Xenin-25 delays gastric emptying and reduces postprandial glucose levels in humans with and without Type 2 diabetes

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The enteroendocrine (EE) system is composed of numerous subtypes of singly dispersed EE cells scattered throughout the gastrointestinal epithelium (6, 51). Peptides released from EE cells regulate gastrointestinal function (6, 51) and glucose homeostasis (5, 14, 33). Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones that are produced predominantly by intestinal L cells in the distal intestine and K cells in the proximal small intestine, respectively. Both are released into the circulation immediately after meal ingestion in response to nutrients present in the lumen of the gut, but not to those in the blood. Both peptides amplify glucose-stimulated insulin secretion and have been coined “incretins” (5, 14, 33).

Xenin-25 (Xen) is a 25-amino acid neurotensin-related peptide produced by a subset of GIP-producing cells (4, 15). Genetic and pharmacological data indicate that effects of Xen are mediated by neurotensin receptor-1 (NTSR1 (19, 24, 35, 57)). In animals, Xen delays gastric emptying (25), reduces food intake (2, 10, 27), increases gut motility (17), augments gall bladder contractions (23), and enhances secretion from the exocrine pancreas (16). Many of these Xen effects are known to be mediated by neurons (9, 10, 23, 25, 27). In vivo mouse studies from our (54) and another (30) laboratory demonstrated that Xen increases the effects of GIP on insulin release. We further showed that Xen does not act directly on beta cells (54). Rather, Xen initiates a cholinergic relay in the periphery without activating regions of the brain associated with afferent or efferent signaling. Thus effects of Xen on insulin release may be independent of the central nervous system. Consistent with this hypothesis, effects of Xen on gall bladder contractions are inhibited by atropine, but not vagotomy (23).

As in our mouse studies, we recently showed that administration of Xen to humans during intravenous graded glucose infusions increased the effects of exogenously administered GIP on insulin secretion rates (ISRs) in humans with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT), but not in those with Type 2 diabetes mellitus (T2DM (53)). Infusion of Xen alone had no effect in any group. With meal ingestion, GIP and other gut-derived factors are released into the circulation, suggesting that exogenously administered Xen may have different effects in conjunction with orally vs. intravenously administered nutrients. The purpose of the present study was to determine whether xenogenously administered Xen modulates gastric emptying and glucose, insulin, C-peptide, glucagon, Xen, GIP, and GLP-1 levels as well as ISRs in response to meal ingestion.
MATERIALS AND METHODS

Studies in human subjects. All protocols were approved by Washington University’s Human Research Protection Office and the FDA (IND no. 103,374) and are registered with ClinicalTrials.gov (NCT00949663). Studies were performed in the Clinical Research Unit of the Institute of Clinical and Translational Sciences of Washington University after obtaining written informed consent. Male and female subjects with NGT, IGT, and mild T2DM were studied (Table 1). Glucose tolerance, inclusion/exclusion criteria, and screening protocols were as previously described (53). With respect to T2DM, subjects were required to have HbA1c ≤9%, could not be using insulin for treatment, and had no known history of symptomatic gastroparesis or peripheral neuropathy. These selection criteria were designed to exclude T2DM subjects with advanced beta cell failure.

Study design. Studies were performed after a 10-h overnight fast. In subjects with T2DM and taking oral diabetes medications, drugs were discontinued for 48 h before each study visit. One intravenous catheter was placed into a hand vein. This hand was kept in a thermostatically controlled box (50–55°C) for sampling arterialized venous blood (7, 31). A second intravenous line was inserted in the contralateral arm for administration of peptide. Subjects with a fasting blood glucose >120 mg/dl were given boluses of intravenous human insulin (~0.01 U/kg) at 20-min intervals as needed to decrease the blood glucose level to 100–120 mg/dl to limit variability of initial glucose levels. Blood glucose was stable (±120 mg/dl) for 20 min before starting the meal tolerance test.

Meal tolerance tests. Boost Plus (Nestle Health Science, Florham Park, NJ) is a liquid mixed meal (360 calories, 14 g of fat, 45 g of carbohydrates, and 14 g of protein). From 0 to 3 min, fasted subjects ingested Boost Plus and liquid acetaminophen (ACM; 1.5 gm/15 ml; Q-PAP Infants’ Drop; Qualitest Pharmaceuticals, Huntsville, AL).

Treatments. Treatments were administered in a crossover design and involved intravenous infusions of albumin alone (Alb; i.e., no peptide) or different duration/doses of Xen. On separate visits, at least 2 wk apart, a primed constant infusion of Alb or Xen alone was administered starting at time zero and continued for 300 min. For the Lo-Xen treatments, infusion rates of 0–2, 3–7, 7–10, and 10–300 min were 10.8, 7.7, 5.6, and 4.0 pmol·kg

Data analysis and statistics. ISRs were derived by stochastic deconvolution of the peripheral C-peptide concentrations as previously described by using population-based estimates of C-peptide clearance kinetics (45, 46, 49). Incremental areas under the curve (iAUCs) relative to baseline were used to estimate effects and were calculated by the trapezoid method. Following meal ingestion, Xen treatment altered the temporal pattern of plasma glucose levels rather than the total amount of glucose in the blood over the 300 min of the study. Thus iAUCs for glucose, ISR, glucagon, GIP, and GLP-1 responses were calculated for the time period of 0–120 min (i.e., the crossover time point for plasma glucose levels in the NGT group). Similarly, plasma ACM levels crossed over at 240 min in all three groups. Thus ACM iAUCs were calculated for the time frame from 0–240 min.

Physiological data were analyzed by the mixed-effects models with subjects as a random effect and peptide as a fixed effect by use of IBM SPSS Version 20. Within each group, comparison of 300-min infusions allowed two degrees of freedom and pairwise comparisons were limited to evaluating the effects of Hi-Xen vs. Alb and Lo-Xen vs. Alb. Within the NGT group, comparison of the 0-, 30-, 90-, and 300-min Hi-Xen infusions allowed three degrees of freedom, and pairwise comparisons were limited to evaluating the effects of J) 300 min Hi-Xen vs. Alb, 2) 90 min Hi-Xen vs. Alb, and 3) 30 min Hi-Xen vs. Alb. Two-tailed t-tests were used for all analyses and P < 0.05 was considered significant. Differences in baseline characteristics, immunoreactive (IR)-Xen, and IR-GIP levels between groups were evaluated by one-way ANOVAs via GraphPad Prism Version 5. Contingency tables with Pearson χ2 were used to evaluate effects of treatments on occurrence of diarrhea. Fisher’s exact test was used when expected cell frequencies in the contingency table were low.

Immunohistochemical studies. Paraffin-embedded blocks of human stomach were obtained from our Department of Pathology. Sections were deparaffinized, subjected to antigen retrieval with EDTA (pH 8), blocked with CAS-Block (Invitrogen, Frederick, MD), and incubated with primary antibodies overnight at 4°C as previously described (52, 57). After washing, bound primary antibodies were detected following incubation for 45 min at room temperature with the appropriate conjugated secondary antibodies. Nuclei were counterstained with bis-benzimide. For double-label studies, single tissue sections were incubated with both primary antibodies and single color images were merged by use of Adobe Photoshop. All antibodies were diluted in Da Vinci Green Diluent (Biocare Medical, Concord, CA). The antibodies including source, catalog number, and dilution were goat anti-NTSR1 (Santa Cruz Biotechnology, Dallas, TX; no. SC-7596, 1:100), rabbit anti-PGP9.5 (Millipore; no. AB1761), and mouse anti-smooth muscle actin (Sigma Chemical; no. A5228, 1:200). Minimal cross-reacting Alexa Fluor 488- and Alexa Fluor 549-conjugated donkey anti-mouse, goat, and rabbit antibodies were obtained from Jackson ImmunoResearch (West Grove, PA) and used at a 1:500 dilution.

RESULTS

Subject characteristics. Two-hour glucose, fasting glucose, and HbA1c levels were progressively higher in subjects with NGT vs. IGT vs. T2DM (Table 1). Body mass index was higher in subjects with T2DM. Subjects with T2DM had mild disease and no history of gastroparesis or other clinically evident neuropathies (e.g., burning or tingling in feet). Six of the 12 subjects with T2DM were treated with oral medications (three with metformin, one with metformin and glipizide, one

Table 1. Group characteristics

<table>
<thead>
<tr>
<th>NGT (n = 10)</th>
<th>IGT (n = 14)</th>
<th>T2DM (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-h Glucose, mg/dl</td>
<td>118 ± 11</td>
<td>162 ± 14</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>95 ± 7.2</td>
<td>97 ± 7.3</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.6 ± 0.3</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>38 ± 2.2</td>
<td>39 ± 1.9</td>
</tr>
<tr>
<td>Sex, men/women</td>
<td>4/6</td>
<td>9/5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>40 ± 11</td>
<td>46 ± 9.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29 ± 5.1</td>
<td>31 ± 5.3</td>
</tr>
</tbody>
</table>

Data are group means ± SD. *,†,‡P < 0.05, 0.01, and 0.001, respectively, by 1-way ANOVA.
XENIN DELAYS GASTRIC EMPTYING IN HUMANS

Fig. 1. Plasma xenin-25 (Xen) levels in response to Xen infusions. Immuno-reactive (IR)-Xen levels were determined in plasma prepared from subjects with normal glucose tolerance (NGT; A), impaired glucose tolerance (IGT; B), and Type 2 diabetes mellitus (T2DM; C) at the indicated time after Boost Plus ingestion with a primed 300-min continuous infusion of albumin (Alb; open circles), 4 pmol·kg⁻¹·min⁻¹ Xen (Lo-Xen; open squares), or 12 pmol·kg⁻¹·min⁻¹ Xen (Hi-Xen; open triangles). Group averages ± SE are shown and n = x, y, z represents the number of Alb, Lo-Xen, and Hi-Xen infusions, respectively, included in the measurements.

with metformin and rosiglitazone, and one with pioglitazone). Four subjects with T2DM required insulin before all three study visits and four required insulin before only one visit. Post hoc analysis of data indicated that two subjects in the IGT group had inappropriately elevated baseline concentrations for plasma glucose, insulin, C-peptide, GIP, and GLP-1 compared with their other study visits, indicating that they had not fasted before the study visit (e.g., “fasting” GIP and GLP-1 levels were elevated ~10-fold). Thus data for these two specific visits were excluded. For the other individuals, basal values for all parameters were similar for each study visit.

Xen infusion results in pharmacological levels of peptide. Consistent with our previous results (53), IR-Xen levels were undetectable (<2 pM) in all three groups following an overnight fast (Fig. 1). After Boost Plus ingestion, IR-Xen levels remained undetectable during infusion with Alb alone. In a preliminary investigation, endogenously released IR-Xen was also undetectable in several nondiabetic subjects following ingestion of a solid meal (pizza) or oral glucose (not shown). In contrast to infusion with Alb, plasma IR-Xen levels rapidly increased to and remained at ~200 pM and ~600 pM during infusion of Lo-Xen and Hi-Xen, respectively, in subjects with NGT, IGT, and T2DM (Fig. 1). During infusions with Lo-Xen and Hi-Xen, the respective iAUCs for IR-Xen levels from 0 to 300 min were not different between groups (P = 0.18 for Lo-Xen and P = 0.06 for Hi-Xen), although there was a trend toward higher levels in T2DM (not shown).

Hi-Xen delays gastric emptying in humans with NGT, IGT, and T2DM. Plasma appearance of orally administered ACM was used to indirectly measure the rate of gastric emptying (55). Compared with Alb, infusion with Hi-Xen for 300 min shifted the postprandial increase in plasma ACM levels to later times in all groups (Fig. 2, A–C). As shown in Fig. 3, A–C, the iAUCs from 0 to 240 min for ACM levels were decreased by 34% in subjects with NGT (812 ± 105 vs. 1,222 ± 105 µg·ml⁻¹·min⁻¹; P = 0.014), 26% in subjects with IGT (821 ± 90 vs. 1,106 ± 66 µg·ml⁻¹·min⁻¹; P = 0.019), and 33% in subjects with T2DM (635 ± 90 vs. 948 ± 81 µg·ml⁻¹·min⁻¹; P = 0.022) for Hi-Xen vs. Alb, respectively. Compared with Alb, infusion with Lo-Xen did not significantly affect the postprandial increase in plasma ACM levels in any group.

Hi-Xen reduces postprandial glucose levels in humans with NGT, IGT, and T2DM. Consistent with delayed gastric emp-
5,094 vs. 61,640
statistically significant only in the group with NGT (36,719 Lo-Xen, compared with Alb. This difference in iAUCs was lower for the first 120 min during infusion with Hi-Xen, but not iAUCs (Fig. 3, G–I). After Boost Plus ingestion, the 120-min GIP iAUC was significantly lower during infusion with Hi-Xen compared with Alb alone in the subjects with NGT (Fig. 5D; 3,459 ± 359 vs. 5,535 ± 663 pmol·min⁻¹; P < 0.05). There was no significant effect of Hi-Xen on the 120-min GIP iAUC in subjects with IGT and T2DM (Fig. 5, E and F).

Hi-Xen has little effect on GIP release. During Alb infusion, fasting plasma IR-GIP levels were ~10 pM, peaked at ~82 pm 30 min after Boost Plus ingestion, and then declined similarly in all three groups (Fig. 5, A–C). In general, infusion with either dose of Xen did not alter the temporal GIP response within or between any groups. However, as noted with ISR and consistent with delayed gastric emptying, the 120-min GIP iAUC was significantly lower during infusion with Hi-Xen compared with Alb alone in the subjects with NGT (Fig. 5D; 3,459 ± 359 vs. 5,535 ± 663 pmol·min⁻¹; P < 0.05). There was no significant effect of Hi-Xen on the 120-min GIP iAUC in subjects with IGT and T2DM (Fig. 5, E and F).

Hi-Xen inhibits GLP-1 release. Fasting plasma levels of intact GLP-1 were ~0.3 pM in all three groups (Fig. 6, A–C). During Alb infusions, intact GLP-1 levels rapidly, transiently, and similarly increased following meal ingestion in all three groups. The average times (in minutes) to peak intact GLP-1 levels were 18 ± 3.0 vs. 37 ± 9.1 vs. 33 ± 6.6 in subjects with NGT vs. IGT vs. T2DM, respectively, and the 0- to 120-min iAUCs were 139 ± 21 vs. 104 ± 15 vs. 92 ± 11 pmol·min⁻¹, respectively (Fig. 6, D–F). However, these differences did not reach statistical significance (P = 0.16 and P = 0.14, respectively, by one-way ANOVA). Compared with Alb alone, infusion of Hi-Xen reduced the postprandial GLP-1 response (Fig. 6, D–F in subjects with NGT (24 ± 21 vs. 139 ± 21 and 3, D–F), the iAUCs for Xen were normalized to those for plasma glucose. As shown in Fig. 3, J–L, differences in the ratios of ISR to plasma glucose iAUCs from 0–120 min were not significant within any group. To further evaluate the ability of Xen to enhance the beta cell sensitivity to glucose, ISRs were plotted as a function of plasma glucose levels (Fig. 4). Within each group, the curves from these plots were virtually identical for albumin, Lo-Xen, and Hi-Xen during the first 30 min after meal ingestion (when both glucose levels and ISRs are generally rising) and from 60–300 min (when glucose and ISRs are generally decreasing).

Hi-Xen improves postprandial glucose levels. As shown in Fig. 4, A–C, fasting glucose levels were similar in all three groups. Within each group, plasma glucose (from 0 –120 min; D–I), ISRs (from 0 –120 min; D–I), plasma glucose. As shown in Fig. 3, J–L, differences in the ratios of ISR to plasma glucose iAUCs from 0–120 min were not significant within any group. To further evaluate the ability of Xen to enhance the beta cell sensitivity to glucose, ISRs were plotted as a function of plasma glucose levels (Fig. 4). Within each group, the curves from these plots were virtually identical for albumin, Lo-Xen, and Hi-Xen during the first 30 min after meal ingestion (when both glucose levels and ISRs are generally rising) and from 60–300 min (when glucose and ISRs are generally decreasing).

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FIG. 5. Plasma levels of IR-glucose-dependent insulinotropic polypeptide (GIP) during Xen infusions. A–C: IR-GIP levels at the indicated times were measured in subjects with NGT, IGT, and T2DM (A–C, respectively) during infusion of Alb (open blue circles), Lo-Xen (open red squares), or Hi-Xen (open green triangles). D–F: for statistical analyses of data in A–C. IR-GIP iAUCs from 0–120 min were determined for each subject during infusion of Alb, Lo-Xen, or Hi-Xen. Group averages ± SE are shown. The number of subjects (n) is shown as described in Fig. 1. P values by 1-way ANOVA are shown in E and F.

Hi-Xen increases the glucagon response in humans with IGT and T2DM. Fasting plasma glucagon levels increased progressively (p = 0.04) from NGT (70 ± 5 pg/ml) to IGT (78 ± 4 pg/ml) to T2DM (90 ± 7 pg/ml). During infusions with Alb, the glucagon response (defined as the change from fasting values) rapidly increased after meal ingestion, peaked at 30 min, and then declined to subfasting levels in all three groups (Fig. 7, A–C). However, the postprandial peaks increased progressively and declined increasingly slower from NGT to IGT to T2DM. As shown in Fig. 7, D–F, iAUCs from 0–120 min were also significantly increased with Hi-Xen vs. Alb in subjects with IGT (882 ± 145 vs. 150 ± 121 pg·ml⁻¹·min; P < 0.001) and T2DM (2,743 ± 391 vs. 1,643 ± 343 pg·ml⁻¹·min; P = 0.05), but not in those with NGT (−32 ± 225 vs. −358 ± 225 pg·ml⁻¹·min). When plotted vs. plasma glucose levels or ISRs, plasma glucagon levels were inappropriately high during infusions with Hi-Xen only in the subjects with IGT and T2DM (not shown). Infusion with Lo-Xen modestly, but significantly, increased the glucagon response only in subjects with IGT.

Mild diarrhea is a side effect of Xen infusions. On the basis of symptom surveys, Xen administration for 300 min was not associated with nausea, vomiting, heart palpitations, chest pain, dizziness, shortness of breath, blurred vision, changes in salivation, sweating, or frequency of urination. Heart rate and blood pressure as well as plasma levels of potassium, bicarbonate, AST/ALT, and amylase were also unaffected (not shown). Infusion with Alb was not associated with diarrhea in any subject. In contrast, mild, self-limited diarrhea occurred in 58% (21 of 36) and 77% (24 of 31) of subjects who received Lo-Xen and Hi-Xen, respectively, for 300 min (Fig. 8A; P < 0.0001 for Lo-Xen/Hi-Xen vs. Alb). The difference between Lo-Xen and Hi-Xen was not significant (P = 0.11). The number of diarrheal episodes per affected individual was not different with Lo-Xen and Hi-Xen infusions (2.0 ± 0.33 and 1.8 ± 0.22, respectively; Fig. 8B). Hi-Xen was also infused for 30 and 90 min in a subset of subjects with NGT. There was a trend for fewer participants to experience diarrhea when the Hi-Xen was administered for shorter times (P = 0.074; Fig. 8C). Although statistically significant effects with different times or doses of Xen were not detected, we do not have sufficient power to say that differences do not exist since only seven subjects were administered 30- and 90-min infusions of Hi-Xen. With respect to the diarrhea, all episodes occurred during the 30-min time points for all infusions (not shown). As with intact GLP-1 levels, total GLP-1 levels were also reduced by Hi-Xen infusion. Thus Hi-Xen reduces GLP-1 release rather than affecting degradation. Compared with Hi-Xen, Lo-Xen had a smaller effect on intact GLP-1 levels.

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the study visit in 83% of the subjects in response to infusion with Hi-Xen. In the remaining subjects, diarrhea occurred within 24 h of the visit. Diarrhea was noted in all three groups. Diarrhea was nonbloody and treated with loperamide 2 mg by mouth in 7 of 31 subjects who received Hi-Xen. Infusion with Hi-Xen was terminated early in 3 of the 31 patients at their request due to diarrhea.

Receptors for Xen are expressed on neurons in the human stomach. As shown in Fig. 9, intense punctate staining for NTSR1 was detected within the actin-positive longitudinal muscle in the human stomach. The NTSR1 staining colocalized with a subset of PGP9.5 nerve fibers (Fig. 10). The density of NTSR1-positive puncta was greatly reduced within the circular muscle (Fig. 9) as well as in regions contacting actin-positive smooth muscle cells lining the basal membranes of glandular epithelial cells (not shown).

DISCUSSION

As detailed in the introduction, Xen, alone and/or in combination with GIP, has been shown to improve glucose homeostasis in animals (2, 9, 10, 25, 27, 30, 54). With the exception of a single publication from our laboratory demonstrating that Xen amplifies the effects of GIP on insulin secretion in humans without T2DM (53), nothing is known concerning the effects of Xen on glucose homeostasis in humans. Thus the present study is extremely novel. The major findings of this paper are that infusion of Hi-Xen, but not Lo-Xen, for 90 or 300 min reduced ACM appearance in the blood and thus delayed gastric emptying in humans with NGT, IGT, and T2DM. Consequently, postprandial plasma glucose levels were lower during Hi-Xen infusions. ISRs, when normalized to plasma glucose levels, were unaffected by Hi-Xen, suggesting that delayed gastric emptying alone mediates the beneficial effects of Hi-Xen on postprandial glucose levels. The GIP response, if normalized to plasma ACM levels (i.e., gastric emptying), was normal (NGT) or even elevated (IGT and T2DM) in all three groups of subjects during infusion of Hi-Xen. In stark contrast, infusion with Hi-Xen reduced postprandial levels of plasma intact GLP-1 to a much greater degree compared with ACM, suggesting that endogenously released GLP-1 may play only a minor role in regulating postprandial insulin secretion in response to ingestion of a liquid mixed meal, especially in those with NGT.

Hyperglycemia itself can delay gastric emptying (37). However, Hi-Xen was a potent antagonist of gastric emptying in all

Fig. 8. Xen effects on diarrhea. The percentage of subjects who experienced diarrhea in response to a particular infusion is shown (A; ****P < 0.0001). The average number of diarrhea episodes ± SE per affected subject is shown (B). Hi-Xen (Hi) infusions for shorter duration were administered only to subjects with NGT. Effects of 30-, 90-, and 300-min Hi-Xen infusions are shown (C). P value by Fisher’s exact test for affect of Xen (any time or dose) vs. albumin is <0.0001. Comparison of dose and time for Xen P = 0.12 (Fisher’s exact test). Lo, Lo-Xen.

Fig. 10. Receptors for Xen are expressed on nerve fibers in the longitudinal muscle in the human stomach. A single paraffin-embedded section of human stomach was stained for PGP9.5 (red) plus NTSR1 (green). Nuclei were counterstained blue. Staining was visualized by confocal microscopy with a ×120 objective. A merged image was generated in Photoshop.

Fig. 9. A high density of Xen receptors is present in the longitudinal muscle (LM) in the human stomach. A single paraffin-embedded section of human stomach was stained for smooth muscle α-actin (green) plus NTSR1 (red). Nuclei were counterstained blue. Staining was visualized by confocal microscopy with a ×40 objective. A merged image was generated in Photoshop.
three groups even though each had varying levels of postprandial glucose. Gastric emptying in humans involves a complex interplay between the central and enteric nervous systems (20). As shown in the present study, a high density of NTSR1 was detected only on enteric neurons residing within the longitudinal muscle in the human stomach (Figs. 9 and 10). Thus the effects of Xen on gastric emptying are most likely mediated by a neural relay. Delaying gastric emptying is a therapeutic target for treating T2DM, and agonists for GLP-1 (26, 28) and amylin (40, 50) receptors work in part via this mechanism. Thus Xen alone, or in combination with other drugs that delay gastric emptying, could have therapeutic benefit for reducing postprandial glucose levels in humans with T2DM. Acute dosing of Xen, as with pramlintide (36) and GLP-1 (47), could overcome potential problems due to tachyphylaxis as noted with chronic administration of GLP-1 (32).

Hi-Xen delayed gastric emptying in all three groups but plasma levels of GIP were reduced only in the group with NGT. The reason for this differential effect on GIP levels is unknown but indicates that GIP levels do not simply reflect the rate of gastric emptying. That Hi-Xen infusion inhibited GLP-1 release is perhaps not surprising since Xen appears to act by exciting neurons, and a host of neurotransmitters and neuropeptides including ACh, bombesin/gastrin-releasing peptide, and calcitonin-related peptide are known to increase GLP-1 release (3, 11, 12, 34).

An important finding from our study is that infusion of Hi-Xen reduced plasma levels of active GLP-1 nearly sixfold without decreasing the incretin response in subjects with NGT. Several studies from other laboratories are consistent with our data since they showed that infusion of exendin-9,39 (a GLP-1 receptor antagonist) had little effect on postprandial plasma glucose, insulin, and C-peptide levels as well as static ISRs in healthy control subjects (13, 39, 44). In contrast, other studies using exendin-9,39 suggest that endogenous GLP-1 does play an important role in regulating the incretin response (38, 43, 56). However, these study protocols were significantly different from ours. For example, some were conducted by administering glucose alone, either orally or by intraduodenal infusion. However, the collective cohort as well as amounts of individual intestinal peptides that are released in response to glucose alone is not the same as that released in response to a mixed meal (6, 51). Moreover, physical ingestion of nutrients would elicit a neural response that would not be recapitulated by intraduodenal administration of the same nutrients (1). Interestingly, oral glucose elicits a greater insulin secretory response compared with an isocaloric load delivered by duodenal perfusion (42). Other studies administered a mixed meal or oral glucose in conjunction with a hyperglycemic clamp, which is quite different than conditions present when a meal is ingested in the fasted state (e.g., plasma glucagon levels would be very different). Exendin-9,39 clearly reduces the incretin response in subjects following gastric bypass surgery (22, 39, 44). However, this surgical procedure alters the anatomy of the gut and results in profoundly increased levels of postprandial plasma GLP-1 and thus does not represent normal physiology. Regardless, even when noted, exendin-9,39 typically inhibits less than 50% of the endogenous incretin response. Thus our results concerning the role of endogenous GLP-1 for the incretin response should be considered complementary rather than contradictory to these other studies but further suggest that GIP may play a more significant role in the endogenous incretin response than previously appreciated. Several alternative explanations for our results with GLP-1 include the possibilities that 1) other incretins and/or additional mechanisms (e.g., neural input) could also be important for the incretin response in humans; 2) residual GLP-1 release from L cells is still sufficient to enhance glucose stimulated insulin secretion in humans with NGT; and 3) central rather than peripheral GLP-1 production and action is important for regulating insulin release.

Glucagon secretion is dysregulated as humans progress from NGT to T2DM (21, 48). In the present study, infusion with Hi-Xen increased glucagon levels in humans with IGT and T2DM. Despite this, Hi-Xen still reduced postprandial glucagon levels by a similar percentage to that noted in subjects with NGT. Although glucagon reportedly delays gastric emptying, infusion of Hi-Xen did not increase the glucagon response in humans with NGT, suggesting that increased glucagon does not account for the Xen-mediated delay in gastric emptying.

Plasma glucose, intra-islet insulin, incretins, and neural signaling regulate glucagon release. Interestingly, glucagon responses to Hi-Xen in subjects with IGT and T2DM could not be accounted for simply by changes in plasma glucose levels or ISRs. Moreover, GLP-1 levels were profoundly decreased by infusion with Hi-Xen in subjects with NGT whereas glucagon levels did not increase. Collectively, these results suggest that changes in neural input to islets may be responsible for the Xen-mediated increase in the glucagon response in humans with IGT and T2DM.

A prior study reported that postprandial Xen levels were ~120 pM (18). However, this study measured plasma Xen by a RIA using a single antibody directed against the COOH terminus of Xen. Consistent with our previous human (53) and mouse (54) studies, fasting and/or postprandial levels of endogenous Xen were undetectable (Fig. 1, A–C). Similarly, we have not detected endogenous Xen in plasma following ingestion of a solid meal (pizza) or oral glucose. The ELISA we developed utilizes different capture and detection antibodies, requires at least 16 COOH-terminal residues of Xen, and can detect less than 2 pM peptide (54). Thus it will be important to determine the basis for the differences in endogenous Xen levels when levels are measured by different assays.

Mild diarrhea was the only side effect associated with Xen administration. Interestingly, diarrhea persisted in some subjects long after the Xen infusions were terminated despite the fact that Xen has a circulating half life of only 2.5 min in humans (53). In conscious dogs, Xen increases gall bladder contractions, which indirectly increases intestinal motility since this latter response is lost following cholecystectomy (23), which could explain the long-acting effects of Xen on diarrhea.

It is important to address several limitations to our study. First, scintigraphy is considered the gold standard for measuring gastric emptying. However, plasma appearance of orally administered ACM has been used as an indirect estimate of the rate of gastric emptying (55) since pharmacokinetic data indicate that ACM is rapidly absorbed in the duodenum, but not stomach (8). Although this assumes that gastric emptying is the rate-limiting step in ACM appearance in plasma, AUCs for plasma levels of ACM are highly correlated with the rates of gastric emptying of a liquid, but not solid, meal as measured by
scintigraphy (55). However, potential errors from the ACM method can arise from large variations in ACM metabolism between individuals (41). Our results also assume that Xen infusion itself has no effect on ACM absorption or clearance. ACM clearance could also be reduced in subjects with impaired renal or liver function. Owing to selection of subjects with normal renal and liver function and the crossover design of our study, these potential artifacts are unlikely to explain how Xen reduced ACM appearance in the blood. That Hi-Xen infusion concomitantly reduced postprandial glucose levels and ACM iAUCs is also consistent with the conclusion that Hi-Xen delays gastric emptying. Secondly, it is important to note that solid, liquid, and oil phases of a meal are emptied from the stomach at different rates (29). Moreover, the specific components of each phase may also affect gastric emptying. Thus the overall effect of Hi-Xen on gastric emptying of a “normal” mixed solid-liquid meal may be different from that noted in the present study. Finally, insulin was administered to several subjects with T2DM to lower basal glucose levels before the study meal was administered, which potentially could have lowered basal glucagon levels in these subjects. However, blood glucose was stable for at least 20 min before start of the meal tolerance test. Moreover, our results suggest that neither glucagon nor insulin mediate the effects of Xen on gastric emptying, and thus insulin administration is unlikely to have affected our results.

Overall, results obtained by infusing Xen are yielding important insights concerning the differential regulation of glucose homeostasis in humans with NGT, IGT, and T2DM. Studies are currently underway to determine the role of cholinergic, noncholinergic, vagal, and nonvagal signaling for regulating Xen action in humans.

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DISCLOSURES

Washington University is pursuing a patent related to the use of xenin-25 to treat T2DM. In the future, this could lead to personal financial benefit to B. M. Wice, K. S. Polonsky, and the University.

AUTHOR CONTRIBUTIONS


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