The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent upon the length of small intestine exposed

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The known regulatory gut peptides have differing distributions along the small intestine. For example, GIP is secreted from endocrine K cells (7) and CCK by endocrine I cells (33) in the proximal small intestine, whereas peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are secreted from endocrine L cells in the distal small intestine (6, 28). Whether these peptides are released primarily, or exclusively, by direct local contact with specific nutrients in their regions of storage or are released reflexively from the distal small intestine by signals arising from the proximal small intestine, or vice versa, is still debatable. For example, in rats, intravenous infusion of GIP has been shown to stimulate the secretion of GLP-1 (34). In dogs, Lin and colleagues (18, 19) demonstrated that cholecystokinin (CCK) was released equipotently whether fat contacted the proximal, or distal, small intestine and that the release of PYY was dependent, at least in part, on the stimulation of CCK by fat confined to proximal small intestine. On the other hand, Plichiewicz et al. (31) concluded recently that, in humans, CCK was released only by fat in the proximal small intestine and PYY was much more potently released by fat locally in the distal small intestine than in the jejunum. These last observations suggest that the nutrient-stimulated release of gut peptides in humans may differ substantially from the patterns observed in experimental animals.

Because the “incretin” peptides, GIP and GLP-1, play an important role in glucose homeostasis, both in health (26) and diabetes (22), it is important to establish how the release of each peptide is controlled by luminal glucose, specifically, whether in humans, GLP-1 is released only by local, distal jejunal, or ileal contact with glucose when dietary carbohydrates enter stomach at rates high enough to overcome the absorptive capacity of the proximal jejenum and thus to contact more distal small intestine. The observations of Schirra et al. (38) strongly suggest that, in humans, as opposed to rats, the release of GLP-1 is dependent on direct local contact of glucose with endocrine L cells. When human volunteers ingested 50 or 100 g of glucose in 400 ml of water, rates of gastric emptying of glucose in the first postcibal hour ranged, respectively, from 3.0 to 1.5 and from 4.5 to 3.0 kcal/min. During that first postcibal hour, plasma concentrations of GLP-1 were more than twice as high after the 100 g, compared with the 50 g, glucose load, whereas plasma concentrations of GIP were similar after the two loads for most of that time (38). Thus the observations of Schirra et al. are consistent with the idea that, in humans, GLP-1 is released only by local contact of glucose with GLP-1-bearing L cells in the distal jejenum and ileum when the loads of glucose entering the duodenum exceed the absorptive capacities of the proximal gut. Yet, to date, this hypothesis has not been directly tested.

The major aim of the present study was to compare the effect of limiting glucose to the proximal 60 cm (“short-segment infusion”) of the small intestine during duodenal infusion with the effect of allowing access of this duodenal load to the jejunum beyond 60 cm (“long-segment infusion”). Limiting glucose to the short (0–60 cm) segment was accomplished by...
inflating an occluding balloon 60 cm distal to the pylorus and draining glucose from the segment from an aspiration port just proximal to the balloon. On the long-segment day, the blocking balloon was inflated, and glucose was infused beyond 60 cm at a rate determined by the rate of glucose recovery from the distal end of the 60-cm segment on the day of the short-segment infusion. On the basis of the observations of Schirra et al., we postulated that release of GIP would be similar whether the short, or long, segments were perfused, whereas GLP-1 secretion would be observed only during the long-segment infusion.

The secretion of the orexigenic peptide ghrelin, by the oxyntic mucosa of the gastric fundus, is suppressed when nutrients are ingested (29, 30). In animals, this suppression is dependent upon exposure of the distal small intestine to nutrients (29). In humans, the relatively prolonged time required for the suppression of plasma ghrelin by enterally administered glucose (30) as well as a marked suppression of ghrelin observed following Roux-en-Y gastric bypass (3, 16), which shunts nutrients to the distal bowel, suggest that exposure of the distal intestine is required. Our short- vs. long-segment design afforded the opportunity to determine whether confining glucose to 60 cm of the proximal small bowel would blunt the suppression of ghrelin by glucose.

Because we aimed to identify regional effects of glucose on the basis of the distribution of the two “incretin” peptides along the small intestine, the ideal experimental design for this purpose would have been to compare the effects of glucose confined to the proximal 0–60 cm, with glucose confined to 60–120 cm, of the small intestine. However, because we found that we could not consistently, or easily, intubate the full 120 cm of the small intestine in the 6 h or so that ambulatory volunteers were willing to give to this study on each day, we compromised to use only a 0- to 60-cm intubation. This comparison allowed us to look at the effect of extending the length of glucose contact with sensors along the small intestine on antral and pyloric motility. In dogs, glucose has been demonstrated to increasingly inhibit gastric emptying of liquid meals, as the length of contact was extended from 15 to 60 to 150 cm of the small intestine (20). This inhibition was maximal when 150 cm of the proximal small intestine were contacted, and maximal inhibition was not significantly different whether the proximal 150 cm (jejunum), or the distal 150 cm (ileum), were contacted (20). Thus the inhibition of gastric emptying of a liquid meal by intestinal glucose appeared to be length, but not region, dependent. Although we did not measure gastric emptying in the present experiment, we were able to determine whether the effects of luminal glucose on antral and pyloric motility were maximal during the short-segment infusion or were increased by lengthening glucose contact with the small intestine during the long-segment infusion.

MATERIALS AND METHODS

Subjects. Eight healthy males with a mean age of 30.4 ± 13.7 (range 18–59) yr and with a mean body mass index of 26.2 ± 3.0 (range 22–32) kg/m² were studied. Subjects were questioned prior to the study to exclude any medical conditions, gastrointestinal symptoms or surgery, the use of medications known to affect gastrointestinal function, consumption of >20 g of alcohol or intake of >10 cigarettes per day. The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee. All subjects provided informed, written consent prior to their enrolment.

Study conditions. Each subject was studied on two occasions separated by 3–10 days in single-blind, nonrandomized fashion. This nonrandomized study design was required to determine the rate at which glucose should be infused into the distal small intestine during the second, long-segment study (a rate equal to the amount of glucose aspirated from the distal end of the proximal segment during the short-segment study). On both days, subjects received an infusion of glucose into the small intestine over 60 min. On the first day, glucose was infused into an isolated 60-cm segment of the proximal small intestine (short-segment infusion), and on the other, the same amount of glucose was infused with access to the entire small intestine (long-segment infusion).

Catheter design. The manometric assembly (outer diameter 4.5 mm; Dentsleeve, Adelaide, South Australia, Australia) included 5 manometric channels located at 1.5-cm intervals, with three channels positioned in the antrum, a 4.5-cm pyloric sleeve sensor (4), with two pyloric channels located on the back of the sleeve, and one duodenal channel, two small intestinal infusion ports, an aspiration channel at 60 cm distal to the pylorus, and a balloon port containing three air channels (Fig. 1). An inflatable polypropylene balloon (5 cm in length, 4 cm in diameter, with a maximum volume of 40 ml) was attached to the balloon port (41) and, when inflated with air, created an “isolated” 60-cm segment of the proximal small intestine. The first of the two infusion ports was located 2 cm distal to the sleeve and used to infuse glucose into the isolated 60-cm segment of the proximal small intestine, whereas the second, located 75 cm distal to the sleeve, was used to infuse saline, or glucose, distal to the occluding balloon. The aspiration port with an air channel (to facilitate suction) was located 1 cm proximal to the balloon to enable aspiration from the isolated 60-cm segment of the proximal small intestine.

Study design. Subjects attended the Department of Medicine at 0830 following an overnight fast (14 h for solids, 12 h for liquids). On each day, the catheter was inserted into the stomach via an anesthetized nostril (or orally if nasoduodenal intubation was not tolerated (3 of 8 subjects)) and allowed to pass into the small intestine by peristalsis. The correct positioning of the assembly, with the sleeve sensor straddling the pylorus, was monitored by continuous measurement of the antroduodenal transmucosal potential difference (TMPD).

![Fig. 1. Schematic drawing of catheter use for small intestinal infusion. Assembly included 5 manometric channels spaced at 1.5-cm intervals [including 3 channels in the antrum (A1, A2, and A3) and a 4.5-cm pyloric sleeve sensor, with 2 channels located on the back of the sleeve (P1 and P2)]. A channel in the duodenum (D1) was used to monitor the position of the catheter. An inflatable polypropylene balloon (5 cm in length, with a maximum volume of 40 ml) was attached to the catheter 60 cm distal to the pylorus. When inflated with air through the balloon port, the balloon created an isolated 60-cm segment of proximal small intestine. The catheter also contained 2 infusion ports, the first located 2 cm distal to the pylorus and the second 75 cm distal to the pylorus, and an aspiration port with an air channel located 1 cm proximal to the balloon, to facilitate suction and to enable aspiration from the isolated 60-cm segment of the proximal small intestine.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00034.2006)
between the most distal antral channel (~40 mV) and the duodenal channel (0 mV), using a reference electrode (a 20-gauge intravenous cannula filled with sterile saline) placed subcutaneously in the left forearm (9). The manometric channels were perfused with degassed, distilled water, except for the TMPD channels, which were perfused with degassed 0.9% saline at 0.15 ml/min (9). An intravenous cannula was placed into a right antecubital vein for blood sampling.

Following correct positioning of the catheter, the balloon was slowly inflated with air to a volume of ~30–40 ml. In all cases, balloon inflation ceased when subjects reported a sensation of pressure, without discomfort. Intraballoon pressure (~20 mmHg) was monitored continuously throughout each study with a pressure gauge. A previous study (41) had validated this technique by achieving complete recovery of the nonabsorbable marker, polyethylene glycol 4000 (PEG 4000), from the isolated segment; i.e., total occlusion of the small intestine was achieved. “Baseline” blood samples were collected at \( t = -5 \) and 0 min and baseline antropyloric pressures recorded between \( t = -1 \) to 0 min. At \( t = 0 \) min, intraduodenal infusion of 1 M glucose (4.9 ml/min, 3.5 kcal/min) was commenced in a single-blind, nonrandomized fashion. Thus, on the first study day (short-segment infusion), 1 M glucose was always infused into the isolated 60-cm segment of the proximal small intestine. During the infusion, the intestinal perfusate was continuously aspirated by gentle suction using a hand-held syringe from this segment via the aspiration port. The aspirate collected over each 10-min period was weighed and the glucose concentration determined. Between \( t = 10 \) and 60 min, the small intestine distal to the balloon was infused with 0.9% saline at a rate corresponding to the weight of the aspirate collected from the proximal segment in the preceding 10-min interval; i.e., the volume infused into the distal small intestine approximated that removed from the proximal segment. On the second study day (long-segment infusion), 1 M glucose was infused into the isolated proximal segment of the small intestine at the same rate as on the first day, whereas glucose was infused into the distal small intestine. The concentration of glucose for the distal infusion was calculated from the mean glucose concentration of the aspirate recovered on the first study day, i.e., the amount of glucose not absorbed from the proximal segment, and was infused according to the weight of the aspirate collected in the preceding 10 min (i.e., at each successive 10-min period on the second, long-segment day) (Table 1), as described for day 1. Venous blood samples were obtained at \( t = 5, 10, 15, 20, 30, 45, \) and 60 min for the determination of blood glucose and plasma insulin, GIP, GLP-1, CCK, and ghrelin concentrations. At \( t = 60 \) min, the infusion was ceased, the subject extubated, and the intravenous cannula removed. The subject was then free to leave the laboratory.

**Preparation of glucose solutions.** The 1 M glucose solution was prepared by dissolving 90 g of glucose in water, made up to a total volume of 500 ml. The glucose infusion rate of 210 kcal/h (3.5 kcal/min) is somewhat less than the initial rate of gastric emptying for the first 30 min following a glucose meal of moderate to high volume and energy density but greater than the slower, steady rates of gastric emptying (1, 15).

**Blood glucose and plasma hormone concentrations.** Blood glucose concentrations (mmol/l) were determined immediately using a portable glucose meter (Medisense Precision QLD; Abbott Laboratories, Bedford, MA). The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (14). Venous blood samples (20 ml) were collected into ice-chilled EDTA-treated tubes containing 400 KIU aprotinin/ml blood (Trasylol; Bayer Australia, Pymble, Australia). Plasma was separated by centrifugation at 3,200 rpm for 15 min at 4°C within 30 min of collection and stored at −70°C until assayed.

Plasma insulin concentrations (mIU/l) were measured by ELISA (Diagnosis Systems Laboratories, Webster, TX). The sensitivity of the assay was 0.26 mIU/l; the intra-assay and interassay coefficients of variation (CVs) were 2.6 and 6.2%, respectively (13). Plasma GIP concentrations (pmol/l) were measured by radioimmunoassay (42). The sensitivity of the assay was 2 pmol/l, and both the intra-assay and interassay CVs were 25%. Plasma GLP-1 concentrations (pmol/l) were measured by radioimmunoassay (43). The sensitivity of the assay was 1.5 pmol/l, the intra-assay and interassay CVs were 17 and 18%, respectively. Plasma CCK concentrations (pmol/l) were measured by radioimmunoassay (43). The sensitivity of the assay was 2 pmol/l, the intra-assay and interassay CVs were 9 and 27%, respectively. Plasma ghrelin concentrations (ng/l total ghrelin) were measured by radioimmunoassay (30). The sensitivity of the assay was 40 ng/l, and the intra-assay and interassay CVs were 17 and 23%, respectively.

**Antropyloric pressures.** Pressures in the antrum and pylorus were digitized and recorded on a computer-based system running commercially available software [Flexisoft, version 3; Prof. G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)] and stored for subsequent analysis. Pressures were analyzed for 1) number and amplitude of antral

**Table 1. Total glucose absorbed from the proximal 60-cm small intestinal segment during infusion into either “short-segment infusion” or “long-segment infusion”**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Infused</th>
<th>Recovered</th>
<th>Absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>210</td>
<td>36.3</td>
<td>173.7</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>64.8</td>
<td>145.2</td>
</tr>
<tr>
<td>3</td>
<td>210</td>
<td>110.2</td>
<td>99.8</td>
</tr>
<tr>
<td>4</td>
<td>210</td>
<td>72.3</td>
<td>137.7</td>
</tr>
<tr>
<td>5</td>
<td>210</td>
<td>42.3</td>
<td>167.7</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>48.4</td>
<td>161.6</td>
</tr>
<tr>
<td>7</td>
<td>210</td>
<td>81.9</td>
<td>128.1</td>
</tr>
<tr>
<td>8</td>
<td>210</td>
<td>74.2</td>
<td>135.8</td>
</tr>
</tbody>
</table>

Means ± SE 210 ± 0 66 ± 8.5 143.7 ± 8.5

"Short-segment infusion,” a 60-cm segment of the proximal small intestine; “long-segment infusion,” >60 cm of the small intestine. Total amount of glucose (kcal/h) infused distal to the occluding balloon and %glucose recovered from the proximal segment that was infused into the distal segment during the long-segment infusion. Calculated by subtracting the sum of glucose recovered in the aspirate from the proximal small intestinal segment/10 min over the 60 min of perfusion from the total amount of glucose infused; %glucose was infused at a concentration equivalent to that which was not absorbed in the proximal small intestinal segment during the short-segment infusion. The rate of infusion was determined by the amount of glucose recovered from the proximal segment during the long-segment infusion. %Short-segment recovery is the percentage of glucose that was recovered from the proximal small intestinal segment infused into the distal segment.
pressures, and IPPWs were analyzed by repeated-measures analysis of variance (ANOVA), with time and treatment as factors. The AUCs for plasma variables were analyzed by one-way ANOVA. Post hoc paired comparisons, corrected for multiple comparisons by Bonferroni’s correction, were performed if ANOVAs revealed significant effects. Correlations, corrected for repeated measures, were performed to determine the relationship between the amount of glucose (kcal/h) infused into the “distal segment” with the AUCs of plasma GLP-1 and ghrelin. Statistical significance was accepted at \( P < 0.05 \), and data are presented as means ± SE.

### RESULTS

The study protocol was tolerated moderately well by all subjects. The average time taken from intubation to the commencement of the infusion was 3.5 ± 2.4 (range 1–8) h. The amount of glucose infused on the 2 study days for each subject was variable due to the aspiration process; i.e., it was dependent on the amount of glucose recovered from the proximal segment on each day. Figure 2 shows the amount of glucose (kcal) recovered per 10 min from the proximal 60-cm segment, and Table 1 shows the absolute amount of glucose infused into both the proximal and distal segments during the short- and long-segment infusions, respectively. Whereas glucose was infused at the average concentration of the aspirate recovered on the short-segment day, the rate of the distal glucose infusion was determined by the volume of glucose recovered from the proximal segment on the long-segment day. Hence, given that the volume of glucose absorbed from the proximal segment was greater on that day, an average of only 60% of the glucose recovered from the proximal segment during the short-segment infusion was infused distally during the long-segment infusion.

### Blood glucose

There was a treatment-by-time interaction \( (P < 0.05) \) and an effect of time \( (P < 0.001) \), but no effect of treatment, for blood glucose (Fig. 3A). Although blood glucose increased progressively on both days between \( t = 15 \) and \( 60 \) min \( (P < 0.05) \), the magnitude of the rise was greater during the long- than during the short-segment infusion between \( t = 30 \) and \( 60 \) min \( (P < 0.05) \). There was no effect of treatment on the AUC for blood glucose.

### Insulin

There was a treatment by interaction \( (P < 0.001) \) and an effect of time \( (P < 0.001) \), but no effect of treatment, for plasma insulin concentrations (Fig. 3B). Although plasma insulin concentrations increased progressively from baseline during both treatments between \( t = 20 \) and \( 60 \) min \( (P < 0.05) \), the magnitude of the rise was greater during the long- than during the short-segment infusion, between \( t = 30 \) and \( 60 \) min \( (P < 0.05) \). There was a strong trend for the AUC for plasma insulin to be greater during the long- than during the short-segment infusion \( (P = 0.08) \).

### GIP

There was an effect of time \( (P < 0.001) \), but no effect of treatment and no effect of time interaction, for plasma GIP concentrations (Fig. 3C). During both conditions, plasma GIP increased rapidly from baseline at \( t = 10 \) min \( (P < 0.001) \) and plateaued at ~30 min. There was no effect of treatment on the AUC for plasma GIP.

### GLP-1

There was a treatment by interaction \( (P < 0.001) \) and an effect of time \( (P < 0.001) \), but no effect of treatment, for plasma GLP-1 concentrations (Fig. 3D). During the short-segment infusion, plasma GLP-1 did not change from baseline, whereas during the long-segment infusion, plasma levels of GLP-1 increased progressively from baseline between \( t = 30 \) and \( 60 \) min \( (P < 0.01) \). At \( t = 20 \) min and between \( t = 45 \) and \( 60 \) min, plasma GLP-1 was greater during the long- than during the short-segment infusion \( (P < 0.01) \). There was a strong trend for the AUC for plasma GLP-1 to be greater during the long- than during the short-segment infusion \( (P = 0.06) \). There was no relationship between the amount of glu-
CCK. There was an effect of time \((P < 0.001)\) but no effect of treatment and no treatment-by-time interaction, for plasma CCK concentrations (Fig. 4A). On both days, plasma CCK increased rapidly from baseline at \(t = 5\) min \((P < 0.001)\) and over the first 20 min of infusion and plateaued thereafter. There was no effect of treatment on the AUC for plasma CCK.

Ghrelin. There was a trend for an effect of treatment \((P = 0.06)\) but no effect of time and no treatment-by-time interaction, for plasma ghrelin concentrations (Fig. 4B). Plasma ghrelin tended to be lower during the long- than during the short-segment infusion. Moreover, there was an effect of treatment on the AUC for plasma ghrelin \((P < 0.05)\). There was no relationship between the amount of glucose (kcal/h) infused distal to the balloon and the AUC for plasma ghrelin \((r = 0.42, P = 0.1)\).

Antral and pyloric pressures. There was a treatment by time interaction \((P < 0.05)\), but no effect of time or treatment, for the number of antral PWs (Fig. 5A). The number of antral PWs was greater during the short- than during the long-segment infusion between \(t = 30\) and 60 min \((P < 0.05)\). There was no effect on the amplitude of antral PWs.

There was an effect of time \((P < 0.05\) for both) but no effect of treatment and no treatment-by-time interaction, for basal pyloric pressure (tone) and the number of IPPWs (Fig. 5, B and C). Basal pyloric pressure increased from baseline between \(t = 20\) and 60 min during the short- \((P < 0.05)\) but not the long-segment infusion. In contrast, the number of IPPWs increased from baseline between \(t = 20\) and 60 min on both days \((P < 0.05)\). There was no effect on the amplitude of IPPWs.

**DISCUSSION**

This study has provided new insights into the small intestinal regulation of gastrointestinal hormone secretion and antropyloric motility in humans. Our observations reveal that exposure of \(>60\) cm of the small intestine to glucose is required for the secretion of GLP-1 and the suppression of ghrelin but not for the secretion of GIP and CCK. Insulin secretion was also greater during the long-segment infusion, likely reflecting the higher blood glucose concentrations. Exposure of \(>60\) cm of the small intestine to glucose was also required to suppress antral PWs, whereas both infusions equally stimulated isolated pyloric PWs and increased basal pyloric pressure. Given that there was no significant relationship between the amount of glucose infused and either the AUC for plasma GLP-1 or ghrelin, the length of small intestine exposed to nutrient, rather than the amount of glucose infused, appears to be the important factor in determining GLP-1 and ghrelin release.

Although previous studies \((27, 30)\) have established that intraduodenal infusion of glucose stimulates the release of GIP, GLP-1, insulin, and CCK and suppresses ghrelin secretion, it has, hitherto, been unknown whether the release of these hormones is dependent on the length of small intestine exposed to glucose. In the present study, plasma GLP-1 concentrations increased promptly (i.e., within 20 min) and progressively only
GLP-1 from the distal small intestine by direct, local contact with glucose. By contrast, the release of GIP and CCK, both distributed mainly in the duodenum and proximal jejunum (7, 33), was similar on both days in which the duodeno-jejunal mucosa was contacted by glucose. Despite the fact that GIP was released similarly on both days, the failure of GLP-1 to rise significantly above basal concentration on day 1 suggests that, in humans, unlike the rat (34), the release of GLP-1 is not stimulated by GIP. This is supported by the observation that, in humans, intravenous infusion of GIP did not stimulate the
during exposure of >60 cm of the small intestine to glucose. This observation is consistent with the localization of endocrine L cells in the distal small intestine (6). In humans, GLP-1 is detectable in the duodenum (which extends 30 cm in length from the pylorus), has a moderate concentration in the jejunum (a segment that spans ~30–80 cm from the pylorus), and has high concentrations in the ileum (beyond 80 cm from the pylorus) (40). Although it has been postulated that the early rise in plasma GLP-1 following a glucose load may be explained by the release of GLP-1 from duodenal L cells (40), our observations argue against this possibility. On both study days, most of the glucose infused in the proximal segment was absorbed during transit through the 60-cm duodenojejunal segment (Table 1); but on the second day, ~20% of the infused glucose was given access to the jejunum beyond 60 cm distal to the pylorus. Although we cannot say precisely how much jejunal length was exposed to glucose, contact surely extended to the GLP-1-bearing mucosa. For example, it has been demonstrated in human volunteers that glucose transport per centimeter of the proximal jejunum reaches a maximum at loads above inflows of 1.4 kcal/min but that absorption is considerably slower at inflows of ~0.6 kcal/min, as was the case beyond the blocking balloon on day 2 (Table 1) (12, 24). Our observations are, therefore, consistent with the release of

Fig. 4. Plasma cholecystokinin (CCK; A) and ghrelin (B) concentrations during a 60-min infusion of 1 M glucose to either 1) a 60-cm segment of the proximal small intestine (short-segment infusion) or 2) >60 cm of the small intestine (long-segment infusion). A: there was no effect of treatment on plasma CCK concentrations. B: there was a trend for an effect of treatment on plasma ghrelin concentrations (P = 0.06). Data are means ± SE; n = 8.

Fig. 5. Number of antral pressure waves (PWs; A), basal pyloric pressure (B), and number of isolated pyloric pressure waves (IPPWs; C) during a 60-min infusion of 1 M glucose into either 1) a 60-cm segment of the proximal small intestine (short-segment infusion) or 2) >60 cm of the small intestine (long-segment infusion). A: there was a treatment-by-time interaction for the number of antral PWs (P < 0.05). The number of antral PWs was greater during the short-segment infusion between t = 30 and 60 min compared with the long-segment infusion (P < 0.05). B and C: there was no effect of treatment on basal pyloric pressure (tone) or the number of IPPWs. Data are means ± SE; n = 8. *Short-segment infusion vs. long-segment infusion, P < 0.05.
secretion of GLP-1 (25). Despite the above considerations, our design does not allow us definitively to distinguish between 1) a length-dependent release of GLP-1 reflexively from L cells (that is, some reflex mechanism, not involving GIP, that depends on an increasing number of glucose sensors contacted along a length of small intestine) and 2) a region-specific response that depends on local contact of luminal glucose with L cells in the distal small intestine, rather than the total length of small intestine contacted. Given the distribution of the L cells along the gut, we believe the second possibility is more likely.

Our conclusion, that the release of the incretin hormones by glucose is dependent on direct nutrient contact with GIP- or GLP-1-bearing mucosa in the duodenum vs. mid jejunum, respectively, is consistent with the observations of Schirra et al. (38). When human volunteers ingested 50 and 100 g of glucose in water, glucose emptied from the stomach initially (i.e., over the first 30 postcibal min) at rates of 3.0 and 4.5 kcal/min, respectively. Plasma concentrations of GIP rose somewhat sooner than those of GLP-1 after both glucose drinks, but both hormones peaked within 30 min during higher rates of duodenal entry of glucose. However, the peak GIP concentration was ~800 pg/ml following both glucose loads, whereas the peak GLP-1 concentrations were significantly different at ~3.5 and 7.0 pmol/l, for the 50- and 100-g loads, respectively. After both drinks, gastric emptying of glucose at or above 3.0 kcal/min was sufficient to expose the proximal 60 cm of small intestine to glucose (as in our case with infusion of 3.5 kcal/min) to release GIP, whereas only after the larger glucose load and faster inflow rate was there sufficient unabsorbed glucose in the proximal and distal jejunum to release GLP-1. It is important to understand how gastrointestinal hormone release is regulated in this way by interactions between ingested load, gastric emptying rate, and length of small intestine contacted by nutrient, because this has important implications for the regulation of gastric emptying (20) and energy intake (23). The observations relating to GIP and GLP-1 also have major implications for the regulation of postprandial glycemia and insulinemia (17).

Insulin secretion was also enhanced during exposure of >60 cm of the small intestine to glucose when compared with infusion into the isolated 60-cm segment of the proximal small intestine; this observation is most likely to be predominantly attributable to the relative hyperglycemia on this day. Both GIP and GLP-1 stimulate the release of insulin (17, 25). GLP-1 stimulates the secretion of insulin in a glucose-dependent manner (17). Hence, it is likely that the greater blood glucose and GLP-1 response observed during exposure of >60 cm of the small intestine interacted to enhance insulin secretion. These observations suggest that contact of glucose with the distal small intestine may be an important determinant of the postprandial insulin response.

Plasma ghrelin was also suppressed during the long-segment infusion compared with the short-segment infusion. Animal studies (29) have demonstrated that the inhibitory effects of intraduodenal nutrients on ghrelin secretion are not discretely dependent on exposure of the stomach or duodenum to nutrient and have suggested that exposure of the small intestine distal to the duodenum may play a role. This is also supported by observations that Roux-en-Y gastric bypass in morbidly obese subjects, an operation that markedly increases exposure of the ileum to nutrients, rapidly decreases plasma ghrelin, and increases PYY (16). Again, however, our design does not allow us to distinguish a length-dependent from a region-specific process. One possible explanation for our observations is that ghrelin suppression requires the exposure of >60 cm of the small intestine to glucose. It must, however, be noted that ghrelin secretion is also suppressed during intravenous infusion of glucose (39). Hence, the increased blood glucose response during exposure of >60 cm of the small intestine to glucose may have mediated the suppression of ghrelin. Hyperinsulinemia has also been shown to suppress plasma ghrelin concentrations under eu-, hypo-, and hyperglycemic conditions in humans (8), which suggests that insulin may mediate ghrelin suppression independently of glucose. Other studies (2, 36) have, however, failed to demonstrate an inhibitory effect of intravenous insulin on circulating ghrelin concentrations, except at supraphysiological concentrations. Our observations suggest that either elevated blood glucose or plasma insulin may indeed play a role in mediating the suppression of plasma ghrelin, since the greater glycemic and insulminemic responses during exposure of >60 cm of the small intestine to glucose was associated with lower concentrations of plasma ghrelin compared with glucose confined to the isolated 60-cm segment of the proximal small intestine. However, it should be noted that there was no change in plasma ghrelin when glucose was infused into the short segment despite significant rises in both blood glucose and plasma insulin, suggesting that the length of small intestinal exposure was the most important variable. This concept is further supported by the lack of relationship between the amount of glucose infused into the distal segment with the AUC for plasma ghrelin.

Antral motility was suppressed only during the long-segment infusion, whereas IPPWs and pyloric tone were stimulated to a similar degree whether glucose was confined to the proximal 60 cm or whether it was allowed access to intestinal lengths >60 cm. Both CCK and GLP-1, when administered exogenously, have been shown to potently suppress antral PWs and stimulate phasic and tonic pyloric pressures (32, 37). Therefore, the additional release of GLP-1 may have enhanced the suppression of antral PWs during the long-segment infusion. Because isolated pyloric PWs were potently stimulated by the 3.5 kcal/min glucose infusion on both study days, it is likely that the response that we observed was supramaximal (i.e., approaching >2–3 IPPWs/min) (11). Hence, the effect of increasing the length of small intestine exposed to nutrient may have been obscured. It should, however, be noted that duodenal distension has been shown to stimulate isolated pyloric PWs and increase basal pyloric pressure in humans (5). Hence, our observations may be confounded by the presence of the intraintestinal balloon, a possibility we cannot discount because of the lack of a control day of saline perfusions during balloon distension. Moreover, although the suppression of antral motility and stimulation of pyloric pressures is associated with the slowing of gastric emptying (9), it is unclear whether these measures completely characterize the control of gastric emptying. Clearly, additional investigation is required to understand how the control of gastric emptying, antropyloric motor responses, and length-dependent, glucose-driven intestinal feedback may be interrelated in humans.

In conclusion, this study has demonstrated that increasing the length of small intestine exposed to glucose stimulates the
release of GLP-1 and suppresses ghrelin secretion but has no effect on GIP and CCK. These observations have implications for the regulation of gastric emptying, postprandial glycaemia, appetite, and energy intake.

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