

# Effect of Glucagon-Like Peptide 1 (7-36) Amide on Glucose Effectiveness and Insulin Action in People With Type 2 Diabetes

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Although it is well established that glucagon-like peptide 1(7-36) amide (GLP-1) is a potent stimulator of insulin secretion, its effects on insulin action and glucose effectiveness are less clear. To determine whether GLP-1 increases insulin action and glucose effectiveness, subjects with type 2 diabetes were studied on two occasions. Insulin was infused during the night on both occasions to ensure that baseline glucose concentrations were comparable. On the morning of study, either GLP-1 ( $1.2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) or saline were infused along with somatostatin and replacement amounts of glucagon. Glucose also was infused in a pattern mimicking that typically observed after a carbohydrate meal. Insulin concentrations were either kept constant at basal levels ( $n = 6$ ) or varied so as to create a prandial insulin profile ( $n = 6$ ). The increase in glucose concentration was virtually identical on the GLP-1 and saline study days during both the basal ( $1.21 \pm 0.15$  vs.  $1.32 \pm 0.19 \text{ mol/l per 6 h}$ ) and prandial ( $0.56 \pm 0.14$  vs.  $0.56 \pm 0.10 \text{ mol/l per 6 h}$ ) insulin infusions. During both the basal and prandial insulin infusions, glucose disappearance promptly increased after initiation of the glucose infusion to rates that did not differ on the GLP-1 and saline study days. Suppression of endogenous glucose production also was comparable on the GLP-1 and saline study days during both the basal ( $-2.7 \pm 0.3$  vs.  $-3.1 \pm 0.2 \text{ } \mu\text{mol/kg}$ ) and prandial ( $-3.1 \pm 0.4$  vs.  $-3.0 \pm 0.6 \text{ } \mu\text{mol/kg}$ ) insulin infusions. We conclude that when insulin and glucagon concentrations are matched, GLP-1 has negligible effects on either insulin action or glucose effectiveness in people with type 2 diabetes. These data strongly support the concept that GLP-1 improves glycemic control in people with type 2 diabetes by increasing insulin secretion, by inhibiting glucagon secretion, and by delaying gastric emptying rather than by altering extrapancreatic glucose metabolism. *Diabetes* 49:611-617, 2000

People with type 2 diabetes have defects in insulin secretion, insulin action, and glucose effectiveness (defined as the ability of glucose to stimulate its own uptake and suppress its own release at basal insulin). Numerous studies have established that glucagon-like peptide 1(7-36) amide (GLP-1) is a potent stimulus for insulin secretion in both people with type 2 diabetes and healthy volunteers (1-5). It also inhibits glucagon secretion and delays gastric emptying (3,6,7). However, GLP-1's effects on insulin action and glucose effectiveness are more controversial (8,9). GLP-1 receptors have been reported to be present in fat, muscle, and liver (10-13). Some (14-17), but not all (18), in vitro studies have shown that GLP-1 can stimulate glucose uptake and enhance insulin action in these tissues. In vivo studies have been even more contradictory. Gutniak et al. (19) and Sandhu et al. (20) have reported that GLP-1 increases glucose uptake during a hyperinsulinemic-euglycemic clamp in people with type 1 diabetes and in pancreatectomized dogs, respectively. On the other hand, other investigators using the same technique failed to detect an effect of GLP-1 on insulin action in healthy volunteers, pancreatectomized dogs, and people with type 2 diabetes (21-24). In contrast, D'Alessio and colleagues (25,26), using the cold minimal model, has reported that GLP-1 increases both insulin action and glucose effectiveness in healthy volunteers.

The present studies were undertaken in an effort to resolve this uncertainty. We chose to study people with type 2 diabetes because these are the individuals who are likely to be treated with GLP-1 or one of its analogs in the future. We measured glucose effectiveness directly by determining the glycemic excursion and changes in glucose uptake and production observed during a glucose infusion when insulin concentrations were maintained at constant basal levels (27,28). This approach has been shown to avoid the errors associated with the cold minimal model (29,30). Because under physiologic conditions, insulin concentrations rarely increase unless glucose concentrations also increase, we also assessed the effects of GLP-1 on glucose metabolism during a concurrent prandial insulin infusion that resulted in circulating insulin concentrations mimicking those commonly observed following food ingestion (29,31). We infused somatostatin to inhibit endogenous insulin secretion along with replacement amounts of glucagon to ensure that hepatic sinusoidal insulin and glucagon concentrations were the same in the presence and absence of GLP-1. We used the variable tracer infusion approach to maintain a constant

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GLP-1, glucagon-like peptide 1(7-36) amide.

glucose specific activity, thereby enabling accurate measurement of glucose turnover throughout the experiment (32). We report that under these carefully controlled conditions, GLP-1 does not appreciably increase the ability of glucose to stimulate its own uptake and has no effect on either insulin action or the ability of glucose to suppress its own production in people with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

**Subjects.** After approval by the Mayo Institutional Review Board, 12 subjects with type 2 diabetes gave informed written consent to participate in the study. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. Three of the 12 volunteers were treated with oral hypoglycemic agents; 3 were treated with insulin in combination with metformin; and the remaining 6 were treated with insulin alone. Oral agents were discontinued 3 weeks before the study, whereas long-acting insulin was discontinued 48 h before each study.

**Experimental design.** Each subject was studied on two occasions separated by at least 1 week. All subjects were admitted to the Mayo Clinic General Clinical Research Center at 1800 the evening before the study. After ingestion of a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein), subjects fasted until the end of the study. After the meal, an 18-gauge cannula was inserted into each forearm, and human insulin (0.1 U/ml Humulin R; Eli Lilly, Indianapolis, IN) was infused in an amount sufficient to maintain euglycemia throughout the night.

At 0530 (–270 min) the next morning, an 18-gauge cannula was inserted in a retrograde fashion into a dorsal hand vein. The hand was then placed in a heated box to obtain arterialized venous blood samples. An infusion containing somatostatin ( $120 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), human growth hormone ( $3 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), and glucagon ( $0.65 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was started at 0600 (–240 min). A primed continuous infusion of [ $3\text{-}^3\text{H}$ ]glucose ( $12 \mu\text{Ci}$  bolus,  $0.12 \mu\text{Ci}/\text{min}$  continuous; New England Nuclear, Boston, MA) was started at 0800 (–120 min) and continued until the end of the study. The insulin infusion rate was varied so as to maintain glucose at  $\sim 5.5 \text{ mmol}/\text{l}$  until 0900 (–60 min), after which it was maintained constant.

A prandial glucose infusion was started at 1000 (0 min), and insulin was either maintained constant ( $n = 6$ ) or infused in a pattern mimicking a nondiabetic postprandial insulin profile ( $n = 6$ ), as previously described (42). On one occasion, a GLP-1 ( $1.2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) infusion and on the other occasion a saline infusion was started at the same time, with the order of study being random. All infused glucose contained [ $3\text{-}^3\text{H}$ ]glucose in amounts equal to the calculated baseline plasma glucose specific activity. In addition, the basal infusion of [ $3\text{-}^3\text{H}$ ]glucose was altered so as to approximate the anticipated pattern of fall of glucose production (–120 to 0 min: 100%; 0 to 10 min: 90%; 10 to 20 min: 80%; 20 to 30 min: 65%; 30 to 40 min: 55%; 40 to 50 min: 45%; 50 to 75 min: 35%; 75 to 120 min: 40%; 120 to 300 min: 45%; 300 to 360 min: 50%) in an effort to maintain plasma glucose specific activity constant throughout the experiment.

**Analytical techniques.** Arterialized plasma samples were placed in ice, centrifuged at  $4^\circ\text{C}$ , separated, and stored at  $-20^\circ\text{C}$  until assay. Plasma glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin, cortisol, and growth hormone concentrations were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay using reagents supplied by Linco Research (St. Charles, MO). Body composition was measured using dual-energy X-ray absorptiometry (DEXA scanner; Hologic, Waltham, MA). Plasma glucose specific activity was measured using liquid scintillation counting. Plasma concentrations of GLP-1 were measured using antisera specific for the  $\text{NH}_2$ - and  $\text{COOH}$ -terminus of GLP-1(7-36) amide, as previously described (33).

**Calculations.** Glucose specific activity was smoothed using the method of Bradley et al. (34). Glucose appearance and disappearance were calculated using the non-steady-state equations of Steele et al. (35) using the actual tracer infusion rate for each interval. The volume of distribution of glucose was assumed to equal  $200 \text{ ml}/\text{kg}$  and the pool correction factor to equal 0.65. Endogenous glucose production was determined by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance. All rates of infusion and turnover were expressed per kilogram of lean body mass.

**Statistical analysis.** Data are expressed as means  $\pm$  SE. Rates of glucose turnover are expressed per kilogram of lean body mass. The mean of the values during the 30 min prior to the start of the prandial glucose infusion (–30 to 0 min) was considered baseline. Areas were calculated using the trapezoidal rule. Responses observed in the presence of the basal insulin infusion were considered to be due to the ability of glucose to regulate its own metabolism (referred to as glucose effectiveness), whereas those observed in the presence of the prandial

insulin infusion were considered to be an index of insulin action and glucose effectiveness. The one-tailed paired Student's *t* test was used to test the hypothesis that GLP-1 improves glucose effectiveness or insulin action. A *P* value of  $<0.05$  was considered statistically significant.

## RESULTS

Insulin concentrations. Insulin concentrations from –30 to 0 min were comparable in both groups on both study days. Insulin concentrations remained constant throughout the experiment during the basal insulin infusion (Fig. 1A) and increased during the prandial insulin infusion, reaching a peak of  $\sim 240 \text{ pmol}/\text{l}$  within 30 min followed by a return to basal values over the next 2 h (Fig. 1B). Insulin concentrations on the GLP-1 and saline study days did not differ during either the basal or prandial insulin infusions.

C-peptide, glucagon, growth hormone, and cortisol. C-peptide concentrations from –30 to 0 min were comparably suppressed in both groups on both study days (Fig. 2). C-peptide remained suppressed during the glucose infusion on the saline study days but increased slightly on the GLP-1 study days in three subjects during the basal insulin infusion and one subject during the prandial insulin infusion. Despite these increases, the mean C-peptide concentrations did not differ on the GLP-1 and saline study days in either group.

Glucagon and growth hormone concentrations remained constant and equal both before and during the glucose infusion in both groups on both study days (Figs. 2 and 3, respectively). In contrast, GLP-1 infusion increased cortisol to levels that were greater than those observed during the saline infusion (Fig. 3) during both the basal ( $502.2 \pm 96.6$  vs.  $422.1 \pm 35.9 \text{ nmol}/\text{l}$ ) and the prandial ( $397.3 \pm 52.4$  vs.  $286.9 \pm 19.3 \text{ nmol}/\text{l}$ ) insulin infusions. However, the differences were only significant during the latter ( $P = 0.015$ ).

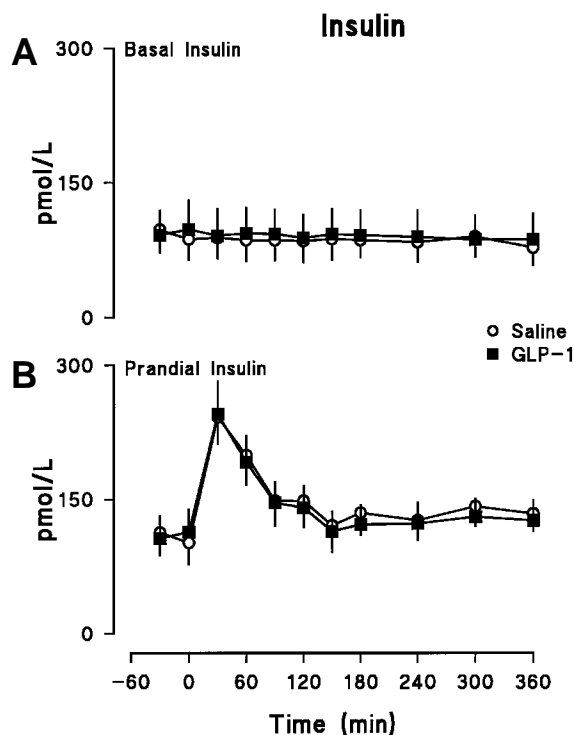


FIG. 1. Plasma insulin concentrations observed during the basal (A) and prandial (B) insulin infusions on the GLP-1 and saline study days.

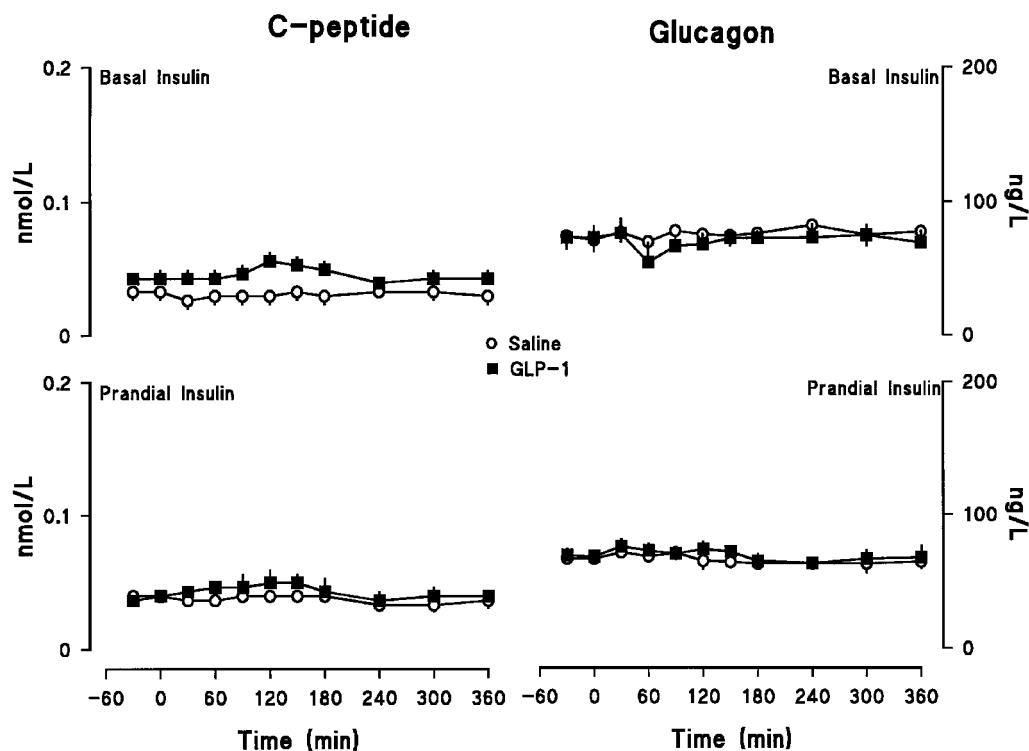


FIG. 2. Plasma C-peptide and glucagon concentrations observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

GLP-1 concentrations. GLP-1 concentrations from -30 to 0 min did not differ on either study day in either group (Fig. 4). GLP-1 infusion resulted in an increase in total GLP-1 immunoreactivity (as measured by COOH-terminal assay) to  $103 \pm 4$  vs.  $8 \pm 3$  pmol/l ( $P < 0.001$ ) during the basal insulin

study and to  $95 \pm 4$  vs.  $7 \pm 1$  pmol/l ( $P < 0.001$ ) during the prandial insulin study. On the other hand, GLP-1 infusion resulted in an increase in intact (as measured with an NH<sub>2</sub>-terminal immunoassay) GLP 1(7-36) immunoreactivity to  $26 \pm 3$  vs.  $14 \pm 2$  pmol/l ( $P < 0.01$ ) during the basal insulin study and to  $26 \pm 2$

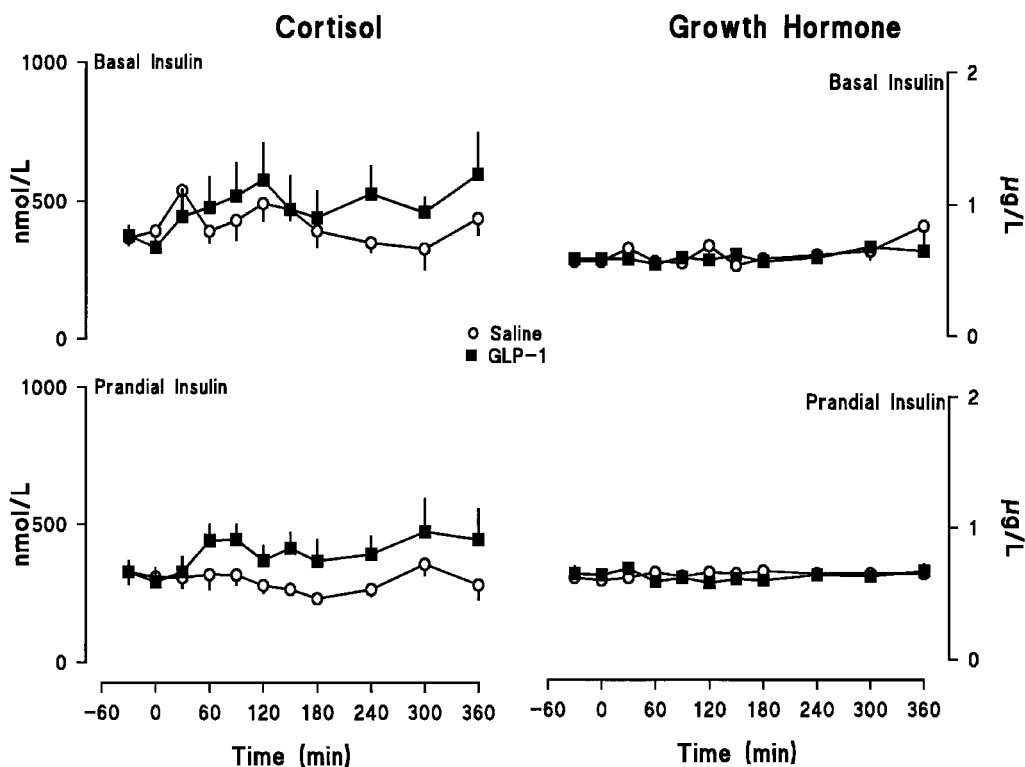


FIG. 3. Plasma cortisol and growth hormone concentrations observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

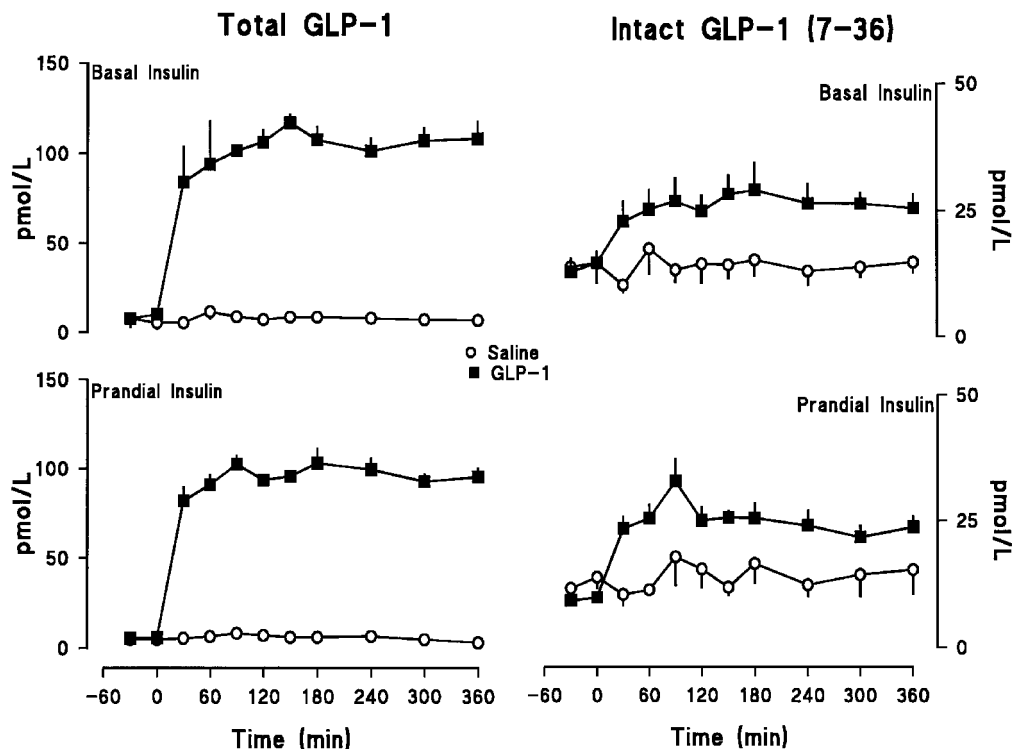


FIG. 4. Total and intact (7-36) plasma GLP-1 concentrations observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

vs.  $15 \pm 3$  pmol/l ( $P < 0.05$ ) during the prandial insulin study. In contrast, neither total nor intact GLP-1 activity changed on the saline study day.

**Glucose concentrations.** Plasma glucose concentrations from -30 to 0 min were comparable in both groups on both study days (Fig. 5). Plasma glucose concentrations rose during the glucose infusion on the basal insulin study days to peak levels that were greater ( $10.5 \pm 0.3$  vs.  $8.4 \pm 0.4$  mmol/l;  $P < 0.001$ ) and occurred later ( $101 \pm 5$  vs.  $68 \pm 9$  min;  $P < 0.01$ ) than those observed on the prandial insulin study days. However, the peak glucose concentrations ( $10.5 \pm 0.4$  vs.  $10.6 \pm 0.5$  mmol/l and  $8.1 \pm 0.7$  vs.  $8.2 \pm 0.5$  mmol/l) and the glycemic areas above basal ( $1.21 \pm 0.16$  vs.  $1.31 \pm 0.19$  mol/l and  $0.47 \pm 0.16$  vs.  $0.55 \pm 0.11$  mol/l per 6 h) did not differ on the GLP-1 and saline study days during either the basal or the prandial insulin infusions, respectively.

**Glucose specific activity.** Glucose specific activity was comparable in both groups on both study days and changed minimally during the glucose infusion (Fig. 6).

**Glucose disappearance and endogenous glucose production.** Glucose disappearance promptly increased after initiation of the glucose infusion during both the basal and prandial insulin infusions (Fig. 7) to rates that did not differ on the GLP-1 and saline study days. Suppression of endogenous glucose production also was comparable on the GLP-1 and saline study days during both the basal ( $-2.7 \pm 0.3$  vs.  $-3.1 \pm 0.2$  mmol/kg) and prandial ( $-3.1 \pm 0.4$  vs.  $-3.0 \pm 0.6$  mmol/kg) insulin infusions (Fig. 8).

#### DISCUSSION

GLP-1 potently stimulates insulin secretion and inhibits glucagon secretion in people with type 2 diabetes (8,9). Its effects on insulin action and glucose effectiveness have been

less clear, in large part because of the difficulty in measuring these parameters under conditions in which glucose, insulin, and glucagon concentrations differ and/or in which they are rapidly changing (32,36,37). In an effort to circumvent these

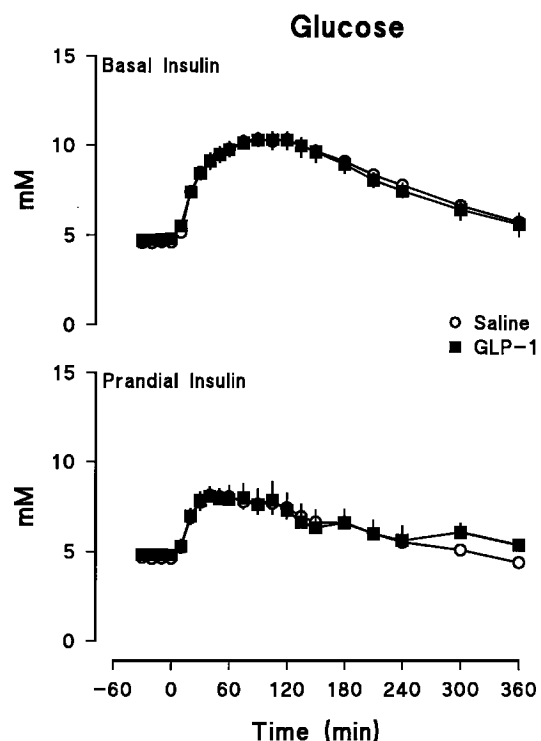


FIG. 5. Plasma glucose concentrations observed during the basal or prandial insulin infusion on the GLP-1 and saline study days.

## Specific Activity

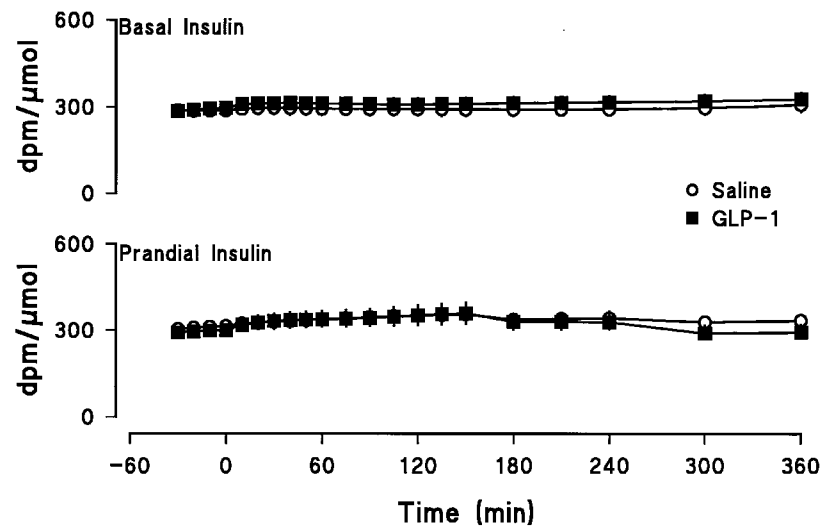


FIG. 6. Plasma specific activity observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

problems, the ability of glucose to stimulate its own uptake and to suppress its own production was measured in people with type 2 diabetes when insulin and glucagon concentrations were kept constant and equal (27). Under these conditions, the glycemic excursion above basal was identical during the GLP-1 and saline infusions, indicating no change in net glucose effectiveness. This observation in type 2 diabetic subjects is consistent with the report of Toft-Nielsen et al. (38), who observed in nondiabetic humans that GLP-1 does not alter the rate of decay of glucose concentrations after a bolus injection of glucose when insulin and glucagon secretion is suppressed by a somatostatin infusion. However, the obser-

vation differs from that of D'Alessio and colleagues (25,26), who reported that GLP-1 increased net glucose effectiveness in nondiabetic subjects. This result is likely due to the fact that the cold minimal model used in those experiments systematically overestimates glucose effectiveness (37) when insulin concentrations are high (i.e., as occurred during the GLP-1 infusion). In the present experiments, glucose concentration, disappearance, and production curves were virtually superimposable in the presence and absence of GLP-1. These data therefore indicate that if, indeed, GLP-1 enhances the ability of glucose to stimulate its own uptake and suppress

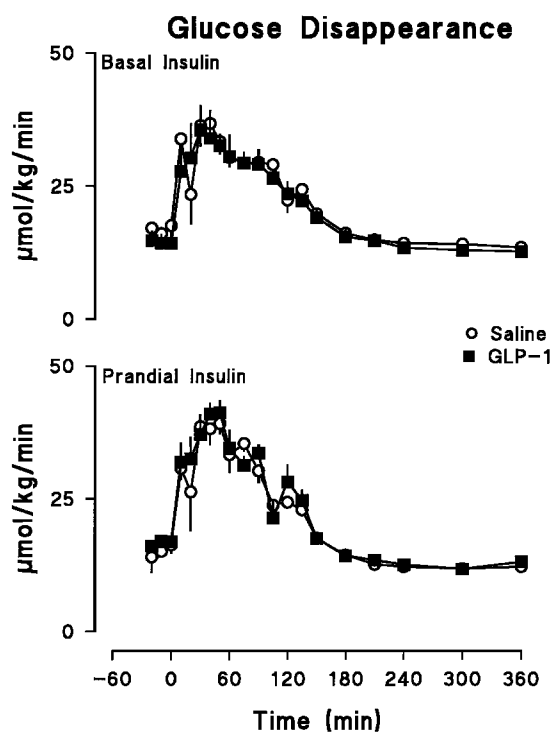


FIG. 7. Rates of glucose disappearance observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

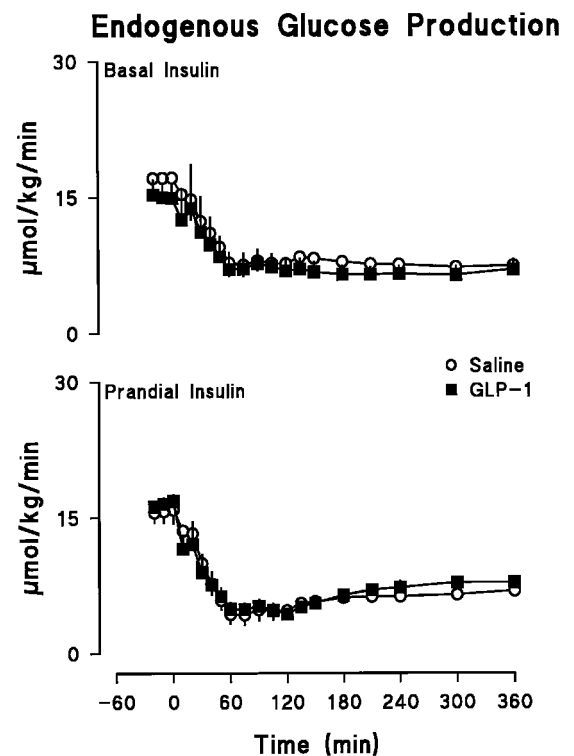


FIG. 8. Rates of endogenous glucose production observed during the basal and prandial insulin infusions on the GLP-1 and saline study days.

its own release, the magnitude of this effect is negligible in people with type 2 diabetes.

A number of studies have examined the effect of GLP-1 on insulin action (20–23). Some have reported enhanced insulin action (20), and others have not (21–23). Most of these studies have used the euglycemic-hyperinsulinemic clamp technique to assess insulin action. Because under conditions of daily living, insulin rarely increases unless glucose also increases, we examined the effects of GLP-1 on insulin action during concordant changes in plasma insulin and glucose concentrations. Using this approach, the increment in both glucose concentration and glucose disappearance and the decrement in glucose production were virtually identical in the presence and absence of GLP-1. These data strongly argue against a substantial extrapancreatic effect of GLP-1 on glucose tolerance in people with type 2 diabetes.

Interpretation of these data obviously depends on the experimental conditions used. We elected to study people with type 2 diabetes because they are the individuals who likely will be treated with GLP-1 or one of its analogs. We infused insulin during the night so that fasting glucose concentrations would be comparable before each study. We have previously demonstrated that overnight insulin infusion in people with type 2 diabetes enhances insulin-induced suppression of glucose production but does not alter either insulin-induced stimulation of glucose uptake or glucose effectiveness (39,40). Therefore, it is unlikely that this approach precluded detection of a further improvement in insulin action or glucose effectiveness during the GLP-1 infusion. We only examined one infusion rate of GLP-1 ( $1.2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). We chose this rate because it has been used in most in vivo studies of GLP-1 actions (8,9) and because higher rates frequently cause gastrointestinal distress, especially when infused with high doses of somatostatin (38).

We took considerable care to be sure that glucose specific activity remained constant, thereby enabling accurate measurement of glucose turnover (32). We also infused somatostatin with replacement amounts of glucagon and growth hormone so that the hormonal milieu would be the same in the presence and absence of GLP-1. Despite this, GLP-1 resulted in a slight but nonsignificant increase in insulin secretion in some of the volunteers. If anything, this should have resulted in an overestimate of the ability of insulin and glucose to suppress endogenous glucose production. On the other hand, GLP-1 increased serum cortisol concentrations consistent with previous reports of a stimulatory effect of GLP-1 on ACTH release (22). Since glucocorticoids can impair insulin action (41,42) and possibly glucose effectiveness, higher circulating cortisol levels could have antagonized GLP-1's ability to enhance glucose uptake, perhaps accounting for the positive impact of GLP-1 on insulin action in vitro but not in vivo. If so, the same offsetting effect of cortisol on insulin action is likely to be observed when people with type 2 diabetes are treated with GLP-1.

In summary, when insulin and glucagon concentrations are matched, GLP-1 has negligible effects on either insulin action or glucose effectiveness in people with type 2 diabetes. These data strongly support the concept that GLP-1 improves glycemic control in people with type 2 diabetes by increasing insulin secretion, by inhibiting glucagon secretion, and by delaying gastric emptying rather than by altering extrapancreatic glucose metabolism.

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