Interaction of insulin, glucagon-like peptide 1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate\textsuperscript{1–3}

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ABSTRACT  The relation between gastrointestinal incretin hormones and insulin release was investigated in 8 fasted, healthy male volunteers. Insulin, gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and appetite ratings were measured during, and food intake was measured after, intraduodenal infusions of glucose or saline. Studies were conducted under fed and euglycemic conditions. Raising plasma insulin with intravenous insulin infusion to concentrations slightly above usual postprandial concentrations (356.4 ± 4.8 pmol/L) had no effect on GIP, GLP-1, or appetite ratings before the intraduodenal infusions began. Intraduodenal glucose infusion resulted in a further increase in plasma insulin to a peak of 779.4 ± 114.0 pmol/L, caused an early increase in plasma GIP and a later increase in GLP-1 concentrations (P < 0.01), suppressed appetite (P < 0.05), and reduced energy intake (P < 0.01) compared with intraduodenal infusion of saline. There was a close association between the increase in GLP-1 and decrease in appetite. Infusion of octreotide to suppress the release of gastrointestinal hormones prevented the rise in insulin, GIP, and GLP-1 induced by intraduodenal glucose infusion and reversed the suppression of appetite and reduction in energy intake. These results suggest that 1) when infused to result in plasma concentrations slightly above usual postprandial concentrations, insulin does not inhibit its own release and 2) the effects of intraduodenal glucose on appetite may be mediated through the release of GLP-1 and not insulin.  Am J Clin Nutr 1998;68:591–8.

KEY WORDS  Glucose, small intestine, satiety, somatostatin, gastric inhibitory polypeptide, glucagon-like peptide 1, GIP, GLP-1, incretin hormones, insulin, men

INTRODUCTION

The presence of carbohydrate in the gastrointestinal tract plays a powerful modulating effect on both insulin release and appetite. The reason for the greater response to gastrointestinal than to intravenous carbohydrate has only been partially delineated and appears to involve gastrointestinal hormones. The incretin peptides, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), increase insulin secretion (1), causing a greater rise in plasma insulin after oral than after intravenous glucose administration in normal subjects (2). Although it has been established that ∼50% of the insulin release after an oral glucose load is mediated by incretin hormones (3), the relative roles of GLP-1 and GIP are uncertain. Oral and small-intestinal carbohydrate administration may have different effects on the release of GLP-1 and GIP (4). It is also uncertain whether gastrointestinal incretins influence eating behavior and whether this is independent of insulin release, although intracerebroventricular injection of GLP-1 was shown recently to inhibit feeding in rats deprived of food (5).

The mechanisms by which carbohydrate consumption inhibits appetite are not fully understood, but are likely to be multifactorial. Early theories implicated the postabsorptive role of hyperglycemia, suggesting that glucose utilization by specific receptors in the hypothalamus (6, 7) or liver (8) conveys information regarding the availability of glucose to central feeding and satiety systems. Other studies, however, emphasized the importance of preabsorptive signaling from the digestive tract. VanderWeele et al (9) showed in rabbits that intraduodenal infusion of glucose reduced food intake, whereas infusions of the same amounts of glucose into the hepatic portal circulation had no effect. We showed recently in healthy human subjects that intraduodenal administration of glucose reduces hunger and energy intake compared with an intravenous infusion of glucose that resulted in the same blood glucose concentrations (2). These studies therefore suggest that the rise in blood glucose per se after absorption of glucose from the intestine is not the crucial factor in signaling satiety, and that the presence of glucose in the digestive tract may be of greater importance.

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The presence of glucose in the small intestine may signal satiation through the stimulation of afferent vagal activity (10), the release of gastrointestinal hormones (11), or both of these mechanisms. Our recent study (2), in which we compared the effects of intraduodenal infusions of glucose and saline on feeding behavior, suggested that the release of insulin or incretin hormones (GLP-1 and GIP) or both plays a major role in the satiating effect of small-intestinal carbohydrate. In particular, after administration of the somatostatin analogue octreotide (12), a peptide known to inhibit the release of gastrointestinal hormones, intraduodenal glucose no longer suppressed appetite or reduced food intake.

There is a lack of consensus concerning the effect of insulin on eating behavior. Some animal studies indicated that increases in plasma insulin enhance the satiating effects of a meal (13–18), whereas other studies suggested that exogenous administration of insulin may stimulate appetite (19–21). The discrepancies in these observations are unlikely to reflect differences in blood glucose concentrations because Rodin et al (22) reported that hyperinsulinemia increased hunger and food intake in humans under both hypo- and hyperglycemic conditions. Lovett and Booth (13) observed a biphasic response to insulin injection—an initial enhancement of satiation and a subsequent increase in hunger—and concluded that insulin hastened the end of satiety of the previous meal, leading to premature resumption of hunger.

This study was designed to examine the relation between plasma insulin, GIP, and GLP-1 and the control of short-term satiety in response to small-intestinal carbohydrate administration. Using a euglycemic-hyperinsulinemic clamp technique, we evaluated the effects of intraduodenal infusions of glucose on insulin response, GIP and GLP-1 release, and eating behavior when 1) both plasma insulin and glucose were maintained by intravenous infusion at postprandial concentrations and 2) the release of gastrointestinal hormones was inhibited by infusion of octreotide.

SUBJECTS AND METHODS

Subjects

Subjects were 8 healthy, male volunteers (aged 19–33 y) who had a body mass index (in kg/m²) in the normal range (20–25). All volunteers scored <8 (± SE: 2.5 ± 0.7) on the restraint factor of the Eating Inventory Questionnaire (23), indicating that they were not restrained eaters. All subjects were nonsmokers and none were taking medication. Each subject gave his written, informed consent to participate in the study and the protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

Experimental protocols

Three single-blind experimental studies were carried out in random order. Each experiment was separated by ≥ 5 d (range: 5–36 d; median: 8 d). Subjects were instructed to consume a weight-maintaining diet containing ≥200 g carbohydrate/d for 3 d before each experiment. They were also instructed to not ingest any alcohol or indulge in strenuous exercise for 24 h before each experiment. Subjects fasted from 2000 on the night before each study and attended the Department of Medicine at 0800.

All 3 studies were carried out under hyperinsulinemic conditions designed to yield plasma insulin concentrations of ≈360 pmol/L through a primed, continuous infusion of insulin (24). Plasma glucose was maintained in the range of 5.0–9.5 mmol/L, commensurate with postprandial concentrations, by using intravenous infusion of glucose when necessary. In the first study, glucose was infused into the duodenum (study A); in the second, saline was infused intraduodenally (study B); and in the third, intraduodenal glucose was given while octreotide was infused intravenously (study C). Study C was conducted in 6 of the 8 subjects, who were selected at random.

After the subjects arrived at the laboratory, a small-diameter (4 mm) tube was inserted through the nostril into the intestine and positioned with its tip 15 cm distal from the pylorus by using a technique described previously (25). An intravenous cannula was inserted into an antecubital vein for infusion of insulin, potassium chloride, and glucose. An additional intravenous cannula was inserted into the opposite hand for obtaining blood samples. Venous blood was arterialized by wrapping the hand in a heated pad.

After placement of the nasaenteric tube and intravenous lines (t = −75 min), subjects rested quietly for 30 min. They were then (t = −45 min) asked to complete a visual analogue scale (VAS) questionnaire assessment of hunger and fullness. The questionnaire was adapted from one published previously (26). The scales were administered every 15 min thereafter throughout the study. After 5 min, a 12-mL venous blood sample was obtained. Subsequently, 2-mL samples were taken every 5 min throughout the experiment for immediate measurement of blood glucose and 10-mL samples every 10 min for laboratory measurement of plasma concentrations of glucose, insulin, GIP, and GLP-1.

At t = −30 min, an intravenous infusion of insulin (Actrapid; NovoNordisk, Australia) was given at a rate to raise and maintain plasma insulin concentrations at ≈360 pmol/L (Figure 1). An intravenous infusion of 25% glucose was also commenced at a rate (between 25 and 130 mL/h) designed to maintain blood glucose in the euglycemic range, and 5 mmol KCl/h (in 0.9% saline) was administered to offset the transport of potassium into the intracellular fluid space induced by the high concentrations of insulin and glucose. In study C an intravenous infusion of octreotide [Sandostatin (in 0.9% saline); SANDOZ Pharma Ltd, Basel, Switzerland] was also commenced at a rate of 50 µg/h. After a further 30 min (t = 0 min), an intraduodenal infusion of either 20% glucose (studies A and C) or 0.9% saline (study B) was given at a rate of 4 mL/min (13.4 kJ/min for glucose) and continued for 90 min. In studies A and C the intravenous infusion of glucose was stopped when blood glucose concentrations began to rise as a result of absorption of glucose from the small intestine. At t = 90 min all intravenous infusions were discontinued and the nasoenteric tube and intravenous infusion lines were removed.

Subjects were then presented (t = 100 min) with a cold, buffet-style meal (Table 1) prepared in excess of what they would normally be expected to eat, and invited to eat as much as they wished. Subjects could select from several food and drink items of various macronutrient contents. The time it took subjects to eat their meal and the amount of food consumed were recorded. Energy and macronutrient intakes were subsequently calculated by using COMP-EAT diet analysis software (LifeLine Nutritional Services Ltd, London). Subjects were allowed 30 min to consume their meal and at this time a venous blood sample was taken and a VAS questionnaire administered. Subjects remained in the laboratory for a further 30 min, after
which time we took a final blood sample and the subjects completed another VAS questionnaire.

Blood measurements

Blood glucose was measured immediately in the 2-mL blood samples with a portable blood glucose meter (Companion 2 Blood Glucose System; MediSense, Inc, Waltham, MA). Plasma from the samples taken at 10-min intervals was separated within 30 min of collection and stored at 2–7°C for later analysis. Plasma glucose was measured by using a hexokinase enzymatic reagent (Trace Scientific Proprietary Ltd, Baulkham Hills, Australia) and plasma insulin by radioimmunoassay (Phadeseph Insulin RIA; Pharmacia Diagnostics, Uppsala, Sweden). The intraassay CV for the insulin assay was < 5%.

Blood samples for the measurement of GLP-1 and GIP were collected into chilled EDTA-coated tubes containing 400 × 10³ kallikrein inhibitory units of aprotinin/L blood. GIP was measured with a previously described radioimmunoassay (27). GLP-1(7-36) was measured by using an antibody provided by SR Bloom (Hammersmith Hospital, London) that did not cross-react with glucagon, GIP, or other gut or pancreatic peptides (28). The radioimmunoassay method used was an adaptation of that used by Orskov et al (29). The interassay CV was 18%. [125 I]GLP(7-36) was prepared by the lactoperoxidase method and purified by reversed-phase HPLC on a 5-μm Techsil C18 column (HPLC Technology, Macclesfield, United Kingdom) with a 120-min, 30–50% gradient of acetonitrile in water containing 0.1% trifluoroacetic acid (29).

Before radioimmunoassay, plasma samples and standards (made up in serum treated by charcoal stripping) were extracted in 70% ethanol, dried under nitrogen, and resuspended to their original volumes in assay buffer (0.1 mol phosphate buffer/L, pH 7.4; 1 g human serum albumin/L; 0.6 mmol thiomerusal/L; 3.9 g EDTA/L; 1.3 g α–amino caproic acid/L). Aliquots of 100 μL of the reconstituted samples and standards were incubated for 48 h with 100 μL diluted antisera and 100 μL labeled peptide (≈10000 cpm). The bound fraction was separated from the free by adding dextran-coated, charcoal-containing gelatin (0.015 g gelatin, 0.09 g dextran, and 0.15 g charcoal in 30 mL assay buffer) and the radioactivity of the supernate was measured.

Statistical analysis

Statistical analysis was performed by using SPSS for WINDOWS (version 6.0; SPSS Inc, Chicago). To determine whether the hyperinsulinenic clamp technique itself had any effect on appetite ratings, one-way repeated-measures analysis of variance (ANOVA), with time as a main factor, was applied to ratings of hunger and fullness measured during the 45 min before the intraduodenal infusions began. Because all 3 experimental conditions (A, B, and C) were identical up to the time of intraduodenal infusion, the data from the 3 studies were grouped for this

<table>
<thead>
<tr>
<th>Food</th>
<th>Weight (g)</th>
<th>Energy (kJ)</th>
<th>Fat (g)</th>
<th>Carbohydrate (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sliced ham</td>
<td>100</td>
<td>502</td>
<td>5.1</td>
<td>0.0</td>
<td>18.4</td>
</tr>
<tr>
<td>Sliced chicken</td>
<td>100</td>
<td>665</td>
<td>9.0</td>
<td>4.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Sliced cheese</td>
<td>100</td>
<td>1693</td>
<td>34.0</td>
<td>0.1</td>
<td>24.7</td>
</tr>
<tr>
<td>Whole-meal bread</td>
<td>228</td>
<td>2048</td>
<td>5.7</td>
<td>94.9</td>
<td>21.0</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>100</td>
<td>71</td>
<td>0.3</td>
<td>3.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Cucumber</td>
<td>100</td>
<td>42</td>
<td>0.1</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Lettuce</td>
<td>100</td>
<td>59</td>
<td>0.5</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Potato salad</td>
<td>250</td>
<td>1463</td>
<td>20.5</td>
<td>40.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Bean salad</td>
<td>150</td>
<td>539</td>
<td>0.0</td>
<td>21.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>50</td>
<td>1513</td>
<td>39.7</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Margarine</td>
<td>50</td>
<td>1526</td>
<td>40.5</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Tomato sauce</td>
<td>50</td>
<td>205</td>
<td>0.0</td>
<td>1.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Canned peaches</td>
<td>280</td>
<td>1020</td>
<td>0.0</td>
<td>64.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Canned pears</td>
<td>280</td>
<td>903</td>
<td>0.0</td>
<td>56.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Ice cream</td>
<td>200</td>
<td>1622</td>
<td>19.6</td>
<td>48.8</td>
<td>7.2</td>
</tr>
<tr>
<td>Chocolate-custard</td>
<td>400</td>
<td>1585</td>
<td>12.0</td>
<td>61.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>16737</td>
<td>198.7</td>
<td>451.5</td>
<td>128.4</td>
</tr>
</tbody>
</table>

1 Amounts given are as served. The total percentages of energy from fat, carbohydrate, and protein were 44.7%, 42.3%, and 12.8%, respectively.
analysis. Changes in hunger and fullness from baseline, during the intraduodenal infusions, were evaluated by using two-way repeated-measures ANOVA, with infusion condition and time as main factors. The means of 2 measurements taken for each condition when insulin concentrations had been raised and stabilized (t = −15 and 0) were used as baseline values. Differences in plasma glucose, insulin, GIP, and GLP-1 concentrations during the intraduodenal infusions were evaluated by using two-way repeated-measures analysis of covariance (ANCOVA); plasma measurements taken immediately before the intraduodenal infusions were used as covariates (glucose and insulin at t = 0 and GIP and GLP-1 at t = −10). After repeated-measures ANOVA, post hoc tests were carried out by using Student’s t test to compare data between 2 studies (A compared with B); Tukey’s procedure was used for comparisons involving 3 studies (A compared with B compared with C). Differences in energy and macronutrient intakes were tested by using Student’s t test (A compared with B) or ANOVA followed by Tukey’s procedure (A compared with B compared with C).

Because study C was conducted in 6 of the 8 subjects, the effects of intraduodenal glucose during infusion of octreotide were evaluated on the data for the 6 subjects who completed all 3 studies. Data are shown as means ± SEMs. P < 0.05 was considered significant.

RESULTS

Intraduodenal glucose (study A) compared with intraduodenal saline (study B)

Plasma glucose, insulin, GLP-1, and GIP concentrations

Infusion of insulin alone had no effect on plasma GIP or GLP-1. Plasma glucose concentrations increased from 5.3 ± 0.1 mmol/L during the baseline period to 7.7 ± 0.4 mmol/L (study A) and 6.8 ± 0.3 mmol/L (study B) during the intraduodenal infusions. Plasma glucose concentrations were higher during infusion of intraduodenal glucose than during infusion of intraduodenal saline (F[1,7] = 9.00, P < 0.05; Figure 1). Plasma insulin concentrations increased to 356.4 ± 4.8 pmol/L as a result of intravenous infusion of insulin and a further increase to 779.4 ± 114 pmol/L occurred during infusion of intraduodenal glucose, so that concentrations were higher than those during infusion of intraduodenal saline [F[1,7] = 14.08, P < 0.01; Figure 1]. Post hoc tests revealed that this increase occurred within 20 min of the intraduodenal infusions.

Plasma GLP-1 concentrations increased during the intraduodenal glucose infusion but not during the saline infusion (F[1,7] = 24.10, P < 0.01). This increase did not occur until 30 min after the start of the intraduodenal infusions and concentrations continued to rise progressively throughout the infusion period (Figure 2). Plasma GIP concentrations also increased in response to intraduodenal glucose, but not intraduodenal saline, infusion (F[1,6] = 31.03, P < 0.01) and post hoc tests indicated that this increase was evident within the first 10 min of the intraduodenal infusions, when there was a rapid rise, before concentrations plateaued within ≈30 min (Figure 2). (One subject had GIP concentrations > 2 standard SDs from the mean of the group at all times and therefore his data were excluded from this part of the analysis.)

FIGURE 2. Mean (±SEM) plasma concentrations of gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) during infusion of intraduodenal glucose (study A), intraduodenal saline (study B), and intraduodenal glucose plus intravenous octreotide (study C). Significantly higher in A than in B and C, P < 0.05 (Tukey’s procedure). In studies A and B, n = 8 for GLP-1 and n = 7 for GIP; in study C, n = 6 for GLP-1 and n = 5 for GIP.

Appetite ratings

Raising insulin concentrations with the hyperinsulinemic clamp had no effect on hunger (F[3,63] = 0.48) or fullness (F[3,63] = 1.21) before the intraduodenal infusions began. Moreover, there were no changes in ratings of hunger (F[9,63] = 0.98) or fullness (F[9,63] = 0.92) throughout the study while saline was infused intraduodenally.

Compared with intraduodenal saline infusion, intraduodenal glucose infusion decreased hunger (condition × time interaction, F[5,35] = 4.33, P < 0.01) and increased fullness (condition × time interaction, F[5,35] = 4.74, P < 0.01). These effects became evident during the last 30 min of the infusions, when there was a further decrease in hunger and increase in fullness during the intraduodenal glucose infusion, whereas ratings began to return to baseline when saline was being infused (Figure 3). These
changes resulted in a significant difference in ratings of fullness by 90 min ($P < 0.02$) and a trend for a difference in hunger at this time ($P = 0.08$).

**Energy and macronutrient intakes**

Energy intake from the test meal was less after infusion of intraduodenal glucose than intraduodenal saline ($t_{10} = 3.58, P < 0.01$). Fat and protein intakes were reduced after infusion of intraduodenal glucose ($t_{10} = 3.64, P < 0.01$) and there was a trend toward a decreased intake of carbohydrate ($t_{10} = 2.18, P = 0.07$).

Effect of octreotide on responses to intraduodenal glucose (study C)

**Plasma glucose, insulin, GLP-1, and GIP concentrations**

Plasma glucose concentrations increased from $5.3 \pm 0.1$ mmol/L during the baseline period to $7.4 \pm 0.2$ mmol/L during the intraduodenal glucose infusion with octreotide (study C). There was no change in the plasma glucose response to intraduodenal glucose when octreotide was infused intravenously (Figure 1). Plasma insulin concentrations, however, did differ between the 3 studies during the intraduodenal infusions ($F_{2,10} = 14.07, P < 0.001$). Post hoc tests indicated that the rise in plasma insulin concentrations during the intraduodenal glucose infusion was abolished by octreotide ($P < 0.05$ for all time points from $t = 20$; Figure 1) and that, in fact, plasma insulin concentrations were slightly lower during octreotide infusion than during intraduodenal infusion of saline ($P < 0.05$, from $t = 60$ min).

There were also significant differences in GIP and GLP-1 concentrations between the 3 studies ($condition \times time interaction, F_{2,10} = 9.00, P < 0.001$, and $F_{2,10} = 5.68, P < 0.001$, for GIP and GLP-1, respectively; Figure 2). Post hoc tests revealed that infusion of octreotide prevented the increase in the concentrations of both GIP and GLP-1 that occurred during the intraduodenal glucose infusion ($P < 0.05$ for all time points from $t = 10$ min and $P < 0.05$ for all time points from $t = 30$ min for GIP and GLP-1, respectively), such that concentrations were no different from those during the intraduodenal saline infusion. Octreotide had no effect on basal (nonstimulated) concentrations of GIP or GLP-1. The total amount of energy provided from the intraduodenal and intravenous infusions of glucose was greater in study C ($1960 \pm 113$ kJ) than in study A ($1588 \pm 75$ kJ, $P < 0.05$) or study B ($1083 \pm 100$ kJ, $P < 0.01$).

**Appetite ratings**

There was a significant effect of the infusions on ratings of hunger (condition $\times$ time interaction, $F_{2,10} = 1.96, P < 0.05$) and fullness (condition $\times$ time interaction, $F_{2,10} = 2.33, P < 0.02$), which was apparent 90 min after the infusions began. Post hoc tests indicated that infusion of octreotide reversed the trend in hunger and fullness ratings observed in response to intraduodenal glucose alone: octreotide abolished the decrease in hunger and increase in fullness, resulting in ratings no different from those observed during the intraduodenal saline infusion (Figure 4).

**Energy and macronutrient intakes**

There was a significant difference in energy intake from the test meal after the 3 studies ($F_{2,10} = 5.79, P < 0.05$). Post hoc tests showed that the reduction in energy intake after intraduodenal glucose infusion compared with intraduodenal saline ($P < 0.05$) was no longer observed when octreotide was administered intravenously. Similarly, ANOVA revealed significant differences in fat ($F_{2,10} = 6.07, P < 0.02$) and protein ($F_{2,10} = 4.72, P < 0.05$) intakes from the test meal between the 3 studies and

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**TABLE 2**

Energy and macronutrient intakes from a test meal after intraduodenal infusion of glucose (study A) and intraduodenal infusion of saline (study B)†

<table>
<thead>
<tr>
<th></th>
<th>Study A</th>
<th>Study B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>4757 ± 627†</td>
<td>6433 ± 635†</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>47.1 ± 8.0†</td>
<td>69.8 ± 7.1†</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>139.5 ± 17.3</td>
<td>177.4 ± 18.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>47.4 ± 8.4†</td>
<td>60.6 ± 7.4†</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>36.1 ± 2.8</td>
<td>40.9 ± 1.5</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>47.4 ± 3.9</td>
<td>43.3 ± 2.1</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>16.3 ± 1.6</td>
<td>15.6 ± 0.8</td>
</tr>
<tr>
<td>Time taken to eat (min)</td>
<td>24.8 ± 1.5</td>
<td>25.0 ± 1.3</td>
</tr>
</tbody>
</table>

†$\bar{x} \pm$ SEM; $n = 8$.

*Significantly different from study B, $P < 0.01$.

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![Figure 3](https://academic.oup.com/ajcn/article-abstract/68/3/591/4648638/2414646338/1)

**FIGURE 3.** Mean ($\pm$SEM) changes in hunger and fullness during infusion of intraduodenal glucose (study A) and intraduodenal saline (study B). There was a decrease in hunger ($P < 0.01$) and increase in fullness ($P < 0.01$) in A compared with B (condition $\times$ time interactions). *Significantly higher in A than in B, $P < 0.05$ (Student’s $t$ test). $n = 8$ in each study.
subjects to consume the meal (octreotide and there was also no difference in the time it took percentage of energy provided by the macronutrients after differences in carbohydrate intake between the 3 studies. The longer seen with the administration of octreotide. There were no after the intraduodenal glucose infusion (study A) compared with intraduodenal saline infusion (study B). post hoc tests showed that the decreases in fat and protein intakes after the intraduodenal glucose infusion (P < 0.05) and increase in fullness (P < 0.05) seen in study A (condition × time interactions). There was no difference in changes in hunger or fullness between studies C and B. n = 6 in each study.

post hoc tests showed that the decreases in fat and protein intakes after the intraduodenal glucose infusion (P < 0.05) were no longer seen with the administration of octreotide. There were no differences in carbohydrate intake between the 3 studies. The percentage of energy provided by the macronutrients after intraduodenal glucose infusion was not affected by infusion of octreotide and there was also no difference in the time it took subjects to consume the meal (Table 3).

DISCUSSION

This study showed that insulin, when infused to concentrations above the normal postprandial range, did not decrease appetite and did not inhibit its own release in response to an intraduodenal glucose infusion. In response to intraduodenal glucose, plasma GIP concentrations increased rapidly, whereas GLP-1 concentrations responded more slowly and were associ- ated with an increase in insulin and decrease in appetite and subsequent food intake.

The results of this study confirm our previous observations that when postprandial concentrations of glycemia are maintained by intravenous glucose infusion, intraduodenal infusion of glucose decreases appetite in humans compared with intraduodenal infusion of saline (2). This was observed as a reduction in ratings of hunger, an increase in ratings of fullness, and a decrease in energy intake from a test meal with no change in macronutrient selection. The administration of 72 g glucose in 90 min may be slightly supraphysiologic, albeit within the normal range for gastric emptying (30). Although plasma glucose concentrations in the current experiment were slightly higher during infusion of intraduodenal glucose than during infusion of intraduodenal saline, this difference is unlikely to have affected our observations because it was small (<1 mmol/L) and our previous study showed that the effects of intestinal glucose on appetite are not mediated via an increase in blood glucose per se (2).

The observation that changes in appetite occurred only during the last 30 min of the duodenal infusion also agrees with our previous study (2). Although this might suggest a postabsorptive mechanism influencing satiety, a similar delay was observed in the responses of gastrointestinal motor activity to intestinal nutrient infusion, which was explained by the time taken to recruit sufficient intestinal receptors (31, 32). Lin et al (33, 34) showed that inhibition of gastric emptying is influenced more by the extent of exposure of the small intestine to nutrients than by the concentration of nutrients. Thus, it is possible that stimulation of a critical number of receptors to release sufficient peptide was required to induce the satiating effect of the intraduodenal glucose in the present study.

Although the intravenous insulin infusion elevated plasma insulin concentrations to slightly above the normal postprandial range (35), it was not possible to match the plasma insulin concentrations during intraduodenal saline infusion with those obtained during intraduodenal glucose infusion because, in contrast with published data (36, 37), raising plasma insulin artificially did not suppress endogenous insulin release. Marchetti et al (36) found that 200 or 400 μU/mL (1200 or 2400 pmol/L) insulin decreased first- and second-phase secretion of insulin from perifused human islets. In a study on human subjects, Piatti et al (37) found that second-phase, but not first-phase, arginine-induced insulin release was suppressed by both high-dose (1.20 μU·kg⁻¹·min⁻¹, or 7.2 pmol·kg⁻¹·min⁻¹) and low-

![FIGURE 4. Effect of intravenous octreotide (study C) on mean (±SEM) changes in hunger and fullness during intraduodenal glucose infusion (study A) compared with intraduodenal saline infusion (study B). In study C there was a reversal of the decrease in hunger (P < 0.05) and increase in fullness (P < 0.05) seen in study A (condition × time interactions). There was no difference in changes in hunger or fullness between studies C and B. n = 6 in each study.](https://academic.oup.com/ajcn/article-abstract/68/3/591/4648638)

![TABLE 3](https://academic.oup.com/ajcn/article-abstract/68/3/591/4648638) Energy and macronutrient intakes from a test meal after intraduodenal infusion of glucose without (study A) and with (study C) intravenous octreotide infusion and intraduodenal infusion of saline without intravenous octreotide infusion (study B).

<table>
<thead>
<tr>
<th></th>
<th>Study A</th>
<th>Study B</th>
<th>Study C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>5020 ± 822²</td>
<td>6759 ± 748</td>
<td>5685 ± 761</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>50.8 ± 10.3²</td>
<td>73.8 ± 8.0</td>
<td>62.1 ± 9.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>139.0 ± 22.9</td>
<td>181.4 ± 23.0</td>
<td>150.1 ± 21.3</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>54.9 ± 9.3²</td>
<td>67.2 ± 7.7</td>
<td>59.1 ± 8.1</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>36.7 ± 3.4</td>
<td>41.4 ± 2.0</td>
<td>40.7 ± 3.2</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>44.9 ± 4.5</td>
<td>41.8 ± 2.5</td>
<td>41.9 ± 3.9</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>18.3 ± 1.3</td>
<td>16.6 ± 0.6</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Time taken to eat (min)</td>
<td>25.5 ± 1.9</td>
<td>26.3 ± 0.8</td>
<td>23.8 ± 2.1</td>
</tr>
</tbody>
</table>

1x ± SEM; n = 6.

2Significantly different from study B, P < 0.05.
dose (0.33 mU · kg$^{-1} ·$min$^{-1}$, or 1.98 pmol · kg$^{-1} ·$min$^{-1}$) insulin infusion. The high-dose infusion resulted in mean plasma insulin concentrations of 455 pmol/L, similar to those obtained in the current study.

Although we were unable to match plasma insulin concentrations between the experiments, 2 observations suggest that, by itself, an increase in the plasma insulin concentration did not influence appetite in the present study. First, raising insulin concentrations by intravenous infusion had no effect on appetite ratings before the intraduodenal infusions began. Second, there was no significant effect of intravenous insulin on hunger and fullness during the control intraduodenal saline infusion. These results support the observations of Woo et al (38) that raising plasma glucose and insulin to normal postprandial concentrations with intravenous infusions does not affect food intake from a test meal. The decrease in appetite during the intraduodenal glucose infusion did, however, occur while insulin concentrations were further raised because of endogenous secretion. It is not possible, therefore, to exclude a role for this increase in plasma insulin in the suppression of appetite. However, Rodin et al (22) reported that raising plasma insulin to much higher concentrations through endogenous secretion in fact increased hunger in healthy subjects after a delay of ≈60 min. Moreover, our group reported recently that the infusion of insulin either at or higher than postprandial concentrations (to approximate postprandial insulin concentrations in the portal vein) under euglycemic conditions had no effect on sensations of appetite or subsequent food intake (39). Therefore, on the basis of current evidence, insulin does not appear to be a physiologic mediator of satiation.

In contrast, our data do support the concept that gastrointestinal incretin peptides, in particular GLP-1, play a role in the suppression of appetite by intraduodenal glucose. Hunger and food intake were suppressed and GLP-1 and GIP concentrations were increased in response to intraduodenal glucose but not intraduodenal saline. GLP-1, when given as an intracerebroventricular injection, inhibits feeding in both rats (5) and chickens (40). Peripheral administration of GLP-1 does not decrease food intake in rats (5); however, peripherally released GLP-1 has been shown to cross the blood-brain barrier (41) and a recent study in healthy rats (5) that the release of gastrointestinal peptides; (2) the slightly lower plasma insulin concentrations with octreotide than with intraduodenal saline and intravenous glucose may have been related to an effect of basal circulating concentrations of GIP and GLP-1 to stimulate insulin release in the presence of exogenous glucose. Although octreotide abolished the increase in hunger and food intake, any separate actions of insulin and other gastrointestinal hormones in the suppression of appetite could not be discriminated because octreotide also prevented the further increase in plasma insulin induced by the intraduodenal glucose. It is, however, clear that stimulation of intestinal receptors by glucose per se is not involved in the production of satiety, at least not without the involvement of gastrointestinal hormones. Moreover, the observation that greater amounts of intravenous glucose are required to maintain plasma glucose concentrations in this condition also contradicts the notion that the increases in appetite were solely related to increased glucose metabolism.

Taken together, these studies (1) confirm that intraduodenal glucose suppresses appetite and that the effect is dependent on the release of gastrointestinal peptides; (2) show that insulin, at concentrations slightly above the upper postprandial range, does not inhibit its own release; and (3) support the concept that GLP-1, rather than insulin or GIP, may be involved in mediating the suppression of appetite.

REFERENCES
