Insulinotropic Action of Glucagonlike Peptide-I-(7–37) in Diabetic and Nondiabetic Subjects

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OBJECTIVE — Whether glucagonlike peptide-I-(7–37) (GLP-I-[7–37]), a naturally occurring intestinal peptide, is insulinotropic in nondiabetic and non-insulin-dependent (type II) diabetic subjects.

RESEARCH DESIGN AND METHODS — GLP-I-(7–37) or saline placebo was infused (1–5 $ng \cdot kg^{-1} \cdot min^{-1}$ for 30 min) in 4 nondiabetic and 11 type II diabetic subjects in the fasting and prandial state. Glucose, insulin, and GLP-I-(7–37) levels were measured.

RESULTS — GLP-I-(7–37) infusion resulted in a 3- to 10-fold increase in peak insulin levels and in insulin area under the curve in nondiabetic and diabetic subjects. In diabetic subjects, infusion concurrent with a standard meal eliminated the postprandial glucose excursion for 60 min after the meal. Insulin-releasing potency of GLP-I-(7–37) was attenuated at decreased glucose levels.

CONCLUSIONS — GLP-I-(7–37) has potent insulinotropic effects in nondiabetic and diabetic subjects. Whether GLP-I-(7–37) is useful as a therapeutic medication in type II diabetes requires further investigation.

Identification of several highly conserved glucagonlike peptides (GLPs), related in structure to glucagon (1–3) and processed from the glucagon prohormones (4), prompted a search for a biological function for these peptides. Studies of the aminoterminally truncated peptides, the 31- and 30-amino acid peptides, GLP-I-(7–37) (5–8) and GLP-I-(7–36) amide (9), revealed potent insulinotropic activities in the perfused rat pancreas in situ (5,8,9) and in insulinoma cell lines in vitro (7,10). In the perfused rat pancreas (9), the insulinotropic actions of GLP-I-(7–37) are glucose dependent. The GLP-I-(7–36) amide was identified by Kreymann et al. (11) to fit the role of an incretin because it is secreted in response to oral but not intravenous glucose and is associated with an increase in insulin secretion in humans. In this study, we find potent insulinotropic effects of GLP-I-(7–37) in patients with non-insulin-dependent (type II) diabetes mellitus and in nondiabetic subjects.

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RESEARCH DESIGN AND METHODS

— Four healthy nondiabetic and nine diet-treated type II diabetic men and two diabetic women between 18 and 65 yr of age and with no known history of other diseases were recruited to participate in the studies. The nondiabetic subjects had no history of glucose intolerance or obesity and no family history of diabetes. There was no attempt to match the nondiabetic and type II diabetic subjects for age or weight in these preliminary experiments. The baseline characteristics of the nondiabetic subjects included mean ± SD age of 26.5 ± 3.0 yr, ideal body weight 99.0 ± 1.5%, and HbA_{1c} 4.88 ± 0.40%. The type II diabetic subjects were 54.5 ± 7.5 yr of age, 129 ± 23% of ideal body weight, had 4.9 ± 5.1 yr diabetes duration, and HbA_{1c} 8.07 ± 1.40.

Subjects were requested to fast for at least 10 h and were admitted to the General Clinical Research Center at the Massachusetts General Hospital on the morning of testing. Intravenous lines were placed in each arm, one for withdrawal of blood specimens and one for the administration of GLP-I-(7–37). Blood specimens were obtained every 15 min for 45 min and then either GLP-I-(7–37) or saline (randomized order on different admissions) was infused for 30 min (Harvard infusion pump 22, Harvard Apparatus, South Natick, MA). Blood samples were obtained every 5 min during the 30-min infusion, at 2-min intervals for 10 min after cessation of the GLP-I-(7–37) in-

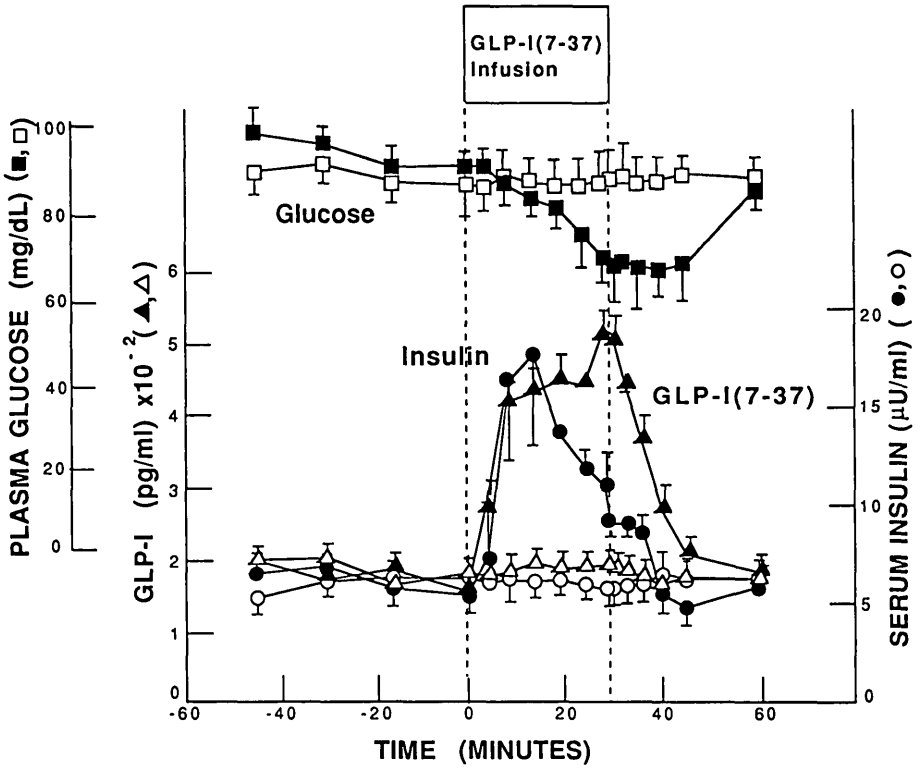

Figure 1—*Insulinotropic actions of glucagonlike peptide (GLP)-I(7–37) infusion in nondiabetic fasting subjects. Saline (□, ○) or GLP-I(7–37) (■, ●) at a rate of 5 ng · kg^{-1} · min^{-1} was infused into 3 subjects for 30 min after overnight fast. Values are means ± SE.*

fusion to allow determination of the peptide disappearance rates, and at 15- to 30-min intervals for another 120 min.

Studies were performed with the subjects (3 nondiabetic, 5 diabetic) continuing to fast during the entire study or with a standard breakfast meal (1 nondiabetic, 5 diabetic) (450 calories—50% carbohydrate, 30% fat, and 20% protein) consumed during the first 15–20 min of the GLP-I-(7–37) infusion. All meal studies were performed with a 5-ng · kg^{-1} · min^{-1} GLP-I-(7–37) infusion. GLP-I-(7–37) was synthesized by the stepwise solid-phase method (12).

Plasma glucose levels were determined with a hexokinase assay. GLP-I-(7–37) (4) (detection limit 5 pg/ml) and insulin (13) were measured with radioimmunoassays in plasma and serum samples, respectively. The GLP-I-(7–37) assay has <0.001% cross-reactivity with glucagon, gastric inhibitory polypeptide, GLP-II, and insulin. The inter- and intraassay coefficients of variation for the GLP-I-(7–37) and insulin assays were <10 and 5%, respectively. HbA_{1c} was measured with a high-performance liquid chromatography with inter- and intraassay coefficients of variation <2.5% and a nondiabetic range of 3.8–6.4% (14).

RESULTS — Fasting nondiabetic subjects responded to GLP-I-(7–37) infusions (5 ng · kg^{-1} · min^{-1}) with an approximately threefold increase in insulin levels from 6.5 ± 1.6 at time 0 to a mean peak value of 17.7 ± 8.6 μU/ml at 15 min after the beginning of the peptide infusion (Fig. 1). The insulin area under the curve (μU/ml · 30 min) was 219 ± 76. Glucose concentrations declined from a mean baseline value of 5.00 ± 0.33 mM to a nadir of 3.78 ± 0.33 mM 34 min after the infusion began. GLP-I-(7–37) levels rose by ~2.5-fold during the infusion.

Type II diabetic subjects experienced a threefold increase in insulin response to GLP-I-(7–37) infusions, similar to those of the nondiabetic subjects during the fasting studies (Fig. 2). Unlike the responses in nondiabetic subjects, the diabetic subjects experienced a more prolonged and variable period of increased insulin levels lasting until the termination of the GLP-I-(7–37) infusion (Fig. 2). Glucose levels of the diabetic subjects decreased progressively (10–31% decrease from baseline) during the GLP-I-(7–37) infusion and for 30–90 min after termination of the infusion. During meal studies in type II diabetic subjects, GLP-I-(7–37) infusion (5 ng · kg^{-1} · min^{-1}) essentially eliminated the expected postprandial rise in plasma glucose for the first 60 min after beginning the meal in association with a significant, threefold increase in insulin levels. Mean insulin levels peaked 10 min after beginning the infusion of GLP-I-(7–37) and remained elevated during the remainder of the infusion. With saline infusion, insulin levels did not increase significantly until 45 min after the meal (Fig. 3). Note that glucose levels increased after cessation of the GLP-I-(7–37) infusion and reached the same level as with the placebo infusion by 120 min after the meal.

To determine whether the insulinotropic actions of GLP-I-(7–37) are glucose dependent in human subjects, we studied the relative insulinotropic responses of GLP-I-(7–37) in two type II diabetic subjects both before and after lowering their fasting glucose levels by the administration of an overnight insulin infusion (Fig. 4). The insulin infusion, discontinued 2 h before GLP-I-(7–37) infusion, resulted in

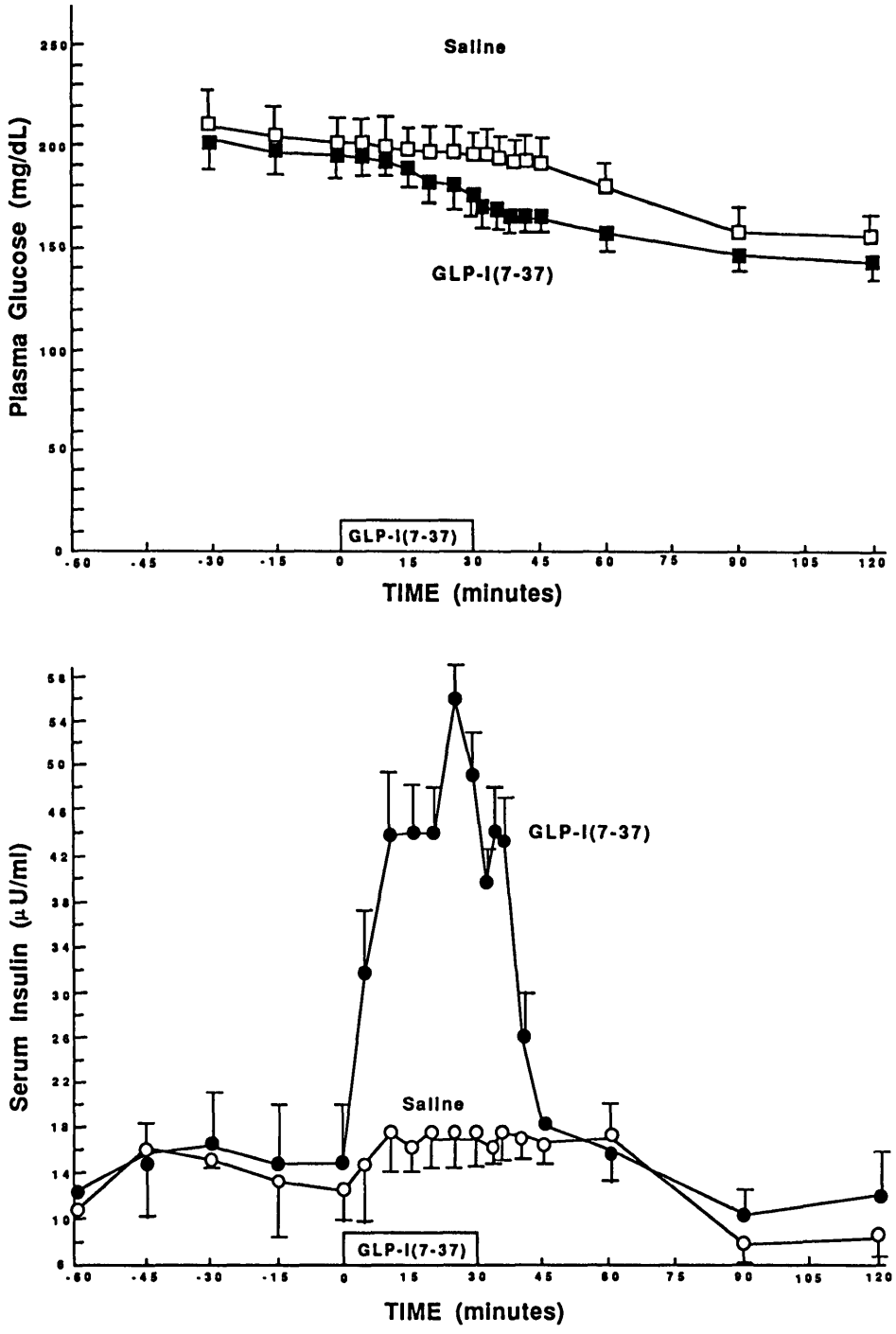

Figure 2—*Glucagonlike peptide (GLP)-1(7–37) (■, ●; 5 ng · kg^{-1} · min^{-1} for 30 min) or saline (□, ○) infusion in 3 fasting non-insulin-dependent diabetic subjects.*

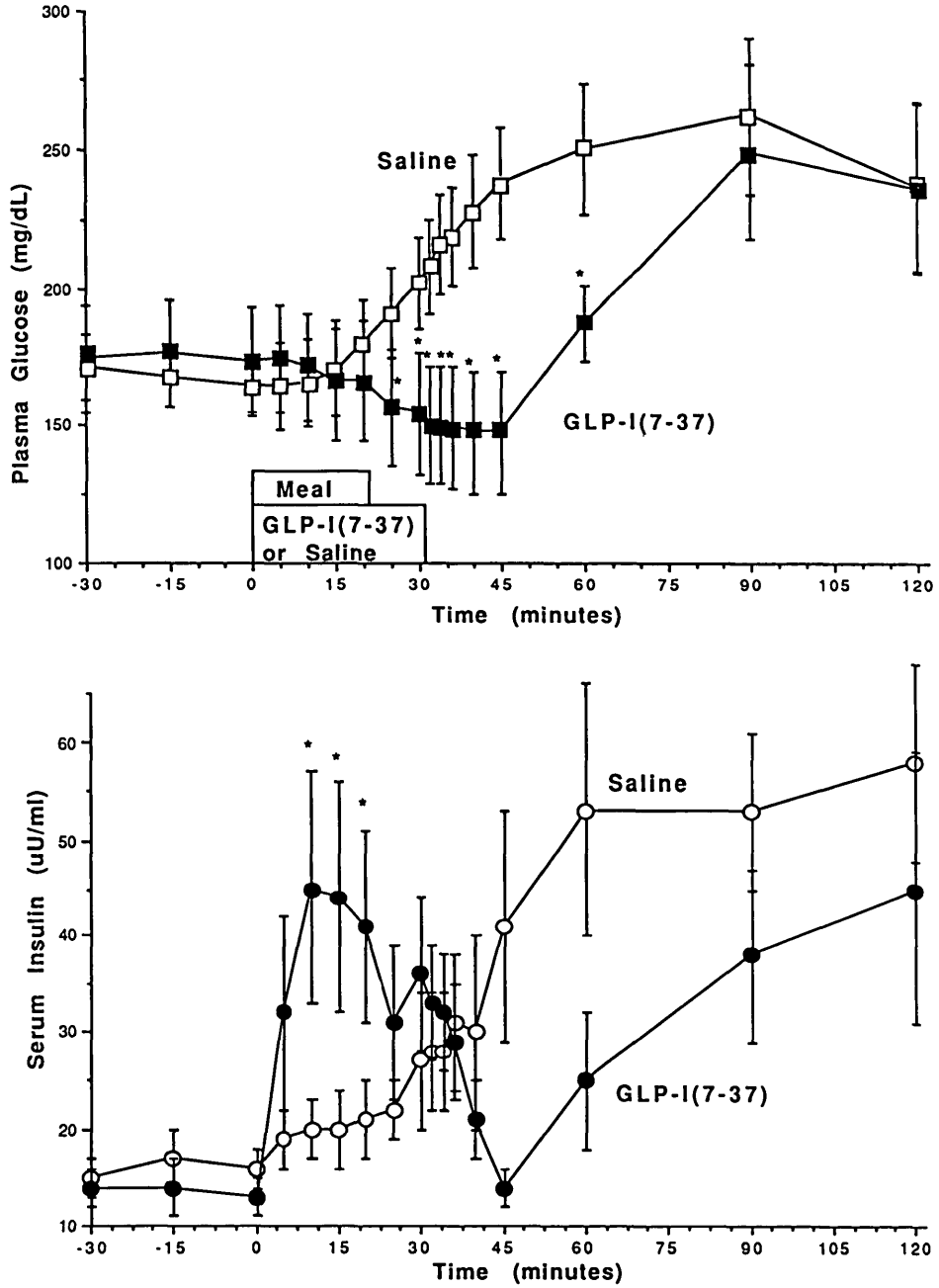

Figure 3—*Glucagonlike peptide (GLP)-I(7–37) (■, ●; 5 ng · kg^{-1} · min^{-1}) or saline (□, ○) infusion in 5 non-insulin-dependent diabetic subjects concurrent with the ingestion of a standard test meal. *P < 0.05 GLP-I(7–37) vs. placebo.*

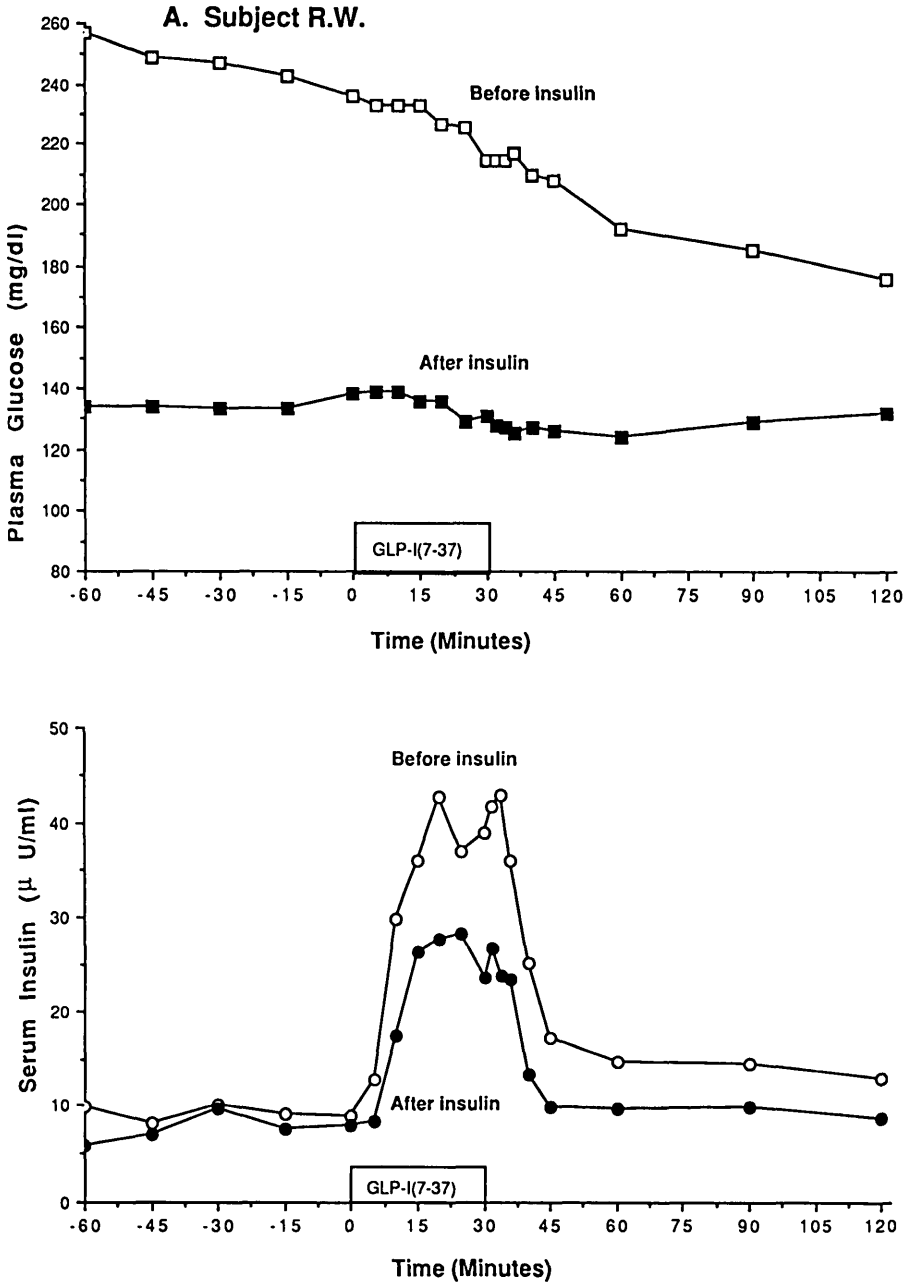

Figure 4—*Glucagonlike peptide (GLP)-I(7–37) infusion (5 ng · kg^{-1} · min^{-1} for 30 min) in 2 fasting non-insulin-dependent diabetic subjects (A and B) on 2 separate days. On 1st occasion (before insulin), GLP-I(7–37) was infused with fasting protocol (○, □; see METHODS). On 2nd occasion (after insulin), intravenous CZI insulin was given overnight to lower fasting plasma glucose levels. Insulin infusion was discontinued for 2 h before repeat GLP-I(7–37) infusion (●, ■).*

a lowering of the fasting glucose in both subjects compared with fasting levels the day before (Fig. 4). The peak increments in insulin and areas under the curve, in response to GLP-I-(7–37), were reduced by >50% in the studies in which insulin infusion lowered fasting glucose.

CONCLUSIONS— The results of these studies extend those of earlier studies in which GLP-I-(7–37) stimulated insulin release in the perfused rat pancreas at concn as low as 5×10^{-12} M (5,8,9) and stimulated cAMP formation and proinsulin mRNA levels in insulinoma cell lines (7,11). Our studies establish that GLP-I-(7–37) stimulates insulin secretion in type II diabetes. The insulinotropic actions of GLP-I-(7–37) are near maximal at dosages of 2.5–5 ng · kg^{-1} · min^{-1} given for 30 min; infusion rates that raise the blood level of immunoreactive GLP-I-(7–37) to ~1.5- to 2.5-fold above background levels. The meal studies demonstrated a similar insulinotropic effect of GLP-I-(7–37) as in the fasting studies. Insulin levels increased by threefold compared with placebo infusion and led to a decrease in postprandial glucose levels. In retrospect, the concurrent administration of GLP-I-(7–37) with meal ingestion was not optimal in limiting postprandial glycemia; insulin secretion preceded meal absorption and subsequent rise in glucose levels.

The extent of the insulin-releasing potency of GLP-I-(7–37) appears to be dependent on ambient glucose concentration (9). It seems reasonable to ascribe the decrease in insulin levels observed after the first 10–15 min of GLP-I-(7–37) infusion in nondiabetic subjects to glucose dependency of the insulinotropic actions. Glucose dependency is supported by the studies in two type II subjects in which fasting glucose was lowered with an overnight insulin infusion. The insulinotropic effect of GLP-I-(7–37) was considerably

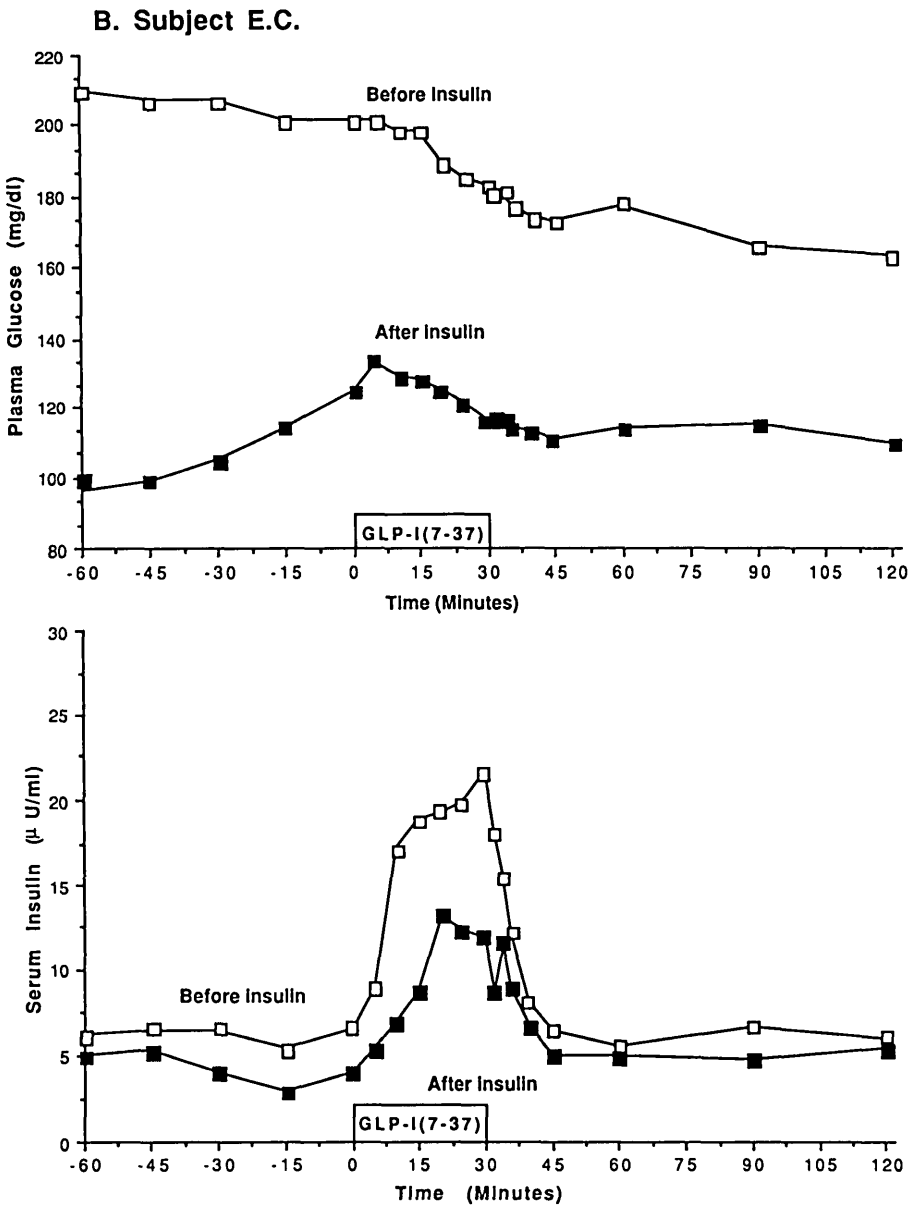

Figure 4—*Continued.*

less when glucose levels were lower, similar to the effects of glucose on other insulin secretagogues.

The demonstration that GLP-I-(7–37) stimulates insulin secretion from the pancreas in subjects with type II diabetes raises possibilities for the potential use of GLP-I-(7–37) as a therapeutic agent for the treatment of this disease. It is desirable to treat patients with type II diabetes with an agent that stimulates endogenous insulin secretion rather than administering insulin by subcutaneous injections because the endogenous insulin is delivered directly to the liver through the portal blood flow rather than from the systemic circulation. GLP-I-(7–37), unlike the sulfonylureas, is a naturally occurring substance that could potentially be exploited as an effective medication for the treatment of type II diabetes. Because of the postulated glucose dependency of the insulinotropic actions of GLP-I-(7–37), hypoglycemia, as occurs in the treatment of patients with subcutaneously administered insulin or oral sulfonylureas, may not be a side effect of treatment with GLP-I-(7–37).

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