Effect of Glucagon-Like Peptide 1 (7-36 Amide) on Insulin-Mediated Glucose Uptake in Patients With Type 1 Diabetes

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OBJECTIVE — To examine the insulinomimetic insulin-independent effects of glucagon-like peptide (GLP)-1 on glucose uptake in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — We used the hyperinsulinemic-euglycemic clamp (480 pmol · m^{-2} · min^{-1}) in paired randomized studies of six women and five men with type 1 diabetes. In the course of one of the paired studies, the subjects also received GLP-1 at a dose of 1.5 pmol · kg^{-1} · min^{-1}. The patients were 41 ± 3 years old with a BMI of 25 ± 1 kg/m^2. The mean duration of diabetes was 23 ± 3 years.

RESULTS — Plasma glucose was allowed to fall from a fasting level of ~11 mmol/l to 5.3 mmol/l in each study and thereafter was held stable at that level. Plasma insulin levels during both studies were ~900 pmol/l. Plasma C-peptide levels did not change during the studies. In the GLP-1 study, plasma total GLP-1 levels were elevated from the fasting level of 31 ± 3 to 150 ± 17 pmol/l. Plasma glucagon levels fell from the fasting levels of ~14 pmol/l to 9 pmol/l during both paired studies. Hepatic glucose production was suppressed during the glucose clamps in all studies. Glucose uptake was not different between the two studies (~40 μmol · kg^{-1} · min^{-1}).

CONCLUSIONS — GLP-1 does not augment insulin-mediated glucose uptake in lean type 1 diabetic patients.

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Glucagon-like peptide (GLP)-1 is a hormone released from the enteroendocrine cells of the gut. Plasma levels of GLP-1 increase after eating, and it has already been shown that GLP-1 augments insulin secretion in response to meals. In vitro studies have found that GLP-1 augments insulin-mediated glucose uptake (1–7), but the results of in vivo studies are conflicting (8–15). The exogenous administration of GLP-1 lowers blood glucose levels in both type 1 and type 2 diabetic subjects (9,11–14,16). It has been proposed that a component of the glucose-lowering effects of GLP-1 occurs via insulin-independent mechanisms—so-called insulinomimetic actions (8,17). When taking this possible component of the action of GLP-1 into account, experimental designs are always confounded by the fact that GLP-1 induces endogenous insulin release. Recently, we have shown in elderly type 2 diabetic subjects that GLP-1 increases both insulin-mediated and non–insulin-mediated glucose uptake when somatostatin is used to suppress endogenous insulin release in response to GLP-1 (14,18). This led us to postulate that in insulin-resistant states (e.g., aging), we see insulinomimetic properties of GLP-1 that we did not appreciate in an earlier study of young lean subjects where glucose uptake was normal (11). In another model of insulin resistance, obese subjects with a BMI of >30 kg/m^2, we found that GLP-1 infusions increased glucose uptake by 25% above that due to insulin alone (19).

In this study, we again looked for insulinomimetic effects of GLP-1 during a hyperinsulinemic-euglycemic clamp. Because the subjects had type 1 diabetes, endogenous insulin release by GLP-1 was not an issue. Euglycemia was maintained by using varying infusions of glucose by clamp methodology. All subjects were studied twice: once under hyperinsulinemic-euglycemic conditions alone and once under hyperinsulinemic-euglycemic conditions in the presence of infusion of GLP-1. The order of the studies was randomized.

RESEARCH DESIGN AND METHODS

Experimental subjects

Six women and five men with type 1 diabetes participated in the study. They were all Caucasian and nonsmokers. Patients were recruited from the Vancouver Hospital Diabetes Center. The mean age of the patients was 41 ± 3 years, and their mean BMI was 25 ± 1 kg/m^2. The mean duration of diabetes was 23 ± 3 years and their mean HbA_{1c} was 7.9 ± 0.4%. Three patients were being treated with ACE inhibitors for microalbuminuria. There were

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Abbreviations: GLP, glucagon-like peptide; HGP, hepatic glucose production; rGLP-1, recombinant GLP-1(7-36) amide.

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

no other significant illnesses. The University of British Columbia and Massachusetts General Hospital Committees on Human Investigation approved this study. All patients provided written informed consent before participating in the study.

All patients were asked to consume a weight-maintaining diet without carbohydrate restriction and to maintain their usual level of physical activity. Each patient was admitted to the General Clinical Research Center for two separate hyperinsulinemic-euglycemic clamp studies, which were conducted at least 4 weeks apart. The tests were performed in random order. All testing was performed after a 12-h overnight fast and began at 0700. In each study, glucose production and utilization rates were determined by means of the primed constant infusion technique with tritiated glucose (20). A priming dose of 8.5 kilobecquerels per kilogram sterile and pyrogen-free (^{3}H) glucose (NEN Life Science Products, Boston, MA) was administered at −120 min, followed by a constant intravenous infusion of 85 becquerels · kg^{-1} · min^{-1} for the duration of the experiment (240 min). To assess basal metabolic parameters, four arterialized blood samples (21) were taken from a dorsal hand vein, which was enclosed in a box heated to 68–70°C, at 10-min intervals starting at −30 min. At 0 min, using the euglycemic clamp technique (22), a 10-min falling priming of insulin (Humulin R; Eli Lilly, Indianapolis, IN), followed by a continuous infusion of insulin (10–240 min, 480 pmol · m^{-2} · min^{-1}), was started as previously described (23). Plasma glucose was allowed to fall from fasting levels to 5.3 mmol/l and then maintained stable at that level with an infusion of 20% dextrose in water for the duration of the study. The coefficient of variation of plasma glucose did not exceed 5% in any patient after the plasma glucose level had fallen to 5.3 mmol/l. The 20% glucose infusion (Travenol, Deerfield, IL) was spiked with tritiated glucose ("hot G_{inf}") to maintain a constant glucose-specific activity as previously described (24). The actual concentration of the 20% glucose solution measured was 10.2 mmol/l, which was 92% of its stated concentration. In one clamp study, insulin and glucose were infused; in the other study, in addition to insulin and glucose, GLP-1 was also infused in a 10-min falling prime, followed by a continuous infusion (10–240 min, 1.5 pmol · kg^{-1} · min^{-1}) as previous described (11).

Recombinant GLP-1(7-36) (rGLP-1) amide was produced by prokaryotic fermentation, and COOH-terminal amide was added to the peptide by transamidation. This preparation is >99% pure and displays a single peak on high-performance liquid chromatography. rGLP-1 was formulated and inserted into vials at a concentration of 1 mg peptide/ml, purity >99%, and was stored frozen at −20°C until use (Restoragen, Lincoln, NE). Net peptide content was used for dose calculations. Samples were analyzed and shown to be sterile, pyrogen free, and biologically active.

Analytical techniques

Blood samples were collected in heparinized syringes. Blood tests for complete blood count, HbA_{1c}, and hepatic and renal functions were performed using standard laboratory techniques. During the glucose clamp studies, plasma glucose was measured immediately at the bedside using a YSI Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was placed in prechilled test tubes containing diprotin A, 0.1 μmol/ml blood (a protease inhibitor that prevents the action of dipeptidyl peptidase IV; manufactured by Bachem, Torrence, CA, for measurement of active GLP-1), aprotonin (400 kallikrein inhibitor units per milliliter), and EDTA (1.5 mg/ml) (for measurement of C-peptide, glucagon, and insulin) and centrifuged at 4°C. Samples were stored in a −70°C freezer until analysis. Insulin, C-peptide, glucagon, and GLP-1 (total and active) were measured as previously described (11). The active GLP-1 assay is 100% specific for the intact molecule [both GLP-1(7-37) and GLP-1(7-36) amide] and does not detect GLP-1(9-36 amide) at all. The lower level of detection is 3 pmol/l. HbA_{1c} was measured with a Bio-Rad DiaSTAT (Bio-Rad, Hercules, CA), which has an upper limit of 12.6%.

Statistical analysis

The rates of total appearance and disappearance of glucose were calculated according to the non–steady-state equations of Steele, as modified for the use of hot G_{inf} (20,24). This eliminates the implausible negative rate of appearance that results when only unlabeled glucose is used during a hyperinsulinemic-euglycemic clamp procedure (24). The volume of distribution of glucose was assumed to be 210 ml/kg (25). Endogenous glucose production was estimated as the difference between the calculated total appearance rate and the exogenous glucose infusion for the appropriate time interval during the clamp. The trapezoidal rule was used to calculate the integrated responses over 30-min intervals. The integrated responses were divided by the time interval, which resulted in mean concentrations or values. All data were analyzed using Statistical Analysis System (SAS) version 6.12 (SAS Institute, Cary, NC). Standard methods were used to compute means, SEs, and Pearson correlation coefficients. A mixed-model analysis for repeated-measures design was used to analyze hormone and metabolite responses. Differences between clamps were evaluated using the paired *t* test. All statistical tests were two-tailed. Except where otherwise stated, data are means ± SE, and *P* values <0.05 were regarded as statistically significant.

RESULTS — Plasma glucose levels and the glucose infusion rates necessary to maintain euglycemia during both the GLP-1/insulin and insulin studies are illustrated in Fig. 1. Fasting glucose levels during the GLP-1/insulin and insulin studies were 10.1 ± 1.5 and 12.6 ± 1.2 mmol/l (range 4.3–21.9 and 8.3–17.6 mmol/l, respectively). The euglycemic clamp creates a square wave of hyperinsulinemia. In patients whose fasting plasma glucose level was >5.3 mmol/l, glucose infusion was not started at 4 min, as required in volunteers with normal plasma glucose levels. Rather, plasma glucose was allowed to fall and glucose infusion was started when plasma glucose levels approached 5.3 mmol/l. In both studies, plasma glucose levels were identical from ~90 min to the end of the study (Fig. 1*A*). Glucose infusion rates during the GLP-1 and insulin studies were 37.9 ± 4.0 and 32.4 ± 3.0 μmol · kg^{-1} · min^{-1} during the 90- to 240-min period ($P = 0.28$). The difference in glucose infusion rates between the two studies during the first 90 min occurred because two patients had normal fasting glucose levels during the GLP-1 study and it was necessary to start the glucose infusion at 4 min to maintain euglycemia (Fig. 1*B*).

Plasma insulin and C-peptide levels

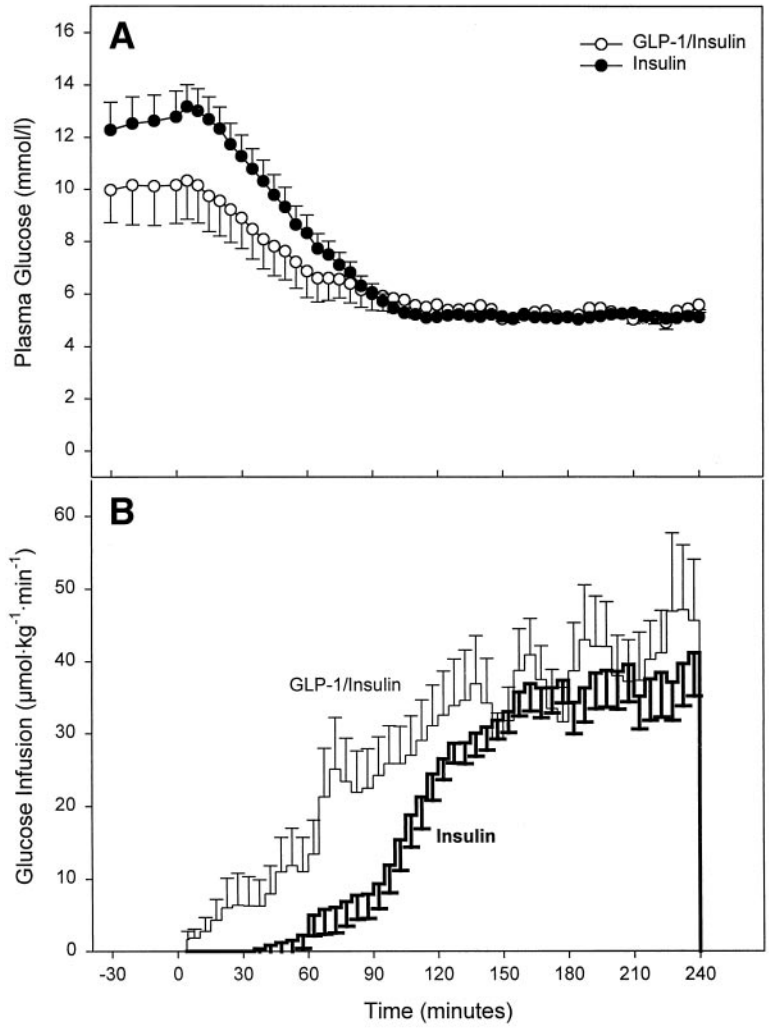

Figure 1—*Plasma glucose (*A*) and glucose infusion (*B*) rates during euglycemic clamp studies in the 11 patients with type 1 diabetes. All patients participated in both studies (means ± SE).*

are shown in Fig. 2. Fasting insulin levels were 63 ± 15 and 40 ± 6 pmol/l in the GLP-1/insulin and insulin studies (P = 0.17). The corresponding plasma insulin levels during the 0- to 240-min period were 973 ± 61 and 879 ± 65 pmol/l (P = 0.30). All patients had fasting C-peptide levels below 0.14 and many were as low as 0.03 pmol/l (the lower limit of the assay) on both occasions. The fasting levels for the GLP-1/insulin and insulin infusion studies were 0.05 ± 0.01 pmol/l in each study. C-peptide levels remained at the fasting basal level throughout the insulin infusion period in both studies.

Fasting total GLP-1 levels were similar in the two studies and were 31 ± 3 and 28 ± 2 pmol/l in the GLP-1/insulin and insulin studies (Fig. 3*A*). In the GLP-1/insulin study, a square wave of GLP-1 was created and the 0- to 240-min levels were 150 ± 17 pmol/l. In the insulin infusion study, GLP-1 levels did not change during the insulin infusion period and the 0- to 240-min levels were 29 ± 2 pmol/l. Fasting plasma glucagon levels in the two studies were 13.6 ± 1.02 and 14.8 ± 0.92 pmol/l (Fig. 3*B*). In response to the hyperinsulinemia, plasma glucagon levels began to fall and reached a plateau from 90 to 240 min. The levels during this period were 9.2 ± 0.82 and 9.5 ± 0.68 pmol/l in the two studies.

Basal hepatic glucose production (HGP) (~20 ± 1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was suppressed to 0 by 60 min in both the control and GLP-1 studies, and the glucose infusion rate was taken to represent insulin-mediated glucose disposal rates (Fig. 1). Glucose infusion rates were used to calculate glucose utilization (M), and the 180- to 240-min rates were not different between the two studies (control: 40.8 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; GLP-1: 44.4 ± 4.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; P = 0.49).

CONCLUSIONS — We examined the insulinomimetic effects of GLP-1 in type 1 diabetic patients and could not demonstrate an effect. Only one previous study has assessed the effect of GLP-1 on insulin-mediated glucose disposal in patients with type 1 diabetes. Gutniak et al. (9) studied eight patients with type 1 diabetes. They examined the effect of GLP-1 infusion compared with that of saline after a standard lunch. All patients were connected to a closed-loop insulin infusion system and were infused with insulin intravenously to keep the postprandial blood glucose level at ~6–7 mmol/l. They reported that during the GLP-1 infusion, the requirement of insulin dropped to 2.0 ± 0.5 units. The requirement during saline infusion was 17.4 ± 2.8 units. They attributed this decrease to increased glucose utilization during GLP-1 infusion. This decrease may have been due, in large part, to the well-documented delayed gastric emptying of the meal during the GLP-1 infusion (26). Gutniak et al. also performed hyperinsulinemic-euglycemic clamps in type 1 diabetic patients using a closed-loop insulin system (Biostater Miles, Elkhart, IN). All patients were well controlled (HbA_{1c} 6.2%) and had their blood glucose levels normalized (~5.0 mmol/l) before the start of the clamp. In one study, GLP-1 was administered and in the other saline was given. They used half of the dose of GLP-1 and one-fourth of the dose of insulin used in our study and reported that the rate of glucose infusion necessary to maintain euglycemia was increased by 20% during the GLP-1 study. There are several plausible explanations for the differences between the two studies. In our study, the level of plasma insulin was higher and the GLP-1 effect, if any, could not be displayed, despite a higher dose of GLP-1. Their estimate of glucose utilization is incorrect if there was a difference in HGP between the two studies. They do not provide any C-peptide data for their patients, and GLP-1 may have stimulated endogenous insulin release sufficiently to have an effect on HGP. There is also the

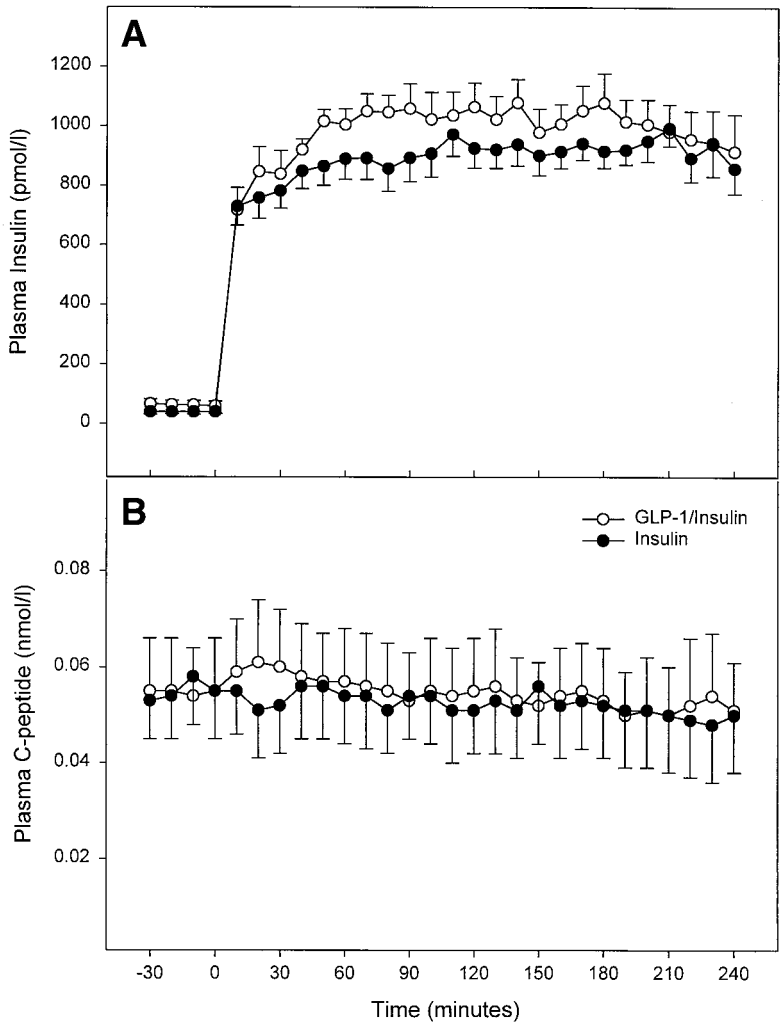

Figure 2—*Plasma insulin* (A) *and C-peptide* (B) *levels in the two euglycemic studies (means ± SE).*

possibility of a type II error in our study or their study.

Dupre et al. (27) examined the effects of GLP-1 on glycemic excursion after a mixed meal in eight C-peptide–positive type 1 diabetic volunteers. GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) inhibited the increments of plasma glucose observed compared with saline infusion and this was also attributed to delayed gastric emptying. Vella et al. (28) also examined the effects of GLP-1 on splanchnic glucose uptake in seven type 1 diabetic volunteers. GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) or saline was infused for 240 min during constant infusion of glucose (20 μmol · kg^{-1} · min^{-1}) delivered via a nasoduodenal tube. Glucose was maintained at ~8.3 mmol/l with additional intravenous administration of glucose (hyperglycemic clamp) along with a concomitant constant infusion of somatostatin, glucagon, growth hormone, and insulin—the pancreatic clamp technique (40 pmol · kg^{-1} · min^{-1}). Total glucose uptake was not different between the two studies during the first 3 h but was higher during the fourth hour in the GLP-1 study. Splanchnic glucose uptake was lower during GLP-1 infusion. Vella et al. conclude that under their experimental design (hyperglycemia, hyperinsulinemia, intravenous, and intraduodenal glucose infusions), GLP-1 increases total body glucose uptake in a time-dependent manner in patients with type 1 diabetes through unknown mechanisms. More recently, these authors have again examined the effects of both GLP-1 and exendin-4 using the pancreatic clamp technique in a three-step hyperinsulinemic-euglycemic clamp in eight healthy young nonobese volunteers (15). One of the aims of the study was to evaluate whether the rapid degradation of GLP-1 could alter the GLP-1 response, because exendin-4 has a much longer half-life. Although glucose uptake was higher at the highest insulin level in the GLP-1 and exendin-4 studies, the differences were not significant. Thus, they conclude that neither GLP-1 nor exendin-4 has insulinomimetic effects and that the degraded product of GLP-1 does not alter the effects of insulin in healthy volunteers.

Orskov et al. (29) also examined insulinomimetic effects of GLP-1(7-36) amide during hyperinsulinemic-euglycemic clamps in healthy young male volunteers using the pancreatic clamp technique. There was no difference between the two studies with respect to glucose infusion requirements for maintenance of euglycemia or with respect to HGP, and the authors concluded that GLP-1 does not have insulinomimetic effects in healthy males.

We previously examined the insulinomimetic effects of GLP-1 in volunteers with normal glucose tolerance (11). The design in that study was such that we matched the hyperinsulinemia produced by infusion of GLP-1, during a euglycemic clamp, with infusion of regular insulin in a second study. Thus, all parameters were equal, except that in one clamp study, a square wave of GLP-1 was present, whereas in a second study, GLP-1 remained at fasting levels. The difference in glucose uptake between the two studies was not statistically significantly different. We then thought that to show insulinomimetic effects of GLP-1, an insulin-resistant state must be present. We subsequently repeated the studies with the same design as in the lean young volunteers in obese individuals (BMI >30%) and showed that GLP-1 augments glucose disposal by 25%, independent of insulin (19). The type 1 diabetic patients used in the present study were not obese and were not insulin resistant. The glucose uptake in the type 1 diabetic patients was comparable to the glucose uptake of the young volunteers and did not increase significantly with GLP-1. Thus, the present study corroborates our initial hypothesis in the beginning of the study that to demonstrate an insulin-independent insulinomimetic effect of GLP-1, a state of

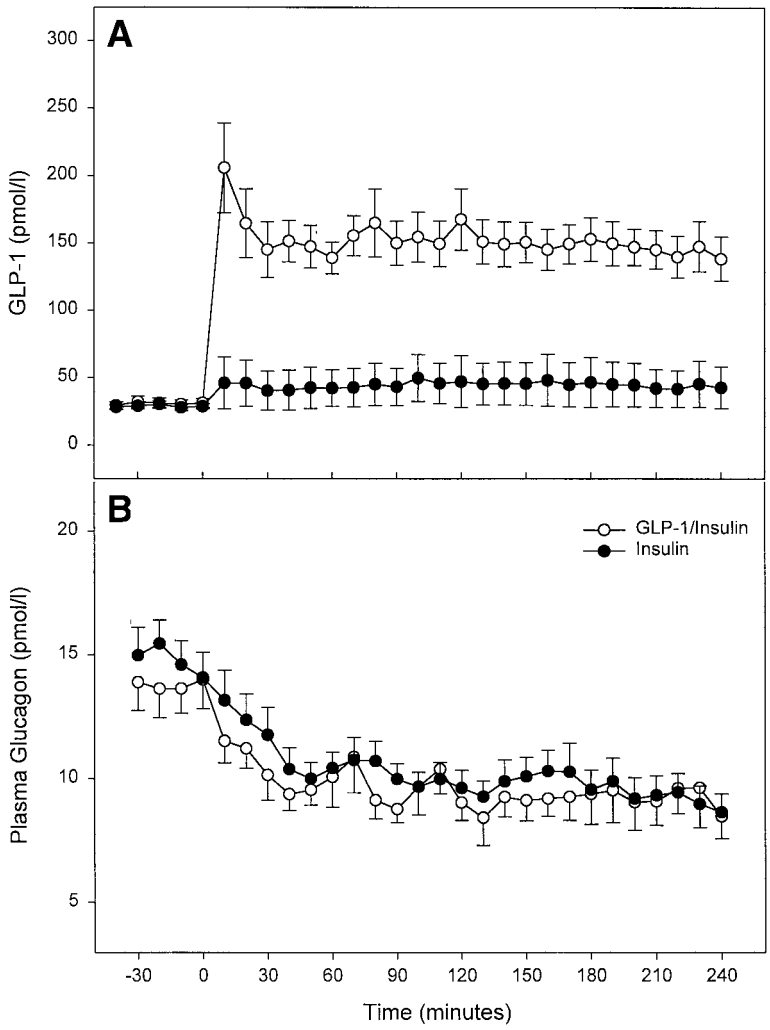

Figure 3—*Plasma GLP-1* (A) *and glucagon* (B) *levels in the two euglycemic studies (means ± SE).*

insulin resistance must be present. Taking into account all the data published since the first description of the GLP-1 effect on glucose disposal per se, we conclude that there may be an as yet undescribed GLP-1–like receptor that is functional in insulin-resistant states, such as those in obese and elderly individuals, and that is below the level of detection by the current experimental designs in healthy or non–insulin-resistant states. In conclusion, our data suggest that GLP-1 does not enhance insulin-mediated glucose disposal in normal-weight type 1 diabetic patients. However, to establish this unequivocally, dose-response studies with at least three doses each of GLP-1 and insulin, in at least two levels of glycemia, are required.

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