Effect of Glucagon-Like Peptide-1(7-36)-Amide on Initial Splanchnic Glucose Uptake and Insulin Action in Humans With Type 1 Diabetes

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In vitro studies indicate that glucagon-like peptide-1(7-36)-amide (GLP-1) can enhance hepatic glucose uptake. To determine whether GLP-1 increases splanchnic glucose uptake in humans, we studied seven subjects with type 1 diabetes on two occasions. On both occasions, glucose was maintained at ~5.5 mmol/l during the night using a variable insulin infusion. On the morning of the study, a somatostatin, glucagon, and growth hormone infusion was started to maintain basal hormone levels. Glucose (containing [³H]glucose) was infused via an intraduodenal tube at a rate of 20 μmol · kg⁻¹ · min⁻¹. Insulin concentrations were increased to ~8.8 mmol/l for the next 4 h by means of a variable intravenous glucose infusion labeled with [6,6-²H₂]glucose. Surprisingly, the systemic appearance of intraduodenally infused glucose was higher (P = 0.01) during GLP-1 infusion than saline infusion, indicating a lower (P < 0.05) rate of initial splanchnic glucose uptake (1.4 ± 1.5 vs. 4.8 ± 0.8 μmol · kg⁻¹ · min⁻¹). On the other hand, flux through the hepatic uridine-diphosphate-glucose pool did not differ between study days (14.2 ± 5.5 vs. 13.0 ± 4.2 μmol · kg⁻¹ · min⁻¹), implying equivalent rates of glycogen synthesis. GLP-1 also impaired (P < 0.05) insulin-induced suppression of endogenous glucose production (6.9 ± 2.9 vs. 1.3 ± 1.4 μmol · kg⁻¹ · min⁻¹), but caused a time-dependent increase (P < 0.01) in glucose disappearance (93.7 ± 10.0 vs. 69.3 ± 6.3 μmol · kg⁻¹ · min⁻¹; P < 0.01) that was evident only during the final hour of study. We conclude that in the presence of hyperglycemia, hyperinsulinemia, and enterally delivered glucose, GLP-1 increases total body but not splanchnic glucose uptake in humans with type 1 diabetes. Diabetes 50: 565–572, 2001

G lucagon-like peptide-1(7-36)-amide (GLP-1) and its analogues are being actively evaluated as a potential therapy for humans with type 1 diabetes (1–5). GLP-1 enhances insulin secretion, decreases glucagon secretion, and delays gastric emptying (6,7). In vitro studies also suggest that GLP-1 may have extrapancreatic effects (8,9). GLP-1 receptors are present in muscle (10,11), fat (8,12), and liver (13). In vitro studies have shown that GLP-1 can increase glucose uptake in each of these tissues and that its effects are additive to those of insulin (14,15). In contrast, results from in vivo studies have been less consistent. GLP-1 has been reported to increase glucose uptake during hyperglycemia and hyperinsulinemia in diabetic rats (16) and pancreatectomized dogs (17) and during euglycemia and hyperinsulinemia in humans with type 1 diabetes (18). On the other hand, GLP-1 has been reported to have no effect on insulin action during euglycemia and hyperinsulinemia in nondiabetic humans (19) and dogs (20) or during “prandial” insulin and glucose infusions in humans with type 2 diabetes (21).

All of these studies evaluated insulin action during intravenous glucose infusion. This approach contrasts with the situation that occurs under the conditions of daily living, in which carbohydrate is ingested either alone or as part of a meal. Glucose delivery into the duodenum at a rate in excess of ~1.4 kcal/min stimulates secretion of GLP-1 into the portal venous system (22), presumably resulting in exposure to higher concentrations of GLP-1 in the liver than in peripheral tissues. Therefore, when GLP-1 secretion is stimulated by ingestion of a meal, portal venous glucose, insulin, and GLP-1 concentrations are simultaneously increased.

Numerous experiments have shown that hyperglycemia and hyperinsulinemia together result in greater hepatic glucose uptake than either stimulus alone (23–28). We hypothesized that the elevated GLP-1 concentrations that typically occur after ingestion of food further enhance uptake of enterally delivered glucose. If this is true, it could account for previous reports by some (25,29,30) but not all (31) investigators that splanchnic glucose uptake is greater when glucose is administered enterally than when it is infused intravenously.

The present experiments were undertaken to determine whether GLP-1 increases initial splanchnic uptake of glu-
Glucose (i.e., that which occurs when enterally administered glucose crosses from the intestinal lumen into the systemic circulation). Splanchnic extraction of intraduodenally infused glucose was measured during GLP-1 or saline infusion. Somatostatin was infused along with replacement amounts of glucagon and growth hormone to maintain constant portal venous hormone concentrations. Glucose and insulin were clamped at equal but elevated levels on both occasions to stimulate hepatic glucose uptake. We studied patients with type 1 diabetes to preclude the possibility that endogenous insulin secretion, in the presence of sustained hyperglycemia and GLP-1, would lead to higher hepatic sinusoidal insulin concentrations during GLP-1 infusion. Flux through the uridine diphosphate (UDP)–glucose pool was measured using the aceterminophen glucuronide method to determine whether GLP-1 increases hepatic glycogen synthesis as well as splanchnic glucose uptake (32).

**RESEARCH DESIGN AND METHODS**

**Subjects.** After approval by the Mayo Institutional Review Board, seven patients with type 1 diabetes gave informed written consent to participate in the study. All subjects were in good health and at a stable weight. None of the subjects regularly engaged in vigorous physical exercise. Three of the seven subjects were treated with continuous subcutaneous insulin infusion therapy, and the remaining four were treated with multiple daily injections of insulin. The glycosylated hemoglobin concentration at the time of study averaged 8.5 ± 0.4%. Long-acting insulin was discontinued 24 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 6:00 AM the day before the study. After ingestion of a standard 10 kcal/kg meal (50% carbohydrate, 30% fat, 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/mU Humulin R; Eli Lilly, Indianapolis, IN) was infused in an amount sufficient to maintain euglycemia throughout the night (35).

At 6:00 AM (~120 min) the following morning, subjects were taken to the fluoroscopy unit, where an 8-French Flexiflo enteral feeding tube (Ross Laboratories, Columbus, OH) was passed under fluoroscopic guidance via the nasopharynx into the fourth part of the duodenum. The average duration of tube placement was 15 min; the average fluoroscopy time was ~2 min. The subjects were returned to the General Clinical Research Center. At 7:45 AM, subjects were asked to void, after which 2 g of aceterminophen suspension were administered over ~3 min via the nasoduodenal tube. Urine was then collected for the duration of the study for measurement of urinary aceterminophen glucuronide, as previously described (32).

At 8:00 AM (0 min), either a GLP-1 infusion (1.2 pmol · kg⁻¹ · min⁻¹) or saline infusion was started; the order of study was random. At the same time, a constant infusion of glucose (20 pmol · kg⁻¹ · min⁻¹) containing [3-³H]glucose (DuPont-NEN, Boston, MA), at a specific activity of 590 ± 37 dpm/µmol glucose, was started via the nasoduodenal tube. Intravenous infusions of insulin (6 pmol · kg⁻¹ · min⁻¹), somatostatin (60 ng · kg⁻¹ · min⁻¹), human growth hormone (3 mg · kg⁻¹ · min⁻¹), glucagon (0.65 mg · kg⁻¹ · min⁻¹), and [6,6-²H₂]glucose (33 µmol/kg prime, 0.53 µmol/kg · min⁻¹ · min⁻¹ constant; Masstrace, Woburn, MA) were also started at 8:00 AM. [³C]lactate (15 µCi prime, 0.15 µCi/min constant; DuPont-NEN) was also infused as part of a separate study examining the effects of type 1 diabetes on UDP-glucose flux. Additional glucose (enriched with [6,6-²H₂]glucose) was infused intravenously in amounts sufficient to maintain plasma glucose concentration at ~8.3 mmol/l. The rate of the “basal” intravenous [6,6-²H₂]glucose infusion was altered as follows in an effort to approximate the anticipated pattern of decrease in glucose production and thereby maintain plasma glucose-specific activity constant: (0–30 min, 100%; 31–60 min, 86% 61–90 min, 62%; 91–120 min, 40%; 121–150 min, 38%; 151–180 min, 30%; 181–210 min, 20%; 211–240 min, 24%) (34).

**Calculations.** Glucose disappearance (Rₐ) was calculated using the steady state equations of Steele (38):

\[ R_\alpha = \frac{F[D_{6\alpha}]}{MPE\{6,6-\text{²H₂}\} \text{glucose}} - \frac{F[D_{6\alpha}]}{3\text{H} \text{glucose}} \]

where \( F[D_{6\alpha}] \) is the [6,6-²H₂] tracer infusion rate and MPE [6,6-²H₂]glucose is the plasma [6,6-²H₂]glucose enrichment.

The systemic rate of appearance of the intraduodenally infused [3-³H]glucose was calculated as

\[ R_a[3-\text{³H}] \text{glucose} = \frac{F[D_{6\alpha}]}{[6,6-\text{²H₂}] \text{glucose}/[3-\text{³H}] \text{glucose}} \]

where [6,6-²H₂]glucose is the concentration (in µmol/ml) of [6,6-²H₂]glucose in plasma and [3-³H]glucose is the concentration (in dpm/ml) of [3-³H]glucose in the plasma.

The systemic rate of appearance of the intraduodenally infused glucose was calculated as

\[ R_a \text{intraduodenal glucose} = R_a[5-\text{³H}] \text{glucose} \times SA \text{intraduodenal glucose} \]

where SA intraduodenal glucose equals specific activity (in dpm/µmol) of the glucose infused intraduodenally.

Initial splanchnic glucose uptake (SGU) was calculated as

\[ SGU = G_{\text{intrad}} - R_a \text{intraduodenal glucose} \]

where \( G_{\text{intrad}} \) is the intraduodenal infusion rate of glucose.

Endogenous glucose production was calculated by subtracting the sum of the intravenous glucose infusion rate and the systemic rate of appearance of intraduodenally infused glucose from glucose appearance.

**Statistical analysis.** Data in the text and figures are expressed as means ± SE. All rates are expressed as µmol · kg⁻¹ · lean body mass · min⁻¹. To compare turnover, steady-state conditions from 210 to 240 min were measured and used for statistical analysis. Two-tailed, paired Student’s t test was used to test the hypothesis that GLP-1 alters splanchnic glucose uptake and insulin action. A P value of less than 0.05 was considered statistically significant.

**RESULTS**

**Glucose, insulin, and C-peptide concentrations.** Baseline glucose, insulin, and C-peptide concentrations did not differ on the two study days (Fig. 1). The glucose and insulin infusions resulted in a prompt increase in glucose and insulin concentrations. Glucose (8.7 ± 0.3 vs. 8.8 ± 0.2 pmol/l) and insulin (536 ± 94 vs. 557 ± 88 pmol/l) concentrations reached a comparable plateau within 2 h on the GLP-1 and saline study days, respectively. C-peptide concentrations remained at the limits of detection both before and during the glucose and insulin infusions on both study days.

**Glucagon, growth hormone, and cortisol concentrations.** Glucagon and growth hormone concentrations remained constant and equal on both study days (Fig. 2). Basal cortisol concentrations also did not differ on the two study days. However, GLP-1 blunted the normal diurnal decrease in cortisol concentration, resulting in levels that were higher (P < 0.05) during the final 30 min of the GLP-1 infusion (443 ± 76 vs. 277 ± 47 nmol/l).

**Glucose infusion rates.** The intravenous glucose infusion rates necessary to maintain plasma glucose concentrations at target levels (Fig. 3A) were slightly higher on the GLP-1 study day (P < 0.03) at the end of the first 3 h...
of the experiment (56.1 ± 9.4 vs. 50.5 ± 8.2 μmol·kg⁻¹·min⁻¹). Thereafter, the intravenous glucose infusion rate necessary to maintain plasma glucose concentrations at target levels increased more rapidly during the GLP-1 infusion than during the saline infusion, resulting in significantly higher (P < 0.02) rates during the final 30 min of the study (68.7 ± 6.9 vs. 53.4 ± 6.4 μmol·kg⁻¹·min⁻¹). By design, the intraduodenal glucose infusion rate did not differ on the two study days (Fig. 3).

**GLP-1 concentrations.** GLP-1 concentrations at baseline did not differ on either study day. GLP-1 infusion resulted in an increase in total GLP-1 immunoreactivity (as measured by COOH-terminal assay) to 215 ± 26 vs. 19 ± 2 pmol/l (P < 0.001) during the GLP-1 study day as compared with the saline study day (Fig. 4A). Similarly, GLP-1 infusion resulted in an increase in intact (as measured with an NH₂-terminal immunoassay) GLP-1(7-36) immunoreactivity to 24 ± 2 vs. 12 ± 1 pmol/l (P < 0.01) during the GLP-1 study day as compared with the saline study day (Fig. 4B). Total and intact GLP-1 immunoreactivity did not change on the saline study day, which is consistent with the ability of somatostatin to inhibit intestinal GLP-1 secretion (39).

**Glucose enrichment and specific activity.** Plasma [6,6-²H₂]glucose enrichment and the ratio of [6,6-²H₂]glucose to [3-²H]glucose concentration both achieved steady state within 120 min on both study days (Fig. 5). This allowed accurate measurement of glucose disappearance and the systemic rate of appearance of intraduodenally infused glucose, respectively, during the final 2 h of both study days (40).

**Total body glucose disappearance and endogenous glucose production.** Total glucose disappearance did not differ on the two study days during the first 3 h of the glucose and insulin infusions (Fig. 6A). Thereafter, glucose disappearance increased on the GLP-1 study day to rates that were higher (P < 0.01) than those observed on the saline study day (93.7 ± 10.0 vs. 69.3 ± 6.3 μmol·kg⁻¹·min⁻¹). Infusion of glucose and insulin resulted in near complete suppression of endogenous glucose production on both study days. However, endogenous glucose production remained slightly higher (P < 0.05) during the final 30 min of the GLP-1 infusion than during the final 30 min of the saline infusion (6.9 ± 2.9 vs. 1.3 ± 1.4 μmol·kg⁻¹·min⁻¹) (Fig. 6B).

**Systemic rate of appearance of intraduodenally infused glucose, initial SGU, and UDP-glucose flux.** The systemic rate of appearance of intraduodenally infused glucose was higher after 120 min on the GLP-1 study day as compared with the saline study day (Fig. 7). This difference was maintained so that the systemic rate of appearance of the intraduodenally infused glucose during the final 30 min of study was greater (P < 0.02) during the GLP-1 study day (18.7 ± 1.2 vs. 15.0 ± 0.9 μmol·kg⁻¹·min⁻¹). Because the amount of glucose infused into the duodenum was the same on both occasions, this resulted in a lower (P < 0.02) rate of initial SGU on the GLP-1 study days than on the saline study days (1.4 ± 1.3 vs. 4.8 ± 0.8 μmol·kg⁻¹·min⁻¹).

**FIG. 1.** Plasma glucose, insulin, and COOH-peptide concentrations observed during the GLP-1 and saline study days.

**FIG. 2.** Plasma glucagon, growth hormone, and cortisol concentrations observed during the GLP-1 and saline study days.

![Graphs of Glucose, Insulin, and C-Peptide](image1)

![Graphs of Glucagon, Growth Hormone, and Cortisol](image2)
UDP-glucose flux (an index of hepatic glycogen synthesis) did not differ on the GLP-1 and saline study days (14.2 ± 5.1 vs. 13.0 ± 3.9 μmol·kg⁻¹·min⁻¹; P = 0.86).

DISCUSSION
The effects of GLP-1 on insulin action and glucose effectiveness have been the subject of much controversy (5,7). In vitro studies have suggested that GLP-1 alone or in combination with insulin can enhance liver, muscle, and fat glucose uptake (8–10,14–16). We (21) and others (19,20,41–43) have been unable to detect an effect of GLP-1 on insulin action or glucose effectiveness in either nondiabetic subjects or subjects with type 2 diabetes. However, all these experiments involved intravenous infusion of insulin and glucose. The purpose of the present experiment was to determine whether pharmacological concentrations of GLP-1 further enhanced SGU under conditions that optimize SGU, namely when glucose is administered enterally and glucose and insulin concentrations are elevated. Unexpectedly, these experiments rejected this hypothesis and demonstrated that prolonged infusion of GLP-1 at rates sufficient to achieve pharmacological concentrations paradoxically decreased initial SGU in all subjects studied. Furthermore, GLP-1 impaired the suppression of endogenous glucose production by hyperglycemia and hyperinsulinemia. Interestingly, this was accompanied by an increase in total body glucose disposal, which only became evident after 3 h of GLP-1 infusion.

Several possible explanations exist regarding the decreased initial SGU caused by GLP-1 infusion. For example, GLP-1 may alter intestinal transport and/or metabolism rather than hepatic glucose uptake. Studies in both animals and humans have shown that the gut metabolizes ~20% of the glucose presented to it during the process of absorption (44–46). GLP-1 is a potent inhibitor of gut motility (47,48). If GLP-1 decreases intestinal glucose metabolism during absorption, then a greater proportion of the intraduodenally infused glucose would reach the portal vein, and subsequently the hepatic vein, during GLP-1 infusion. If decreased intestinal glucose metabolism indeed is the explanation for the present observations, then SGU presumably also will be decreased by dipeptidylpeptidase IV inhibitors or long-acting GLP-1 analogues, which have identical effects on gut motility and, presumably, intestinal glucose metabolism.

As reported previously, cortisol concentrations were higher during the GLP-1 infusion as compared with the saline infusion (21,42). Cortisol impairs insulin-induced suppression of endogenous glucose production (49). However, cortisol (50) enhances rather than suppresses hepatic glycogen synthesis (51,52). In addition, there was no correlation between cortisol concentration and SGU during either the saline or GLP-1 infusions, making it unlikely that cortisol had a major effect on either of these processes. Elevated free fatty acids decrease rather than increase muscle glucose uptake (53–55). We are unaware of data indicating that they also decrease hepatic glucose uptake.

FIG. 3. Intravenous glucose infusion rates necessary to maintain a constant plasma glucose concentration (A) and the rate of intraduodenal glucose delivery (B) during the GLP-1 and saline study days.

FIG. 4. Total (A) and intact (7-36) plasma GLP-1 (B) concentrations observed during the GLP-1 and saline study days.
Although we did not measure free fatty acid concentrations in the present study, previous experiments have shown that GLP-1 does not alter fatty acid concentrations in either humans or animals (17). Tracer steady state was not achieved until \( \approx 2 \) h after the start of the enteral glucose infusion. This precluded accurate measurement of SGU before that time. Therefore, we cannot exclude the possibility that SGU may have been increased during the early part of the GLP-1 study. If so, this effect obviously was transient, because it was decreased after 120 min.

The effects of GLP-1 on glucose disappearance also are intriguing. Because SGU was lower during GLP-1 infusion than during saline infusion, this implies that the increased glucose disposal observed during the GLP-1 study day was caused by increased extrahepatic glucose uptake. However, the effects of GLP-1 on glucose disappearance were not evident until the fourth hour of the study. This observation may explain some of the contradictions in the literature. Toft-Nielsen et al. (41), Ryan et al. (42), and Orksov et al. (19) all have reported that GLP-1 does not alter insulin-induced stimulation of glucose disposal in nondiabetic humans. However, GLP-1 was infused for \( \leq 3 \) h in all of those experiments. We have recently demonstrated that GLP-1 has no effect on glucose disappearance or glucose effectiveness in subjects with type 2 diabetes during "prandial" insulin and glucose infusions mimicking concentrations commonly observed after food ingestion (21). However, although we infused GLP-1 for 6 h in those experiments, glucose disappearance was only transiently increased in response to the continuously changing glucose and insulin concentrations. We may have missed an effect of GLP-1 on glucose disposal if this effect requires a sustained increase in glucose concentrations.

Yang et al. (56) have presented evidence suggesting that GLP-1 enhances insulin-stimulated glycogen synthesis in L6 myotubules via a receptor that is different from the classical pancreatic GLP-1 receptor. If this receptor is also present in human muscle, then it will be of considerable interest to determine whether sustained elevations of GLP-1 increase the concentration and/or activity of either this receptor or the conventional GLP-1 receptor. If so, this could explain the time-dependent increase in glucose uptake observed in the present studies. Alternatively, the time-dependent increase may have occurred in extrahepatic tissues other than muscle (e.g., fat). Adipocytes have been reported to have GLP-1 receptors (12), and GLP-1 stimulates glucose uptake in adipocytes (8,9). However, the effect of GLP-1 on glucose uptake in those in vitro experiments was rapid. Furthermore, under the conditions of the present study, muscle rather than fat was the primary site of glucose uptake (57). Future experiments will be required to determine the site and mechanism of this time-dependent increase in uptake.

Insulin and glucose have been shown to stimulate glucose uptake via different intracellular pathways; the former involves the phosphoinositol kinase pathway, and the latter involves a calcium-dependent pathway (58). Therefore, GLP-1 may have a different effect on glucose metabolism when both glucose and insulin are elevated than when either is elevated alone. Of note, the present experiments were conducted in humans with type 1 dia-

![FIG. 5. Plasma \([6,6^{2}H_{2}]\)glucose enrichment and the ratio of \([6,6^{2}H_{2}]\)glucose to \([3-3H]\)glucose observed during the GLP-1 and saline study days.](image)

![FIG. 6. Rates of glucose disappearance and endogenous glucose production observed during the GLP-1 and saline study days.](image)
Assuming the liver took up at least a portion of the glucose at a rate as low as 20 μmol · kg⁻¹ · min⁻¹. However, because those experiments were performed in dogs, it remains possible that higher rates of portal glucose delivery may be necessary to fully elicit the “portal signal” in humans. Nevertheless, the systemic appearance rates (and by implication, the portal delivery rates) achieved in the present experiments approximate those commonly observed in humans within 1 h after ingestion of a carbohydrate-containing meal (60–63).

In summary, the current experiments indicate that GLP-1 does not enhance initial SGU during enteral glucose delivery. On the contrary, they suggest that in the presence of comparable glucose and insulin concentrations, GLP-1 impairs initial SGU. However, in most circumstances, this is likely to have a minor effect on glucose tolerance because of the ability of GLP-1 to potently stimulate insulin secretion, inhibit glucagon secretion, and delay gastric emptying. In addition, the current study presents evidence for a novel time-dependent effect of GLP-1 on glucose disposal. The mechanism and physiological significance of these effects requires further study.

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