The Influence of GLP-1 on Glucose-Stimulated Insulin Secretion

Effects on β-Cell Sensitivity in Type 2 and Nondiabetic Subjects

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The intestinally derived hormone glucagon-like peptide 1 (GLP-1) (7-36 amide) has potent effects on glucose-mediated insulin secretion, insulin gene expression, and β-cell growth and differentiation. It is, therefore, considered a potential therapeutic agent for the treatment of type 2 diabetes. However, the dose-response relationship between GLP-1 and basal and glucose-stimulated prehepatic insulin secretion rate (ISR) is currently not known. Seven patients with type 2 diabetes and seven matched nondiabetic control subjects were studied. ISR was determined during a graded glucose infusion of 2, 4, 6, 8, and 12 mg · kg^{-1} · min^{-1} over 150 min on four occasions with infusion of saline or GLP-1 at 0.5, 1.0, and 2.0 pmol · kg^{-1} · min^{-1}. GLP-1 enhanced ISR in a dose-dependent manner during the graded glucose infusion from 332 ± 51 to 975 ± 198 pmol/kg in the patients with type 2 diabetes and from 711 ± 123 to 2,415 ± 243 pmol/kg in the control subjects. The β-cell responsiveness to glucose, expressed as the slope of the linear relation between ISR and the glucose concentration, increased in proportion to the GLP-1 dose to 6 times relative to saline at the highest GLP-1 dose in the patients and 11 times in the control subjects, but it was 3 to 5 times lower in the patients with type 2 diabetes compared with healthy subjects at the same GLP-1 dose. During infusion of GLP-1 at 0.5 pmol · kg^{-1} · min^{-1} in the patients, the slope of ISR versus glucose became indistinguishable from that of the control subjects without GLP-1. Our results show that GLP-1 increases insulin secretion in patients with type 2 diabetes and control subjects in a dose-dependent manner and that the β-cell responsiveness to glucose may be increased to normal levels with a low dose of GLP-1 infusion. Nevertheless, the results also indicate that the dose-response relation between β-cell responsiveness to glucose and GLP-1 is severely impaired in patients with type 2 diabetes. ***Diabetes*** **52:380–386, 2003**

Glucagon-like peptide 1 (7-36) amide (GLP-1) is an incretin hormone, secreted from the L-cells of the small intestine in response to ingestion of a meal (1–3). Together with the related hormone glucose-dependent insulinotropic polypeptide (GIP), GLP-1 is responsible for the augmentation of insulin secretion that results from oral, compared with intravenous, stimulation of insulin secretion with nutrients (4). Unlike GIP, the insulinotropic effect of GLP-1 is preserved in patients with type 2 diabetes (5), in whom intravenous infusion of ~1 pmol · kg^{-1} · min^{-1} of GLP-1 may actually normalize fasting hyperglycemia (6). Part of this effect may be due to simultaneous inhibition of glucagon secretion, resulting in a diminished hepatic glucose production (6,7). However, despite multiple studies examining the effect of GLP-1 in healthy volunteers (8,9) and in subjects with type 1 and type 2 diabetes (8,10–12), a dose-response relationship for GLP-1 and insulin secretion has not been established, even though this is relevant for the long-term treatment studies with GLP-1.

Therefore, we decided to investigate the dose-response relationship for GLP-1 with respect to glucose-induced insulin secretion in patients with type 2 diabetes and in control subjects matched for age, sex, and weight, using a graded intravenous glucose infusion protocol (13,14). The relationship between plasma glucose and the prehepatic rate of insulin secretion, calculated from C-peptide data by a deconvolution method (15,16), was determined at each of four rates of infusion (0, 0.5, 1.0, and 2.0 pmol · kg^{-1} · min^{-1} GLP-1). Thus, it was possible to establish for both healthy control subjects and patients with type 2 diabetes the insulinotropic effect of GLP-1 and its influence on the sensitivity of the β-cells to changes in glucose.

RESEARCH DESIGN AND METHODS

Subjects. Seven patients with type 2 diabetes (five men and two women) with a fasting plasma glucose of 8.9 ± 0.9 mmol/l and seven control subjects (four men and three women) with a fasting plasma glucose of 4.4 ± 0.2 mmol/l were studied. All subjects were Caucasian, mean age 61.4 ± 1.3 vs. 52.3 ± 3.7 years (patients versus control subjects, P = NS), and the mean BMI was 31.6 ± 1.1 vs. 28.7 ± 1.2 kg/m^2 (patients versus control subjects, P = NS). None of the control subjects had a family history of diabetes, they had no medical illnesses, and they were not receiving any medication. The patients (duration of disease 2–10 years) did not suffer from any diabetic complications or any other medical illnesses. Three of the seven patients were treated with only diet/exercise, two were treated with sulfonylureas, and two patients were

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AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; ISR, insulin secretion rate.

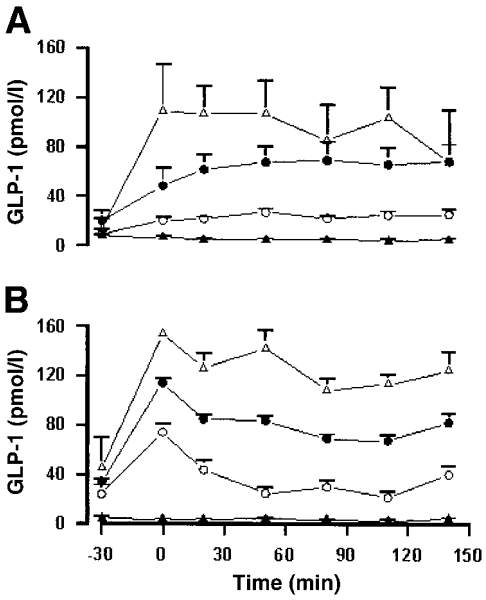

FIG. 1. Mean plasma GLP-1 (7-36) amide concentrations during saline (▲) or intravenous infusion of GLP-1 (7-36 amide) at a dose of 0.5 (○), 1.0 (●), or 2.0 (△) pmol · kg^{-1} · min^{-1}. The GLP-1 infusion was initiated −30 min at a doubled infusion rate for the first 10 min and thereafter continued throughout the study at 0.5, 1.0, or 2.0 pmol · kg^{-1} · min^{-1}. The type 2 diabetic subjects, are depicted in *A* and the control subjects are depicted in *B*.

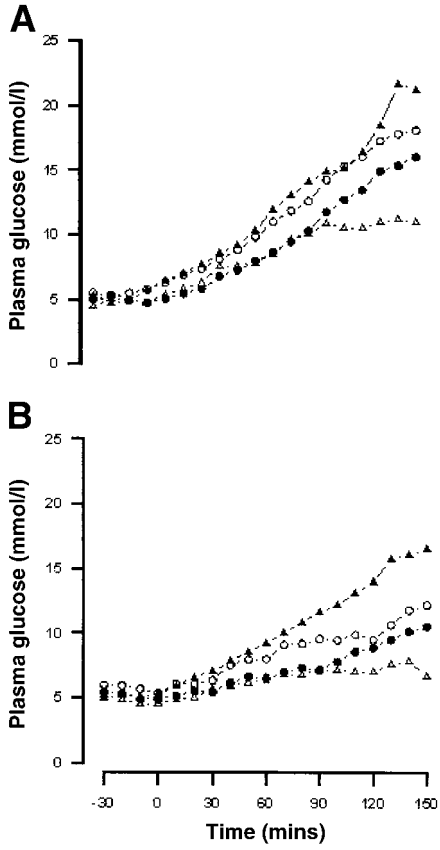

FIG. 2. Mean plasma glucose concentrations during the graded glucose infusion. From −30 min, saline (▲) or intravenous GLP-1 (7-36 amide) at a dose of 0.5 (○), 1.0 (●), or 2.0 (△) pmol · kg^{-1} · min^{-1} were infused. At *t* = 0 min, glucose was infused at a rate of 2 mg · kg^{-1} · min^{-1}, followed by 4, 6, 8, and 12 mg · kg^{-1} · min^{-1}. Each infusion rate was maintained for a period of 30 min. Samples were drawn 10, 20, and 30 min into a 30-min period for the measurement of plasma glucose. The type 2 diabetic subjects are depicted in *A*, and the control subjects are depicted in *B*.

treated with metformin. The patients were not taking any other drugs known to affect carbohydrate metabolism. The oral hypoglycemic agents were discontinued at least 3 days before each experiment. All studies were carried out at the Department of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark. Subjects gave written informed consent, and the protocols were approved by the local committees of ethics in Copenhagen and were performed in accordance with the Helsinki Declaration, II.

GLP-1. Synthetic, human GLP-1 (7-36 amide) was purchased from Peninsula Europe (Merseyside, U.K.). The same lot number of GLP-1 was used in all studies. GLP-1 was checked for sterility by use of standard bacterial culture methods and for pyrogens by use of a Limulus assay (Pyroquant 50; Walldorf, Germany). Net peptide content (76%) was used for dose calculations. The peptide was dissolved in a 0.9% saline solution containing 1% human serum albumin guaranteed to be free of hepatitis B surface antigen and HIV antibody (Novo Nordisk, Bagsvaerd, Denmark), subjected to sterile filtration, checked for sterility, and kept at −20°C until use.

Experimental protocol. The present study was designed to establish, during a graded glucose infusion, the dose-response effect of GLP-1 on insulin secretion. The peptide was infused on three separate days at rates expected to give rise to physiological, supraphysiological, and clearly pharmacological concentrations. On the fourth day, saline was infused as control. During GLP-1/saline administration, a graded glucose infusion was carried out. At least 1 week was allowed to pass between experiments, which were performed in random order. All subjects completed all four experiments. The subjects attended the ward in the morning after fasting (including abstinence from smoking) from 10:00 P.M. the evening before. Studies were performed in recumbent position, and an intravenous catheter was placed in each forearm, one for blood sampling and one for infusion. In all experiments, the hand and arm with the sampling catheter was wrapped in a heating blanket for arterialization of the venous samples (17).

To achieve similar glucose concentrations in the two groups at the start of the GLP-1 infusion, all studies in patients with type 2 diabetes began with the administration of a small bolus of intravenous insulin (0.007 units/kg) followed by a continuous intravenous infusion of 0.1 unit insulin · kg^{-1} · h^{-1} (Actrapid; Novo Nordisk). When the blood glucose level had decreased to 6.0 mmol/l, the insulin infusion was stopped (time −60 min, relative to start of the graded glucose infusion). During the following 30 min, the exogenously administered insulin was allowed to decay, and samples were drawn at −60, −50, −40, −35, and −30 min. Baseline insulin, C-peptide, and glucose levels were defined at −30 min. The control subjects were studied at their individual fasting plasma glucose levels (4.4 ± 0.2 mmol/l), whereas the patients had a mean baseline plasma glucose of 5.1 ± 0.2 mmol/l.

Glucose and GLP-1 infusion. The intravenous GLP-1 infusion was initiated at −30 min, at a doubled infusion rate for the first 10 min, to reach a plateau quickly, and thereafter continued throughout the study at the rates of 0.5, 1.0, and 2.0 pmol · kg^{-1} · min^{-1} using an automatic pump (B. Braun; Melsungen, AG, Germany). Blood samples were drawn every 30 min for measurement of GLP-1 concentrations.

A stepwise graded intravenous infusion of glucose (20% dextrose) was started at 0 min at a rate of 2 mg · kg^{-1} · min^{-1}, followed by 4, 6, 8, and 12 mg · kg^{-1} · min^{-1}. Each infusion rate was maintained for a period of 30 min. Samples were drawn at 10, 20, and 30 min during each 30-min period for the measurement of plasma insulin, C-peptide, and glucose.

Assays. Blood samples for insulin, C-peptide, and GLP-1 determination were drawn into heparinized chilled tubes containing an additional 20,000 KIU/ml aprotinin (Novo Nordisk) and placed on ice. All samples were centrifuged immediately at 4°C, 3,000 rpm for 20 min, and plasma was pipetted into separate tubes for measurement of GLP-1, insulin, and C-peptide and frozen at −20°C until analysis in duplicate.

Glucose. The blood glucose samples were drawn into tubes containing heparin and sodium fluoride, centrifuged, and measured immediately by the glucose oxidase technique using an automated analyzer (YSI model 23 A; YSI, Yellow Springs, OH).

Insulin. Plasma insulin concentrations were measured by a two-site insulin enzyme-linked immunosorbent assay method as previously described (18). The assay is based on two monoclonal murine antibodies (Novo Nordisk) specific for insulin. There was no cross-reactivity with intact proinsulin, any of the four intermediates, or C-peptide. The working range for the assay is 5–600 pmol/l and the intra- and interassay coefficients of variation were 2 and 4%, respectively.

C-peptide. Plasma C-peptide measurements were performed with a commercially available kit (K6218; DAKO, Cambridgeshire, U.K.). The method is based on two monoclonal murine antibodies and uses the same principles referred

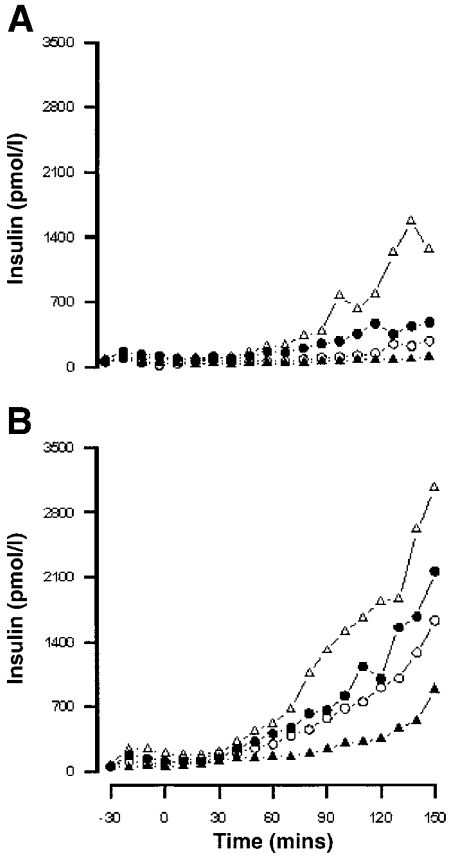

FIG. 3. Plasma insulin concentrations during the graded glucose infusion. From −30 min, saline (▲) or intravenous GLP-1 (7-36 amide) at a dose of 0.5 (○), 1.0 (●) or 2.0 (△) pmol · kg^{-1} · min^{-1} were infused. At t = 0 min, glucose was infused at a rate of 2 mg · kg^{-1} · min^{-1}, followed by 4, 6, 8, and 12 mg · kg^{-1} · min^{-1}. Each infusion rate was maintained for a period of 30 min. Samples were drawn 10, 20, and 30 min into a 30-min period for the measurement of plasma insulin. The type 2 diabetic subjects are depicted in *A*, and the control subjects are depicted in *B*.

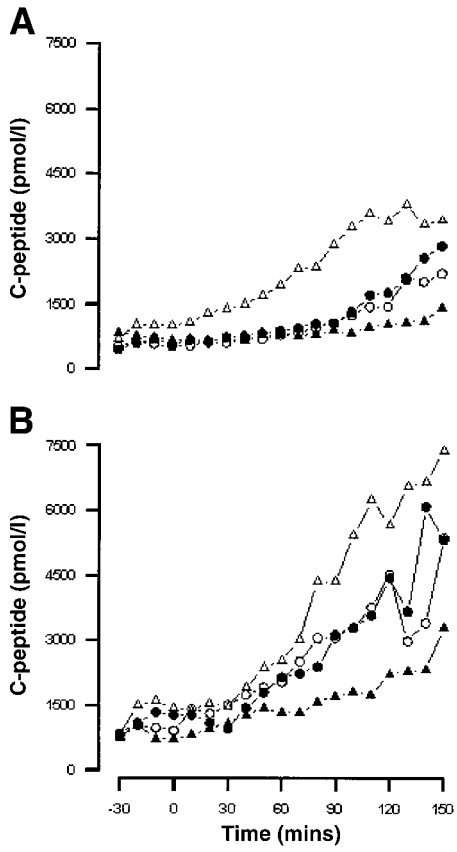

FIG. 4. C-peptide concentrations during the graded glucose infusion. From −30 min, saline (▲) or intravenous GLP-1 (7-36 amide) at a dose of 0.5 (○), 1.0 (●) or 2.0 (△) pmol · kg^{-1} · min^{-1} were infused. At t = 0 min, glucose was infused at a rate of 2 mg · kg^{-1} · min^{-1}, followed by 4, 6, 8, and 12 mg · kg^{-1} · min^{-1}. Each infusion rate was maintained for a period of 30 min. Samples were drawn 10, 20, and 30 min into a 30-min period for the measurement of C-peptide. The type 2 diabetic subjects are depicted in *A*, and the control subjects are depicted in *B*.

to above (18). The detection limit was 17 pmol/l and the intra- and interassay coefficients of variation were 3 and 6%, respectively. The cross-reactivities of intact and split proinsulin were 63–87%.

GLP-1. The plasma concentrations of GLP-1 (7-36 amide) were measured as previously described (19) against standards of human GLP-1 (7-36 amide) using antiserum code no. 89390 raised against synthetic proglucagon 98-107 amide. This antiserum has an absolute requirement for the intact amidated COOH-terminus of GLP-1 (7-36) and cross-reacts <0.01% with COOH-terminally truncated or extended forms. The detection limit of the assay is <1 pmol/l, and the intraassay coefficient of variation is <5% at 20 pmol/l. The assay measures with full reactivity the primary metabolite of GLP-1, GLP-1 (9-36) amide. Because this metabolite is formed in the circulation, it must be codetermined in the analysis if the results are to reflect the total amount of peptide infused.

Data analysis

Glucose, insulin, C-peptide, and GLP-1 concentration data. Fasting plasma glucose, insulin, C-peptide, and GLP-1 concentrations were calculated as the mean of the basal samples from −60 to −30 min (before infusion of saline or GLP-1). The areas under the plasma concentrations curves (AUCs) for glucose, insulin, and C-peptide were calculated by the trapezoidal rule for the periods of GLP-1/saline infusion as well as following the graded glucose infusion, and incremental AUCs were calculated by subtraction of the baseline values.

Assessment of insulin secretion rates. Prehepatic insulin secretion rates (ISR) for each individual during the four experiments were derived by deconvolution of peripheral C-peptide concentrations using a two-compartment model of C-peptide kinetics (15,16) and population-based C-peptide kinetic parameters (20). The population-based parameters are derived from analysis of a large number of individual kinetic parameters allowing calculation of values adjusted for clinical status (normal, obese, or type 2 diabetes and age and sex) (20). ISR is expressed as pmol · kg^{-1} · min^{-1}.

Relationship between glucose concentration and insulin secretion. The calculated ISR values were plotted against plasma glucose to establish the dose response relationship for each individual, experimental day, and dose of GLP-1. The slopes of these approximately linear relations were evaluated by cross-correlation analysis as previously described (13) and expressed as pmol · kg^{-1} · min^{-1}/(mmol/l) and regarded as measures of β-cell responsiveness to glucose.

Statistical analysis. Standard statistical methods were employed for analysis and presentation of the data. Comparison of results from the control subjects and the patients were made by a Mann-Whitney U test. Comparison of the results obtained on the four experimental days were made by use of Friedman's test (21). Unless otherwise stated, results are reported as means ± SE. P values < 0.05 were considered significant.

RESULTS

Fasting glucose levels were higher in the patients with type 2 diabetes than in control subjects (8.9 ± 0.9 vs. 4.4 ± 0.2 mmol/l, P < 0.01) before the studies. After intravenous insulin administration, normoglycemia was achieved in the patients (5.1 ± 0.2 mmol/l).

GLP-1 infusions and side effects. Plasma GLP-1 concentrations increased in a dose-dependent manner in the control subjects as well as in the patients for all three infusion rates of GLP-1 (Fig. 1). The control subjects had a slightly greater initial increase than the patients with type 2 diabetes (P < 0.05) (Fig. 1).

In the control subjects, infusion of GLP-1 from −30 to 0 min before the initiation of the graded glucose infusion

TABLE 1
Incremental areas under the plasma glucose, insulin, and C-peptide curves as well as the total amount of insulin secreted (total ISR) during GLP-1 and saline infusion

Infusion	Saline	GLP-1 (0.5 pmol · kg^{-1} · min^{-1})	GLP-1 (1.0 pmol · kg^{-1} · min^{-1})	GLP-1 (2.0 pmol · kg^{-1} · min^{-1})
Type 2 diabetic subjects				
AUC glucose (mmol/l/150 min)	1,882.0 ± 12.4	1,805.3 ± 10.6	1,567.9 ± 9.4	1,306.0 ± 5.5
AUC insulin (nmol/l/150 min)	8.3 ± 0.4	14.2 ± 3.3	32.4 ± 8.2	63.0 ± 37.5
AUC C-peptide (nmol/150 min)	128.7 ± 19.2	164.7 ± 24.5	323.7 ± 120.4	369.9 ± 120.4
Total ISR (pmol · kg^{-1} · min^{-1})	332.2 ± 50.5	629.5 ± 52.6	910.1 ± 107.4	974.6 ± 197.6
Control subjects				
AUC glucose (mmol/l/150 min)	1,596.1 ± 9.1	1,297.8 ± 4.8	1,093.9 ± 4.3	972.1 ± 2.4
AUC insulin (nmol/l/150 min)	37.8 ± 10.2	81.2 ± 23.6	104.2 ± 26.7	159.7 ± 43.6
AUC C-peptide (nmol/l/150 min)	238.9 ± 49.8	380.5 ± 88.9	408.0 ± 97.2	587.4 ± 127.6
Total ISR (pmol · kg^{-1} · min^{-1})	710.9 ± 123.2	1,201.2 ± 179.4	1411.7 ± 222.4	2414.6 ± 242.5

Data are means ± SE.

increased fasting plasma insulin and C-peptide concentrations ($P < 0.01$, Friedmann analysis) and decreased plasma glucose concentrations, an effect observed for all three infusion rates of GLP-1 (Figs. 2–4). In contrast, in the patients with type 2 diabetes, only the two higher infusion rates of GLP-1 (1.0 and 2.0 pmol · kg^{-1} · min^{-1}) enhanced plasma insulin and C-peptide, whereas infusion of 0.5 pmol · kg^{-1} · min^{-1} had no significant effect on the fasting hormone levels (Figs. 2–4).

No side effects, such as nausea or profuse sweating, were registered during the GLP-1 infusions.

Responses to graded glucose infusion. Time courses for the changes in plasma glucose, insulin, and C-peptide concentrations are shown in Figs. 2, 3, and 4, respectively.

In patients with type 2 diabetes, a graded glucose infusion without GLP-1 elicited the expected delayed and impaired increment in plasma insulin and C-peptide levels compared with the control subjects (Figs. 3*A* and 4*A*). The glucose response was exaggerated in the patients with type 2 diabetes, with a mean glucose concentration during the graded glucose infusion of 12.6 ± 1.2 mmol/l and a mean peak plasma glucose concentration of 21.7 ± 0.7 mmol/l (Fig. 2*A*). The corresponding glucose values in control subjects were 10.7 ± 1.4 and 16.5 ± 0.8 mmol/l and were associated with much greater insulin and C-peptide responses (Figs. 2*B*, 3*B*, and 4*B*).

In the patients, infusion of increasing doses of GLP-1 enhanced the insulin and C-peptide responses to the graded glucose infusion (Figs. 3*A* and 4*A*). The high infusion rate of GLP-1 (2.0 pmol · kg^{-1} · min^{-1}) had a marked effect on the secretory response, with mean plasma insulin and C-peptide values of 502.8 ± 118.6 and 2,450.4 ± 383.5 pmol/l, respectively (Figs. 3*A* and 4*A*), approaching those of the control subjects during glucose with the low GLP-1 infusion (0.5 pmol · kg^{-1} · min^{-1}) (Figs. 3*B* and 4*B*). Corresponding to the enhanced insulin secretion, decreasing plasma glucose profiles were observed, which were most pronounced during the high GLP-1 infusion (2.0 pmol · kg^{-1} · min^{-1}) (Fig. 2*A*). In the diabetic patients, the insulinotropic effect of GLP-1 was reflected in the incremental AUC of plasma insulin and C-peptide (Table 1). At the high infusion rate of GLP-1 infusion (2.0 pmol · kg^{-1} · min^{-1}), the incremental insulin and C-peptide AUC increased to 600 and 300% of the responses on the saline infusion day ($P < 0.001$, GLP-1 [2.0 pmol · kg^{-1} · min^{-1}] vs. saline; Table 1), respectively.

In the control subjects, small increments in glucose resulted in large increments in plasma insulin and C-peptide during saline and graded glucose infusions (Figs. 2*B*, 3*B*, and 4*B*). The insulin and C-peptide responses to the graded glucose infusion were markedly increased in a dose-dependent manner by intravenous infusion of GLP-1, with a left and upward shift in the insulin and C-peptide profiles (Figs. 3*B* and 4*B*). During the high-rate GLP-1 infusion (2.0 pmol · kg^{-1} · min^{-1}), integrated, incremental insulin and C-peptide AUCs were increased by 400 and 250%, respectively ($P < 0.001$, GLP-1 vs. saline; Table 1). Decreases in the plasma glucose profiles were observed for all three GLP-1 doses and were most pronounced during the high dose of GLP-1 infusion (Fig. 2*B*).

Insulin secretion rates. On the day of saline infusion, the basal ISRs were comparable between the patients with type 2 diabetes and the control subjects (1.9 ± 0.3 vs. 1.8 ± 0.1 pmol · kg^{-1} · min^{-1}, patients versus control subjects, P = NS).

In the patients, infusion of GLP-1 at the two highest doses (1.0 and 2.0 pmol · kg^{-1} · min^{-1}) 30 min before glucose infusion increased basal ISR ($P < 0.01$), whereas no significant effect was seen during the 30-min infusion of the low GLP-1 dose (0.5 pmol · kg^{-1} · min^{-1}) (Friedman analysis, see Fig. 4*A* for the underlying C-peptide responses). The graded glucose infusion without GLP-1 only marginally stimulated insulin secretion, whereas the insulin secretory response was significantly enhanced during infusion of GLP-1. Increasing rates of intravenous GLP-1 infusion enhanced the total ISR gradually from 332.2 ±

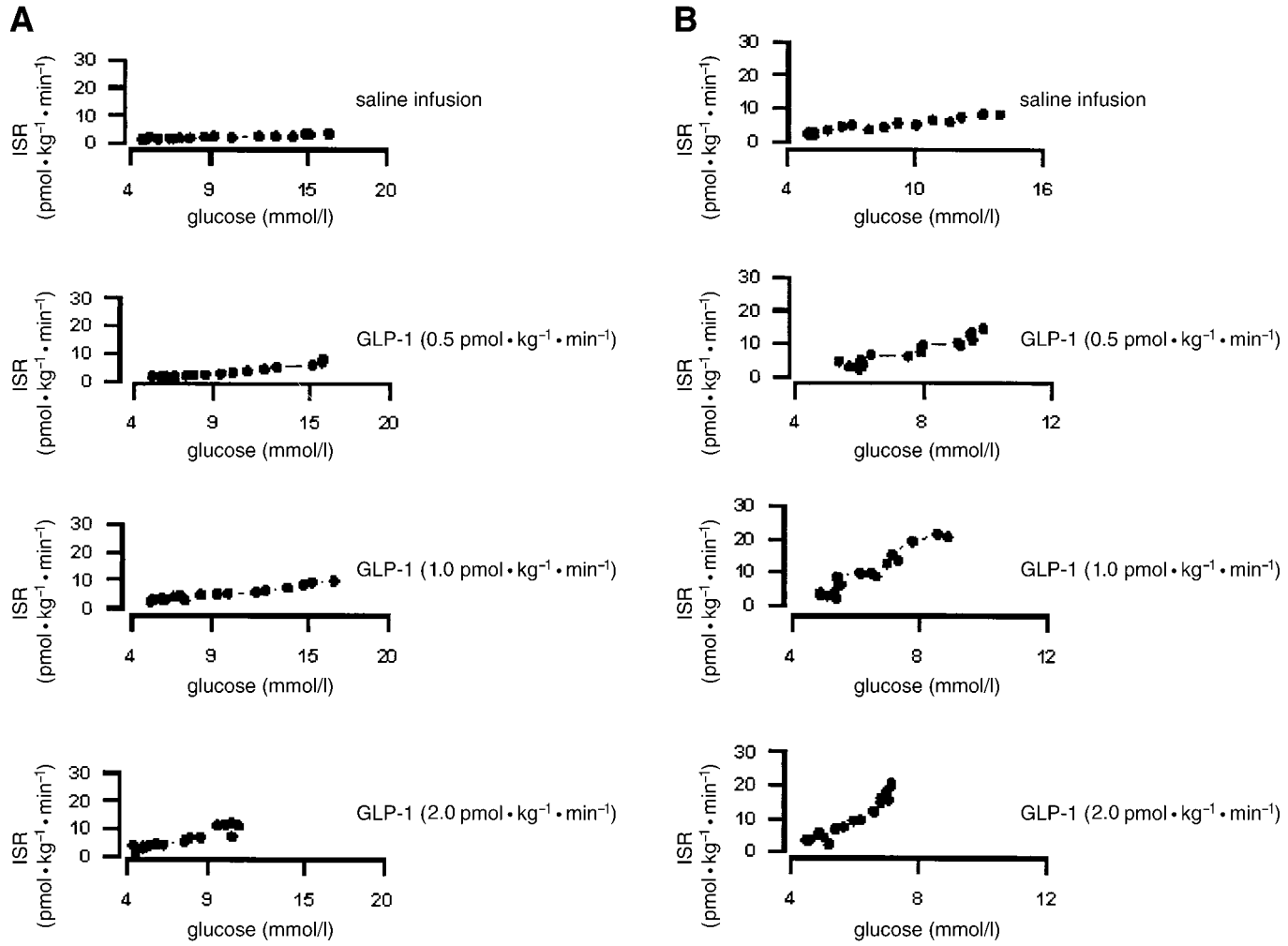

FIG. 5. Dose-response relationship between plasma glucose and prehepatic insulin secretion rates during the graded glucose infusion with intravenous saline or GLP-1 (7-36 amide) at a dose of 0.5, 1.0, or 2.0 pmol · kg^{-1} · min^{-1}. *A*: Mean curves for the type 2 diabetic subjects. *B*: Mean curves for the control subjects. All relationships were tested by regression analysis, and the significance of effect of GLP-1 ($P < 0.001$, for both diabetic patients and control subjects, respectively) was evaluated by comparison of the individual slopes using Friedman's test.

50.5 pmol · kg^{-1} · 150 min^{-1} during saline infusion to 974.6 ± 197.6 pmol · kg^{-1} · 150 min^{-1} at 2.0 pmol · kg^{-1} · 150 min^{-1} GLP-1 infusion with a left and upward shift in the secretion profiles (Table 1).

In the control subjects, infusion of GLP-1 before glucose infusion increased the basal ISR compared with basal ISR during saline infusion, an effect observed for all three doses (Friedmann analysis, see Fig. 4*B* for the underlying C-peptide results). The effect of glucose alone on insulin secretion resulted in a total secretion during the 150 min of 710.9 ± 123.2 pmol · kg^{-1} · 150 min^{-1} (Table 1). GLP-1 augmented the AUC linearly with increasing doses ($P <$ 0.001) (Table 1). In both groups, the high GLP-1 caused a threefold increase in total secretion compared with that observed on the saline infusion day.

Effects of GLP-1 on the relationship between glucose and ISR. The sensitivity of the β-cells to increments in plasma glucose was evaluated as the slope of the relation between ISR and glucose. These relationships are illustrated in Fig. 5, which shows plots of mean values for ISR and plasma glucose at the various sampling times for each of the 4 experimental days in both groups ($P < 0.001$ for both diabetic patients and control subjects). In the patients with type 2 diabetes, infusion of GLP-1 linearly enhanced the slope of ISR versus glucose by a factor of 6, from 0.2 ± 0.1 pmol · kg^{-1} · min^{-1}/(mmol/l) during saline infusion to 1.3 ± 0.4 pmol · kg^{-1} · 150 min^{-1}/(mmol/l) at the highest GLP-1 infusion ($P < 0.001$) (Fig. 6). In the control subjects, infusion of GLP-1 also had significant and dose-dependent effects on the slope of ISR versus glucose (Figs. 5*B* and 6, $P < 0.001$ for all relationships). Thus, the mean slope increased by a factor of 11 from 0.6 ± 0.2 pmol · kg^{-1} · min^{-1}/(mmol/l) with saline to 6.7 ± 1.3 pmol · kg^{-1} · min^{-1}/(mmol/l) (Fig. 6) with the highest GLP-1 dose.

It may be noted that infusion of a low-dose GLP-1 (0.5 pmol · kg^{-1} · min^{-1}) in the diabetic patients resulted in a slope (or β-cell sensitivity to glucose) that was similar to that of control subjects during saline infusion 0.60 ± 0.3 vs. 0.60 ± 0.2 pmol · kg^{-1} · min^{-1}/(mmol/l) (Fig. 5 and 6).

β-cell responses to GLP-1. As illustrated in Fig. 5*A* and *B*, increasing amounts of GLP-1 progressively shifted the ISR/glucose dose-response curves to the left in both groups. However, for the same rate of GLP-1 infusion, the dose-response curve was always and significantly shifted more leftwards and upwards in the control subjects compared with the patients, indicating a reduced β-cell sensi-

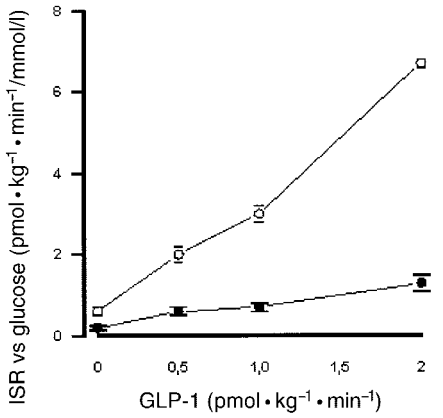

FIG. 6. Alterations in the β-cell responsiveness to glucose in the type 2 diabetic subjects (●) and control subjects (○) during intravenous infusion with saline or GLP-1 (7-36) amide at a dose of 0.5, 1.0, or 2.0 pmol · kg^{-1} · min^{-1}. The β-cell responsiveness is expressed as the slope of the line between ISR and glucose during the graded glucose infusion. The relationship between GLP-1 versus slope was significant (P < 0.001) for diabetic patients as well as control subjects. The significance of the difference between the slopes from the control subjects versus that of the diabetic patients was tested by a Mann-Whitney U test (P < 0.02 control subjects vs. diabetic patients).

tivity to GLP-1 in patients with type 2 diabetes (Fig. 6, $P <$ 0.02 control subjects vs. patients).

DISCUSSION

We studied acute effects of changes in glucose and GLP-1 in patients with type 2 diabetes and in matched control subjects. In both groups, we found that GLP-1 caused a dose-dependent increase in the prehepatic insulin secretion rate when compared with saline infusion. However, since an increment in insulin secretion was still observed between the two highest infusion rates of GLP-1, it is not possible to predict the GLP-1 dose that would elicit maximum β-cell response. Furthermore, we found that β-cell responsiveness to glucose, expressed as the slope of the relationship between ISR and plasma glucose, was enhanced by GLP-1, but less so in the patients.

GLP-1 was infused in doses chosen to result in physiological, supraphysiological, and pharmacological plasma GLP-1 concentrations in matched groups of patients with type 2 diabetes and control subjects. Normal peak GLP-1 concentrations during a meal may range up to 50 pmol/l, corresponding to the level obtained during the lowest GLP-1 infusion rate (22). We have previously demonstrated, using bolus injections of GLP-1, that the dose response relationship for GLP-1 on insulin secretion might differ between patients with type 2 diabetes and healthy subjects and that a higher dose of GLP-1 may be needed to obtain a maximal insulin response in patients with type 2 diabetes (23). However, for the present investigations, we chose not to include infusion rates exceeding 2 pmol · kg^{-1} · min^{-1} of GLP-1 because of the risk of side effects (23,24).

The difference between the patients with type 2 diabetes and the control subjects with respect to ISR was clearly demonstrated on the day of saline infusion (top panels on Figs. 5*A* and *B*). The rise in ISR in response to increments in plasma glucose was markedly attenuated in the patients. Using the slope of the linear relationship between ISR and glucose as an index of β-cell responsiveness to glucose, the β-cell responsiveness was found to be reduced by a factor of 3 in the patients with type 2 diabetes relative to control subjects. However, infusion of 0.5 pmol · kg^{-1} · min^{-1} of GLP-1 resulted in an increased slope and a dose-response curve for the patients that was superimposable on that of the control subjects with saline infusion. Thus, infusion of a low dose of GLP-1 acutely brings about an apparently normal glucose responsiveness of the β-cells in these patients with type 2 diabetes. In view of the central role played by the β-cell function in maintaining normal glucose tolerance, the present finding, that near physiological concentrations of GLP-1 can restore the ability of the β-cell to sense and respond to glucose in patients with type 2 diabetes, is of considerable interest. In 1993, Holz et al. (25) demonstrated that GLP-1 was capable of restoring glucose responsiveness with respect to insulin secretion in single, isolated β-cells, the so-called "glucose competence" concept. Possibly, the effect we are observing in the diabetic patients may be related to this phenomenon.

Only a single previous study has examined the effect of GLP-1 on the dose-response relationship between ISR and plasma glucose (26). In this study, only healthy subjects were investigated and a single infusion rate of 0.4 pmol · kg^{-1} · min^{-1} was employed. This resulted in a 3.5-fold increase in the slop of ISR versus plasma glucose, very similar to our findings in healthy subjects.

However, the present data also indicate that a β-cell defect exists in type 2 diabetes with respect to responsiveness to GLP-1. Thus, the dose-response relation between, on one hand, the slope of ISR versus glucose, and on the other hand, the GLP-1 dose, was markedly impaired and shifted to the right for the diabetic patients relative to the control subjects (Fig. 6). In fact, the highest GLP-1 dose (2 pmol · kg^{-1} · min^{-1}) gave a substantially smaller slope in the patients with type 2 diabetes compared with the lowest GLP-1 dose (0.5 pmol · kg^{-1} · min^{-1}) in the control subjects. This impaired response to GLP-1 in type 2 diabetes is a novel observation that may be of significance for the pathogenesis of the disease. The impaired response could be due to a reduction in the number of β-cells and/or a defect in the function of the β-cells. There is evidence for a reduction in β-cell mass in patients with type 2 diabetes (27), but a combination of defects seems most likely (28–32). There is considerable evidence that prolonged exposure of the β-cell to high glucose levels induces functional defects in insulin secretion in response to both glucose and nonglucose secretagogues in type 2 diabetes (28–32). We do recognize that it is difficult to separate the intertwined effects of glucose and GLP-1 in the current study and that the findings in Fig. 6 could be interpreted as a decreased effect of glucose to modulate the β-cell response to GLP-1. It would, therefore, be of interest to study whether therapeutic interventions that lower blood glucose will improve the β-cell response to GLP-1.

In summary, our results show that GLP-1 increases prehepatic insulin secretion in patients with type 2 diabetes and control subjects in a dose-dependent manner, without any indication of reaching a maximal effect at the highest dose. Infusion of GLP-1 at a low dose could restore insulin secretion and β-cell responsiveness to glucose in patients with type 2 diabetes to the levels observed in normal subjects without GLP-1 infusion. However, the

highest infusion rate of GLP-1 increased the responsiveness of the patients with type 2 diabetes to a lesser degree than the lowest GLP-1 dose did in the control subjects. Thus, the patients with type 2 diabetes exhibit a substantially impaired response to GLP-1.

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