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Exenatide Augments First- and Second-Phase Insulin Secretion in Response to Intravenous Glucose in Subjects with Type 2 Diabetes

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Context: First-phase insulin secretion (within 10 min after a sudden rise in plasma glucose) is reduced in type 2 diabetes mellitus (DM2). The incretin mimetic exenatide has glucoregulatory activities in DM2, including glucose-dependent enhancement of insulin secretion.

Objective: The objective of the study was to determine whether exenatide can restore a more normal pattern of insulin secretion in subjects with DM2.

Design: Fasted subjects received iv insulin infusion to reach plasma glucose 4.4–5.6 mmol/liter. Subjects received iv exenatide (DM2) or saline (DM2 and healthy volunteers), followed by iv glucose challenge.

Patients: Thirteen evaluable DM2 subjects were included in the study: 11 males, two females; age, 56 ± 7 yr; body mass index, 31.7 ± 2.4 kg/m^2; hemoglobin A_{1c}, 6.6 ± 0.7% (mean ± SD) treated with diet/exercise (n = 1), metformin (n = 10), or acarbose (n = 2). Controls included 12 healthy, weight-matched subjects with normal glucose tolerance: nine males, three females; age, 57 ± 9 yr; and body mass index, 32.0 ± 3.0 kg/m^2.

Setting: The study was conducted at an academic hospital.

Main Outcome Measures: Plasma insulin, plasma C-peptide, insulin secretion rate (derived by deconvolution), and plasma glucagon were the main outcome measures.

Results: DM2 subjects administered saline had diminished first-phase insulin secretion, compared with healthy control subjects. Exenatide-treated DM2 subjects had an insulin secretory pattern similar to healthy subjects in both first (0–10 min) and second (10–180 min) phases after glucose challenge, in contrast to saline-treated DM2 subjects. In exenatide-treated DM2 subjects, the most common adverse event was moderate nausea (two of 13 subjects).

Conclusions: Short-term exposure to exenatide can restore the insulin secretory pattern in response to acute rises in glucose concentrations in DM2 patients who, in the absence of exenatide, do not display a first phase of insulin secretion. Loss of first-phase insulin secretion in DM2 patients may be restored by treatment with exenatide. **(*J Clin Endocrinol Metab* 90: 5991–5997, 2005)**

A PROMINENT FEATURE of type 2 diabetes is a dramatic reduction in first-phase insulin secretion, the insulin normally secreted by pancreatic β-cells within 10 min after a sudden rise in plasma glucose concentrations (1). This early insulin response appears to be lost, even in the early stages of the disease, when fasting glucose concentrations are only slightly elevated above normal. This defect is important because first-phase insulin secretion is postulated to have the greatest impact on postprandial plasma glucose excursions (1, 2) and the loss of early-phase insulin release is a common defect that plays a pathogenic role in postmeal hyperglycemia. Specific therapeutic interventions may result in improvements in glycemic control (3); however, it is controversial whether antidiabetic medications that act through a relatively glucose-independent stimulation of insulin secretion (*e.g.* sulfonylureas) have a clinically meaningful influence on first-phase insulin secretion in patients with type 2 diabetes (4, 5). Therefore, other treatments for restoring normal physiological β-cell responses in patients with type 2 diabetes need to be explored.

The mammalian incretin hormone glucagon-like peptide (GLP)-1 augments first-phase insulin secretion in healthy subjects (6, 7), subjects with impaired glucose tolerance (8), and patients with type 2 diabetes (7, 9). It also amplifies the amplitude of pulsatile pattern insulin secretion in all these groups (10–12). Exenatide is a 39-amino acid peptide incretin mimetic that exhibits glucoregulatory activities similar to those of GLP-1 (13–18). These actions include glucose-dependent enhancement of insulin secretion (19–22), suppression of inappropriately high glucagon secretion (20, 21), and slowing of gastric emptying (20, 21). Exenatide's glucose-dependent enhancement of insulin secretion may be mediated by exenatide binding to the pancreatic GLP-1 receptor (23). However, exenatide has a prolonged half-life after sc injections of 2–3 h, compared with approximately 20 min for GLP-1 (21, 24). Thus, exenatide may be suitable for long-term improvement of glycemic control in patients with type 2 diabetes receiving two sc injections per day (15–17). An additional benefit is that exenatide treatment is often accompanied by weight loss (15–17). In addition, in animal

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Abbreviations: AUC, Area under the curve; BMI, body mass index; GLP, glucagon-like peptide; K_g, glucose disappearance constant.

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models of diabetes and in insulin-secreting cell lines, exenatide and GLP-1 improved β-cell function by increasing the expression of key genes involved in insulin secretion and by increasing insulin biosynthesis and augmenting β-cell mass through multiple mechanisms (25, 26). Data obtained in animal models also indicate that exenatide and GLP-1 reduce food intake, cause weight loss, and have an insulin-sensitizing effect (25, 26). It was the aim of the present study to assess the effect of an iv infusion of exenatide on biphasic insulin secretory responses in patients with type 2 diabetes and in comparison with healthy control subjects.

Preliminary results have been published in abstract form.

Subjects and Methods

Study protocol

The study protocol was approved by the ethics committee of the medical faculty of the Georg-August-University, Göettingen, Germany, on August 25, 2003 (registration number 17/8/03) before the study, in accordance with the principles described in the Declaration of Helsinki, including all amendments through the 1996 South Africa revision (27). All subjects provided written informed consent before participation.

Subjects

Patients with type 2 diabetes were studied twice in randomized order. On one occasion, exenatide was administered iv, and saline was administered iv on the other occasion. These subjects were compared with gender-, age-, and weight-matched healthy subjects with normal oral glucose tolerance who were studied once and received saline iv. Exclusion criteria were age younger than 35 or more than 70 yr; body mass index (BMI) less than 25 or more than 35 kg/m^2; blood pressure (measured in the sitting position) more than 165/95 mm Hg; any other clinically significant abnormalities detected during physical examination, laboratory tests, or electrocardiogram; failure to document a negative pregnancy test; and a positive screen for alcohol and drugs. Additional exclusion criteria for patients with type 2 diabetes were a disease duration less than 3 months; any treatment other than diet and exercise alone or in combination with metformin or an α-glucosidase inhibitor; fasting plasma glucose concentration greater than 200 mg/dl (>11.1 mmol/liter); or a hemoglobin A_{1c} greater than 6.0 or more than 8.5% (normal range 4.3–6.1%).

Study medication

Exenatide was supplied by Amylin Pharmaceuticals (San Diego, CA) as a clear, colorless, sterile solution in sodium acetate buffer (pH 4.5), containing 4.3% mannitol as an isoosmolality modifier and 0.22% metacresol as a preservative. The strength of this formulation was 0.25 mg/ml. Study medication was stored at 2–8 C.

Experimental procedures

After providing informed consent, participants attended a screening visit to verify inclusion and exclusion criteria, perform anthropometric measurements (body height and weight, calculation of BMI), and measure safety parameters (physical examination, vital signs, laboratory screening, electrocardiogram, review of medication). A normal oral glucose tolerance (after a 75-g oral glucose load) was confirmed in healthy subjects by measuring capillary blood glucose 120 min after glucose challenge [plasma glucose 108 ± 4 mg/dl (6.0 ± 0.2 mmol/liter)].

Subjects returned to the study site within 14 d of the screening visit and subsequently were hospitalized for the duration of the study. In subjects with type 2 diabetes, two experiments were performed in randomized order on d 2 and 4. No study-related procedures were performed on d 3. All antidiabetic medications (metformin or α-glucosidase inhibitor) were withheld throughout the study duration. In healthy subjects, a single experiment (saline) was performed on d 2. On the days before experiments, food and beverages were allowed until 2200 h and then only water the rest of the night. Study procedures began at 0600 h with the insertion of an indwelling iv catheter and the infusion of insulin (subjects with diabetes) or saline (healthy subjects) for 390 min. The insulin infusion was varied as needed to reach a fasting plasma glucose concentration of 79–101 mg/dl (4.4–5.6 mmol/liter) within approximately 3 h and to maintain this glucose concentration for the duration of the insulin infusion. At approximately 1000 h (*e.g.* after 240 min of exogenous insulin or saline administration and 180 min before injection of the glucose bolus), an iv infusion of exenatide or saline was started and maintained for 300 min. For the initial 30 min, the exenatide dose was 50 ng/min. After this loading dose, infusion was maintained at 25 ng/min. At approximately 1230 h, *i.e.* after 390 min, the infusion of insulin was stopped, 30 min before giving the iv glucose bolus. At approximately 1300 h, the glucose bolus (0.3 g/kg body weight as 50% glucose in water) was administered over 30 sec.

A second indwelling iv catheter was inserted into a contralateral forearm vein for blood sampling at the same time the first catheter was inserted. Blood was sampled at −195, −180, −120, −60, −30, −15, −10, −5, and 0 min before the glucose bolus and at 2, 4, 6, 8, 10, 12, 15, 20, 30, 45, 60, 90, and 120 min after the bolus for the measurement of plasma glucose, insulin, C-peptide, and glucagon. At −195, −180, −165, −150, −120, −90, −60, 0, 60, and 120 min relative to bolus injections, blood was collected for the determination of plasma exenatide concentrations in subjects with type 2 diabetes. Blood samples were kept in ice and centrifuged in a prechilled centrifuge at 1000 × *g* for 15 min at 4 C. Plasma was separated within 15 min of centrifugation and stored frozen at −20 to −70 C until analysis. After each experiment, participants immediately received a mixed meal. Plasma glucose concentration was measured before subject release from the hospital to exclude hypoglycemia due to stimulated insulin secretion. Subjects were dismissed from the hospital after an additional assessment of safety parameters on either d 2 (healthy subjects) or d 4 (subjects with type 2 diabetes).

Laboratory determinations

Glucose was measured using a glucose oxidase method with a glucose analyzer 2 (Beckman Instruments, Munich, Germany). Insulin, C-peptide, and glucagon were determined by specific immunoassays as previously described (28, 29). For the measurement of glucagon (antiserum 4305), plasma was extracted with ethanol. Plasma exenatide was measured as previously described (30).

Statistical analysis

The intent-to-treat population encompassed all subjects who received at least one study infusion. The evaluable population encompassed all intent-to-treat subjects who completed the infusion procedures on d 2 (groups 1 and 2) and d 4 (group 1). The primary study end point was first-phase insulin response [area under the insulin secretion curve (AUC) for the first 10 min after glucose challenge plus additional measures of insulin secretory activity]. Secondary end points were second-phase insulin release, glucose disappearance constant (K_g), and insulin secretion rate.

First-phase insulin response was assessed by calculating the incremental area under the insulin concentration curve relative to basal insulin concentration during the first 10 min after the iv glucose bolus. The basal insulin concentration was the mean insulin concentration obtained between −15 min and 0 min before the glucose bolus. Second-phase insulin release was calculated in a similar manner as first-phase insulin release, as the incremental area relative to basal insulin concentration from 10 min to 120 min after glucose administration. Integrations were carried out using the trapezoidal rule. K_g was calculated as 100 × the slope of the regression line fitted to the natural logarithm of glucose concentrations from time 10 min to 30 min after the glucose bolus. Insulin secretion rates were calculated by deconvolution analysis based on a two-compartment model of C-peptide elimination as described by Eaton *et al.* (31) and Polonsky *et al.* (32) and calculated from C-peptide concentrations using the insulin SECretion (ISEC) computer program (3, 33).

All statistical comparisons were carried out using a mixed-effects model for the two experiments performed in subjects with type 2 diabetes. The mixed-effect model included treatment (exenatide *vs.* saline), treatment sequence (exenatide or saline first), and period (first *vs.* second study) as fixed effects and subject within sequence as a random effect.

Results for the exenatide and saline infusions in subjects with type 2 diabetes were compared with those in healthy controls. The SAS* MIXED procedure (SAS* version 8.2, SAS Institute, Durham, NC) was used for statistical analyses. Except for T_{max}, all other pharmacodynamic parameters were log transformed (base e) before fitting the mixed-effect ANOVA model. Pharmacokinetics parameters were calculated using SAS and verified by WinNonlin* standard version 4.0. $P < 0.05$ was considered a significant difference. Results are reported as mean ± SEM unless otherwise indicated.

Results

Thirteen evaluable subjects with type 2 diabetes (group 1) treated with diet and exercise alone (n = 1), metformin (n = 10), or acarbose (n = 2) were studied in randomized order (one exenatide and one saline infusion) and were compared with 12 healthy weight-matched subjects with normal glucose tolerance (group 2). Demographic characteristics and baseline values are given in Table 1. Subjects with type 2 diabetes who normally received metformin or an α-glucosidase inhibitor were required to withhold antidiabetic medications until study termination. The most common concomitant medications for indications other than diabetes for the evaluable population were from the following classes: angiotensin-converting enzyme inhibitors [eight group 1 subjects (62%); two group 2 subjects (17%)]; β-blocking agents [five group 1 subjects (39%), two group 2 subjects (17%)]; and thiazides [four group 1 subjects (31%), two group 2 subjects (17%)].

Intravenous infusion of exenatide resulted in steady-state plasma concentrations of approximately 130 pg/ml (Fig. 1A), well within the range of concentrations observed after sc injection of exenatide in phase 3 clinical trials (15–17). Plasma glucagon concentrations were similar among group 1 subjects during both exenatide and saline infusions and were similarly suppressed after the iv glucose bolus (Fig. 1B).

In comparison with healthy subjects, first-phase insulin secretion in subjects with type 2 diabetes given saline was significantly blunted (Fig. 2, A–C and Table 2). Treatment with exenatide increased plasma insulin ($P < 0.005$) and C-peptide integrated incremental responses ($P < 0.005$) during the first (0–10 min) and second (10–120 min) phases of glucose-stimulated insulin secretion by approximately 180–310% and also increased insulin secretion rates relative to saline. Exenatide-treated patients with type 2 diabetes had a similar secretory pattern to healthy subjects but with an even higher secretion rate during the second-phase insulin response. The elevation in insulin was accompanied by a significant increase in K_g, the glucose disappearance constant in exenatide-treated subjects with type 2 diabetes (least square mean 1.5 ± 0.1%/min for exenatide *vs.* 0.9 ± 0.1%/min for saline; 95% confidence interval difference 0.3–1.0; $P = 0.0031$). K_g in exenatide-treated patients with type 2 diabetes was not significantly different from K_g in saline-treated, healthy volunteers (1.1 ± 0.1%/min; $P = 0.089$).

The most common adverse event was mild to moderate nausea (Table 3). Only one mild hypoglycemic event [plasma glucose 56 mg/dl (3.1 mmol/liter)] that occurred during exenatide infusion, 70 min after the iv glucose bolus was considered a result of exenatide administration, and the subject recovered without intervention. In addition, one group 1 saline

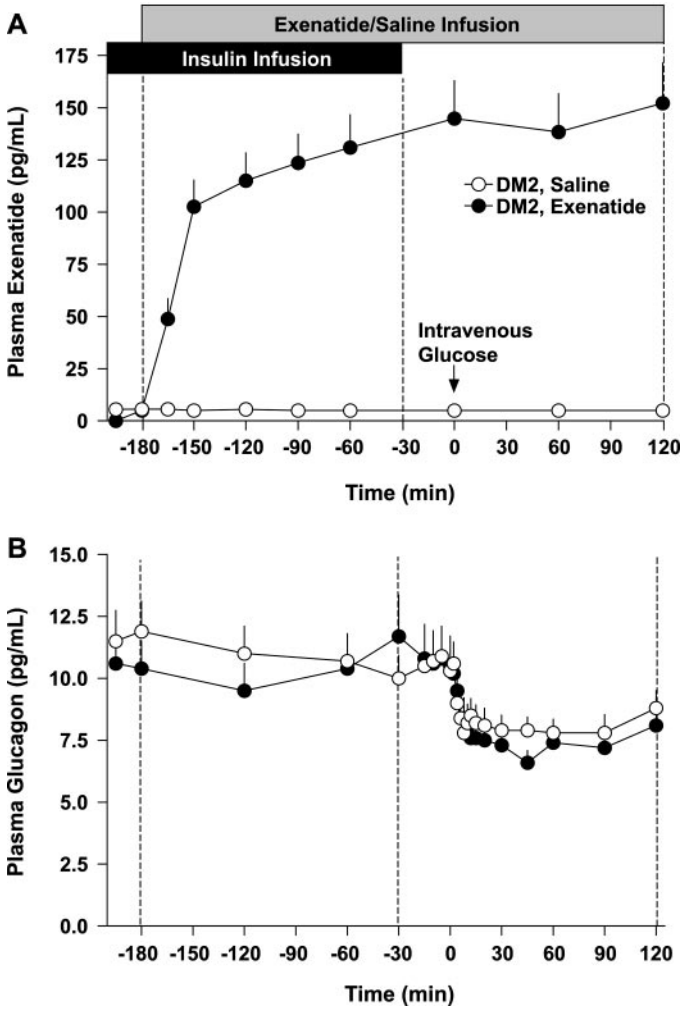

FIG. 1. Plasma exenatide pharmacokinetic (A) and glucagon pharmacodynamic (B) profiles in evaluable subjects with type 2 diabetes (DM2; group 1).

TABLE 1. Intent-to-treat subject disposition with demographics and baseline characteristics for the evaluable population

	Group 1: type 2 diabetes	Group 2: healthy
Enrolled (intent-to-treat) [n (%)]	14 (100)[a]	12 (100)
Completed (evaluable) [n (%)]	13 (93)	12 (100)
Withdrew (adverse event) [n (%)]	1 (7)[a]	0 (0)
Sex, M/F (n)	11/2	9/3
Age (yr)	56 ± 7	57 ± 9
Weight (kg)	94 ± 13	97 ± 10
BMI (kg/m^2)	31.7 ± 2.4	32.0 ± 3.0
HbA_{1C} (%)	6.6 ± 0.7	5.5 ± 0.4
Fasting plasma glucose [mg/dl (mmol/liter)]	145 ± 23 (8.0 ± 1.3)	NA
Diabetes duration (yr)	4 ± 2	NA
Diabetes treatment [n (%)]		
α-Glucosidase inhibitors	2 (15)	NA
Metformin	10 (77)	NA
Diet/exercise	1 (7)	NA

Mean ± SD. NA, Not applicable; HbA_{1C}, hemoglobin A_{1aC}. All subjects were Caucasian.

[a] One subject who had not received exenatide (only saline) withdrew from the study on d 2 due to an elevated white blood cell count, probably unrelated to study procedures.

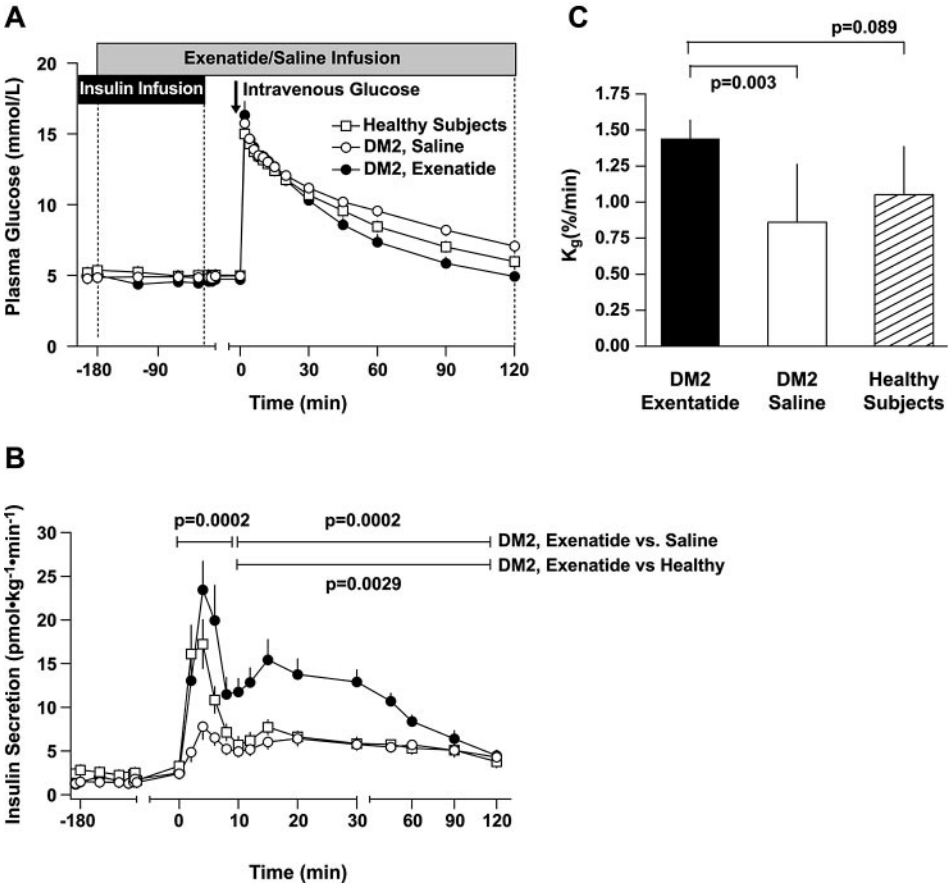

FIG. 2. Plasma glucose concentrations (A) and insulin secretion rates (B) in 13 subjects with type 2 diabetes (DM2) given an iv infusion of exenatide or saline and 12 healthy volunteers given an iv infusion of saline. Repeated-measures ANOVA indicated significant differences in insulin secretion as measured by AUC analysis of the first- (0–10 min) and second-phase (10–120 min) insulin release between subjects with type 2 diabetes treated with exenatide and saline. Exenatide-treated subjects with type 2 diabetes also had significantly increased second-phase insulin release, compared with healthy volunteers. Data represent mean ± SEM. Conversion factor for glucose concentrations: millimoles per liter divided by 0.05551 = milligrams per deciliter. C, Exenatide significantly increased glucose clearance rates in subjects with type 2 diabetes. LS mean ± SEM.

subject who had not received exenatide withdrew from the study on d 2 due to an elevated white blood cell count.

Discussion

To examine whether iv exenatide could stimulate first- and second-phase insulin secretion in response to an iv glucose bolus in subjects with type 2 diabetes, 13 subjects were studied in randomized order (one exenatide infusion and one saline infusion) and compared with 12 healthy weight-matched subjects with normal glucose tolerance.

Exenatide restored both first- and second-phase insulin secretion in response to glucose bolus in subjects with type 2 diabetes (Fig. 2). The insulin secretory response could be augmented using a dose of exenatide that produced few adverse reactions, resulting in a pattern of insulin secretion that was comparable with the responses in healthy control subjects given saline. Whereas in saline-treated subjects with type 2 diabetes, first-phase insulin secretion was barely detectable, compared with healthy control subjects (Fig. 2B), first-phase insulin secretion was clearly delineated and quantitatively important after exenatide administration. These results confirm previous studies demonstrating a prominent and rapid augmentation of insulin secretion by exenatide with metformin and/or sulfonylureas (20, 21) or different regimens of glucose administration (22, 34). They also support the view that iv exenatide has the same potential to improve or even normalize β-cell function that has previously been demonstrated for GLP-1 in healthy subjects (6, 35), subjects with impaired glucose tolerance (8, 11), and patients with type 2 diabetes (7, 9, 11).

TABLE 2. Pharmacodynamic comparisons within and between groups (evaluable population)

	Group 1: type 2 diabetes			Group 2: healthy	
	Saline (n = 13)	Exenatide (n = 13)	Ratio group 1 exenatide to saline	Saline (n = 12)	Ratio group 1 exenatide to group 2 saline
Insulin (mU·min/liter)					
Geometric LS mean $AUC_{0-10min}$	212 ± 38	655 ± 116	3.1 ($P < 0.0001$)	441 ± 78	1.5 ($P = 0.1236$)
Geometric LS mean $AUC_{10-120min}$	2611 ± 355	6923 ± 941	2.7 ($P < 0.0001$)	2374 ± 433	3.0 ($P = 0.0001$)
Geometric LS mean $AUC_{0-120min}$	2830 ± 386	7623 ± 1040	2.7 ($P < 0.0001$)	2880 ± 490	2.7 ($P = 0.0002$)
C-peptide (ng·min/liter)					
Geometric LS mean $AUC_{0-10min}$	26 ± 3	52 ± 6	2.0 ($P < 0.0001$)	52 ± 5	1.0 ($P = 0.9879$)
Geometric LS mean $AUC_{10-120min}$	514 ± 37	908 ± 65	1.8 ($P < 0.0001$)	573 ± 66	1.6 ($P = 0.0014$)
Geometric LS mean $AUC_{0-120min}$	541 ± 39	961 ± 70	1.8 ($P < 0.0001$)	627 ± 70	1.5 ($P = 0.0021$)

Data represent geometric least square (LS) mean ± SE.

TABLE 3. Treatment-emergent adverse events with an overall incidence of 10% or more in any treatment arm (intent-to-treat population)

	Group 1: type 2 diabetes		Group 2: healthy
	Saline (n = 14) n (%)	Exenatide (n = 13) n (%)	Saline (n = 12) n (%)
Nausea	1 (7)	2 (15)	0 (0)
Hypoglycemia	2 (14)	2 (15)	0 (0)

One limitation of our study is that we do not know whether the results can be generalized to all patients with type 2 diabetes, notably those with a longer disease duration and more complex therapy (combination of oral antidiabetic agents, combination of antidiabetic tablets and insulin, or insulin only). The full recovery of first-phase insulin secretion may, accordingly, be limited to early stages of the disease. This, however, can and should be tested in further studies focusing on patients with more advanced stages of type 2 diabetes.

A second limitation is that the results have been achieved under experimental conditions that artificially normalized glucose before the glucose stimulus was administered. It may be questioned whether this is representative of the situation of chronic antidiabetic treatment with sc exenatide because the degree of normalization of glycemia described under such conditions is different. It cannot be determined, based on available data, whether this normalization of glycemia is absolutely necessary to elicit the full effect on early phase insulin secretion in response to a glucose bolus as described in the present communication. In other studies, without the previous normalization of glycemia through a feedback-regulated insulin infusion, slight elevations in fasting glucose were associated with a reduction in early insulin secretion (4, 36). It is not known whether this is brought about by a short-term or a more chronic exposure to elevated glucose concentrations. In the patients with type 2 diabetes studied in the present examination, fasting glucose was higher than the normalized glucose attained before iv glucose was given as an artificial stimulus to elicit a biphasic insulin response. It remains to be determined whether a similar protocol without prior normalization of plasma glucose would result in a similar stimulation of insulin secretion, in particular first-phase response.

Third, it must be stressed that our study examined a single exposure to exenatide, lasting a few hours before and after the glucose stimulus was given. It would be necessary to demonstrate maintenance of this effect over days, weeks, or months in response to repeated or continuous administration of exenatide to demonstrate a lasting recovery of β-cell function.

In the present study, exenatide was administered, and plasma exenatide concentrations were within the therapeutic range well before glucose was administered. When GLP-1 was the agent used in other studies to augment glucose-induced insulin secretion, such a pattern of administration was associated with a greater insulinotropic effect, compared with initiation of GLP-1 infusion immediately before a glucose bolus, especially concerning the first-phase response (7). However, with an immediate start of GLP-1 infusions concomitant with the glucose bolus, it is likely that GLP-1 plasma concentrations might not have reached therapeutically relevant levels during the period when first-phase insulin secretion occurred (7). Other studies do not support the notion that there is time-dependent potentiation or a memory effect for GLP-1 effects on insulin secretion (6), and there is no reason to assume that this might be different with exenatide.

The present results show that exenatide can rapidly, *i.e.* within hours, restore a normal pattern of glucose-stimulated insulin secretion in patients with type 2 diabetes. This may be taken to indicate that it is, in principle, possible to revert the type 2 diabetic phenotype of insulin secretion with its relative inability to quickly respond to experimental stimuli (9, 37, 38) and different metabolic conditions (5, 39–41). This is compatible with the view that this deficient pattern of insulin secretion is mainly functional in nature and that the reduction in pancreatic islet β-cell mass is moderate in patients with type 2 diabetes (42–44). It is also very unlikely that typical effects mediated through the interaction of ligands (like exenatide) with the GLP-1 receptor, such as stimulation of proinsulin biosynthesis and replenishment of insulin secretory granules (45, 46), have contributed to the effects on insulin secretion within the time frame of the present study. These mechanisms may, however, be more important in the long-term treatment with exenatide (15–17) or similar agents (47, 48). We did not test the efficacy of exenatide in healthy volunteers with normal oral glucose tolerance test results. It is possible that the potentiation of insulin secretion by exenatide in patients with type 2 diabetes was only a fraction (<50%) of what would have been achieved in the volunteers with a normal oral glucose tolerance test. In any case, the present study clearly underlines the value of attempts to search for or improve agents that have the potential to acutely or chronically improve pancreatic β-cell function in patients with type 2 diabetes.

In studies using sc injections of GLP-1 (35, 49–52), exenatide (15–17, 30), and GLP-1 derivatives like liraglutide (47, 53–55), nausea was the most common side effect. The occurrence of this side effect appears to be associated with the peak plasma concentration of the insulinotropic agent. Nevertheless, any effects on insulin secretory responses or plasma glucose concentrations tended to be smaller in magnitude than in the present study, albeit given the differences in study designs these comparisons must be considered preliminary in nature. In our study, exenatide was administered in a manner designed to result in continuous exposure to therapeutic concentrations without accompanying peaks (Fig. 1A). This might be the reason that only a modestly higher incidence of nausea was reported in exenatide-treated subjects, compared with saline-treated subjects, and nausea incidence overall was low. Nonetheless, these data suggest it would be helpful to improve the pharmacokinetics of available candidate antidiabetic drugs that interact with the GLP-1 receptor, with the aim of producing steady concentration profiles without prominent peaks. If this were possible, the potential antidiabetic potency of such agents might be further improved.

In conclusion, iv exenatide restored the ability of type 2 diabetic pancreatic β-cells to respond to rapid changes in glycemia in a physiological manner. These results elucidate

another mechanism of exenatide action, support the continued clinical development of exenatide as a treatment for type 2 diabetes, and suggest that the loss of first-phase insulin secretion in patients with type 2 diabetes may be reversible with appropriate treatments, *e.g.* exenatide, GLP-1, or incretin mimetics in general.

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References

1. **Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensinck JW, Bierman EL, Porte D** 1976 Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. J Clin Endocrinol Metab 42:222–229
2. **Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW** 1988 Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. Diabetes 37:736–744
3. **Del Prato S, Tiengo A** 2001 The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. Diabetes Metab Res Rev 17:164–174
4. **Hosker JP, Rudenski AS, Burnett MA, Matthews DR, Turner RC** 1989 Similar reduction of first- and second-phase B-cell responses at three different glucose levels in type II diabetes and the effect of gliclazide therapy. Metabolism 38:767–772
5. **Shapiro ET, Van Cauter E, Tillil H, Given BD, Hirsch L, Beebe C, Rubenstein AH, Polonsky KS** 1989 Glyburide enhances the responsiveness of the β-cell to glucose but does not correct the abnormal patterns of insulin secretion in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 69:571–576
6. **Meier S, Hücking K, Ritzel R, Holst JJ, Schmiegel WH, Nauck MA** 2003 Absence of a memory effect for the insulinotropic action of glucagon-like peptide 1 (GLP-1) in healthy volunteers. Horm Metab Res 35:551–556
7. **Quddusi S, Vahl TP, Hanson K, Prigeon RL, D'Alessio DA** 2003 Differential effects of acute and extended infusions of glucagon-like peptide-1 on first- and second-phase insulin secretion in diabetic and nondiabetic humans. Diabetes Care 26:791–798
8. **Byrne MM, Gliem K, Wank U, Arnold R, Katschinski M, Polonsky KS, Göke B** 1998 Glucagon-like peptide 1 improves the ability of the β-cell to sense and respond to glucose in subjects with impaired glucose tolerance. Diabetes 47: 1259–1265
9. **Rachman J, Gribble FM, Barrow BA, Levy JC, Buchanan KD, Turner RC** 1996 Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1 (7-36) amide in patients with NIDDM. Diabetes 45:1524–1530
10. **Pørksen N, Grofte B, Nyholm B, Holst JJ, Pincus SM, Veldhuis JD, Schmitz O, Butler PC** 1998 Glucagon-like peptide 1 increases mass but not frequency or orderliness of pulsatile insulin secretion. Diabetes 47:45–49
11. **Ritzel R, Schulte M, Pørksen N, Nauck MS, Holst JJ, Juhl C, Marz W, Schmitz O, Schmiegel WH, Nauck MA** 2001 Glucagon-like peptide 1 increases secretory burst mass of pulsatile insulin secretion in patients with type 2 diabetes and impaired glucose tolerance. Diabetes 50:776–784
12. **Holst JJ, Gromada J** 2004 Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab 287:E199–E206
13. **Holst JJ** 2002 Therapy of type 2 diabetes mellitus based on the actions of glucagon-like peptide-1. Diabetes Metab Res Rev 18:430–441
14. **Nauck MA** 2004 Glucagon-like peptide 1 (GLP-1) in the treatment of diabetes. Horm Metab Res 36:852–858
15. **Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD** 2004 Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. Diabetes Care 27:2628–2635
16. **DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD** 2005 Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes mellitus. Diabetes Care 28:1092–1100
17. **Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD** 2005 Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes mellitus treated with metformin and a sulfonylurea. Diabetes Care 28:1083–1091
18. **Nielsen LL** 2005 Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes. Drug Discovery Today 10:703–710
19. **Parkes DG, Pittner R, Jodka C, Smith P, Young A** 2001 Insulinotropic actions of exendin-4 and glucagon-like peptide-1 *in vivo* and *in vitro*. Metabolism 50:583–589
20. **Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, Taylor K, Kim D, Aisporna M, Wang Y, Baron AD** 2003 Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 88:3082–3089
21. **Kolterman OG, Kim DD, Shen L, Ruggles JA, Nielsen LL, Fineman MS, Baron AD** 2005 Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. Am J Health Syst Pharm 62:173–181
22. **Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M, Schmitz O** 2004 Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. Diabetes 53:2397–2403
23. **Göke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B** 1993 Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting β-cells. J Biol Chem 268:19650–19655
24. **Vilsbøll T, Agerso H, Krarup T, Holst JJ** 2003 Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab 88:220–224
25. **Nielsen LL, Young AA, Parkes D** 2004 Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regul Pept 117:77–88
26. **Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes D, Young AA** 2005 Exenatide (exendin-4) improves insulin sensitivity and β-cell mass in insulin-resistant obese *fa/fa* Zucker rats independent of glycemia and body weight. Endocrinology 146:2069–2076
27. 1997 World Medical Association declaration of Helsinki recommendations guiding medical physicians in biomedical research involving human subjects. JAMA 277:925–926
28. **Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hüfner M, Schmiegel WH** 2002 Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. J Clin Endocrinol Metab 87:1239–1246
29. **Holst JJ** 1982 Evidence that peak II GLI or enteroglucagon is identical to the C-terminal sequence (residues 33–69) of glicentin. Biochem J 207:381–388
30. **Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD** 2003 Effect on glycemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. Diabetes Care 26:2370–2377
31. **Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J** 1980 Prehepatic insulin production in man: kinetic analysis using peripheral C-peptide behaviour. J Clin Endocrinol Metab 51:520–528
32. **Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B** 1986 Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type 1 diabetic patients. J Clin Invest 77:98–105
33. **Hovorka R, Soons PA, Young MA** 1996 ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 50:253–264
34. **Egan JM, Clocquet AR, Elahi D** 2002 The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to type 2 diabetes. J Clin Endocrinol Metab 87:1282–1290
35. **Ritzel R, Ørskov C, Holst JJ, Nauck MA** 1995 Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response-relationships. Diabetologia 38: 720–725
36. **Pratley RE, Weyer C** 2001 The role of impaired early insulin secretion in the pathogenesis of type II diabetes mellitus. Diabetologia 44:929–945
37. **Efendic S, Cerasi E, Luft R, Gladnikoff G** 1976 Potentiation of glucose-induced insulin release by glucose in the isolated pancreas of fed and fasted rats. Diabetes 25:949–954
38. **Fasching P, Ratheiser K, Nowotny P, Uurzemann S, Parzer S, Waldhausl W** 1994 Insulin production following intravenous glucose, arginine, and valine: different pattern in patients with impaired glucose tolerance and non-insulin-dependent diabetes mellitus. Metabolism 43:385–389
39. **Seltzer HS, Allen EW, Herron Jr AL, Brennan MT** 1967 Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 46:323–335
40. **Nauck M, Stöckmann F, Ebert R, Creutzfeldt W** 1986 Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 29:46–52
41. **Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E** 1988 Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 318:1231–1239
42. **Rahier J, Goebbels RM, Henquin JC** 1983 Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371

43. **Rahier JRD, Ibrahim M, Channaoui K** 1989 Islet cell populations in obese subjects and type 2 (non-insulin-dependent) diabetic patients. Diabetologia 32(Suppl 1):532
44. **Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC** 2003 β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110
45. **Fehmann H-C, Habener JF** 1992 Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells. Endocrinology 130:159–166
46. **Fehmann HC, Gherzi R, Göke B** 1995 Regulation of islet hormone gene expression by incretin hormones. Exp Clin Endocrinol Diabetes 103(Suppl 2):56–65
47. **Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR** 2004 Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. Diabetes Care 27:1335–1342
48. **Saad M, An B, Santiago O, on behalf of the 2072 study group** 2002 The effect of NN2211, a long-acting GLP-1 derivative, on glycemic control and body weight in obese patients with type 2 diabetes. Diabetologia 45(Suppl 2):A44
49. **Gutniak MK, Linde B, Holst JJ, Efendic S** 1994 Subcutaneous injection of the incretin hormone glucagon-like peptide 1 abolishes postprandial glycemia in NIDDM. Diabetes Care 17:1039–1044
50. **Nauck MA, Wollschläger D, Werner J, Holst JJ, Ørskov C, Creutzfeldt W, Willms B** 1996 Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. Diabetologia 39:1546–1553
51. **Zander M, Taskiran M, Toft-Nielsen MB, Madsbad S, Holst JJ** 2001 Additive glucose-lowering effects of glucagon-like peptide-1 and metformin in type 2 diabetes. Diabetes Care 24:720–725
52. **Zander M, Madsbad S, Madsen JL, Holst JJ** 2002 Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830
53. **Agersø H, Jensen LB, Elbrønd B, Rolan P, Zdravkovic M** 2002 The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. Diabetologia 45:195–202
54. **Elbrønd B, Jakobsen G, Larsen S, Agersø H, Jensen LB, Rolan P, Sturis J, Hatorp V, Zdravkovic M** 2002 Pharmacokinetics, pharmacodynamics, safety, and tolerability of a single-dose of NN2211, a long-acting glucagon-like peptide 1 derivative, in healthy male subjects. Diabetes Care 25:1398–1404
55. **Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agersø H, Veldhuis J, Pørksen N, Schmitz O** 2002 Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 51:424–429

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