Enteral infusion of glucose at rates approximating EGP enhances glucose disposal but does not cause hypoglycemia

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Submitted 6 February 2003; accepted in final form 19 March 2003

Zangeneh, Farhad, Rita Basu, Pankaj Shah, Puneet Arora, Michael Camilleri, and Robert A. Rizza. Enteral infusion of glucose at rates approximating EGP enhances glucose disposal but does not cause hypoglycemia. Am J Physiol Endocrinol Metab 285: E280–E286, 2003; 10.1152/ajpendo.00055.2003.—Portal infusion of glucose at rates approximating endogenous glucose production (EGP) causes paradoxical hypoglycemia in wild-type but not GLUT2 null mice, implying activation of a specific portal glucose sensor. To determine whether this occurs in humans, glucose containing [3,3H]glucose was infused intraduodenally at rates of 3.1 mg·kg⁻¹·min⁻¹ (n = 5), 1.55 mg·kg⁻¹·min⁻¹ (n = 9), or 0.1 mg·kg⁻¹·min⁻¹ (n = 9) for 7 h in healthy nondiabetic subjects. [6,6-2H2]glucose was infused intravenously to enable simultaneous measurement of EGP, glucose disappearance, and the rate of appearance of the intraduodenally infused glucose. Plasma glucose concentrations fell (P < 0.01) from 90 ± 1 to 84 ± 2 mg/dl during the 0/0.1 mg·kg⁻¹·min⁻¹ id infusions but increased (P < 0.001) to 104 ± 5 and 107 ± 3 mg/dl, respectively, during the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions. In contrast, insulin increased (P < 0.05) during the 1.55 and 3.0 mg·kg⁻¹·min⁻¹ infusions, reaching a peak of 10 ± 2 and 18 ± 5 µU/ml, respectively, by 2 h. Insulin concentrations then fell back to concentrations that no longer differed by study end (7 ± 1 vs. 8 ± 1 µU/ml). This resulted in comparable suppression of EGP by study end (0.84 ± 0.2 and 0.63 ± 0.1 mg·kg⁻¹·min⁻¹). Glucose disappearance was higher (P < 0.01) during the final hour of the 3.1 than 1.55 mg·kg⁻¹·min⁻¹ id infusion (4.47 ± 0.2 vs. 2.6 ± 0.1 mg·kg⁻¹·min⁻¹), likely because of the slightly, but not significantly, higher glucose and insulin concentrations. We conclude that, in contrast to mice, selective portal glucose delivery at rates approximating EGP does not cause hypoglycemia in humans.

portal signal; splanchnic glucose extraction; glucose uptake; endogenous glucose production

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in a vein of the contralateral hand. The hand was then placed in a Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. At 0500, a primed continuous infusion of [6,6-2H2]glucose was started and continued until the end of the study. At ~0630, the position of the nasoduodenal tube was confirmed by a portable abdominal radiography. At 0800 (0 min), a primed (12 µCi) continuous infusion (0.12 µCi/min) of [3-3H]glucose was infused in the duodenum via the nasoduodenal tube for the next 7 h. The [3-3H]glucose infusion also contained unlabelled glucose. In five subjects, glucose was infused in the duodenum at a rate of 3.1 mg·kg⁻¹·min⁻¹ on one occasion and 0.1 mg·kg⁻¹·min⁻¹ on the other occasion. In nine subjects, glucose was infused in the duodenum at a rate of 1.55 mg·kg⁻¹·min⁻¹ on one occasion and either saline (n = 4) or glucose (n = 5) at a rate of 1.22 mg·kg⁻¹·min⁻¹ (in error rather than saline) on the other occasion. The results of 1.22 mg·kg⁻¹·min⁻¹ infusion did not differ substantially from the 1.55 mg·kg⁻¹·min⁻¹ infusion and therefore are not presented as part of the current study but are available upon request. In addition, because results did not differ during the 0.1 mg·kg⁻¹·min⁻¹ id or saline alone infusions, these results were combined for purposes of analysis. Blood samples were collected at ~180, ~30, 0, 60, 120, 180, 210, 220, 230, 240, 270, 300, 330, 360, 390, and 420 min for analysis of tracer, hormone, and substrate concentrations.

**Analytical determinations.** Arterialized venous plasma samples were placed on ice, centrifuged at 4°C, and separated and stored at ~20°C until assay. C-peptide and glucagon concentrations were measured using reagents purchased from Linco Research (St. Louis, MO). Insulin and growth hormone were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucose concentration was measured using a Yellow Springs glucose analyzer. Plasma [3-3H]glucose specific activity and [6,6-2H2]glucose enrichment were measured by liquid scintillation counting and gas chromatography-mass spectrometry (16, 19). Percent body fat and fat-free mass were measured using a dual-energy X-ray absorptiometry scan QDR4500 with fan scan technology.

**Calculations.** Rates of glucose appearance and disappearance were calculated using Steele’s non-steady-state equations (22). A pool volume of 200 ml/kg and a pool fraction of 0.65 were assumed. The systemic rate of appearance of the intraduodenally infused [3-3H]glucose was also calculated using Steele’s steady-state equations in which the plasma [3-3H]glucose concentration was substituted for the unlabelled glucose concentration (4). The systemic rate of appearance of [3-3H]glucose (in dpm·kg⁻¹·min⁻¹) was divided by the specific activity of the intraduodenally infused glucose (in dpm/mg) to convert the rates to milligrams per kilogram per minute. EGP was calculated by subtracting the systemic rate of appearance of the intraduodenally infused glucose from the total rate of appearance of glucose. Initial splanchnic glucose extraction was calculated as 1 minus the systemic rate of appearance of [3-3H]glucose divided by the intraduodenal infusion rate of [3-3H]glucose.

**Statistical analysis.** Data in the text and Figs. 1–3 are expressed as means ± SE. All rates are expressed as milligrams per kilogram total body weight per minute. ANOVA followed by a signed-rank test was used to compare the results of the different studies. A P value <0.05 was considered statistically significant.

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**Table 1. Subject characteristics**

<table>
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<th>[3-3H]Glucose, mg·kg⁻¹·min⁻¹</th>
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<th>1.55</th>
<th>3.1</th>
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<td>9</td>
<td>5</td>
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<td>Age, yr</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
<td>24 ± 4</td>
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<tr>
<td>Gender</td>
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<td>4M/5F</td>
<td>2M/3F</td>
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<tr>
<td>BMI, kg/m²</td>
<td>24 ± 3</td>
<td>24 ± 4</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>LBM, kg</td>
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<td>51 ± 13</td>
<td>49 ± 10</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>22 ± 8</td>
<td>24 ± 11</td>
<td>24 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. M, males; F, females; BMI, body mass index; LBM, lean body mass.

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RESULTS

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Fasting plasma glucose concentrations (Fig. 1A) did not differ on the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id study days (90 ± 1 vs. 90 ± 2 vs. 87 ± 1 mg/dl). Plasma glucose concentrations fell (P < 0.001) during the 7 h of study to 84 ± 2 mg/dl on the 0/0.1 mg·kg⁻¹·min⁻¹ id study day. In contrast, plasma glucose increased (P < 0.001) on both the 1.55 mg·kg⁻¹·min⁻¹ (to 104 ± 5 mg/dl) and 3.1 mg·kg⁻¹·min⁻¹ (to 107 ± 3 mg/dl) study days. Although slightly higher on the 3.1 mg·kg⁻¹·min⁻¹ study day, glucose concentrations did not differ significantly from those observed during the final hour of the 1.55 mg·kg⁻¹·min⁻¹ study days.

Fasting plasma insulin concentrations (Fig. 1B) did not differ on the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id study days (0.4 ± 0.0 vs. 0.4 ± 0.1 vs. 0.3 ± 0.0 nmol/l). Plasma insulin concentrations fell (P < 0.001) to 0.3 ± 0 nmol/l during the 7 h of the 0/0.1 mg·kg⁻¹·min⁻¹ id study. In contrast, C-peptide increased (P < 0.01) during 3.1 mg·kg⁻¹·min⁻¹ id infusion to 0.8 ± 0.1 nmol/l. C-peptide tended to increase (0.8 ± 0.2 nmol/l) during the 1.55 mg·kg⁻¹·min⁻¹ id infusion. Plasma C-peptide concentrations did not differ (P = 0.5) during the final hour of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions (0.8 ± 0.2 and 0.8 ± 0.1 nmol/l).

Fasting plasma glucagon concentrations (Fig. 1C) did not differ on the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id study days (134 ± 13 vs. 146 ± 8 vs. 115 ± 6 pg/ml). Glucagon concentrations fell (P < 0.01) during the 3.1 mg·kg⁻¹·min⁻¹ id infusion to 100 ± 6 ng/l and during the 1.55 mg·kg⁻¹·min⁻¹ id infusion to 128 ± 6 ng/l and remained unchanged during the 0/0.1 mg·kg⁻¹·min⁻¹ id infusion (125 ± 8). Growth hormone concentrations also did not differ on the three study days either before or during the intraduodenal infusions (data not shown).

Plasma [6,6-²H₂]glucose enrichment and [3-³H]glucose specific activity. Plasma [6,6-²H₂]glucose enrichment (Fig. 2A) remained essentially unchanged during the 7 h of the 0/0.1 mg·kg⁻¹·min⁻¹ id infusion. Plasma [6,6-²H₂]glucose enrichment fell during the first 2 h of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id glucose infusions and changed minimally thereafter. The fall in plasma [6,6-²H₂]glucose enrichment was greater during the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusions, indicating a higher systemic rate of appearance of unlabeled glucose in the former. The ratio of [6,6-²H₂]glucose to [3-³H]glucose fell during the first 3 h of the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id infusions, indicating progressive absorption of the intraduodenally infused tracer (Fig. 2B). The ratio of [6,6-²H₂]glucose to [3-³H]glucose changed minimally thereafter.

Systemic rate of appearance of intraduodenally infused glucose, EGP, and glucose disappearance. The systemic rate of appearance of intraduodenally infused glucose increased during the first 3 h of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions, reaching a plateau thereafter (Fig. 3A). The appearance of intraduodenal glucose increased during the first 3 h of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions, reaching a plateau thereafter (Fig. 3A).
but not significantly, to 2.3 ± 0.1 during the 0/0.1 mg·kg⁻¹·min⁻¹ id infusions. Glucose disappearance during the final hour of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions (4.47 ± 0.2 vs. 2.6 ± 0.1 vs. 2.3 ± 0.1 mg·kg⁻¹·min⁻¹) was greater (P < 0.001) than that observed over the same interval during the 0/0.1 mg·kg⁻¹·min⁻¹ id infusions. Glucose disappearance was higher (P < 0.001) during the final hour of the 3.1 mg·kg⁻¹·min⁻¹ id infusion than during the final hour of the 1.55 mg·kg⁻¹·min⁻¹ id infusion.

**DISCUSSION**

Portal venous and arterial glucose concentrations do not differ in the fasting state, with both being lower than those in the hepatic vein (2, 15). Ingestion of a carbohydrate-containing meal results in a selective increase in the portal venous glucose concentration, thereby increasing the portal venous-to-hepatic venous glucose gradient (2, 15). This gradient is further accentuated by the increase in hepatic glucose uptake resulting from the accompanying postprandial increase in portal insulin concentrations (2, 3, 9–11, 18, 20, 23). Multiple studies have established that hepatic glucose uptake at any given insulin concentration is greater in the presence than in the absence of a portal venous-to-hepatic venous glucose gradient, implying the existence of so-called portal glucose “sensors” (2, 3, 9–11,

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**Fig. 2.** Plasma [6,6-²H₂]glucose enrichment (A) and the plasma ratio of [6,6-²H₂]glucose to [3-³H]glucose (B) observed before and during intraduodenal infusion of glucose at a rate of either 0/0.1, 1.55, or 3.1 mg·kg⁻¹·min⁻¹. The intraduodenal infusions were started at time 0. glucose was greater (P < 0.001) during the final hour of the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusion (3.66 ± 0.2 vs. 2.65 ± 0.03 mg·kg⁻¹·min⁻¹). No intraduodenal glucose appeared during 0/0.1 mg·kg⁻¹·min⁻¹ id infusions. Systemic appearance of intraduodenally infused tracer averaged 105 ± 14, 108 ± 3, and 113 ± 18% during the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id infusions, respectively, indicating negligible splanchnic tracer extraction.

Fasting EGP (Fig. 3B) did not differ on the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id study days (2.34 ± 0.1 vs. 2.30 ± 0.1 vs. 2.35 ± 0.1 mg·kg⁻¹·min⁻¹). EGP suppressed to 1.98 ± 0.2 mg·kg⁻¹·min⁻¹ during the final hour of the 0/0.1 mg·kg⁻¹·min⁻¹ id infusion (P < 0.05); however, EGP suppressed to a greater degree during the final hour of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions to 0.84 ± 0.1 and 0.63 ± 0.1 mg·kg⁻¹·min⁻¹ (P < 0.001). However, EGP did not differ during the final hour of the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id infusions.

Fasting glucose disappearance (Fig. 3C) did not differ on the 0/0.1, 1.55, and 3.1 mg·kg⁻¹·min⁻¹ id study days (2.32 ± 0.3 vs. 2.1 ± 0.2 vs. 2.3 ± 0.2 mg·kg⁻¹·min⁻¹). Glucose disappearance fell slightly,

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**Fig. 3.** Systemic rate of appearance of intraduodenally infused glucose (A), endogenous glucose production (B), and glucose disappearance (C) observed before and during intraduodenal infusion of glucose at a rate of either 0/0.1, 1.55, or 3.1 mg·kg⁻¹·min⁻¹. The intraduodenal infusions were started at time 0.
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18, 20, 23) Studies in dogs have shown that activation of these receptors also results in a compensatory decrease in extrahepatic glucose uptake (3, 10). These experiments, however, employed relatively high portal venous glucose infusion rates to create gradients similar to those observed after food ingestion. Therefore, the reports by Burcelin et al. (5–7) that infusion of glucose in the portal vein of mice at rates approximating EGP caused marked hypoglycemia were surprising. The demonstration that this response did not occur when portal GLUT2 transporters were knocked out was particularly intriguing, since it implied that hypoglycemia was caused by selective activation of a portal venous glucose sensor (6).

The present experiments were undertaken to determine whether low rates of delivery of glucose in the portal vein also resulted in hypoglycemia. To do so, we infused glucose in the duodenum at rates of 1.55 and 3.1 mg·kg⁻¹·min⁻¹. We chose these rates since we wanted to bracket the normal postabsorptive rate of EGP (i.e., ~2 mg·kg⁻¹·min⁻¹). Both infusions stimulated insulin secretion; however, in contrast to what was observed in mice (5–7), glucose concentrations rose rather than fell. The increase in glucose was evident whether considered as the change from the basal value or compared with the glucose concentrations observed over the same interval during the 0.01 mg·kg⁻¹·min⁻¹ id glucose infusions. The rise in glucose concentration continued until glucose disappearance increased sufficiently to equal the sum of the systemic rate of appearance of the intraduodenally infused glucose and EGP. The increase in glucose and insulin concentrations during the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ infusions resulted in equivalent suppression of EGP. However, despite comparable glucose and insulin concentrations, glucose disappearance increased more during the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusions, thereby compensating for the higher systemic rate of appearance of the intraduodenal glucose. Thus selective intraportal infusion of glucose in humans did not cause hypoglycemia but may have enhanced glucose disposal.

It is interesting to speculate why glucose disappearance was higher during the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusion. Burcelin et al. (6, 7) observed greater rates of glucose clearance during intraportal than during intravenous glucose infusion in mice. This effect did not occur in the GLUT2 knockout mice, suggesting a role for a portal glucose sensor (6). Activation of a portal glucose sensor that in turn enhanced extrahepatic glucose disposal also may have occurred in the present experiments. The higher rates of glucose disappearance during the 3.1 than during the 1.5 mg·kg⁻¹·min⁻¹ id infusion despite comparable glucose, insulin, and C-peptide concentrations is consistent with this possibility. On the other hand, we have previously shown that glucose disappearance was the same when glucose was infused intraduodenally or intravenously in nondiabetic volunteers at a rate of 4.0 mg·kg⁻¹·min⁻¹ (24). If the higher rates of glucose disappearance were mediated by a glucose sensor, this would imply a narrow dose-response curve, since this effect was absent during the current 1.55 and the past 4.0 mg·kg⁻¹·min⁻¹ glucose id infusions but present during the current 3.1 mg·kg⁻¹·min⁻¹ glucose infusion. On the other hand, glucose was clamped at ~150 mg/dl in our previous experiments, and somatostatin was infused to inhibit endogenous insulin secretion (24). Because somatostatin, perhaps by inhibiting GLP-1 secretion (12), blunts the “portal signal” in mice (5), our previous experiments could have missed a stimulatory effect of intraportal glucose delivery on glucose disappearance (24).

Alternatively, the higher rate of glucose disappearance during the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusion could have been because of the residual effect of antecedent higher insulin concentration combined with the slightly (but not significantly) higher glucose concentration. As is evident from Fig. 1, both insulin and C-peptide concentrations tended to be higher during the 3.1 than during the 1.55 mg·kg⁻¹·min⁻¹ id infusions, with the differences being most marked immediately after the start of the intraduodenal infusions. Glucose disappearance more closely reflects interstitial than plasma insulin concentrations, and a change in interstitial insulin concentration lags behind a change in plasma insulin concentration (25). Therefore, the higher plasma insulin concentrations during the first few hours of the 3.1 mg·kg⁻¹·min⁻¹ id infusion could have resulted in higher rates of glucose disappearance several hours later. The present experiments therefore leave open the possibility that selective intraportal infusion at rates bracketing EGP may enhance extrahepatic glucose uptake in humans.

We infused glucose at rates of either 0 (i.e., saline alone) or 0.1 mg·kg⁻¹·min⁻¹ as control experiments. We included these control experiments since we wanted to be able to determine whether a fall in glucose concentration (if observed) during the 1.55 and 3.1 mg·kg⁻¹·min⁻¹ id glucose infusions was greater than that which occurred with fasting alone. We infused [3-3H]glucose and carrier unlabeled glucose at a rate of 0.1 mg·kg⁻¹·min⁻¹ in five of the subjects and [3-3H]glucose with saline alone in four of the subjects. We included the saline-alone experiments since we were concerned that infusion of even a small amount of glucose in the duodenum might evoke an enteral signal, thereby obscuring differences that might occur with the higher intraduodenal glucose infusion rates. This was not the case since the results were the same during infusion of either the 0 (saline) or 0.1 mg·kg⁻¹·min⁻¹ id infusions. Burcelin et al. (5) reported that the GLP-1 antagonist exendin-(9–36) prevented hypoglycemia during intraportal glucose infusion in mice, whereas GLP-1 infusion did not. This suggested that the presence of basal levels of GLP-1 acting alone or in combination with other incretin hormones is required for activation of the portal signal in mice. We presume that incretin levels either remained constant or increased slightly during the intraduodenal glucose infusions. Therefore, the lack of
hypoglycemia during the intraduodenal glucose infusions is unlikely to be because of a deficiency of incretin hormones.

The present experiments have several limitations. We assumed that essentially all of the glucose that was infused in the duodenum was absorbed within the small intestine or upper colon. If some of the glucose was absorbed by the distal colon, then the rate of glucose entry in the portal circulation may have been slightly lower than the rate of intraduodenal infusion, since at least a portion of the venous drainage of the distal colon directly enters the systemic circulation. However, we doubt if substantial amounts of glucose were absorbed by the distal colon, since we infused glucose in the duodenum at a rate that was well below the absorptive capacity of the small intestine and since we would anticipate that the majority of glucose reaching the colon would be metabolized by colonic bacteria rather than absorbed (26, 27). Glucose was infused in the duodenum rather than directly in the portal vein since the latter is not feasible in healthy human volunteers. It therefore remains possible that activation of other control mechanisms (e.g., enteric nervous system, enteric hormone secretion) prevented the development of hypoglycemia. Future studies will be required to address this question.

In summary, intraduodenal glucose infusion at rates bracketing normal EGP does not cause hypoglycemia in nondiabetic humans. Rather, glucose and insulin concentrations increase slightly. The higher glucose and insulin concentrations appropriately suppress EGP and stimulate glucose disappearance, thereby minimizing the rise in glucose concentration. On the other hand, the higher rates of glucose disappearance during the 3.1 than during the 1.55 mg·kg\(^{-1}\)·min\(^{-1}\) infusion in the presence of slightly but not significantly higher glucose and insulin concentrations is consistent with, but does not prove the existence of, a portal signal in humans that enhances extrahepatic glucose disposal. Future studies will be required to specifically address this question in humans.

We thank Duane Burton for help in placing the nasoduodenal tubes; Cheri Etter, Betty Dicke, Robert Rood, and Charles Ford for technical assistance; M. Davis for assistance in the preparation of the manuscript; Jean Feehan, Barbara Norby, and the staff of the Mayo General Clinical Research Center for assistance in performing the studies; and the research volunteers for their willingness to participate in the studies.

DISCLOSURES

This study was supported by National Institutes of Health Grants DK-29053, DK-54681, and RR-00585 and the Mayo Foundation. F. Zangeneh was supported by a grant from the Endocrine Fellows Foundation. P. Shah was supported by a research fellowship from Novo-Nordisk, and R. Basu by an American Diabetes Association Mentor-based fellowship.

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