Characterization of GLP-1 Effects on β-Cell Function After Meal Ingestion in Humans

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OBJECTIVE — Glucagon-like peptide 1 (GLP-1) is an incretin that augments insulin secretion after meal intake and is developed for treatment of type 2 diabetes. As a novel therapeutic agent, characteristics of its β-cell effects are important to establish. Previously, β-cell effects of GLP-1 have been characterized in humans during graded intravenous infusions of glucose, whereas its effects after more physiological stimuli, like meal intake, are not known.

RESEARCH DESIGN AND METHODS — Eight women (aged 69 years, fasting glucose 3.7–10.3 mmol/l, BMI 22.4–43.9 kg/m^2) who had fasted overnight were served a breakfast (450 kcal) with intravenous infusion of saline or synthetic GLP-1 (0.75 pmol · kg^{-1} · min^{-1}), and β-cell function was evaluated by estimating the relationship between glucose concentration and insulin secretion (calculated by deconvolution of C-peptide data).

RESULTS — GLP-1 markedly augmented insulin secretion, despite lower glucose. Total insulin secretion was 29.7 ± 4.2 nmol/m^2 with GLP-1 versus 21.0 ± 1.6 nmol/m^2 with saline (P = 0.048). GLP-1 increased the dose-response relationship between glucose concentration and insulin secretion (70 ± 26 with GLP-1 versus 38 ± 16 pmol insulin · min^{-1} · m^2 · mmol^{-1} glucose · l without, P = 0.037) and augmented the potentiation factor that modulates the dose response (2.71 ± 0.42 with GLP-1 versus 0.97 ± 0.17 without, P = 0.005). The potentiation factor correlated to GLP-1 concentration (r = 0.53, P < 0.001); a 10-fold increase in GLP-1 levels produced a twofold increase in the potentiation factor. These effects of GLP-1 did not correlate with fasting glucose levels or BMI.

CONCLUSIONS — Administration of GLP-1 along with ingestion of a meal augments insulin secretion in humans by a dose-dependent potentiation of the dose-response relationship between plasma glucose and insulin secretion.

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Glucagon-like peptide 1 (GLP-1) is a gut hormone that functions as an incretin, thus stimulating insulin secretion after its release into the circulation after ingestion of a meal (1–4). GLP-1 also inhibits glucagon secretion and delays gastric emptying (1,2,5,6). Because these actions result in lowering of prandial glucose levels and persist in subjects with type 2 diabetes (7–11), the potential use of GLP-1 as a novel modality in the treatment of this disease has been explored a great deal during the last decade (1).

The insulinotropic action of GLP-1 is glucose-dependent, dose-dependent, and to a large degree dependent on the activation of adenylate cyclase, formation of cyclic AMP and activation of protein kinase A, as demonstrated in vivo and in vitro in a variety of experimental animals (1–4), although mechanisms distinct from activation of protein kinase A have also been disclosed (12). An insulinotropic action of GLP-1 has also been documented in humans, in whom the peptide augments insulin secretion in response to glucose or a mixed meal (5,7–11,13–16). However, the β-cell action of GLP-1 has been less well characterized in humans. One study has shown that during graded intravenous infusion of glucose, GLP-1 dose-dependently increases insulin secretion by increasing the slope of the relation between glucose concentration and insulin secretion (16). However, for the development of GLP-1 as a novel treatment in type 2 diabetes, it is of importance to establish the effects of GLP-1 on the β-cell characteristics after ingestion of a meal. Insulin secretion after ingestion of a meal is characterized by a baseline secretion rate; by a direct action on the islet β-cells of insulin secretagogues of importance during meal ingestion, such as glucose, amino acids, gastrointestinal hormones and neurotransmitters; and by the sensitivity in the β-cells to these secretagogues. These processes may be separated and evaluated by modeling of insulin, C-peptide, and glucose data after ingestion of a meal (17,18). In this study, we have therefore examined the influence of GLP-1 on these β-cell characteristics after ingestion of a meal in humans. To examine the dependency of the GLP-1 effects on fasting glucose, we recruited subjects with a broad range of fasting glucose and included subjects with normal and elevated glucose levels.

RESEARCH DESIGN AND METHODS

Subjects

The study was undertaken in eight women, all 69 years of age, who were recruited from a larger cohort of women in the city of Malmö, Sweden and who participated in a population study (19). Be-

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Abbreviations: GLP-1, glucagon-like peptide 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

cause we aimed to establish the glucose dependency of the effects of GLP-1, we included subjects with a range of fasting glucose from 3.7 to 10.3 mmol/l. Six of the subjects were healthy, without diabetes or any known history of cardiovascular, liver, or kidney diseases, and they were not taking any medication known to affect carbohydrate metabolism. Two subjects had type 2 diabetes treated with diet for the past 6 and 10 months, respectively. These patients had no diabetic complications or any cardiovascular, liver, or kidney diseases, and they were not taking any medication known to affect carbohydrate metabolism. The mean (± SD) fasting plasma glucose level of the study subjects was 7.4 ± 2.4 mmol/l (range 3.7–10.3) and the BMI was 28.4 ± 7.6 kg/m^2 (range 22.4–43.9). The Ethics Committee of Lund University, Sweden, approved the study. All subjects gave written informed consent before entrance into the study.

Study protocol

Two meal tests were performed in each subject, in random order, with at least 3 weeks between. Intravenous catheters were inserted after an overnight fast into antecubital veins in both arms and baseline samples were collected at −5 and −2 min. Thereafter, synthetic GLP-1 (Clinalfa, Laeufelingen, Switzerland) was infused intravenously at a rate of 0.75 pmol · kg^{-1} · min^{-1} for 90 min. The dose of GLP-1 was selected from previous reports in which GLP-1 was shown to reduce prandial glucose (5,6). In the control test, saline was infused instead of GLP-1. At the start of GLP-1 or saline infusion, a breakfast was served and eaten within 5 min. The breakfast consisted of 450 kcal with 50% of energy as carbohydrate, 23% as fat, and 27% as protein. Additional samples were taken regularly for 120 min.

Analyses

Blood samples for determination of insulin, C-peptide, and glucagon were collected in chilled tubes containing EDTA (7.4 mmol/l, final concentration) and aprotinin (500 kallikrein inhibitor units/ml blood; Novo Nordisk, Bagsværd, Denmark). They were immediately centrifuged at 4°C and plasma frozen at −20°C until analysis in duplicate. Plasma insulin and C-peptide concentrations were analyzed with double-antibody radioimmunoassay techniques (Linco Research, St. Charles, MO) using guinea pig anti-human insulin antibodies, human insulin standard, mono-^{125}I-Tyr–labeled human insulin, guinea-pig anti-human C-peptide antibody, human C-peptide standard, and ^{125}I-human C-peptide as tracer. Analysis of glucagon was performed with double-antibody radioimmunoassay using guinea-pig anti-glucagon antibodies specific for pancreatic glucagon, ^{125}I-glucagon as tracer, and glucagon standard (Linco). Blood samples for determination of GLP-1 were collected into chilled tubes containing EDTA and aprotinin as above with addition of diprotin A (0.1 mmol/l final concentration; Bachem, Bubendorf, Switzerland). They were kept on ice until centrifugation at 4°C. Plasma was separated and stored at –20°C until analysis for amino-terminal GLP-1 immunoreactivity by radioimmunoassay using antiserum 93242 (20), which has a cross-reactivity of ~10% with GLP-1$_{1–36}$amide and of <0.1% with GLP-1$_{8–36}$amide and GLP-1$_{9–36}$amide. The detection limit is 1 pmol/l. High-performance liquid chromatography supports the use of radioimmunoassays with this specificity for determination of intact GLP-1 (21). The intra-assay coefficient of variation is <6%. It should be emphasized that the assay detects the active form of GLP-1, i.e., GLP-1$_{7–36}$amide, and not the total circulating pool of GLP-1, which to a large degree (>80%) includes the inactive form of GLP-1, GLP-1$_{9–36}$amide. Glucose was determined with the glucose oxidase technique.

Calculations

Parameters of β-cell function were determined by reconstructing insulin secretion from C-peptide data by a deconvolution technique based on a two-compartment model of C-peptide kinetics (22), as previously described (17,18). The model describes the relationship between insulin secretion, $S(t)$, and glucose concentration. Insulin secretion consists of two components, according to the equation $S(t) = S_g(t) + S_d(t)$. The first component, $S_g(t)$, represents the dependency of insulin secretion on the absolute glucose concentration (G) at any time point and is characterized by a dose-response function, $f(G)$, relating these variables. Two characteristic parameters of the dose response are its mean slope (in the observed glucose range) and insulin secretion at a fixed glucose concentration of 7 mmol/l (approximately the mean basal glucose concentration). The dose response is modulated by a potentiation factor, $P(t)$, which incorporates glucose-mediated and non–glucose-mediated potentiation (i.e., by non-glucose substrates, gastrointestinal hormones, and neurotransmitters), i.e., $S_g(t) = P(t)f(G)$. The potentiation factor is, by definition, a positive function of time and averages 1 during the experiment (17,18). The second insulin secretion component, $S_d(t)$, represents a dynamic dependency of insulin secretion on the rate of change of glucose concentration, expressed as $S_d(t) = K_d dG(t)/dt$ if $dG(t)/dt > 0$ and $S_d(t) = 0$ if $dG(t)/dt \leq 0$. This component is called the derivative component and is thus described by a single parameter (K_d). From these estimated model parameters, the dose-response $f(G)$, and the potentiation factor $P(t)$ (17,18), the total and basal insulin secretion were calculated. The excursion of the potentiation factor was quantified using ratios between mean values at time 60 and time 0, i.e., P(60)/P(0).

Statistics

Data are reported as means ± SE if not stated otherwise. Paired Student's t test was used to compare differences with versus without GLP-1.

RESULTS

Glucose, insulin, C-peptide, glucagon, and GLP-1 concentrations

Figure 1 shows the glucose, insulin, C-peptide, glucagon, and GLP-1 profiles before and after meal intake in the eight subjects during intravenous infusion of either saline or GLP-1. It is shown that glucose levels were lower during infusion of GLP-1 than during infusion of saline, whereas insulin and C-peptide levels were higher during GLP-1 than during saline. Glucagon levels were lower during infusion of GLP-1 than during saline. During saline infusion, GLP-1 levels increased from 1.9 ± 0.2 to a maximal level of 5.6 ± 0.3 pmol/l ($P < 0.001$). During infusion with GLP-1, the GLP-1 concentrations increased from 2.0 ± 0.3 to 27.5 ± 2.5 pmol/l ($P < 0.001$).

Insulin secretion in response to meal ingestion

Figure 2 shows the modeled insulin secretion profile before and after the meal. Baseline insulin secretion was not differ-

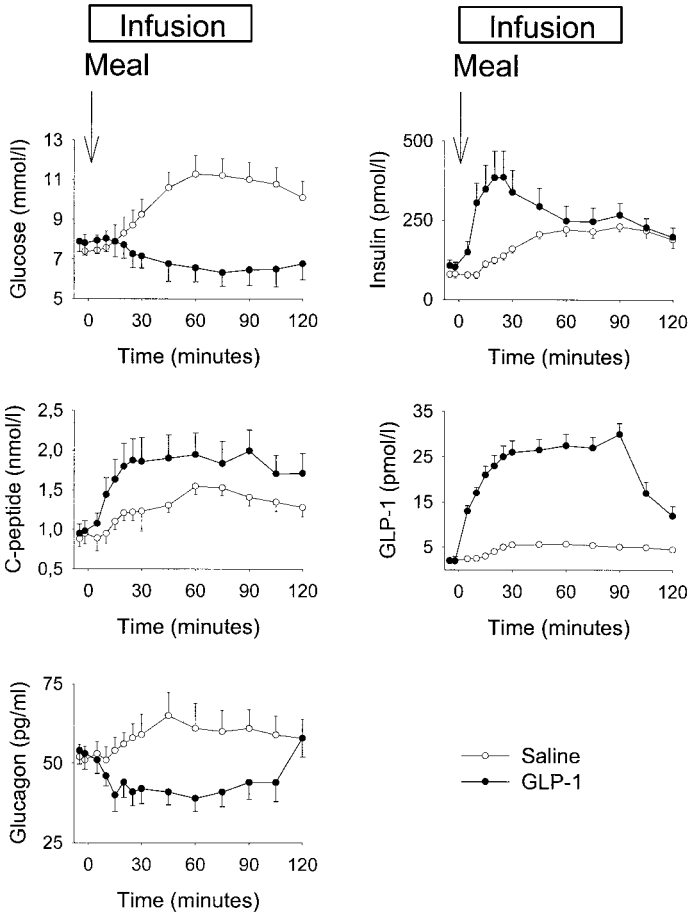

Figure 1—*Plasma levels of glucose, insulin, C-peptide, glucagon, and GLP-1 during the meal test in eight human subjects in the presence of an intravenous infusion of saline or GLP-1, as indicated. Means ± SE are shown.*

ent between the two tests. Meal ingestion alone induced a continuous increase in insulin secretion. GLP-1 markedly augmented this response. The augmentation by GLP-1 was particularly profound during the first 30 min after meal ingestion. Total insulin secretion during the test was higher with GLP-1 than with saline (P = 0.048; Table 1).

Dose response and derivative component

Figure 2 also shows the dose-response relationship between plasma glucose concentrations and insulin secretion as obtained in the model. The curves are obtained by reconstructing the dose-response relationship between glucose and insulin secretion for each subject in the presence and absence of GLP-1. The curves, therefore, represent the calculated insulin secretion as dependent on the glucose concentration without any potentiation. It is seen that the dose response is shifted upward by GLP-1; both insulin secretion at 7 mmol/l glucose and the mean dose-response slope are increased by GLP-1 (Table 1). This shows that for a given glucose concentration, insulin secretion is augmented by GLP-1. The parameter of insulin secretion representing the dynamic dependency on the rate of change of glucose concentration (K_d) was not significantly affected by GLP-1 (Table 1).

Potentiation factor

The potentiation factor obtained from the meal tests with or without infusion of GLP-1 is shown in Fig. 2. The potentiation factor was, by definition, constrained to be 1, on average, during the meal test (17,18). It is seen that during GLP-1 infusion, there was a marked and rapid increase in the potentiation factor already in the beginning of the test. The increase in the potentiation factor by GLP-1, quantified by the ratio of the potentiation factor at time 60 to time 0, was 0.97 ± 0.17 in the control test versus 2.71 ± 0.42 when GLP-1 was infused (P = 0.005). During GLP-1 infusion, the marked increase over the basal level of the potentiation factor (Fig. 2) paralleled that of the GLP-1 concentration (Fig. 1). By pooling the potentiation factor and GLP-1 concentration values of all subjects at all time points, the potentiation factor was positively correlated with GLP-1 levels (r = 0.53, P < 0.0001 after logarithmic transformation). Furthermore, by pooling all experiments, the average increment over basal of the potentiation factor (expressed as ratio) was positively correlated with the corresponding GLP-1 increment (Fig. 2; r = 0.76, P = 0.0006). From the observed nonlinear relationship, a twofold increment in potentiation is predicted for a 10-fold increment in GLP-1 concentration.

Relation to clinical characteristics

In an attempt to relate the effects of GLP-1 on β-cell function to clinical characteristics of the subjects, correlations were performed between the indexes reported in Table 1 with GLP-1 infusion on one hand and fasting plasma glucose or BMI, respectively, on the other hand. However, no significant correlation was found.

CONCLUSIONS — GLP-1 is known to augment insulin secretion in response to intravenous and oral glucose as well as to meal ingestion in humans (7–8,10–11,13–16). Besides the established knowledge that the effect of GLP-1 is dose dependent, the detailed mechanism of the β-cell actions of GLP-1 in humans is not established. Recently, it was shown that the prehepatic insulin secretion was dose dependently potentiated by GLP-1 because the peptide increased the linear slope between glucose concentration and insulin secretion when glucose was administered intravenously as a graded infusion (16). The present study examined the influences of GLP-1 on characteristics of β-cell function as obtained by analyses of the insulin secretion profile after meal ingestion, i.e., when GLP-1 is considered of most importance when evaluated as a therapeutic agent in type 2 diabetes. After meal ingestion, GLP-1 suppressed glucose levels, which is a combined effect of the peptide to stimulate insulin secretion, inhibit glucagon secretion, and inhibit

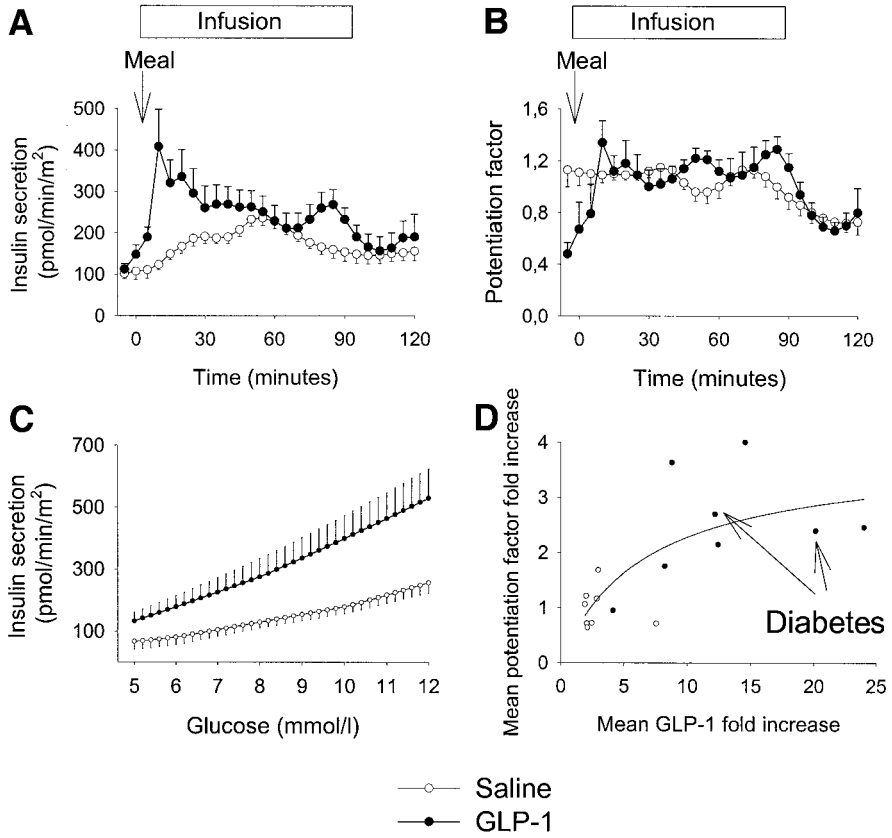

Figure 2—*Insulin secretion profile* (A)*, potentiation factor* (B)*, and static relationship between plasma glucose concentrations and insulin secretion* (C) *during the meal test in eight human subjects in the presence of an intravenous infusion of saline or GLP-1, as indicated. Means ± SE are shown.* D*: The relationship between the average increment over basal of the potentiation factor (expressed as a ratio) and the corresponding GLP-1 increment during the meal tests with saline (○) or with GLP-1 (●). The regression equation (solid line) was* $y = 0.605 \times 0.517$ ($r = 0.76$, $P = 0.0006$).

gastric emptying as demonstrated before (1–11) and in regard to insulin and glucagon confirmed in the present study.

The different glucose profile after the meal ingestion when infusing saline versus GLP-1 requires modeling of the data for estimation of insulin secretion from the β-cells. In this study, we used a model of β-cell function, as recently described in detail (17,18). The modeling disclosed that GLP-1 markedly increased insulin secretion, despite glucose levels being lower, and that this was particularly powerful during the first 30 min after meal ingestion. After the initial 30 min, the action of GLP-1 on absolute insulin secretion was no longer apparent, but this was mainly a consequence of the lowered glucose levels. The main result of the study is that after meal ingestion in humans, the dose-response function representing the static relationship between glucose concentration and insulin secretion is markedly shifted upward by GLP-1, and that potentiation is strongly stimulated. Therefore, GLP-1 increases both glucose dose response and potentiation.

For appreciation of these findings, it is important to consider that the stimulatory effect of glucose on insulin secretion is not constant during the dynamic course of a meal test. The reason for this is, first, that the insulin response to a meal intake is dependent not only on glucose but also on other nutrients, neurotransmitters, and incretins. These factors contribute to the β-cell function by changing (mainly augmenting) the insulinotropic action of glucose. The model showed that GLP-1 augmented the effect of glucose to stimulate insulin secretion, i.e., for a given glucose concentration, the static release of insulin is increased on average during the test. This augmenting effect on glucose-stimulated insulin secretion is expressed as the potentiation factor, which was markedly increased above basal levels with GLP-1 infusion.

The potentiation factor was, by definition, constrained to be 1, on average, during the test (17,18). By doing this, the corresponding dose response represents the average dose response for the experiment and the reported potentiation factor represents a relative potentiation factor. A potentiation factor <1 means, therefore, a potentiation below average during the experiment and, consequently, a potentiation factor >1 means a potentiation above the average. Therefore, by inspecting Fig. 2, the potentiation factor in the GLP-1 experiment was lower in the basal state than in the control meal test, because GLP-1 increased potentiation. By this definition, it is the different shapes of the curves that illustrate the marked effect of

Table 1—***Model indexes of β-cell function as calculated from data obtained after ingestion of a breakfast (450 kcal) in eight human subjects in the presence of an intravenous infusion of saline or GLP-1***

Parameter	Saline	GLP-1	*P*
Insulin secretion at time 0 ($pmol \cdot min^{-1} \cdot m^{-2}$)	108 ± 15	135 ± 20	NS
Total insulin secretion ($nmol \cdot m^{-2}$)	21.0 ± 1.6	29.7 ± 4.2	0.048
Insulin secretion at 7 mmol/l glucose ($pmol \cdot min^{-1} \cdot m^{-2}$)	103 ± 21	249 ± 60	0.036
Slope of the dose response ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mmol^{-1} \cdot l^{-1}$)	38 ± 16	70 ± 26	0.037
Ratio of potentiation factor at time 60 min versus at time 0	0.97 ± 0.17	2.71 ± 0.42	0.005
Parameter of the derivative component ($pmol \cdot m^{-2} \cdot mmol^{-1} \cdot l^{-1}$)	423 ± 127	187 ± 66	NS

Data are means ± SE. *P* indicates probability level of random difference between the two tests.

GLP-1 rather than the actual potentiation factor. The relative change in potentiation factor can, however, be calculated by estimating the excursion of the potentiation factor by making a ratio of the factor at min 60 to baseline. This ratio was 2.71 ± 0.42 in the GLP-1 experiment versus 0.97 ± 0.17 in the control test, i.e., GLP-1 augments potentiation factor by more than 2.5-fold. Appreciating this, it is seen that GLP-1 throughout its infusion provided a high potentiation factor, resulting, therefore, in augmented insulin secretion, as reflected by the glucose dose-response relationship. In the previous study on the characteristics of the effects of GLP-1 during a graded intravenous infusion of glucose, it was found that an ~40-fold increase in GLP-1 levels resulted in an ~11-fold increase in potentiation (16). This higher efficiency when compared with the present study is probably explained by the prolonged (150-min) glucose infusion, which augments time-dependent potentiation. This effect is not seen in the present study because GLP-1 prevented glucose from being increased after ingestion of a meal. Therefore, the present study shows the efficiency of GLP-1 to augment potentiation of insulin secretion when glucose levels show the physiological perturbations after meal ingestion.

The results of the present study also allowed evaluation of a dose response for GLP-1 effects on insulin secretion, which is potentially interesting for GLP-1 dosage. Therefore, we found that a 10-fold increase in active GLP-1 levels resulted in a twofold increase in insulin secretion, as evaluated from the increase in the potentiation factor. Finally, we did not find evidence that the effect of GLP-1 on β-cell function is dependent on the fasting glucose or BMI, based on results in the subjects exhibiting a broad range of fasting glucose and BMI values.

In conclusion, we have characterized the effects of GLP-1 on β-cell function during a meal test in subjects with a broad range of circulating glucose by means of a model of insulin secretion. We found that GLP-1 markedly increased insulin secretion during the meal intake, despite circulating levels of glucose being reduced. This action of GLP-1 was mainly due to an increase in the average β-cell sensitivity to glucose, mediated by a potentiation effect related to GLP-1 levels.

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