Effect of endogenous GLP-1 on insulin secretion in type 2 diabetes

Short title: Endogenous GLP-1 action in type 2 diabetes

Marzieh Salehi¹, Ronald L. Prigeon², Benedict Aulinger¹, David A. D’Alessio¹

1 University of Cincinnati, Department of Internal Medicine, Cincinnati, OH.
2 University of Maryland, Department of Medicine, Division of Gerontology, Baltimore, MD

Corresponding author:
Marzieh Salehi, MD
salehim@uc.edu

Submitted 26 August 2009 and accepted 24 February 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
Objectives: The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) account for up to 60% of postprandial insulin release in healthy people. Previous studies showed a reduced incretin effect in patients with type 2 diabetes (T2DM) but a robust response to exogenous GLP-1. The primary goal of this study was to determine whether endogenous GLP-1 regulates insulin secretion in T2DM.

Methods: Twelve patients with well controlled T2DM (DM) and eight matched nondiabetic subjects (NDM) consumed a breakfast meal containing d-xylose during fixed hyperglycemia at 5 mmol/l above fasting levels. Studies were repeated, once with infusion of the GLP-1 receptor antagonist, exendin-(9-39) (Ex-9), and once with a saline.

Results: The relative increase in insulin secretion after meal ingestion was comparable in DM and NDM groups (44±4% vs. 47±7%). Blocking the action of GLP-1 suppressed postprandial insulin secretion similarly in the DM and NDM subjects (25±4% vs. 27±8%). However, Ex-9 also reduced the insulin response to IV glucose (25±5% vs. 26±7%; DM vs. NDM), when plasma GLP-1 levels were undetectable. The appearance of postprandial ingested d-xylose in the blood was not affected by Ex-9.

Conclusions: These findings indicate that in patients with well controlled diabetes the relative effects of enteral stimuli and endogenous GLP-1 to enhance insulin release is retained and comparable to NDM subjects. Surprisingly, GLP-1 receptor signaling promotes glucose-stimulated insulin secretion independent of the mode of glucose entry. Based on rates of d-xylose absorption, GLP-1 receptor blockade did not affect gastric emptying of a solid meal.
Glucagon-like peptide 1 (GLP-1) is a gut-brain peptide that is a major component of the incretin effect, and is essential for normal glucose tolerance (1). Based on studies in which synthetic GLP-1, or GLP-1 receptor (GLP-1r) agonists, are administered to humans, GLP-1 has a broad range of actions that promote glucose homeostasis, including stimulation of insulin secretion (2), suppression of glucagon release (3-4), delayed gastric emptying (5), and increasing hepatic glucose balance (6-7). Importantly, and unlike other insulinotropic gut peptides, the effects of GLP-1 on glucose metabolism are retained in persons with diabetes (8-10). This has led to the development of novel therapeutic compounds for use in diabetic patients that are based on GLP-1r signaling (11).

The physiologic role of GLP-1 in persons with diabetes has not been determined. However, there are several reasons to question whether the GLP-1 system is fully functional in this patient group. First, there is some evidence that GLP-1 secretion in response to meal ingestion in T2DM is impaired (12-15), although this finding has not been uniform (16-17). Second, the sensitivity of insulin secretion to exogenous GLP-1 is reduced in diabetic individuals (18). Finally, it has long been believed that the augmentation of glucose-stimulated insulin secretion during enteral glucose absorption, the incretin effect, is severely attenuated in T2DM, implying that incretins like GLP-1 are not normally active in this group of subjects.

In this study we tested the hypothesis that the effect of endogenous GLP-1 to promote insulin secretion after meal ingestion is reduced in persons with diabetes. Diabetic and age-and weight-matched nondiabetic subjects were studied with and without infusion of the specific GLP-1r antagonist, exendin-(9-39) (Ex-9), during fixed hyperglycemia before and after a breakfast meal.

**SUBJECTS AND METHODS:**

**Subjects:** Twelve subjects with established T2DM (DM, 5 females and 7 males) and 8 age- and BMI-matched nondiabetic subjects (NDM, 6 females and 2 males) were studied on two separate days (Table 1). All subjects were weight stable for three months prior to the experiments. Diabetic patients had good glycemic control with a mean HbA1c (A1C) level of 6.3 ± 0.1% (range 5.9-7.6%). Normal glucose tolerance was confirmed in the non-diabetic subjects by a 2-hour venous plasma glucose level of <7.8 mmol/l following a 75 gram oral glucose tolerance test (OGTT). The control subjects had no family history of type 2 diabetes mellitus, were free of any chronic medical conditions such as coronary artery disease, uncontrolled dyslipidemia or hypertension, and received no medications for any of these conditions. The studies were approved by the institutional review board of the University of Cincinnati, and all participants provided written informed consent prior to the studies.

**Peptides:** Synthetic exendin-(9-39) (Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) was greater than 95% pure, sterile and free of pyrogens. Lyophilized peptide was prepared in 0.25% human serum albumin on the day of study. The use of synthetic Ex-9 is approved under the U.S. Food and Drug Administration IND 65,837.

**Experimental procedures:** Subjects were instructed to consume greater than 200 g of carbohydrate for 3 days before each visit and not to engage in vigorous physical activity. Subjects with diabetes withheld their oral antidiabetic medication for 3 days before each study. They were admitted to the
General Clinical Research Center at Cincinnati Children’s Hospital on separate occasions after an overnight fast. Intravenous catheters were placed in each forearm for the withdrawal of blood and the infusion of glucose and Ex-9; the arm used for blood sampling was continuously warmed using a heating pad to arterialize the venous blood. After removal of fasting blood samples, a primed continuous infusion of 20% glucose was started to achieve and maintain a target blood glucose concentration of 5 mmol/L greater than fasting levels (19). At 30 minutes subjects received either a) an IV bolus of synthetic Ex-9 (7500 pmol/kg) over one minute followed by a continuous infusion (750 pmol/kg/min) for the remainder of the study, or b) saline, as a control (19). The order of the infusions was balanced so that half the subjects received saline or Ex-9 as their first infusion, and the two experiments were separated by an interval of at least one week. At 90 minutes, subjects consumed a mixed nutrient breakfast (300 kcal with a calorie distribution of 40% carbohydrate, 20% protein, and 40% fat) consisting of scrambled eggs, English muffin, margarine, chocolate pudding mixed with 10 g d-xylose, and milk that was eaten within 10 min. Subjects were instructed to consume the pudding in the middle of the meal. The rate of IV glucose infusion was adjusted to maintain the blood glucose at the target rate throughout the meal and over the remainder of the study. Blood samples were drawn at -10, -5, 0, 15, 30, 45, 60, 70, 75, 80, 85, 90, 95,100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 255, 260, 265, and 270 min, the plasma was separated within 60 minutes of blood withdrawal, and stored at -80º C until assay. Blood samples were collected in tubes containing heparin, 50 mM EDTA, and 500 kallikrein inhibitory units/ml aprotinin.

Assays: Blood glucose concentrations were determined by a glucose oxidase method using a glucose analyzer (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH). Insulin, glucagon, total GLP-1 and C-peptide were measured by radioimmunoassays as described previously (19). D-xylose was measured by colorimetric assay (20), and total GIP was measured by ELISA (Linco Research, St. Charles, MO).

Calculations and analysis: Insulin secretion rates (ISR) were derived from plasma C-peptide concentrations using deconvolution with population estimates of C-peptide clearance (21-22). Fasting concentrations of blood glucose, hormones and ISR were taken as the mean of the three samples drawn at -10, -5 and 0 minutes. Preprandial insulin secretory responses were computed as the mean increments above fasting values of ISR from 60 to 90 minutes; these were used to determine the responses to IV glucose-induced hyperglycemia. Postprandial insulin secretory responses were calculated as the mean increments above the 60-90 minute values of ISR from 95 to 270 minutes. This measure of postprandial insulin secretion reflects the augmentation of ISR, beyond 5 mmol/L hyperglycemia, by meal-induced stimuli, and has been used previously as a surrogate for the incretin effect (23-25). The d-xylose, GLP-1, and GIP concentrations from 80-90 minutes were taken as baseline, and levels following meal consumption (95-270 min for d-xylose and GLP-1; 95-240 min for GIP) were used to compute postprandial areas under the curve (AUC) above baseline. The glucagon levels from 80-90 min (preprandial) were compared with fasting as well as values of glucagon from 250-260 minutes. The stability of the glucose clamps were computed as the mean of coefficients of variation for each study from 60-270 minutes. The reproducibility of the clamps were computed as the difference in mean glucose from 60-270 for each subject.

The contribution of endogenous GLP-1 to insulin secretion was determined by
comparing the mean values of ISR for each subject during infusion of saline or Ex-9. Separate comparisons were made for the preprandial and postprandial periods.

The parameters obtained from each subject in the two studies, and from the diabetic and non-diabetic groups were compared using one and two-way ANOVA with repeated measures. Spearman correlation was used to seek relationships between measured outcomes and subject characteristics such as A1C, and paired t-test to compare measured variables within each study. Data are presented as the mean ± SEM.

RESULTS:
Fasting glucose was significantly higher in the DM subjects than in the NDM group (p < 0.001), but fasting insulin and C-peptide concentrations were comparable, and the values for each subject were similar on the days of the saline and Ex-9 studies (Table 2). Blood glucose concentrations were raised by ~5 mmol/L from fasting values to hyperglycemic levels from 60-270 minutes that were comparable during the Ex-9 and saline studies (Fig.1, Table 2). The average clamped glucose levels (60-270 min) for NDM subjects during the saline and Ex-9 studies were 9.3±0.2 and 9.3±0.2 mmol/l, respectively, with a mean difference of 0.02±0.03 mmol/l. For the DM subjects the mean glucose concentrations (60-270 min) were 12.0±0.4 and 12.1±0.4 mmol/l for the control and Ex-9 clamps, and the mean difference 0.09±0.07 mmol/l. The average coefficient of variation of the glucose concentrations during the hyperglycemic clamp control studies was 4.4±0.4% for the NDM and 4.9±0.5% for the DM subjects.

The glucose infusion rates needed to reach target glycemia were higher in the NDM compared to DM subjects during the preprandial (60-90 min; p<0.01) and postprandial (95-270 min; p <0.05) periods. Infusion of Ex-9 was associated with a significant reduction of the glucose infusion rates necessary to maintain target glycemia from 60-90 min in both groups (p<0.05 for both comparisons). Following ingestion of the breakfast meal, the glucose infusion rate was initially reduced in some subjects to compensate for glucose influx from the gut, but was significantly higher than preprandial infusions by the end of the study (Fig.1, Table 2).

In both the DM and NDM groups, β-cell secretion rose in response to intravenous glucose administration to a plateau from 60-90 minutes (Fig.2, Table 2); preprandial insulin, C-peptide and ISR were greater in the NDM than the DM group (p<0.05). Among the DM subjects, there were significant inverse correlations between the preprandial insulin secretory response and fasting glucose (r = -0.7, p<0.01), and A1C (r = -0.8, p<0.05). Compared to the study with saline infusion, GLP-1r blockade with Ex-9 caused a significant reduction of insulin secretion before meal ingestion (60-90 min) as indicated by significantly lower mean values of insulin, C-peptide and ISR (Table 2). The GLP-1 effect on IV glucose-stimulated insulin secretion, taken as the percent difference in mean ISR from 60-90 minutes with and without Ex-9, was 25±5% in DM subjects, and 26±7% in the NDM group (Fig. 3).

Following meal consumption (95-270 min), β-cell secretion increased significantly over preprandial (60-90 min) values in both the NDM (p<0.01) and DM (p<0.05) subjects; although the absolute responses were significantly lower in the DM group (p < 0.05; Fig.2, Table 2). Among the DM subjects, there were significant inverse correlations between postprandial insulin secretory responses and A1C (r = -0.6, p<0.05). During the saline infusion study, meal ingestion significantly enhanced ISR, by 47±7% in the NDM and 44 ± 4% for DM subjects, above preprandial ISR. During the Ex-9 infusion, postprandial ISR was 44±6%
and 45±4% higher than preprandial ISR in the NDM and DM groups, respectively. Blocking endogenous GLP-1 with Ex-9 diminished postprandial ISR by 27±8 in NDM and 25±4% in DM subjects, compared to the studies with saline infusion (Fig. 3).

Fasting glucagon concentrations in the DM subjects were similar during the saline and Ex-9 studies (Table 2). During both the saline and Ex-9 infusions, the hyperglycemic clamp suppressed glucagon levels from 80-90 minutes (p<0.05). Glucagon values at the conclusion of the experiments remained suppressed during the saline infusion, but were significantly higher when Ex-9 was given (p < 0.05). In NDM subjects, plasma glucagon concentrations decreased slightly, but not significantly, between the fasting and preprandial periods, and did not change further after the meal, with either saline or Ex-9.

Plasma concentrations of d-xylose increased steadily after meal intake and peaked at 60 minutes after meal consumption in both diabetic and non-diabetic subjects during both their saline and Ex-9 studies (Fig.4). The area under plasma d-xylose curve (AUC d-xylose) in the studies with saline and Ex-9 infusion were 105±8.2 and 102±9.1 mmol/l·min in DM, and 71±8.5 and 70±8 mmol/l·min in NDM, respectively indicating that GLP-1 receptor blockade had minimal effects on the passage of d-xylose from the stomach to the intestine. D-xylose AUC was significantly greater in the DM compared to NDM group (p < 0.05).

Fasting plasma GIP levels were comparable before meal ingestion in the studies with saline and Ex-9 infusion in the DM (16±1 and 16±2 pmol/l) and NDM (20±1 and 20±2 pmol/l) subjects. In both the NDM and DM groups, meal consumption caused a similar rise of GIP concentrations in the saline and Ex-9 studies (Fig.4, Table 2), suggesting that GLP-1r blockade had no effect on GIP secretion. The GIP response was significantly greater in the DM compared to NDM group (p < 0.05). Fasting plasma GLP-1 were not different in the DM (3.6±0.3 and 3.6±0.2 pmol/l) and NDM (3.1±0.1 and 3.0±0.0 pmol/l) groups, during the saline and Ex-9 studies, but levels were undetectable in ~80% of the subjects. Plasma GLP-1 increased following meal ingestion in both groups (Fig.4, Table 2), and Ex-9 infusion significantly increased these responses in the NDM and DM subjects (p<0.05).

Subjects tolerated the experiments without notable problems and there were no adverse events associated with Ex-9 infusion.

DISCUSSION:

The current study investigated the role of endogenous GLP-1 on islet hormone secretion and gastric emptying in patients with T2DM. Although previous studies have demonstrated that diabetic patients respond to pharmacologic amounts of GLP-1, the effect of endogenous GLP-1 has not been previously demonstrated. Our results indicate that in diabetic subjects with good glycemic control, there is an enhancement of glucose-stimulated insulin secretion following meal ingestion that is similar on a relative basis to nondiabetic individuals. Moreover, the impact of GLP-1r blockade was also comparable in the groups, indicating that endogenous GLP-1 contributes significantly to postprandial insulin secretion in diabetic subjects. Surprisingly, GLP-1r blockade reduced insulin secretion in response to IV glucose alone and the magnitude of this effect was as great as that on postprandial β-cell responses. Gastric emptying, as reflected by d-xylose uptake, was not affected by blocking GLP-1r. These findings support an important physiologic role for GLP-1 to amplify glucose-stimulated insulin secretion, and demonstrate that this effect is retained in at least a subset of T2DM patients.

Our group and others (4; 19; 26-27) have previously used Ex-9 to demonstrate the
endogenous GLP-1 signaling in human subjects. In our previous report we demonstrated that doses of Ex-9 identical to those used in the present study were effective for blocking supraphysiologic infusions of GLP-1 nearly completely, and for reducing postprandial insulin secretion by ~30%. While no one has established definitively a dose of Ex-9 beyond which no further inhibition of GLP-1 action can be detected, based on previously published results (4; 19; 26) we believe that the dose used in this study was near maximal for what can be achieved in an acute infusion.

The classic method for determining the incretin effect is a two-day method with an oral glucose tolerance test on day 1 followed on day 2 by an IV glucose infusion isoglycemic to arterialized venous glucose levels after glucose ingestion (28). Using this method Nauck and colleagues demonstrated a severe impairment of the incretin effect in subjects with type 2 diabetes (29), a much cited finding that has been very influential in shaping the understanding of enteroinsular physiology. We elected to use an alternative method, the hyperglycemic clamp-meal test, as a means of comparing the relative increase in insulin secretion when carbohydrate is absorbed enterally, because it can be performed in a single morning, reducing day-to-day variability and the time commitment of research subjects. Variations of this method have been used previously by a number of investigators (23-25). Our preliminary data for normal glucose-tolerant subjects demonstrate that the estimation of the incretin effect using the one-day and two-day methods is comparable with a strong and significant within subject correlation (30). In the present study we used a mixed nutrient meal as the enteral stimulus during the hyperglycemic clamp to assess the nutrient, incretin and neural responses activated by typical food consumption. We targeted the clamp as 5 mmol/L above fasting glucose to achieve levels that were slightly greater than what we expected as a result of meal ingestion alone. Overall, this design permitted a measure of the enhancement of insulin secretion by meal ingestion, and the GLP-1 component of it. Without further validation this measure cannot be equated to the classically derived incretin effect, although we believe that the two methods assess similar physiologic processes.

In contrast to what was described originally by Nauck (29), and confirmed in more recent studies (13), the cohort of DM subjects described here had a comparable degree of meal enhancement of insulin secretion to the NDM group. While β-cell secretion was clearly abnormal in DM compared to the NDM subjects, relative to the ISR or plasma insulin levels achieved during stimulation with IV glucose alone, meal ingestion caused equivalent augmentation in the DM and NDM groups (Table 2). This finding is consistent with the results reported by Perley and Kipnis more than 3 decades ago (31) showing that obese subjects with mild T2DM had preserved stimulation of insulin secretion by alimentary factors relative to nondiabetic controls. In contrast to the findings of Nauck (29), the plasma GIP responses were increased in our DM compared to NDM subjects. Increased GIP secretion has been reported previously in some studies of diabetic subjects (reviewed in (32)). While an increased effect of GIP could account for a bigger augmentation of insulin secretion by meal ingestion in our DM subjects, most previous studies indicate that GIP has very modest effects on insulin secretion in T2DM (8).

Just as the relative increase of ISR with meal ingestion were similar in the diabetic and nondiabetic subjects, so too was the contribution of GLP-1 action to postprandial β-cell responses. Based on the effects of Ex-9, GLP-1 accounted for ~25% of the insulin response to the mixed meal at a
fixed level of hyperglycemia. This is consistent with what we reported recently as the GLP-1 effect in healthy lean subjects during an OGTT (19). The finding of intact effects of GLP-1 in the diabetic group was unexpected in that previous work had indicated that T2DM subjects have a blunted response to exogenous GLP-1 infused to supraphysiologic levels (18). While there was a weak trend towards the diabetic group having a greater postprandial GLP-1 response, there was considerable heterogeneity in the AUC among the subjects and no correlation between this measure and insulin secretion or the magnitude of insulin suppression by GLP-1 receptor blockade. These results suggest that circulating GLP-1 may not be a good predictor of the physiologic response to endogenous peptide.

The most surprising finding in this study was that Ex-9 diminished insulin secretion in response to IV-induced hyperglycemia before meal consumption, at a time when circulatory levels of GLP-1 were often undetectable. In fact, the relative effect of GLP-1r blockade on preprandial and postprandial ISR were nearly identical. These findings indicate that GLP-1r effects on the β-cell are not restricted to the potentiation of insulin secretion after enteral nutrient absorption when circulating levels of GLP-1 increase. It is important to note that in previous studies blockade of the GLP-1 receptor did not affect fasting plasma insulin levels (4; 27; 33), but that Schirra and colleagues noted a reduction in plasma insulin, similar to what we describe here, during a hyperglycemic clamp when Ex-9 was infused (27). Thus, when considered together the current evidence supports a role for GLP-1r signaling to potentiate glucose-stimulated but not basal insulin secretion in humans. This is consistent with studies in mice with engineered deletion of the GLP-1r gene where similar degrees of glucose intolerance have been described to both oral and intraperitoneal glucose challenges (34), conditions with elevated and basal plasma GLP-1 levels, respectively. The mechanism whereby Ex-9 affects insulin secretion in the absence of elevated circulating levels of GLP-1 is unclear. However, recent studies raise the possibility of neural (35-36) and paracrine modes of GLP-1 signaling (37), either of which could be affected by GLP-1r blockade. In addition, in vitro work suggests that Ex-9 can act as an inverse agonist in isolated mouse islets (38). Overall, our findings fit with an expanded model of β-cell regulation by GLP-1 that stretches beyond endocrine effects after eating.

Although several investigators have suggested that the predominant effect of GLP-1 on glucose control is based on its effect on gastric emptying (39-40), this conjecture is based on the results of studies using exogenous administration of GLP-1. Given the identical pattern of postprandial plasma d-xylose in our subjects with and without Ex-9, endogenous GLP-1 has no detectable effect on the rate of passage of nutrients from the stomach to the intestine. Appearance of ingested d-xylose followed a similar time-course in DM and NDM subjects, but d-xylose reached higher concentrations in the diabetic group, suggesting more rapid gastric emptying in patients with early or mild diabetes, compatible with previous reports (41-42). The lack of effect of Ex-9 to alter d-xylose appearance is similar to what we have reported previously in studies with GLP-1 blockade in healthy humans and nonhuman primates given liquid glucose solution (19; 33). We have extended these findings in this study by using a solid meal, and our data stand against an important physiologic role for GLP-1 in the regulation of prandial gastric motility in humans. However, Deane and colleagues have recently published evidence that endogenous GLP-1 delays gastric emptying in healthy subjects following a solid carbohydrate meal (43). Since these
investigators used scintigraphy, the gold standard for measuring gastric emptying, our findings must be interpreted cautiously. Nonetheless, our results are consistent with a recent report that gastric emptying in diabetic patients was not affected by administration of a dipeptidyl peptidase-4 inhibitor that enhanced endogenous GLP-1 by three-fold (44).

In previous studies we and others (4; 19) have found that infusion of Ex-9 during GI glucose administration increases postprandial plasma glucagon, supporting an important role for GLP-1 to regulate the α-cell. This effect was seen here, but only in the diabetic group. The diabetic subjects had equivalent suppression of glucagon in response to IV glucose during the Ex-9 and control experiments, but after the meal there was a significant rise in plasma levels when the GLP-1r was blocked. Interestingly we did not see this latter effect in the nondiabetic subjects. While this outcome could indicate differential regulation of prandial α-cell secretion in diabetes, this conclusion needs more rigorous confirmation.

In summary, we report herein that administration of Ex-9 reduces insulin secretion proportionately in response to IV glucose and enteral stimuli, and that this effect is similar in diabetic and nondiabetic subjects. These findings indicate that the effect of endogenous GLP-1 on postprandial insulin release is preserved in patients with well controlled T2DM. Moreover, GLP-1r signaling is important for stimulated insulin secretion independent of the mode of glucose entry. Based on these results it appears that the function of the enteroinsular axis is intact, at least in some persons with T2DM, and that the role of endogenous GLP-1 to regulate islet function is not mediated entirely by an endocrine mechanism.

ACKNOWLEDGEMENTS

We thank Kay Ellis, Clinton Elfers, Ron Bitner for their technical support and Suzanne Summers, RD for assistance with the design and preparation of test meals. We also thank the nursing staff from Clinical Research Center of Cincinnati Children’s Hospital for their expert technical assistance. These studies were supported by grants from the National Institutes of Health, DK 57900 (D.A.D.) and M01-RR-08084 (Cincinnati Children’s Hospital General Clinical Research Center), and the Medical Research Service of the Department of the Veterans Administration.
REFERENCES
Endogenous GLP-1 action in type 2 diabetes


<table>
<thead>
<tr>
<th>Table 1. Characteristics of the study participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with T2DM (DM)</td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
</tr>
<tr>
<td>Duration of diabetes (month)</td>
</tr>
<tr>
<td>Sulfonylurea /metformin/diet</td>
</tr>
</tbody>
</table>

Data are presented as Mean (range) unless otherwise noted.
Table 2. Effect of meal ingestion during hyperglycemic clamp on beta cell hormonal response and gastrointestinal peptides in studies with and without intravenous Ex-9 in subjects with and without diabetes.

<table>
<thead>
<tr>
<th>Time interval (min)</th>
<th>DM</th>
<th>NDM</th>
<th>Statistical effects (p-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>6.7±0.3 6.5±0.3</td>
<td>4.8±0.2 4.9±0.1</td>
<td>NS &lt;0.001 NS</td>
</tr>
<tr>
<td>60-90</td>
<td>12.3±0.3 12.2±0.3</td>
<td>9.3±0.2 9.5±0.2</td>
<td>NS &lt;0.001 NS</td>
</tr>
<tr>
<td>95-270</td>
<td>12.0±0.4 12.1±0.4</td>
<td>9.3±0.2 9.3±0.2</td>
<td>NS &lt;0.001 NS</td>
</tr>
<tr>
<td>GINF (mg/kg/min)</td>
<td>3.9±0.4 3.5±0.4</td>
<td>6.0±0.5 5±0.5</td>
<td>&lt;0.05 &lt;0.01 NS</td>
</tr>
<tr>
<td>95-270</td>
<td>6.1±1.6 4.6±1.4</td>
<td>11.5±0.7 9.6±0.9</td>
<td>&lt;0.01 &lt;0.05 NS</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>128±22 142±28</td>
<td>111±20 116±21</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>60-90</td>
<td>363±69 207±32</td>
<td>719±134 490±107</td>
<td>&lt;0.001 &lt;0.01 NS</td>
</tr>
<tr>
<td>95-270</td>
<td>894±220 577±94</td>
<td>2243±365 1531±287</td>
<td>&lt;0.001 &lt;0.01 &lt;0.05</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>1.2±0.1 1.0±0.1</td>
<td>0.9±0.1 0.9±0.2</td>
<td>0.09 NS NS</td>
</tr>
<tr>
<td>60-90</td>
<td>2.1±0.3 1.8±0.2</td>
<td>3.2±0.4 2.6±0.4</td>
<td>&lt;0.001 &lt;0.05 NS</td>
</tr>
<tr>
<td>95-270</td>
<td>4.6±1.1 3.4±0.6</td>
<td>7.6±0.9 5.9±1.1</td>
<td>&lt;0.01 &lt;0.05 NS</td>
</tr>
<tr>
<td>ISR (nmol/min)</td>
<td>0.34±0.03 0.35±0.04</td>
<td>0.47±0.11 0.49±0.13</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>60-90</td>
<td>0.71±0.1 0.53±0.08</td>
<td>1.20±0.17 0.95±0.2</td>
<td>&lt;0.001 &lt;0.05 NS</td>
</tr>
<tr>
<td>95-270</td>
<td>1.43±0.32 1.03±0.18</td>
<td>2.42±0.32 1.83±0.36</td>
<td>&lt;0.01 &lt;0.05 NS</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>48±4 49±4</td>
<td>46±4 51±5</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>80-90</td>
<td>41±3 41±4</td>
<td>43±2 42±3</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>250-260</td>
<td>43±4 58±8</td>
<td>40±4 41±5</td>
<td>&lt;0.05 NS 0.06</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>3.6±0.3 3.6±0.2</td>
<td>3.1±0.1 3.0±0.0</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>AUC GLP-1 (pmol/l·min)</td>
<td>438±92 1423±340</td>
<td>154±73 560±230</td>
<td>&lt;0.05 0.08 NS</td>
</tr>
<tr>
<td>GIP (µmol/l)</td>
<td>0.07±0.0 0.06±0.0</td>
<td>0.05±0.0 0.04±0.0</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>AUC GIP (µmol/l·min)</td>
<td>12.7±1.4 17.1±1.4</td>
<td>7.9±1.9 7.7±2.3</td>
<td>&lt;0.05 NS NS</td>
</tr>
</tbody>
</table>
Figure Legends

Fig 1. Blood glucose concentrations, and glucose infusion rates during intravenous-meal clamps with Ex-9 (dashed line, open symbols) or saline infusions (solid line, closed symbols) in NDM (left) and DM (right) subjects. Data presented as mean ± SEM.

Fig 2. β-cell secretion in response to hyperglycemia and meal ingestion with (dashed line, open symbols) and without Ex-9 infusion (solid line, closed symbols). A) plasma insulin concentrations, and B) insulin secretion rates for NDM (left) and DM (right) subjects. Data presented as mean ± SEM.

Fig 3. The contribution of GLP-1 to preprandial and postprandial insulin secretion. The percentage reduction of insulin secretion rates by Ex-9 is shown for the preprandial (60-90 min) and postprandial (95-270 min) periods in the NDM (black bars) and DM (grey bars) groups. Data presented as mean ± SEM.

Fig 4. Pre and postprandial concentrations of A) d-xylose, B) GIP and C) GLP-1 in NDM (left) and DM (right) subjects. Data presented as mean ± SEM.
Figure 1

- Blood Glucose (mmol/l) vs. Time (min)
  - NDM: Exendin-(9-39) or saline infusion
  - DM: Saline or Ex-9

- Glucose Infusion Rate (mg/kg/min) vs. Time (min)
  - NDM: IV glucose infusion at variable rates
  - DM: IV glucose infusion at variable rates
Figure 2

![Graph](image)

Figure 3

![Bar chart](image)
Figure 4

A. NDM

B. GIP

C. GLP-1