Xenin-25 Amplifies GIP-Mediated Insulin Secretion in Humans With Normal and Impaired Glucose Tolerance but Not Type 2 Diabetes

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Glucose-dependent insulinotropic polypeptide (GIP) potentiates glucose-stimulated insulin secretion (GSIS). This response is blunted in type 2 diabetes (T2DM). Xenin-25 is a 25–amino acid neurotensin-related peptide that amplifies GIP-mediated GSIS in hyperglycemic mice. This study determines if xenin-25 amplifies GIP-mediated GSIS in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or T2DM. Each fasting subject received graded glucose infusions to progressively raise plasma glucose concentrations, along with vehicle alone, GIP, xenin-25, or GIP plus xenin-25. Plasma glucose, insulin, C-peptide, and glucagon levels and insulin secretion rates (ISRs) were determined. GIP amplified GSIS in all groups. Initially, this response was rapid, profound, transient, and essentially glucose independent. Thereafter, ISRs increased as a function of plasma glucose. Although magnitudes of insulin secretory responses to GIP were similar in all groups, ISRs were not restored to normal in subjects with IGT and T2DM. Xenin-25 alone had no effect on ISRs or plasma glucagon levels, but the combination of GIP plus xenin-25 transiently increased ISR and plasma glucagon levels in subjects with NGT and IGT but not T2DM. Since xenin-25 signaling to islets is mediated by a cholinergic relay, impaired islet responses in T2DM may reflect defective neuronal, rather than GIP, signaling. *Diabetes* 61:1793–1800, 2012

Peptides secreted from the gastrointestinal tract play an important role in regulating insulin secretion (1,2). To date, attention has focused on two intestinal peptides: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is produced predominantly by L cells in the distal bowel, whereas GIP is produced mainly by K cells in the proximal intestine. Both peptides are released into the blood immediately after eating and potentiate glucose-stimulated insulin secretion (GSIS) (3–5). Unlike GLP-1, which stimulates insulin secretion in type 2 diabetes (T2DM), persons with T2DM are thought to be resistant to the actions of GIP (6–8), and increasing GIP signaling has not been pursued as a therapeutic target for T2DM.

To better understand how GIP regulates the incretin response, we generated and characterized transgenic mice that lack GIP-producing cells by driving expression of an attenuated diphtheria toxin transgene with regulatory elements from the GIP gene (9). These GIP/DT mice demonstrated markedly attenuated insulin secretory responses to oral glucose even though GLP-1 release was normal (9). Moreover, the GIP/DT mice exhibited a blunted insulin secretory response to exogenously administered GIP but not GLP-1 (10). Thus, GIP-producing cells may secrete a hormone(s) in addition to GIP that plays a critical role in the incretin response.

Xenin-25 is a 25–amino acid neurotensin-related peptide reportedly produced by a subset of K cells (11,12) and would also be reduced in the GIP/DT mice. In vivo experiments demonstrated that xenin-25 potentiated the insulin secretory response to GIP but had little effect alone (10). Similar results were observed in hyperglycemic NONcNZO10/Ltj mice (10)—a polygenic model of human T2DM in which diabetes spontaneously develops with age (13,14). These studies raise the possibility that xenin-25 could increase the insulin secretory response to GIP in humans. The purpose of the current study was to determine if xenin-25, either alone or with GIP, could amplify the insulin secretory response to glucose in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and/or T2DM.

RESEARCH DESIGN AND METHODS

Studies in human subjects. All protocols were approved by Washington University's Human Research Protection Office and the Food and Drug Administration (IND 103,374) and are registered with ClinicalTrials.gov (NCT00798915). 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 was defined by the 2-h plasma glucose level during a 75-g oral glucose tolerance test using diagnostic criteria of the American Diabetes Association (15). Baseline characteristics were determined during a screening visit after a 10-h fast. HbA$_{1c}$ levels were required to be ≤9.0 in all subjects. Subjects treated with insulin were excluded. Subjects treated with oral antidiabetic medications were enrolled if the agent(s) could be safely discontinued for 48 h preceding each study visit. These selection criteria were designed to exclude T2DM subjects with advanced β-cell failure and to identify participants with residual insulin secretion who have the potential to respond to incretin peptides. Women of childbearing potential were required to use birth control. Subjects were excluded if they 1) had a history of chronic pancreatitis and/or risk factors for chronic pancreatitis, 2) had a history of gastrointestinal disorders, 3) were taking nondiabetes medications known to

TABLE 1
Group characteristics

	NGT ($n = 10$)	IGT ($n = 10$)	T2DM ($n = 9$)
2-h glucose (mg/dL)[1]	117 ± 6.1 (67–132)	175 ± 4.9 (141–193)	255 ± 12.7 (195–303)
Fasting glucose (mg/dL)[2]	93 ± 1.3 (88–102)	103 ± 2.2 (91–113)	122 ± 6.3 (97–163)
HbA$_{1c}$ (%)[2]	5.5 ± 0.1 (4.9–5.9)	5.9 ± 0.15 (5.1–6.7)	6.6 ± 0.14 (6.1–7.2)
Sex, n (males/females)	6/4	6/4	3/6
Age (years)	48.8 ± 3.1 (29–62)	55.9 ± 1.7 (46–63)	55.2 ± 3.0 (37–64)
BMI (kg/m^2)	30.9 ± 2.2 (21–42)	29.2 ± 1.4 (24–37)	32.8 ± 1.3 (28–41)

Data are mean ± SEM (range) unless otherwise indicated. The group means for the 2-h glucose, fasting glucose, and HbA$_{1c}$ were statistically different. [1]$P < 0.001$ for all pairwise comparisons. [2]$P > 0.05$ for NGT vs. IGT and $P < 0.01$ for the other pairwise comparisons.

affect glucose homeostasis, and 4) had significant systemic illness including heart, kidney, liver, inflammatory, or malignant disease.

Study design. Studies were performed after a 10-h overnight fast. One intravenous catheter was placed into a hand vein. This hand was kept in a thermostatically controlled box (50–55°C) to facilitate venous sampling and to provide arterialized venous blood (16,17). A second intravenous line was inserted for administration of glucose/peptides. Subjects with a fasting blood glucose ≥120 mg/dL were given boluses of intravenous human insulin (~0.01 units/kg) at 30-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 for 20 min before starting the graded glucose infusion (GGI).

All subjects were administered four separate GGIs, each separated by at least 2 weeks. During the respective GGIs, along with glucose, subjects also received an intravenous infusion with GIP plus xenin-25, GIP alone, albumin alone (no peptide), or xenin-25 alone (subjects were blinded to treatment). From 0 to 40, 40 to 80, 80 to 120, 120 to 160, 160 to 200, and 200 to 240 min, the glucose infusion rates were 1, 2, 3, 4, 6, and 8 mg · kg^{-1} · min^{-1}, respectively. Peptides were administered by primed-continuous intravenous infusion. Infusions rates from 0 to 3, 3 to 7, 7 to 10, and 10 to 240 min were 10.8, 7.7, 5.6, and 4.0 pmol · kg^{-1} · min^{-1}.

Peptides. GIP and xenin-25 were custom synthesized under good manufacturing practice conditions (Bachem, Torrance, CA), dissolved in water (United States Pharmacopeial Convention, injectable), filter sterilized, aliquoted into cryovials as single doses, and stored at −80°C. Representative vials were analyzed for identity, purity, concentration, sterility, and endotoxins. Peptides were compounded in normal saline containing 1% Flexbumin (Baxter Healthcare Corp., Westlake Village, CA) immediately before infusions.

Peptide dosing. GIP administered by an intravenous infusion at a dose of 4 pmol · kg^{-1} · min^{-1} increases insulin release during a hyperglycemic clamp in healthy individuals approximately fourfold (7,8) but is ineffective in persons with T2DM (7). Similar results were obtained using GIP at a dose of 2.4 pmol · kg^{-1} · min^{-1} (6). Only one human clinical trial with xenin-25 has been published (18). The authors assessed the effects of xenin-25 infused at a dose of 4 pmol · kg^{-1} · min^{-1} on intestinal motility. This dose increased plasma xenin-25 levels and gut motility (18). Effects on insulin secretion and glucose concentrations were not reported. On the basis of these studies, GIP and xenin-25 were each infused at a dose of 4 pmol · kg^{-1} · min^{-1}.

Assays. Plasma glucose was measured bedside by the glucose oxidase technique using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin, C-peptide, glucagon, complete metabolic profiles, and HbA$_{1c}$ levels were determined by Washington University's Core Laboratory for Clinical Studies. Insulin and C-peptide were measured using a solid-phase, two-site chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Glucagon was determined by radioimmunoassay (Millipore Corporation, St. Charles, MO). Immunoreactive (IR)-GIP and IR-xenin levels were determined using an enzyme-linked immunosorbent assay (ELISA) for total GIP (Millipore Corporation) and a custom ELISA developed in our laboratory for IR-xenin (10). Lipase and total amylase were determined by the Barnes Hospital Laboratories.

Insulin secretion rates. Insulin secretion rates (ISRs) were derived by stochastic deconvolution of the peripheral C-peptide concentrations as previously described using population-based estimates of C-peptide clearance kinetics (19–21).

Statistics. Physiologic data were analyzed using mixed-effects models with subject as a random effect and peptide as a fixed effect. Pairwise comparisons were limited to evaluating the effects of 1) xenin-25 alone versus albumin, 2) GIP alone versus albumin, and 3) GIP plus xenin-25 versus GIP alone. One-tailed t tests were used when testing the hypotheses that ISRs were greater during infusion with 1) GIP plus xenin-25 compared with GIP alone and 2) GIP alone compared with albumin. Two-tailed t tests were used when testing the hypothesis that ISRs were similar during infusions with albumin alone and xenin-25 alone. All glucagon data were analyzed using two-tailed t tests.

RESULTS

Subject characteristics. Groups were well-matched and as anticipated, the 2-h and fasting plasma glucose and HbA$_{1c}$ levels progressively increased in groups with IGT and T2DM compared with NGT (Table 1). Subjects with T2DM did not have gastroparesis or clinically evident peripheral neuropathies. Five patients with T2DM were taking metformin and two were also on a sulfonylurea. No subjects were receiving incretin-based therapies. Three subjects with T2DM received insulin to lower basal glucose levels before one, one, and two of their four respective GGIs.

Symptomatic effects of peptide infusions. Plasma total amylase levels (salivary plus pancreatic) were unaffected by any of the infusions (data not shown). On the basis of qualitative surveys taken before, during, and after each infusion, peptides were 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. Mild diarrhea and/or loose stools were observed in 57, 14, 21, and 0% of subjects who received GIP plus xenin-25, GIP alone, xenin-25 alone, or albumin alone, respectively. When experienced, diarrhea occurred within several hours of the infusion and was noted in all three groups. Stools did not contain blood, and diarrhea did not require pharmacologic or medical intervention. Infusion with GIP alone reduced mean arterial blood pressure 10–15 mmHg and increased resting heart rate ~8 bpm in all three groups. In contrast, xenin-25, either alone or with GIP, had no effect on either parameter. Thus, xenin-25 does not appear to affect autonomic function.

GIP and xenin-25 levels during GGIs. In subjects with NGT, IGT, and T2DM (Fig. 1; some data not shown), fasting, preinfusion plasma levels of IR-GIP were <10 pmol/L, and fasting IR-xenin levels were below the limit of detection (typically <2 pmol/L). Infusion of GIP and xenin-25 increased steady-state levels of plasma IR-GIP and IR-xenin to ~400 and ~125 pmol/L, respectively. These levels were unaffected by coinfusion with the other peptide. Peak plasma IR-GIP levels after mixed-meal ingestion were ~80 pmol/L, and plasma IR-xenin was undetectable (<2 pmol/L). Thus, pharmacologic levels of GIP and xenin-25 were maintained throughout the GGIs. On the basis of IR-xenin levels measured after peptide infusions were terminated
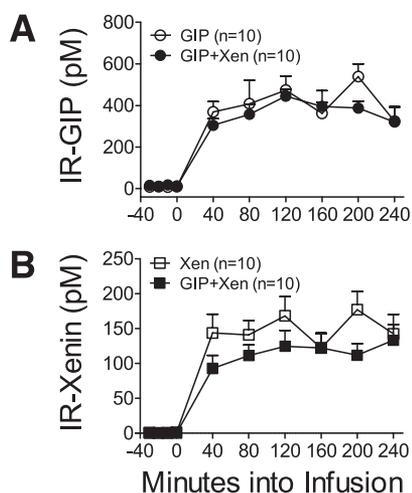
FIG. 1. *A* and *B*: Plasma levels of IR-GIP and xenin-25 during GGIs. Subjects with NGT were administered GGIs with a primed-continuous intravenous infusion of GIP alone, xenin-25 (Xen) alone, or the combination of GIP plus xenin-25. IR peptide levels (group average ± SEM) in subjects with IGT and T2DM were similar to those shown for subjects with NGT.

($n = 6$), the circulating half-life of IR-xenin is 2.5 ± 0.3 min (data not shown).

Xenin-25 alone does not affect ISR or glucose levels in humans. During infusion with albumin alone, plasma insulin, C-peptide, and glucose levels, as well as ISRs (Fig. 2), progressively increased in response to the stepwise increases in glucose infusion rates. Maximal plasma glucose levels were higher in the group with T2DM (313 ± 13 mg/dL) versus IGT (240 ± 12 mg/dL) and NGT (186 ± 11 mg/dL) ($P < 0.003$ for IGT vs. NGT, $P < 0.0001$ for T2DM vs. NGT). In spite of the progressively higher plasma glucose levels, plasma insulin and C-peptide levels, as well as ISRs, tended to reach lower maximal levels (in pmol/min) in subjects with T2DM (838 ± 224) versus IGT ($1,003 \pm 118$) and NGT ($1,037 \pm 141$). As shown in Fig. 3, ISRs increased linearly as a function of plasma glucose level during infusion with albumin alone. However, the slope of the line relating ISR to glucose declined in the progression from NGT to IGT to T2DM (slopes in pmol/min per mg/dL are 9.0, 6.1, and 3.1, respectively), reflecting worsening β-cell insulin secretory responses to glucose with IGT and T2DM.

Compared with albumin, xenin-25 did not significantly affect plasma insulin, C-peptide, or glucose levels or ISRs in any group (Fig. 2). Small and transient, but nonsignificant, increases in insulin, C-peptide, and ISR were noted at the 10- and 20-min time points during infusion of xenin-25 alone (see below). Plots of ISRs versus plasma glucose levels in the presence and absence of xenin-25 were also nearly identical within each group (Fig. 3). Incremental areas under the curve (iAUCs) were calculated for ISR and plasma glucose for each individual during the 240-min GGI with and without infusion of the xenin-25. The ratio of these iAUCs represents a summary measure of β-cell sensitivity (ISR per glucose concentration) over the entire range of glucose levels. As shown in Fig. 3, the means of the ratios were not different within each group during infusions with albumin versus xenin-25 alone in subjects with NGT (8.7 ± 1.4 vs. 9.5 ± 1.4), IGT (6.2 ± 0.8 vs. 6.9 ± 0.8), and T2DM (3.6 ± 1.8 vs. 3.4 ± 1.8). Thus, infusion of xenin-25 alone does not significantly affect ISR over a broad range of plasma glucose levels in humans with NGT, IGT, or T2DM.

Xenin-25 amplifies the effects of GIP on ISRs in humans. Subjects were next administered GGIs with GIP or GIP plus xenin-25. GIP plus or minus xenin-25 increased insulin secretory responses that varied as functions of time, plasma glucose level, and glucose tolerance (Figs. 2 and 3). At all glucose levels studied (Fig. 3), ISRs were greater within each group during GGIs with GIP and GIP plus xenin-25 compared with albumin alone or xenin-25 alone. Since the effects of GIP plus or minus xenin-25 on ISRs were not linear as a function of plasma glucose levels, iAUCs rather than slopes were used to quantify ISRs. Compared with albumin, infusion with GIP increased the 240-min ISR iAUC/glucose iAUC (Fig. 3) 1.7-, 1.5-, and 1.9-fold in subjects with NGT, IGT, and T2DM, respectively (all P values ≤ 0.007 vs. albumin), and these ratios were further increased during infusion with GIP plus xenin-25 in subjects with IGT (1.7-fold further increase vs. GIP alone; $P = 0.002$). In subjects with NGT and T2DM, the differences were not significant. Since the 240-min summary measures are taken over differing ranges of glucose levels between groups, these fold increases do not directly compare peptide effects between the three groups.

Xenin-25 amplifies a rapid and transient increase in GIP-mediated ISR in humans with NGT and IGT but not T2DM. Infusion with GIP alone at the start of the GGI caused rapid and transient increases in insulin, C-peptide, and ISRs in all groups (Figs. 2 and 3). This initial response occurred with little change in plasma glucose levels and was absent during infusions with albumin alone. Since this transient response was not anticipated, a post hoc analysis of the first 40 min was conducted (Fig. 4). In subjects with NGT, this early insulin secretory response to GIP peaked within the first 10 min of the GGI. ISRs then declined until ~40 min but remained greater than levels during infusion with albumin or xenin-25 alone. A similar response to GIP was noted in subjects with IGT and T2DM, except peak ISRs were not reached until 20 min into the infusions and then decreased until ~60–70 min into the GGI. Within each group, the iAUCs for the ISR during the first 40 min of the GGIs (pmol insulin secreted/40 min) were greater during infusion with GIP alone compared with albumin (iAUCs increased 4.6-, 6.1-, and 8.7-fold in subjects with NGT, IGT, and T2DM, respectively; all P values ≤ 0.0005). Moreover, the incremental insulin secretory responses to GIP during the first 40 min of the GGI were remarkably similar in humans with NGT ($7,998 \pm 1,263$), IGT ($10,016 \pm 1,524$), and T2DM ($10,062 \pm 2,290$).

Compared with albumin, infusion with xenin-25 alone had no statistically significant effect on ISRs or plasma glucose levels in any group (Figs. 2–4). In contrast, ISR iAUCs (pmol insulin secreted/40 min) during infusions with GIP plus xenin compared with GIP alone were increased during this period in subjects with NGT ($10,185 \pm 1,263$ compared with $7,998 \pm 1,263$; $P = 0.048$) and IGT ($15,199 \pm 1,524$ compared with $10,016 \pm 1,524$; $P = 0.002$). However, xenin-25 did not significantly increase the effects of GIP in subjects with T2DM ($12,128 \pm 2,290$ with GIP plus xenin-25 compared with $10,062 \pm 2,290$ with GIP alone; $P = 0.18$). Because plasma glucose fell below basal levels for some subjects during the initial 40-min period, ISRs were normalized to glucose AUC rather than to glucose iAUC for this time period. As shown in Fig. 4D and E, the magnitudes of the early insulin secretory responses to GIP remained similar in subjects with NGT, IGT, and T2DM, and ISRs were still significantly amplified by xenin-25 in subjects with NGT and IGT but not T2DM.
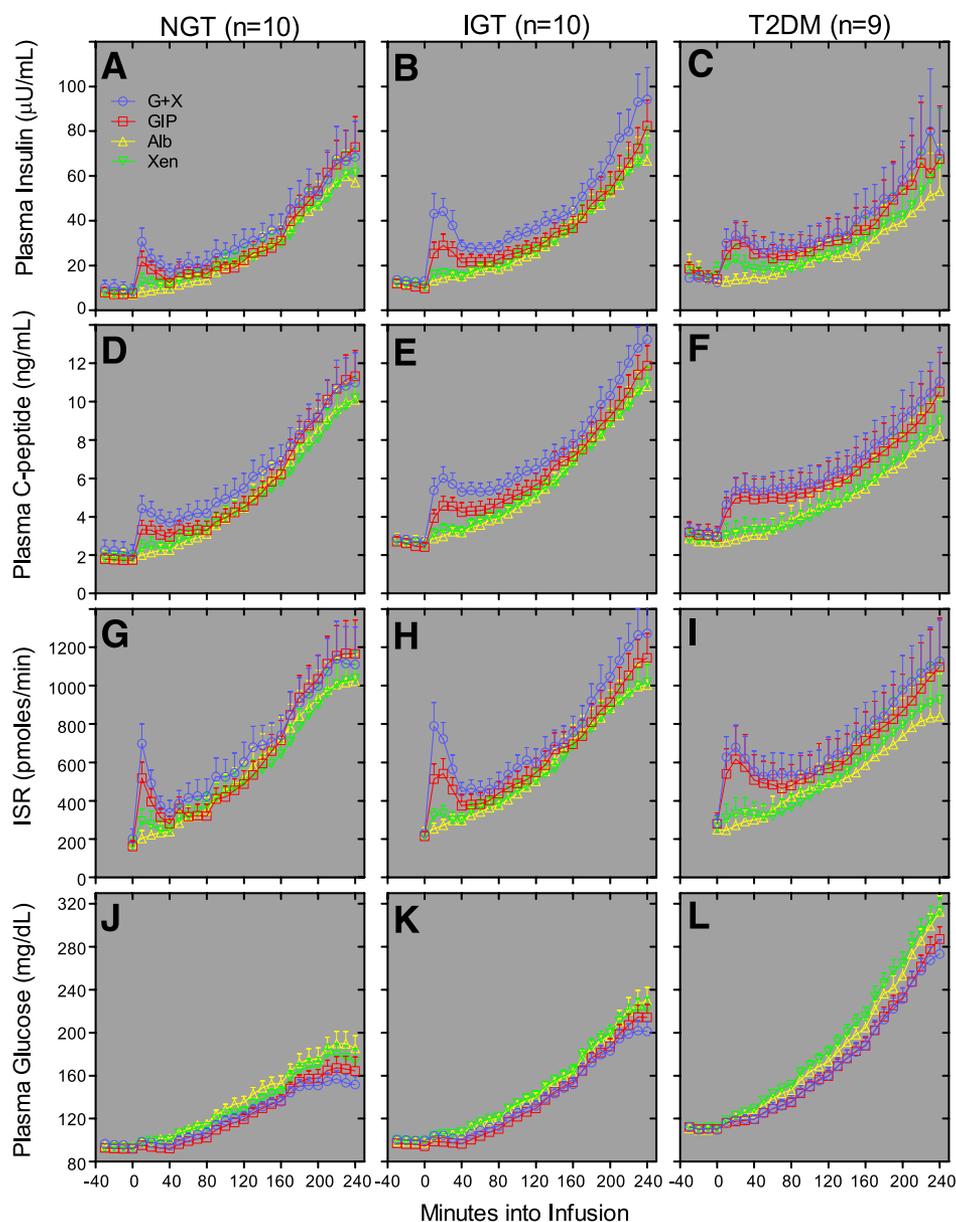
FIG. 2. *A–L*: Xenin-25 amplifies the effects of GIP on plasma insulin, C-peptide, and glucose levels and ISRs in humans with NGT and IGT but not T2DM. Subjects with NGT, IGT, or T2DM were administered GGIs with albumin alone (Alb), GIP alone (GIP), xenin-25 alone (Xen), or the combination of GIP plus xenin-25 (G+X). Group average values ± SEM for insulin, C-peptide, ISR, and glucose are shown.

Xenin-25 amplifies the effects of GIP on glucagon levels in humans with NGT and IGT. Glucagon plays a central role in the regulation of insulin secretion and glucose metabolism. Fasting, basal plasma glucagon levels were similar in all three groups and progressively decreased in humans with NGT, IGT, and T2DM during the GGI when albumin alone was infused (Fig. 5). As expected, the ability of plasma glucose to suppress plasma glucagon levels was attenuated in subjects with T2DM since glucagon levels at the end of the GGI declined by 31, 34, and 21 pg/mL in subjects with NGT, IGT, and T2DM, respectively. Compared with albumin, infusion with xenin-25 alone had no statistically significant effect on glucagon levels in any group. However, compared with GIP alone, infusion with GIP plus xenin-25 increased plasma glucagon levels in subjects with NGT and IGT during the first 40 min and during the entire 240 min of the GGI. In contrast,

xenin-25 failed to amplify the effects of GIP on plasma glucagon levels in subjects with T2DM during either time period. Thus, xenin-25 amplified the glucagon response to GIP in the subjects with NGT and IGT but not T2DM.

DISCUSSION

GIP is an incretin hormone that in people with NGT, significantly increases the insulin secretory response to glucose. However, since GIP-stimulated insulin release is reportedly blunted in humans with T2DM, this peptide has not been pursued as a therapy for T2DM. The current study was undertaken to further our understanding of attenuated β-cell responsiveness to GIP in T2DM and to elucidate the mechanisms involved in GIP signaling in the β-cell.

Previous studies from our laboratory show that xenin-25 potentiates GIP-mediated insulin release in two mouse
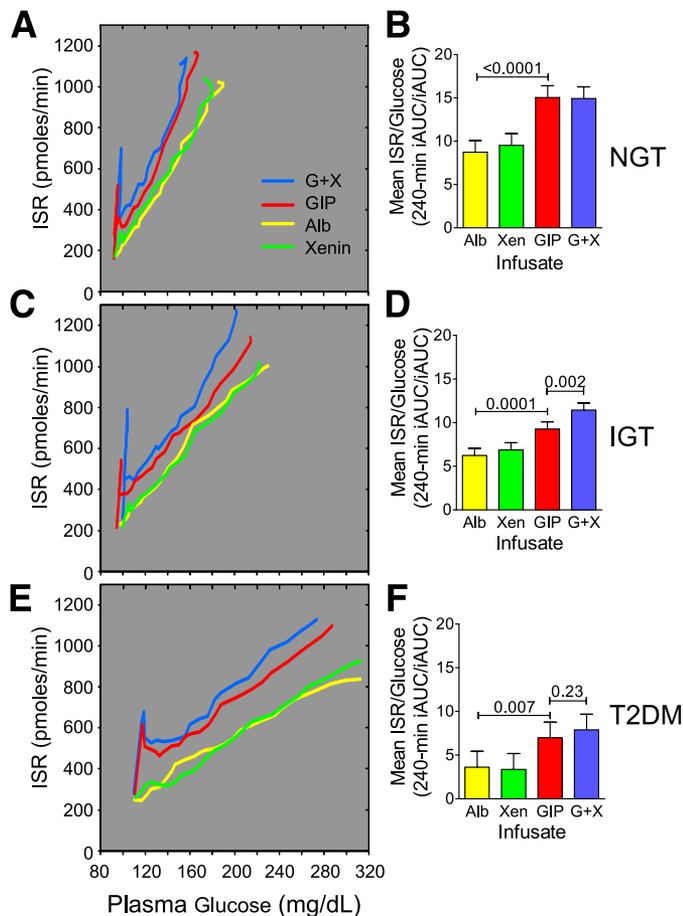
FIG. 3. A–F: Xenin-25 amplifies the insulin secretory response to GIP in humans with NGT and IGT but not T2DM. In A, C, and E, group average ISRs (from G–I in Fig. 2) were plotted vs. group average plasma glucose levels (from J–L in Fig. 2) for the indicated infusions. To simplify plots, error bars and symbols are not shown but are the same for ISR and plasma glucose as those shown in Fig. 2G–I for ISR and Fig. 2J–L for plasma glucose. In B, D, and F, the ratios of ISR iAUC to glucose iAUC are shown for subjects with NGT, IGT, and T2DM. Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.

FIG. 4. Xenin-25 rapidly and transiently amplifies GIP-mediated ISR in humans with NGT and IGT but not T2DM. Data are shown for the first 40 min of each GGI. A–C: ISR iAUCs. D–F: Values for ISR total AUC (tAUC) divided by the glucose tAUC. Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.

models that exhibit blunted insulin secretory responses to GIP (10). In contrast, xenin-25 alone had no effect on insulin secretion in either mouse model. Moreover, xenin-25 did not act directly on islet β-cells. Rather, xenin-25 activated a cholinergic relay in the periphery that did not require neurons in the central nervous system. The results presented in this study extend these observations and show that during GGIs at the peptide and glucose levels studied, xenin-25 alone does not affect ISR in humans but does potentiate GIP-mediated insulin secretion in humans with NGT and IGT but curiously, not with T2DM (see below).

A xenin-25–specific receptor has not been identified, and there is strong evidence that the effects of xenin-25 are mediated by neurotensin receptor-1 (22–27). Similar to our results with xenin-25 alone, intravenous infusion of neurotensin alone in healthy humans caused no significant change in blood pressure or plasma levels of glucose, insulin, or glucagon (28). Thus, our results are consistent with those for another neurotensin receptor-1 agonist.

The GGI is designed to detect subtle changes in the insulin secretory response to glucose. As anticipated, ISRs during the GGIs with albumin alone increased linearly as a function of plasma glucose levels, but the slopes of lines

relating ISR to plasma glucose were progressively reduced in the groups with IGT and T2DM compared with NGT. Infusion of xenin-25 alone did not significantly affect insulin secretion compared with the rates measured during control infusions (Figs. 2–4). In contrast, all subjects, including those with T2DM, exhibited a rapid and transient increase in ISR in response to GIP alone as soon as the peptide/glucose infusions were initiated. Xenin-25 significantly amplified this early response to GIP in subjects with NGT and IGT but not T2DM. After this initial response, ISRs progressively increased as a function of plasma glucose level and were greatest during infusions with GIP plus xenin-25. However, this later response was statistically significant only in subjects with IGT. Unfortunately, plasma glucose levels in the subjects with NGT remained lower during the GGI compared with the other groups. Since glucose-dependent increases in the incretin response were observed starting at ~160 mg/dL glucose (see Fig. 3), the effects of GIP plus or minus xenin-25 may be underestimated in this group. Alternatively, GIP-mediated ISR may already be near maximal and cannot be further amplified by xenin-25 in subjects with NGT.

It is generally assumed that the insulinotropic effects of GIP are blunted in T2DM. However, many studies supporting this conclusion were conducted using hyperglycemic clamps (6,8,29). In a study of fasting nondiabetic humans, plasma insulin levels rapidly (5 min) and transiently increased immediately after GIP was administered as a single bolus in spite of the fact that plasma glucose remained euglycemic (30). This pattern of insulin secretion is very similar to that noted during the initial 40-min response during our GGIs in which GIP rapidly and transiently increased ISRs in subjects with NGT, IGT, and T2DM with little change in plasma glucose levels. Thus, it is possible that some effects of GIP are masked during hyperglycemic clamps. Of importance, even though the β-cell response to glucose (i.e., the slopes for ISR vs. plasma glucose during infusion with albumin alone) was progressively decreased in subjects with NGT, IGT, and T2DM (Fig. 3), the magnitudes of the early responses to GIP were similar in all groups (Fig. 4). Our results are thus
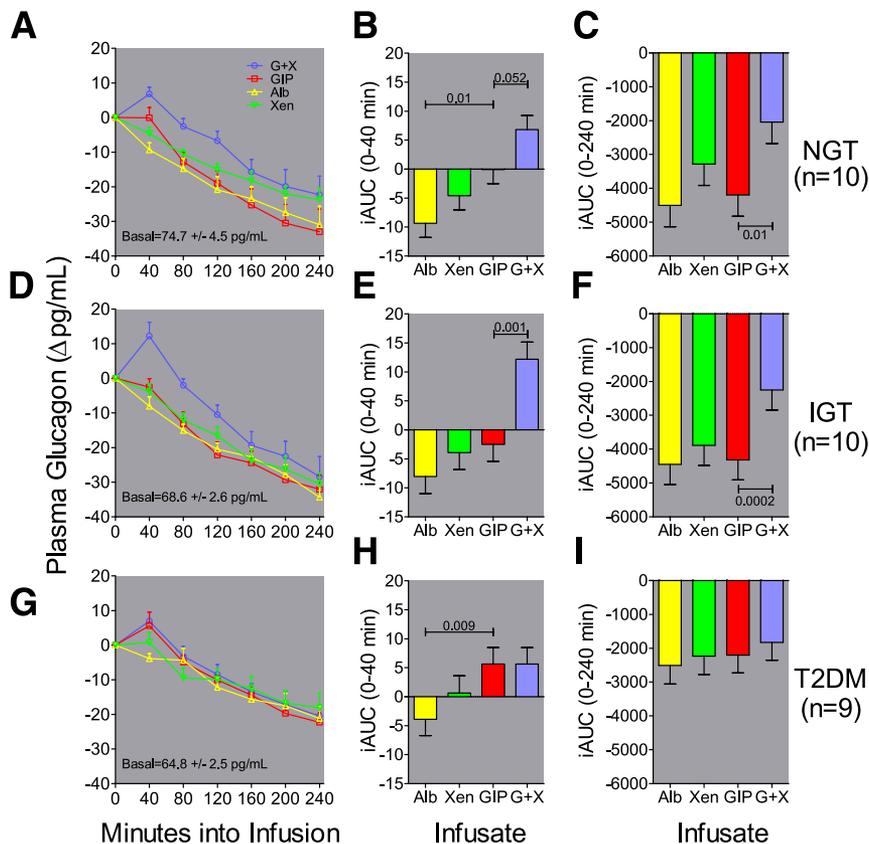
FIG. 5. Xenin-25 amplifies the effects of GIP on plasma glucagon levels in humans with NGT and IGT but not T2DM. *A*, *D*, and *G*: Group average changes in plasma glucagon ± SEM. Changes in plasma glucagon levels during the first 40 min (*B*, *E*, and *H*) or the entire 240 min (*C*, *F*, and *I*). Albumin alone, Alb; GIP alone, GIP; xenin-25 alone, Xen; combination of GIP plus xenin-25, G+X.

consistent with the idea proposed by Meier and Nauck (31) that humans with T2DM may retain the ability to respond to GIP, but reduced GIP responses in these subjects may reflect worsening insulin secretory responses to glucose rather than defective GIP signaling. This is further supported by the fact that the effects of GIP on blood pressure and heart rate were not blunted in humans with T2DM. Moreover, the effects of GIP on ISRs remained elevated even after this initial transient response, suggesting that like the cephalic response (32), this early response to GIP may play an important role in amplifying subsequent insulin secretion.

As with ISR, xenin-25 also amplified the early and transient GIP-mediated increase in glucagon levels in humans with NGT and IGT but not T2DM. GIP rapidly and transiently increased plasma glucagon levels in healthy humans when administered during euglycemic conditions (33) but not during hyperglycemic clamps (34). The very early postprandial glucagon response is thought to prevent hypoglycemia as cells recruit Glut4 and amino acid transporters to the cell surface in anticipation of increased levels of nutrients in the blood (35). Thus, the rapid and transient amplification of GIP-mediated glucagon release by xenin-25 may reflect a normal islet secretory response to nutrients after an overnight fast. These results also suggest that in humans with T2DM, islets exhibit a blunted response to xenin-25 signaling.

Mouse studies indicate that pharmacologic doses of xenin-25 increase GIP-mediated insulin release via a cholinergic relay requiring muscarinic receptors in the periphery (10), and M3 muscarinic receptors in islets are required for both insulin and glucagon release (36). Results in this study suggest that at the dose required to amplify GIP-mediated insulin secretion in humans, xenin-25 does not increase parasympathetic activity since it had no effect on heart rate, blood pressure, salivation, or need to urinate. Thus, an analogous xenin-25–mediated cholinergic relay is the most likely mechanism for increasing insulin and glucagon release in humans. It is worth noting that in contrast to a previous report (37), we have been unable to detect endogenous IR-xenin in either mouse or human plasma prepared under fasting, fed, or postprandial conditions using an ELISA that can detect <2 pmol/L peptide, raising the possibility that xenin-25 is not a physiologic peptide. Moreover, we have not seen other reports where endogenous xenin has been measured in plasma or purified K cells. Alternatively, endogenous xenin-25 may be modified such that it is not detected by our ELISA. Characterization of xenin-25 purified from plasma or isolated K cells would be required to address this issue. If xenin-25 is a physiologic peptide, it is unclear if local levels (e.g., at nerve endings near the K cell) would be high enough to modify islet function in vivo. However, our results with exogenously administered xenin-25 provide important insights into β-cell function during the progression from NGT to IGT to T2DM even if xenin-25 is not a physiologic peptide or if physiologic levels do not modify islet function. These results also suggest that defects in cholinergic or neuronal signaling in the periphery may contribute to defective insulin secretion in humans with T2DM. For example, xenin-25–responsive cells involved in this relay may

produce and/or release reduced amounts of acetylcholine, or islet endocrine cells may exhibit reduced sensitivity to acetylcholine. Alternatively, transmitters produced and released by xenin-25–responsive cells in humans with T2DM may be different from those in humans with NGT and IGT. Studies are underway to distinguish between these different mechanisms.

REFERENCES

1. Lovshin JA, Drucker DJ. Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 2009;5:262–269
2. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007;132:2131–2157
3. Brand SJ, Schmidt WE. Gastrointestinal hormones. In *Textbook of Gastroenterology*. 2nd ed. Yamada T, Ed. Philadelphia, JB Lippincott Company, 1995, p. 25–71
4. Walsh JH. Gastrointestinal hormones. In *Physiology of the Gastrointestinal Tract*. 3rd ed. Johnson LR, Ed. New York, Raven Press, 1994, p. 1–128
5. Fehmann HC, Göke R, Göke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 1995;16:390–410
6. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301–307
7. Elahi D, McAloon-Dyke M, Fukagawa NK, et al. The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects. Regul Pept 1994;51: 63–74
8. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese type II diabetic patients. Diabetologia 2002;45:1111–1119
9. Althage MC, Ford EL, Wang S, Tso P, Polonsky KS, Wice BM. Targeted ablation of glucose-dependent insulinotropic polypeptide-producing cells in transgenic mice reduces obesity and insulin resistance induced by a high fat diet. J Biol Chem 2008;283:18365–18376
10. Wice BM, Wang S, Crimmins DL, et al. Xenin-25 potentiates glucose-dependent insulinotropic polypeptide action via a novel cholinergic relay mechanism. J Biol Chem 2010;285:19842–19853
11. Feurle GE. Xenin—a review. Peptides 1998;19:609–615
12. Anlauf M, Weihe E, Hartschuh W, Hamscher G, Feurle GE. Localization of xenin-immunoreactive cells in the duodenal mucosa of humans and various mammals. J Histochem Cytochem 2000;48:1617–1626
13. Leiter EH, Reifsnyder PC. Differential levels of diabetogenic stress in two new mouse models of obesity and type 2 diabetes. Diabetes 2004;53(Suppl. 1): S4–S11
14. Reifsnyder PC, Leiter EH. Deconstructing and reconstructing obesity-induced diabetes (diabesity) in mice. Diabetes 2002;51:825–832
15. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197
16. McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 1976;41:565–573
17. Brooks DC, Black PR, Arcangeli MA, Aoki TT, Wilmore DW. The heated dorsal hand vein: an alternative arterial sampling site. JPEN J Parenter Enteral Nutr 1989;13:102–105
18. Feurle GE, Pfeiffer A, Schmidt T, Dominguez-Munoz E, Malfertheiner P, Hamscher G. Phase III of the migrating motor complex: associated with endogenous xenin plasma peaks and induced by exogenous xenin. Neurogastroenterol Motil 2001;13:237–246
19. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377
20. Sparacino G, Cobelli C. A stochastic deconvolution method to reconstruct insulin secretion rate after a glucose stimulus. IEEE Trans Biomed Eng 1996;43:512–529
21. Sparacino G, Pillonetto G, Capello M, De Nicolao G, Cobelli C. WINSTODEC: a stochastic deconvolution interactive program for physiological and pharmacokinetic systems. Comput Methods Programs Biomed 2002;67:67–77
22. Nustede R, Schmidt WE, Horstmann O, Sikovec N, Schemminger R, Becker H. On the effect of xenin and xenin fragments on exocrine pancreas secretion in vivo. Regul Pept 1999;81:61–66
23. Kim ER, Mizuno TM. Role of neurotensin receptor 1 in the regulation of food intake by neuromedins and neuromedin-related peptides. Neurosci Lett 2010;468:64–67
24. Feurle GE, Klein A, Hamscher G, Metzger JW, Schuurkes JA. Neurokinetic and myokinetic effects of the peptide xenin on the motility of the small and large intestine of guinea pig. J Pharmacol Exp Ther 1996;278:654–661
25. Feurle GE, Metzger JW, Grudinki A, Hamscher G. Interaction of xenin with the neurotensin receptor of guinea pig enteral smooth muscles. Peptides 2002;23:1519–1525
26. Clemens A, Katsoulis S, Nustede R, et al. Relaxant effect of xenin on rat ileum is mediated by apamin-sensitive neurotensin-type receptors. Am J Physiol 1997;272:G190–G196
27. Pettibone DJ, Hess JF, Hey PJ, et al. The effects of deleting the mouse neurotensin receptor NTR1 on central and peripheral responses to neurotensin. J Pharmacol Exp Ther 2002;300:305–313
28. Blackburn AM, Fletcher DR, Adrian TE, Bloom SR. Neurotensin infusion in man: pharmacokinetics and effect on gastrointestinal and pituitary hormones. J Clin Endocrinol Metab 1980;51:1257–1261
29. Meier JJ, Gallwitz B, Kask B, et al. Stimulation of insulin secretion by intravenous bolus injection and continuous infusion of gastric inhibitory polypeptide in patients with type 2 diabetes and healthy control subjects. Diabetes 2004;53(Suppl. 3):S220–S224
30. Meier JJ, Nauck MA, Siepmann N, et al. Similar insulin secretory response to a gastric inhibitory polypeptide bolus injection at euglycemia in first-degree relatives of patients with type 2 diabetes and control subjects. Metabolism 2003;52:1579–1585
31. Meier JJ, Nauck MA. Is the diminished incretin effect in type 2 diabetes just an epi-phenomenon of impaired beta-cell function? Diabetes 2010;59:1117–1125

32. Ahrén B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes 2001;50:1030–1038

33. Meier JJ, Gallwitz B, Siepmann N, et al. Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. Diabetologia 2003;46:798–801

34. Elahi D, Andersen DK, Brown JC, et al. Pancreatic alpha- and beta-cell responses to GIP infusion in normal man. Am J Physiol 1979;237:E185–E191

35. Young A. Inhibition of glucagon secretion. Adv Pharmacol 2005;52:151–171

36. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. Diabetes 2004;53:1714–1720

37. Feurle GE, Ikonomu S, Partoulas G, Stoschus B, Hamscher G. Xenin plasma concentrations during modified sham feeding and during meals of different composition demonstrated by radioimmunoassay and chromatography. Regul Pept 2003;111:153–159