is shown. The non-surgical group includes all subjects from our earlier study (36 subjects with NGT, impaired glucose tolerance, and T2DM). * and ** represent *p* values of 0.0018 and 0.038, respectively.

Figure 3. Xen has no significant effect on glucose homeostasis after RYGB

On 3 separate occasions, fasted patients with RYGB received an infusion with Alb alone or Xen at a rate of 4 or 12 pmol/kg/min (Lo-Xen and Hi-Xen, respectively) beginning at minus 15 minutes. The meal (Boost Plus) was ingested from 0 to 30 minutes. Values for glucose, insulin, C-peptide, ISR, GLP-1, and GIP (Panels A–F, respectively) are shown for the indicated times. *P* values for time-Xen interactions are shown and were determined by repeated measures two-way ANOVA. Data for all subjects were incorporated in plots because outcomes had returned to near baseline levels by this time point but data for one subject were not included in the analysis that extended to 300 minutes.

Figure 4. Secretion of GIP and insulin precedes GLP-1 release after RYBG

Selected data from Figure 3 were re-plotted to allow comparison of ISR, GIP, and GLP-1 during infusion with Alb (Panel A), Lo-Xen (Panel B) and Hi-Xen (Panel C). Symbols were omitted for clarity. Numerical p values are for interactions between time and all outcomes (for each infusate) and a, b, c, and d represent $p = 0.05, 0.01, 0.001$, and 0.0001 , respectively. Yellow and red letters represent p values for GLP-1 versus ISR and GIP versus ISR, respectively for individual time points. GIP was not measured in the samples collected at 15 minutes and this time point was not included in the statistical analysis. Data for all subjects were included because all studies went to 75-minutes, the endpoint of this analysis.

Figure 5. Xen increases PP and NPY responses in humans with and without RYGB

Plasma levels of PP for the indicated times after starting meal ingestion are shown for subjects with (Panel A) and without (Panel B) RYGB. PP AUCs (Panels C,E) and iAUCs (Panels D,F) from 0 to 300 minutes are shown for subjects with (Panels C,D) and without (Panels E,F) RYGB. Plasma levels of NPY for the indicated times after starting meal ingestion are shown for subjects with (Panel G) and without (Panel H) RYGB. NPY AUCs (Panels I,K) and iAUCs (Panels J,L) from 0 to 300 minutes are shown for subjects with (Panels I,J) and without (Panels K,L) RYGB. All values represent group means $\pm$ SEM. The effects of Xen on PP iAUCs ($p=0.46$), PP AUCs ($p=0.58$), NPY iAUCs ($p=0.16$), and NPY AUCs ($p=0.34$) were not different in groups with and without RYGB. Thus, differences in AUCs and iAUCs for Lo-Xen versus Alb and Hi-Xen versus Alb were analyzed collectively for both groups and were highly significant (p values ranged from 0.0079 to <0.0001). Data for one subject with RYGB were not included because Xen infusions were terminated at 75-minutes.

Table 1**Baseline Characteristics**

Group values $\pm$ SD are shown for the patients with RYGB and participants without RYGB. *p* values were determined by one-way ANOVA for continuous variables and by Fisher exact test for categorical variables.

	RYGB (n=8)	No RYBG (n=10)	p Value
Age, years	47.2 $\pm$ 9.2	40 $\pm$ 11	0.18
Gender, men/women	0/8	4/6	0.09
BMI kg/m ²	29.2 $\pm$ 5.8	29 $\pm$ 5.1	0.98
Months since surgery	73 $\pm$ 48.5	N/A	N/A
Fasting glucose, mg/dl	81.7 $\pm$ 8.8	95 $\pm$ 7.2	0.003
Fasting insulin, μ U/ml	6.5 $\pm$ 2.9	6.3 $\pm$ 1.4	0.91
HOMA-IR	1.4 $\pm$ 0.7	1.5 $\pm$ 0.4	0.77
HbA1C, %	5.3 $\pm$ 0.4	5.6 $\pm$ 0.3	0.10