Acute metabolic effects of exenatide in patients with Type 1 Diabetes with and without residual insulin to oral and IV glucose challenges

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Abstract:

**Objective:** Glucagon-like peptide-1 (GLP-1) is an incretin hormone, released from the GI-tract. Treatment with GLP-1 analogs has proven to be of clinical use for patients with Type 2 diabetes. Patients with Type 1 diabetes, particularly those with residual β-cell function, may also respond to treatment, but the acute metabolic effects of GLP-1 analogs in these patients to both oral and IV glucose challenges are not well understood.

**Research Design and Methods:** Seventeen patients with type 1 diabetes, half of whom had residual insulin production, underwent two mixed meal tolerance tests (MMTT) and two intravenous glucose tolerance tests (IVGTT), with and without pretreatment with exenatide. No exogenous bolus insulin was administered for the studies. Glucose excursions, insulin secretion rates (ISR), glucagon, endogenous GLP-1, and GIP levels were measured following the meal or glucose loads.

**Results:** During the MMTT, glucose levels were suppressed with exenatide in patients with or without residual insulin production (p=0.0003). Exenatide treatment did not change the absolute ISR but the ISR to glucose levels were increased (p=0.0078). Gastric emptying was delayed (p=0.0017) and glucagon was suppressed (p=0.0015). None of these hormonal or changes in glucose were detected during the IVGTT with exenatide administration.

**Conclusion:** Exenatide showed a significant antidiabetogenic effect prior to an oral meal in patients with type 1 diabetes involving glucagon suppression and gastric emptying, while preserving increased insulin secretion. GLP-1 analogs may be useful as an adjunctive treatment in Type 1 diabetes.
Glucagon-like peptide-1 (GLP-1) is an incretin secreted from the L-cells of the GI-tract in response to nutrient ingestion. In healthy control subjects, its physiologic effects control glucose levels by stimulating glucose-dependent insulin secretion, inhibiting glucagon secretion and delaying gastric emptying (1). GLP-1 analogs have been developed to mimic the incretin response (2, 3). Extensive studies of the mechanisms of GLP-1 analogs in patients with type 2 diabetes have confirmed their physiologic actions (4, 5). These drugs as well as DPPIV inhibitors, such as sitagliptin, saxagliptin, linagliptin, alogliptin and vildagliptin, which inhibit the degradation of GLP-1, are routinely used for treatment of this form of diabetes (6, 7).

GLP-1 analogs may also have a role in treatment of type 1 diabetes. Brown et al have demonstrated a progressive rise in meal-stimulated glucagon response associated with declining endogenous insulin production (8). GLP-1 analogs have been shown to inhibit glucagon levels and therefore insulin deficient individuals with type 1 diabetes may show a beneficial response on the basis of reduced glucagon secretion. Moreover, animal studies have suggested that GLP-1 therapy may promote proliferation of β-cells, enhance β-cell recovery and suppress β-cell apoptosis (9, 10), suggesting there may be long term primary benefit to its use.

In patients with type 2 diabetes GLP-1 analogs have been shown to augment glucose dependent insulin secretion (11, 12), but the significance of this action is not clear since detailed analyses of GLP-1 receptor agonists in patients with residual insulin production are limited. While older studies have highlighted the progression of type 1 diabetes to complete insulin deficiency, more recent studies have identified subjects with long standing type 1 diabetes with residual insulin production (13, 14). In these subjects GLP-1 analogs may improve glucose control and reduce the need for exogenous insulin (15) since there may be a significant
functional component to the loss of insulin secretion, possibly due to β-cell exhaustion from hyperglycemia (16). To assess whether combination therapies aimed at promoting β-cell growth in addition to agents that decrease the autoimmune destruction of β-cells, 20 subjects with long-standing type 1 diabetes were enrolled in a trial and randomized to exenatide with or without daclizumab (17). While C-peptide secretion increased with exenatide treatment, the difference failed to reach statistical significance. Other studies suggest that residual insulin production is not a significant contributor to the effects of GLP-1 receptor agonists. Infusion of GLP-1 has been shown to reduce postprandial glycemic excursions in half in subjects with type 1 diabetes regardless of residual endogenous insulin production (18). Furthermore, GLP-1 infusion was found to delay gastric emptying. However, this study was conducted with infusions of GLP-1 and assessment was solely done with orally administered glucose which may not reflect physiologic stimuli.

To determine the metabolic effects of GLP-1 in patients with type 1 diabetes, we studied the acute effects of exenatide, a short acting GLP-1 analog, on glucose tolerance to a mixed meal tolerance test (MMTT) and to an intravenous glucose tolerance test (IVGTT). We analyzed insulin secretion rates (ISR), gastric emptying, and hormonal responses including glucagon, GIP, and endogenous GLP-1 release. We studied individuals with and without residual insulin production to determine the importance of insulin secretion on mediating the metabolic effects of the analog.
RESEARCH DESIGN AND METHODS

Human subjects

We studied 17 patients with type 1 diabetes with (C-peptide positive) (n=8) and without (C-peptide negative) (n=9) residual insulin production that were recruited from the Yale Diabetes Center. The presence of residual insulin production was identified post-hoc after completion of the metabolic studies and this information was used for the comparative data analysis. Patients were considered C-peptide positive if they had a C-peptide value of $\geq 0.017$ nmol/L at any time point during MMTT. To enhance our detection of individuals with residual insulin production we recruited individuals between the ages of 18-56, with diabetes duration of at least 2 years, HbA1c <9%, insulin usage <0.9U/Kg/d. Table 1 shows the baseline characteristics of the study participants. The study protocol was approved by Yale University Institutional Review Board. Written informed consent was obtained from all patients.

Study procedures

Subjects underwent two MMTTs and two IVGTTs. During all studies, subjects received basal insulin (either as basal insulin through an insulin pump or as long acting insulin); however no short acting insulin was administered either for the MMTT or IVGTT. The tests were performed in a randomized manner under two study conditions, with and without pretreatment with exenatide 5 mcg sc 15 minutes prior to start of the test.

A standard 4 hr MMTT was extended to 5-hours since previous studies had shown delayed gastric emptying with GLP-1 use. For the MMTTs patients drank a liquid meal (Boost High Protein, 6cc/kg) and blood samples were collected at 13 time points (-10, 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes) for measurement of glucose, C-peptide and
glucagon, and, on a subset of subjects (n=8), GLP-1, and GIP. Gastric emptying was evaluated by measuring the plasma levels of acetaminophen after a dosage of 20 mg/kg to a maximum of 1300 mg at the start of the meal. IVGTTs were performed by infusing 20% dextrose solution at a dosage of 0.5g / Kg up to a maximum of 35 grams. Blood samples for measurement of glucose, C-peptide were collected at -10, -4, 1, 3, 5, 7, and 10 minutes. GLP-1 (n=6) and GIP (n=4) samples were collected at the start and the end of the test. The IV glucose bolus was given at -3 minutes over 3 minutes.

Laboratory measurements

Plasma C-peptide levels were measured at Northwest Lipid Metabolism and Diabetes Research Laboratories (Seattle, WA) using Tosoh AIA 1800 assay. The lower limit of detection was 0.017nmol/L with intra- and interassay coefficients of variation of 1.71% and 4.68% respectively. Glucagon samples were collected in EDTA tubes containing aprotinin but not DPP-IV inhibitor. Glucagon levels were measured by radioimmunoassay (Millipore, St. Charles Missouri). The lower limit of detection was 19pg/ml, with intra- and interassay coefficients of variation of 6.58% and 6.64%. Total GLP-1 and GIP were collected in tubes containing EDTA and DPP-IV inhibitor and measured by ELISA (Alpco Diagnostics and Millipore, Inc). The GLP-1 assay did not detect exenatide and therefore measured endogenous GLP-1 production. The inter assay CVs for low and high GLP-1 level controls are 8.8% and 7.0% respectively and for the low and high GIP level controls were 9.2% and 8.1% respectively. Acetaminophen was measured by Roche Modular P analyzer using Syva Emit reagents (Siemens Healthcare Diagnostics Ltd, Newark, DE). HbA1c was measured using a Siemens DCA vantage
analyzer machine. Plasma glucose levels were measured at the bedside using an YSI biochemistry analyzer (Model 2700 Select, YSI Incorporated and Xylem, Inc.).

**Insulin Secretion Rates**

To determine insulin secretory rates (ISR), the C-peptide levels obtained during MMTT and IVGTT were deconvoluted using a two-compartment model for hormone clearance with the Chronobiological Series Analyzer (CSA) software (19, 20). Standard kinetic parameters for C-peptide were employed based on the findings of Van Cauter et al., who estimated rate constants, based on extrapolations from C-peptide decay curves of 200 subjects (21). Parameters used for ISR calculation accounted for the patient’s age, sex, height, weight, and C-peptide values (nmol/L). Undetectable levels of C-peptide were assigned a value of 0.017 nmol/L, the lower limit of detection in the assay.

**Statistical analyses and calculations**

The baseline insulin dose was calculated as the average number of units used per day for 3 days prior to the first visit. Subjects without detectable fasting C-peptide levels were classified as not having residual insulin production and we did not observe detectable levels after the mixed meal or IV glucose. Total area under the curve (AUC) was calculated for ISR, glucose, glucagon, GLP-1, GIP and acetaminophen using the trapezoidal rule. The ratio of the ISR/glucose was calculated using the ISR calculated from the time interval initiated with the glucose level. Statistical analyses were performed with Graph Pad (San Diego, CA) Prism® version 5.04. Data are presented as means ± SE, unless indicated otherwise. For comparison between the two groups, a Wilcoxon matched-pairs signed rank test was used. Differences between groups resulting in two-tailed p values <0.05 were considered statistically significant.
RESULTS

Metabolic profiles of subjects with long standing type 1 diabetes

We studied 17 subjects with type 1 diabetes with (n=8) and without (n=9) endogenous C-peptide secretion. Their baseline characteristics are presented in table 1. Although all of the subjects had type 1 diabetes for at least 2 years, those with residual insulin production tended to have a shorter duration of diabetes (p=0.11). The HbA1c and total daily insulin doses were not significantly different between the groups.

The insulin production that was detectable in C-peptide+ subjects was responsive to the metabolic stimuli. ISRs increased approximately 4-fold in those with residual insulin production during the MMTT without exenatide pretreatment, but only 1.3 fold in response to IV glucose (Figure 1, 2A).

Effects of exenatide on the metabolic responses in MMTTs and IVGTTs:

The glucose, ISR, glucagon and acetaminophen responses of representative subjects with and without endogenous C-peptide secretion are shown in Figure 1. When all subjects with and without residual insulin production were considered together, the glucose excursions after the liquid meal was reduced by 33% with exenatide pretreatment (without 79626±3869 mg/dL vs. with 4665±3427mg/dL, p<0.001) (Figure 2A). We did not find a difference in the exenatide effects on glucose excursions in subjects with vs. without residual insulin production. In contrast, we did not detect an effect of exenatide on glucose responses to IVGTT (without 3661±133.2 mg/dL vs. with 3686±158.2 mg/dL) (p=0.8501) (Figure 2B).
Exenatide is known to delay gastric emptying. To assess this effect in patients with type 1 diabetes, we measured absorption of acetaminophen during the mixed meal performed with or without exenatide pretreatment. Gastric emptying was delayed and the total acetaminophen absorption was reduced from $2058\pm196\ \mu g/mL$ to $686\pm138\ \mu g/mL$ ($p=0.0017$). The effects on gastric emptying were also similar in subjects with and without residual insulin production.

**Effects of exenatide on hormonal responses**

We analyzed the ISR in the 8 subjects with detectable levels of C-peptide. Of these, 5 showed a reduction in the absolute ISR AUC when exenatide was given and the remainder showed an increase. Thus, overall the absolute levels of ISR were not changed by the exenatide (Figure 2C) ($p=1.0$). Likewise, we did not see an effect of exenatide treatment on the insulin secretory response to IVGTT (Figure 2D). Interestingly, when we evaluated the relationship of ISR to the glucose levels (AUC ISR/AUC glucose) in C-peptide positive patients, we found that the levels were significantly higher in the exenatide-treated group (without $0.1134\pm0.0388$ vs. with $0.2318\pm0.08855$) ($p=0.0078$) (Figure 2E). We did not see an effect of exenatide treatment on the insulin secretory response to IVGTT (without $209.5\pm102.1$ nmol/L vs. with $297\pm217.1$ nmol/L) ($p=0.875$) (Figure 2D), or an effect on the relationship between ISR and glucose in C-peptide positive patients during the IVGTTs (without $0.0640\pm0.03675$ vs. with $0.07925\pm0.05906$) ($p=1.0$) (Figure 2F).

Glucagon levels were significantly suppressed in the presence of exenatide (without $15909\pm945.8$ pg/mL vs. with $12124\pm1182$ pg/mL) ($p=0.0015$) (Figure 2G). However, the ratio of glucagon:glucose was significantly increased after exenatide (without $0.2116\pm0.01925$ vs.
with $0.2875 \pm 0.0446$ (p=0.006) suggesting that the decrease in glucose excursion involved factors in addition to the effects on glucagon itself (Figure 2H). The responses in patients with and without residual insulin were similar.

Using an assay that was specific for endogenous GLP-1 and did not cross react with the exenatide that was administered, we found that there were reduced GLP-1 levels in six of the eight subjects, while the levels increased in 2 subjects when they received exenatide of 8 although the differences in the hormone levels did not reach statistical significance (without $662.6 \pm 270$ pmol/L vs. with exenatide $444.2 \pm 302.1$ pmol/L, p=0.055) (Figure 3A). We did not find a significant change in the levels of GIP when exenatide was administered (without $1000 \pm 177.8$ pg/mL vs. with exenatide $944.5 \pm 304.2$ pg/mL, p=0.875). (Figure 3B-D).
CONCLUSION

We studied the acute effects of exenatide, a short acting GLP-1 receptor agonist, in individuals with type 1 diabetes with and without residual insulin production. The primary objective of our study was to determine whether exenatide would affect the acute metabolic responses to an MMTT or an IVGTT in subjects with established type 1 diabetes, and to determine the significance of residual insulin production on those responses. We calculated ISR rather than using raw C-peptide values, which gives a more accurate assessment of β-cell function, since the C-peptide levels may not reflect the true levels of insulin secretion because of the relatively long t½ of C-peptide. Moreover, we were able to study the effects of GLP-1 analogs on endogenous GLP-1 and GIP in a subset of subjects. We found that administration of exenatide reduced glucose excursion and absolute glucagon secretion during a mixed meal and delayed gastric emptying, similar to previous reports (22-25). The total absorption of acetaminophen was reduced and gastric emptying was delayed with exenatide administration. On average we did not find an absolute increase in insulin secretion in subjects who were able to secrete insulin but the relative amount of insulin secreted for the glucose was increased.

Since we did not find an increase in the absolute amount of insulin that was secreted, the proportional increase in insulin for the glucose most likely is a reflection of the reduced glucose levels in subjects who were already maximally secreting insulin. The changes that we found in the glucagon/glucose ratio are consistent with the major effect on reducing glucose levels. Therefore our studies indicate that the metabolic effects of exenatide involve 2 mechanisms including its effects on absorption of nutrients and glucagon inhibition, but based on our study design we cannot exclude an effect on augmenting insulin production as well.
The metabolic effects require oral absorption of nutrients since we found no glycemic or hormonal effects of exenatide on the responses to intravenous glucose. The stimuli of a mixed meal and intravenous glucose are different – the former includes protein and fat. In addition, the route of administration may be important since DPP-IV inhibitors have been shown to regulate glycemia by local inhibition of intestinal DPP-IV activity, activation of incretin receptors, and activation of the gut-to-pancreas neural axis (26).

Endogenous GLP-1 levels were decreased with exenatide administration in six of eight patients which may have been due to the effects of the drug on gastric emptying or a feedback inhibition of GLP-1 secretion (27). Kielgast et al (15) studied the effects of exenatide prior to a MMTT in 8 subjects with and 8 without residual insulin production. In these subjects, ½ of the usual dose of fast acting insulin was administered together with exenatide. They reported that the incretin responses were similar in patients compared to healthy control subjects and found that the responses were also similar between those with and without residual insulin production. Similarly, Gutniak et al. (28) studied hormonal responses in insulin deficient patients with T1D and found reduced insulin requirements and glucagon responses when GLP-1 was infused. In addition to a similar reduction in glucagon release to a mixed meal that was seen by these previous authors, our data suggest a reduced level of GLP-1 in most patients but the number of subjects that we studied was small.

Despite the profound effect of the GLP-1 receptor agonist on glycemic excursion in our acute studies, the effects of therapy with GLP-1 receptor agonist on the clinical management of patients with type 1 diabetes have been relatively modest (29). The most significant metabolic consequence of exenatide administration appears to involve the delay in absorption of nutrients.
and reduced rise in glucose as a consequence, but testing for additional benefits to individuals with residual insulin production may require further experience with larger numbers of subjects and longer use of an agonist. In addition to consideration of GLP-1 receptor agonists in the chronic metabolic management, these agents might be considered in combination with other agents including immune modulators in the new onset period (30).

In summary, in patients with long standing type 1 diabetes with and without residual insulin production, we observed a marked reduction in glycemic excursion during a MMTT with exenatide pretreatment, but no changes were observed in glucose excursion in response to an IV glucose challenge with exenatide pretreatment. In patients with residual insulin production, the insulin secretion was preserved even with reduced glucose levels. The value of GLP-1 receptor agonists in chronic management of type 1 diabetes, particularly those with residual insulin production, will require long term studies with larger numbers of subjects.
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Author contributions: T.G. researched data, contributed to the discussion, wrote manuscript and reviewed/edited manuscript. J.L.S. researched data and reviewed/edited manuscript. L.R. researched data. K.C.H. researched data, contributed to the discussion and reviewed/edited manuscript. T.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References


Figure Legends

**Figure 1:** Metabolic effects of exenatide during MMTT: The kinetics of glucose, ISRs, acetaminophen and glucagon levels are shown in a representative patient with (a-d) or without (e-h) detectable C-peptide.

**Figure 2:** Changes in hormonal responses during MMTT and IVGTT with exenatide.  
A) Area under the curve (AUC) for glucose (mg/dL) during a MMTT without and with administration of exenatide 5 µg sc (p=0.0003).  
B) AUC for glucose (mg/dL) during intravenous glucose tolerance test without and with exenatide (p=0.8501).  
C-F) Only patients with detectable C-peptide are shown.  
C) AUC for insulin secretion rate (nmol/L) during MMTT without and with sc injection of exenatide (p=1.0).  
D) AUC for ISR during an IVGTT (p=0.875).  
E) AUC ISR/AUC glucose during MMTT with and without exenatide (P=0.0078).  
F) ISR AUC/glucose AUC at the time of IVGTT with and without pretreatment with exenatide (p=1.0).  
G) AUC for glucagon (pg/mL) during MMTT with and without administration of exenatide (p=0.0015) in all subjects.  
H) AUC ratio for glucagon:glucose in all patients during MMTT, which shows a significant increase with exenatide (p=0.006). Open circles (○) indicate C-peptide negative patients and closed circle (●) indicate C-peptide positive patients.

**Figure 3:** Changes in incretins with exenatide.  
A) AUC for GLP-1 (pmol/L) during MMTT without 662.6± 270 pmol/L vs. with exenatide 444.2±302.1 pmol/L in eight subjects (p=0.055).  
B) AUC for GLP-1 (pmol/L) during IVGTT (without 25.28±12.48 pmol/L vs. with 46.76±32.83 pmol/L) in six subjects (p=0.625).  
C) Area under the curve for GIP (pg/mL) during
MMTT without and with exenatide in eight subjects. D) Area under the curve for GIP (pg/mL) during IVGTT (p=0.875).
Table 1-Patient baseline characteristics

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<th>C-peptide positive (n=8)</th>
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<td>Age</td>
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<td>HbA1c (%)</td>
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<td>Baseline GIP(pg/mL)</td>
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<td>Baseline Glucose(mg/dL)</td>
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Baseline characteristics at the start of the study for all subjects subdivided into C-peptide negative and C-peptide positive. C-peptide positive subjects were defined as those with C-peptide levels ≥0.017 nmol/L at any time point during MMTT Insulin usage was calculated from daily total dosage the last 3 days prior to 1st visit. Baseline ISR, glucagon, GLP-1, GIP and glucose levels in both the insulin depleted (C-peptide negative) and insulin producing (C-peptide positive) subjects at the time of MMTT, without exenatide pretreatment. Data presented are mean ± SE.
Figure 1
Figure 3

A. MMTT

B. IV GTT

C. MMTT

D. IV GTT

Figure legends:

- **MMTT**: Graphs A and C depict the AUC Total GLP-1 (pmol/L) for MMTT. In graph A, there is a significant decrease in GLP-1 levels with exenatide treatment, indicated by a p-value of 0.055. Graph C shows a trend towards decreased GLP-1 levels, although it is not statistically significant (p = 0.1953).

- **IV GTT**: Graphs B and D represent the AUC Total GLP-1 (pmol/L) for IV GTT. Graph B shows a slight increase in GLP-1 levels with exenatide, with a p-value of 0.625, indicating no significant difference. Graph D shows a similar trend without a significant p-value (p = 0.875).

These findings suggest that exenatide may have a differential effect on GLP-1 levels depending on the test used, with MMTT showing more pronounced differences.