Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men

Amelia N. Pilichiewicz,1 Reawika Chaikomin,1 Ixchel M. Brennan,1 Judith M. Wishart,1
Christopher K. Rayner,1 Karen L. Jones,1 Andre J. P. M. Smout,2 Michael Horowitz,1 and
Christine Feinle-Bisset1

1University of Adelaide Discipline of Medicine, Royal Adelaide Hospital, Adelaide, Australia; and 2Department of Gastroenterology, University Hospital Utrecht, Utrecht, The Netherlands

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The rate of gastric emptying is a major determinant of the glycemic response to a meal; even relatively minor changes in small intestinal glucose delivery may have major effects on glycemic and insulinemic responses in healthy subjects (7) and non-insulin-treated type 2 diabetics (41). In type 1 diabetes, the initial postprandial insulin requirement is less when gastric emptying is slower (28, 42). These observations have substantial implications for dietary and pharmacological strategies to minimize postprandial glycemic excursion in diabetes (45) and thereby reduce the risk of microvascular (6) and macrovascular (49) complications. Hence, it is important to define the relationship between glycemic and insulinemic responses with enteral glucose delivery. The secretion of GLP-1 and GIP accounts for ~50% of the insulin response to oral glucose in healthy subjects (40). It has been suggested that a threshold of small intestinal glucose delivery of ~1.8 kcal/min needs to be exceeded to stimulate GLP-1 release (51). However, this observation is inconsistent with our two recent studies demonstrating that duodenal glucose infusion at 1 kcal/min was sufficient for the, albeit transient, stimulation of GLP-1 in healthy subjects and type 2 patients (7, 41). In contrast, there is evidence that the release of GIP is load dependent; however, the load that elicits the maximum response is not known (7, 41). The presence of glucose in the proximal small intestine also stimulates the release of CCK (35), although this response is less marked than that to protein or fat (33). To our knowledge, whether the release of CCK by glucose is load dependent remains to be determined.

The slowing of gastric emptying by glucose in the small intestine is associated with suppression of antral and duodenal pressure waves and stimulation of phasic and tonic pressures localized to the pylorus (11, 21). Studies in animals indicate that small intestinal feedback on gastric emptying is load, but not concentration, dependent (34). In humans, intraduodenal infusions of glucose at 2.4 and 4 kcal/min for 10 min stimulate phasic pressure waves localized to the pylorus (isolated pyloric pressure waves; IPPWs) and increase basal pyloric pressure, with a greater response to 4 kcal/min, indicative of load dependence (21). However, in another study, duodenal infusion of glucose at 2.4 kcal/min for 120 min increased the number of IPPWs and basal pyloric pressure during the first 20 min, but after this time there was a decrease, with a subsequent decrease in gastric motility with the duodenal glucose load, particularly the time course of these effects, are poorly defined.

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return to baseline (11), suggesting that there may be “adaptation” in the pyloric motor response during more sustained small intestinal glucose exposure. In contrast, antral pressure waves remained suppressed throughout the glucose infusion (11). The effects of enteral glucose on gastric motility may also be secondary to the consequent rise in blood glucose; acute hyperglycemia (blood glucose 12–16 mmol/l) is associated with suppression of antral waves (18) and stimulation of pyloric contractions (15). Changes in blood glucose that are within the normal postprandial range also affect gastrointestinal motility and gastric emptying (18, 53).

Perceptions of appetite and energy intake are suppressed by small intestinal infusion of glucose in young obese and healthy older subjects (8, 9, 32). These effects may relate to the release of CCK and GLP-1 (29, 59). It has recently been suggested that the suppression of energy intake may relate to changes in antrpyloroduodenal motility, particularly that of the pylorus (5, 61). In a recent study, intravenous infusion of CCK was shown to markedly stimulate IPPWs and basal pyloric pressures, and this was associated with suppression of energy intake, whereas GLP-1, at least in the dose used, failed to suppress energy intake or stimulate pyloric pressures (5). A further study in animals also supports the concept that the stimulation of pyloric motility may, per se, reduce energy intake (61). It is not known whether the effects of enteral glucose on energy intake and antrpyloroduodenal motility are related or whether the suppression of energy intake by glucose is load dependent in humans.

The aims of this study were to evaluate, in healthy subjects, the effects of different intraduodenal glucose loads on glycemia, insulin, incretin and CCK release, antrpyloroduodenal motility, and energy intake as well as the relationships among these parameters. It was hypothesized that 1) the effects of intraduodenal infusion of glucose at loads lower than (1 kcal/min), comparable with (2 kcal/min), and higher than (4 kcal/min) the rate of normal gastric emptying on these parameters would be load dependent (and in the case of GLP-1, be evident early); and 2) the effects of small intestinal glucose on energy intake would be related to those on antrpyloroduodenal motility.

METHODS

Subjects

Ten healthy males (age, 32 ± 4 yr; body mass index, 25.1 ± 0.4 kg/m²) were studied. None was a restrained eater [score ≤12 on the eating restraint component (factor 1) of the three-factor eating questionnaire (55)], had a history of gastrointestinal disease, or was taking medication known to affect gastrointestinal motility or appetite. No subject was a smoker or habitually consumed >20 g of alcohol per day. The study protocol, which conformed to the standards set by the Declaration of Helsinki, was approved by the Royal Adelaide Hospital Research Ethics Committee, and all subjects provided written, informed consent before their inclusion. The number of subjects included was based on power calculations derived from previous work (37, 38).

Protocol

Each subject was studied on four occasions, each separated by 3–7 days, on which they received in randomized, double-blind fashion an intraduodenal infusion of a 25% glucose (1,390 mosmol/l) solution at 1 kcal/min (“G1”), 2 kcal/min (“G2”), or 3 kcal/min (“G4”) or 4) intraduodenal hypertonic (4.2%, 1,390 mosmol/l) saline (“control”) for 120 min. The intraduodenal glucose solutions were prepared by dissolving glucose powder (Glucodin; Boots Healthcare, North Ryde, NSW, Australia) in distilled water and diluting with hypertonic saline to achieve the specific loads. Thus all infusions had a concentration of 1,390 mosmol/l and were administered at a rate of 4 ml/min, so that the total volume infused was 480 ml in all study conditions. The infusions were prepared by an investigator who was not otherwise involved in either the performance of the studies or data analysis. During studies, the infusion apparatus was covered at all times.

Each subject arrived at the University of Adelaide Discipline of Medicine at 0830 after an overnight fast (14 h for solids, 12 h for liquids). A manometric catheter (diameter, 3.5 mm; Dentsleeve International, Mut Scientific, ON, Canada), used to measure pressures in the antpyloroduodenal region, was inserted into the stomach via an anesthetized nostril and then allowed to pass into the duodenum by peristalsis. The catheter incorporated 16 manometric side holes located at 1.5-cm intervals. Six side holes (channels 1–6) were located in the antrum, a 4.5-cm sleeve sensor (channel 7) with two side holes on the side opposite the sleeve (channels 8 and 9) was positioned across the pylorus, and seven side holes (channels 10–16) were located in the duodenum. An additional channel, used for intraduodenal infusion, was located 11.75 cm distal to the end of the sleeve sensor (i.e., ~14.5 cm from the pylorus). The correct positioning of the catheter, with the sleeve straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference at the most distal antral (channel 6) (approximately ~40 mV) and the most proximal duodenal (channel 10) (~0 mV) channel, as described (20). An intravenous cannula was placed in a forearm vein for blood sampling.

Once the catheter was positioned correctly, fasting motility was monitored until the occurrence of a phase III of the interdigestive migrating motor complex (14). Immediately after the cessation of phase III activity (at time t = −15 min), a baseline blood sample was taken. At t = 0 min, during phase I of the migrating motor complex (14), the intraduodenal infusion commenced and was continued for 120 min. Antpyloroduodenal pressures were recorded during the 15-min baseline period (i.e., t = −15–0 min) and the infusion period (i.e., t = 0–120 min). Blood samples were taken at 15-min intervals between t = 0 and 60 min and then at 30-min intervals between t = 60 and 120 min. At t = 120 min, the infusion was terminated and the manometric catheter removed. Each subject was then presented with a standard cold buffet-style meal and allowed 30 min (i.e., t = 120–150 min) to eat freely until comfortably full (14). Further blood samples were taken at t = 150 and 180 min. At t = 180 min, the intravenous cannula was removed, and the subject was allowed to leave the laboratory.

Measurements

Blood glucose, plasma GLP-1, GIP, and CCK concentrations. Venous blood glucose concentrations (mmol/l) were determined immediately by the glucose oxidase method using a portable glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA). The accuracy of this method has been confirmed in our laboratory using the hexokinase technique (27).

For measurement of plasma insulin, GLP-1, GIP, and CCK, blood samples (~10 ml) were collected in ice-chilled EDTA tubes containing 400 kIU aprotinin (Trasylol; Bayer Australia, Pymble, Australia) per liter of blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at ~70°C until assayed. Plasma insulin was measured by ELISA (Diagnostics Systems Laboratories, Webster, TX). The intra-assay coefficient of variation (CV) was 2.6%, and the interassay CV was 6.2%, with a sensitivity of 2.6 mU/l. Total plasma GLP-1 was measured by radioimmunoassay (41). The intra-assay CV was 17% and the interassay CV was 18%, with a sensitivity of 1.5 pmol/l. Plasma GIP was

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measured by radioimmunoassay (41). The sensitivity was 2 pmol/l, and both the intra- and interassay CVs were 15%. Plasma CCK was measured after ethanol extraction, using an adaptation of a previously described radioimmunoassay (48). The intra-assay CV was 9%, and the interassay CV was 27%, with a sensitivity of 2.5 pmol/l.

Antropyloroduodenal pressures. Antropyloroduodenal pressures were recorded and digitized using a computer-based system running commercially available software [Flexisoft version 3; G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia; written in Labview 3.1.1 (National Instruments)] and stored for subsequent analysis. Antropyloroduodenal pressures were analyzed for 1) number and amplitude of antral and duodenal pressure waves (PWs), 2) number and amplitude of IPPWs, 3) basal pyloric pressure, and 4) number and length of pressure wave sequences (PWSs), as described previously (14).

Energy intake. Energy intake was assessed by measuring consumption at the buffet-style meal, the composition of which has been described (14). The amount (g) and energy (kJ) consumed and the macronutrient distribution (%energy from fat, carbohydrate, and protein) were evaluated using commercially available software (Foodworks version 3.0; Xyris Software, Highgate Hill, QLD, Australia) (14).

Statistical Analysis
Baseline values (“0”) were calculated as the total values obtained for the number of IPPWs, antral and duodenal PWs, and PWSs and the mean values obtained for amplitude of IPPWs, antral and duodenal PWs, and basal pyloric pressure between 0 and 0 min, and the mean values for plasma hormone concentrations at 0 and 0 min. Antral and duodenal PWs were expressed as total numbers in all six antral and all seven duodenal side holes, respectively. Basal pyloric pressures and number and amplitude of IPPWs were expressed as the mean of 15-min periods over the 120-min infusion period. Antropyloroduodenal PWSs were expressed as the total number of PWs spanning over 2 (1.5–3 cm), 3 (3–4.5 cm), . . . , 15 (21–22.5 cm) channels during the 120-min infusion period. All motility data were expressed as changes from baseline, while blood glucose and plasma insulin, GLP-1, GIP, and CCK were expressed as absolute values. Blood glucose and plasma hormone data were evaluated a priori for the trapezoidal rule (4). Only r values >0.5 were considered physiologically relevant. When correlations between the above variables were found, multiple regression analysis was performed to establish determinants of insulin release and energy intake at the buffet meal. Statistical significance was accepted at P < 0.05, and data are presented as means ± SE.

RESULTS
The studies were well tolerated by all except for one subject, who experienced quite marked nausea during the control infusions. That study was completed, and all data were included in the analysis. Fasting concentrations of blood glucose and plasma insulin, GIP, GLP-1, and CCK did not differ among the four study days (Table 1). For the analysis of GLP-1, data in 9 of the 10 subjects were available for analysis.

Gastrointestinal Hormone Concentrations
Blood glucose concentrations. See Fig. 1A.

INFUSION PERIOD. Blood glucose concentrations were higher during all glucose infusions, from t = 15 to 120 min, compared with control (P < 0.05). The increase in blood glucose between t = 15 and 60 min was greater during G2 and G4 compared with G1 (P < 0.05 for both). There was no difference between G2 and G4. Peak glucose concentrations were higher during G2 and G4 compared with G1 (P < 0.01), with no difference between G2 and G4 (Table 2). After ~t = 60 min, blood glucose progressively fell (P < 0.01) during G2 and G4 to concentrations close to baseline by t = 120 min (P = 0.07 for both). The AUC between t = 0 and 120 min was greater for G1, G2, and G4 compared with control (Table 2). There was no relationship between the AUC for blood glucose and the load of glucose administered.

POSTMEAL PERIOD. Blood glucose concentrations decreased after all glucose infusions compared with both control and levels immediately before the meal (i.e., t = 120 min) (P < 0.001); the magnitude of the fall was greater for G4 than for G1 and G2 (P < 0.05). In contrast, there was an increase in blood glucose (P < 0.001) after the control infusion.

Plasma insulin. See Fig. 1B.

INFUSION PERIOD. There was a rise in insulin with all glucose infusions compared with control (P < 0.05), between t = 60 and 120 min for G1, between t = 45 and 120 min for G2, and between t = 15 and 120 min for G4, with no difference between G1 and G2. Insulin concentrations were substantially greater during G4 compared with G1 and G2 between t = 30 and 120 min (P < 0.05). The AUC for plasma insulin was greater for G2 and G4 compared with control and for G4 compared with G1 and G2 (P < 0.001), with no difference between G1 and G2 (Table 2). The AUC for plasma insulin and the load of glucose administered were related (r = 0.89, P < 0.01).

POSTMEAL PERIOD. Plasma insulin concentrations fell after G4 (P < 0.001) to levels comparable with those at G1 and G2. In contrast, there was an increase in plasma insulin after control (P < 0.001); at t = 180 min, insulin concentrations after control were greater than those after the glucose infusions (P < 0.05).

Plasma GLP-1. See Fig. 1C.

INFUSION PERIOD. Between t = 0 and 30 min, there was a prompt rise (i.e., within 15 min) in plasma GLP-1 with all glucose infusions compared with control (P < 0.05), with no

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difference between G1 and G2. GLP-1 concentrations were higher during G4 compared with G1 and G2 \((P < 0.01)\). Between \(t = 15\) and 30 min, plasma GLP-1 fell during G1 and G2, and there were no longer any differences among G1, G2, and control. At \(t = 30\) min, plasma GLP-1 remained higher during G4 compared with control, G1, and G2 \((P < 0.001)\).

Between \(t = 0\) and 120 min, G2 and G4 but not G1 increased plasma GLP-1 compared with control, G2 at \(t = 15\) min and G4 between \(t = 15\) and 120 min \((P < 0.05)\). During G4, there was a progressive rise in plasma GLP-1 \((P < 0.001)\), and levels were much higher compared with G1 between \(t = 15\) and 120 min and with G2 between \(t = 30\) and 120 min \((P < 0.05)\) for both. There was no difference among control, G1, and G2 except at \(t = 15\) min. The AUC for plasma GLP-1 was greater for G4 compared with control, G1, and G2 (Table 2). The AUC for plasma GLP-1 and the load of glucose administered were related \((r = 0.89, P < 0.01)\).

**POSTMEAL PERIOD.** Plasma GLP-1 decreased after G4 and increased with control, and both these concentrations were higher than those following G1 and G2 \((P < 0.01)\). At \(t = 180\) min, there was no difference between control and any glucose infusion.

**Plasma GIP.** See Fig. 1D.

**INFUSION PERIOD.** All treatments increased plasma GIP between \(t = 15\) and 120 min compared with control \((P < 0.001\) for all), with rapid rises followed by relatively stable levels. G2 and G4 increased plasma GIP compared with G1 between \(t = 15\) and 120 min and \(t = 30\) and 120 min, respectively \((P < 0.05)\) for all. Plasma GIP was higher during G4 compared with G2 between \(t = 30\) and 90 min \((P < 0.05)\). Peak GIP concentrations were greater in response to G2 and G4 compared with G1 \((P < 0.001)\), and G4 compared with G2 \((P < 0.05)\) (Table 2). The AUC for plasma GIP was greater for all glucose treatments compared with control (Table 2). The AUC for plasma GIP and the load of glucose administered were related \((r = 0.91, P < 0.01)\).

**POSTMEAL PERIOD.** There were no changes in GIP for G2 and G4, with levels remaining elevated compared with control and G1 \((P < 0.01)\), but there were increases after control and G1 (i.e., \(t = 150\) min). At \(t = 180\) min, there was no difference between control and any glucose infusion.

**Plasma CCK.** See Fig. 1E.

**INFUSION PERIOD.** There was a rapid increase in CCK during all glucose treatments, after which concentrations remained relatively stable. G1 and G2 increased plasma CCK compared with control at \(t = 15\) min \((P < 0.01)\). CCK remained elevated compared with baseline during G1 and G2 between \(t = 15\) and 120 min \((P < 0.01)\). G4 increased plasma CCK compared with control, G1, and G2 between \(t = 15\) and 120 min \((P < 0.001\) for all). Both peak plasma CCK and the AUC for plasma CCK were greater for G4 compared with control, G1, and G2 \((P < 0.01)\) (Table 2). The AUC for plasma CCK and the load of glucose administered were related \((r = 0.82, P < 0.01)\).

**POSTMEAL PERIOD.** CCK concentrations increased after control, G1, and G2 \((P < 0.001\) for all) but not after G4, immediately after consumption of the buffet meal (i.e., \(t = 150\) min). There were no differences among the treatments.

### Antropyloroduodenal Pressures

**Antral PWs.** There was a treatment effect for the number, but not the amplitude, of antral PWs \((P < 0.01)\) (Table 3). G1 \((P < 0.05)\), G2 \((P < 0.05)\), and G4 \((P < 0.001)\) decreased the number of antral PWs compared with control, with no significant difference among them, although the mean value was least for G4. There was no significant relationship between the

### Table 1. Baseline values for antral and duodenal PWs, basal pyloric pressure, number and amplitude of IPPWs, and blood glucose and plasma insulin, GLP-1, GIP, and CCK concentrations*

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Saline (Control)</th>
<th>G1</th>
<th>G2</th>
<th>G4</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antral PWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>1.7±0.7</td>
<td>4.2±2.0</td>
<td>4.0±2.1</td>
<td>3.3±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>7.7±2.1</td>
<td>7.5±2.6</td>
<td>12.0±2.0</td>
<td>10.4±5.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Pyloric pressures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal, mmHg</td>
<td>−0.2±1.4</td>
<td>2.1±2.3</td>
<td>−3.0±5.6</td>
<td>5.6±6.1</td>
<td>NS</td>
</tr>
<tr>
<td>IPPW, no.</td>
<td>0.1±0.1</td>
<td>1.6±1.6</td>
<td>0±0</td>
<td>0±0</td>
<td>NS</td>
</tr>
<tr>
<td>IPPW, mmHg</td>
<td>3.0±3.0</td>
<td>4.2±4.2</td>
<td>0±0</td>
<td>0±0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Duodenal PWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>32.2±10.1</td>
<td>41.1±11.2</td>
<td>41.4±9.0</td>
<td>46.8±18.1</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>14.1±2.8</td>
<td>19.9±2.5</td>
<td>17.4±3.1</td>
<td>16.4±2.3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Blood glucose and plasma hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2±0.3</td>
<td>5.0±0.2</td>
<td>5.4±0.2</td>
<td>5.1±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>2.3±0.3</td>
<td>2.9±0.3</td>
<td>2.6±0.3</td>
<td>2.6±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>GLP-1, pmol/l</td>
<td>8.6±1.4</td>
<td>9.1±1.9</td>
<td>8.8±0.6</td>
<td>10.9±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>GIP, pmol/l</td>
<td>7.4±1.3</td>
<td>13.4±3.3</td>
<td>8.9±1.9</td>
<td>7.3±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>CCK, pmol/l</td>
<td>2.9±0.3</td>
<td>3.3±0.9</td>
<td>3.0±0.2</td>
<td>3.0±0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 10\). \*Before commencement of intraduodenal infusions of 25% \((1,390\, \text{mosmol/l})\) glucose at 1 \("G1")\, 2 \("G2")\, or 4 \("G4")\ kcal/min or 4.2% \((1,390\, \text{mosmol/l})\) saline. CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IPPWs, isolated pyloric pressure waves; NS, not significant; PWs, pressure waves.
number or amplitude of antral PWs and the load of glucose administered.

**Pyloric pressures.** **BASAL PRESSURES.** See Fig. 2A. There was a treatment × time interaction for basal pyloric pressures (P < 0.001). G1, G2, and G4 stimulated basal pyloric pressure compared with control, G1 and G2 between t = 90 and 105 min (P < 0.05) and G4 between t = 15 and 120 min (P < 0.01). During G4, basal pyloric pressure progressively rose until t = 60 min (P < 0.001), after which it fell slightly. G2 stimulated basal pyloric pressure between t = 15 and 45 min (P < 0.05) and G4 between t = 15 and 120 min (P < 0.01) compared with G1. G4 stimulated basal pyloric pressure between t = 30 and 120 min compared with G2 (P < 0.01). The AUC for basal pyloric pressure and the load of glucose administered were related (r = 0.83, P < 0.01).

**PHASIC PRESSURES.** There was no effect of treatment on the number (Fig. 2B) or amplitude of IPPWs (data not shown). All infusions initially stimulated IPPWs (P < 0.05), and this increase was greater during G4 compared with control (between t = 15 and 45 min), G1 (between t = 0 and 45 min), and G2 (between t = 15 and 30 min) (P < 0.05 for all). With all infusions, there was a subsequent decline in the number of IPPWs. There were no significant relationships between the number or amplitude of IPPWs and the load of glucose administered.

**Duodenal pressures.** There was a treatment effect for the number, but not the amplitude, of duodenal PWs (P < 0.001) (Table 3). G4 decreased the number of duodenal PWs compared with control (P < 0.001), G1 (P < 0.001), and G2 (P < 0.01), with no difference among G1, G2, and control. There was an inverse relationship between the number, but not the amplitude, of duodenal PWs and the load of glucose administered (r = −0.75, P < 0.001).

**PWSs.** Only PWSs that spanned 2–9 channels (1.5–13.5 cm) were analyzed statistically, as PWSs spanning over 10–15 channels were infrequent (control, 3.4 ± 0.3; G1, 1.6 ± 0.2;
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Table 3. Antral and duodenal PWs during intraduodenal infusions of 25% glucose (at G1, G2, or G4) or 4.2% saline between t = 0 and 120 min

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Saline (Control)</th>
<th>G1</th>
<th>G2</th>
<th>G4</th>
<th>P Value (ANOVA)</th>
</tr>
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<tbody>
<tr>
<td><strong>Antral PWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>70±16</td>
<td>40±10*</td>
<td>40±12*</td>
<td>24±7*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>26±6</td>
<td>27±7</td>
<td>8±3</td>
<td>22±11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Duodenal PWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>928±78</td>
<td>1,132±25</td>
<td>865±118</td>
<td>390±93*†‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>15±3</td>
<td>11±4</td>
<td>9±4</td>
<td>7±2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10. Intraduodenal infusions: 25% (1,390 mosmol/l) glucose or 4.2% (1,390 mosmol/l) saline. Significant differences: from control (*), from G1 (†), and from G2 (‡).
between basal pyloric pressure ($r = 0.84$, $P < 0.05$) and the AUC for plasma GLP-1. There was an inverse relationship between the number ($r = -0.63$, $P < 0.05$) of antral PWs, the number ($r = -0.57$, $P < 0.01$) and amplitude ($r = -0.67$, $P < 0.01$) of duodenal PWs, and the number of PWSs ($r = -0.57$, $P < 0.05$) and a direct relationship between basal pyloric pressure ($r = 0.68$, $P < 0.001$) and the AUC for plasma GIP. There was an inverse relationship between the number of antral PWs ($r = -0.59$, $P < 0.05$) and the number ($r = -0.76$, $P < 0.01$) and amplitude ($r = -0.62$, $P < 0.05$) of duodenal PWs and a direct relationship between basal pyloric pressure ($r = -0.81$, $P < 0.05$) and the AUC for plasma CCK.

Relation of blood glucose and plasma insulin, GLP-1, GIP, and CCK with energy intake and amount eaten. There was an inverse relationship between the AUC for blood glucose ($r = -0.50$, $P < 0.05$), insulin ($r = -0.77$, $P < 0.01$), GLP-1 ($r = -0.71$, $P = 0.06$), and GIP ($r = -0.61$, $P = 0.01$) and the values at $t = 120$ min for insulin ($r = -0.76$, $P < 0.05$) and GLP-1 ($r = -0.71$, $P < 0.71$) and the amount eaten. There was an inverse relationship between the AUC for insulin ($r = -0.62$, $P < 0.01$) and GLP-1 ($r = -0.80$, $P = 0.07$) and the values at $t = 120$ min for insulin ($r = -0.62$, $P < 0.01$) and CCK ($r = -0.52$, $P < 0.05$) and energy intake.

Relation of antropyloroduodenal motility with energy intake and amount eaten. There was an inverse relationship between the amount eaten at the meal and the AUC for basal pyloric pressure ($r = 0.70$, $P < 0.05$) and energy intake but no relationships between any other motility parameter and energy intake.

Relation of blood glucose and plasma insulin, GLP-1 and GIP. There was a direct relationship between the AUC for blood glucose ($r = 0.76$, $P < 0.001$) and plasma GLP-1 ($r = 0.85$, $P < 0.001$) and GIP ($r = 0.77$, $P < 0.01$) and plasma insulin.

Predictors of Insulin Concentrations and Energy Intake

Multiple regression analysis of the combined data for blood glucose and incretin hormones demonstrated that blood glucose ($\beta = 9.18$, $P < 0.05$), plasma GLP-1 ($\beta = 2.08$, $P < 0.001$), and plasma GIP ($\beta = 0.65$, $P < 0.05$) were independent predictors of plasma insulin concentrations.

Multiple regression analysis of the combined data for blood glucose, all hormones, and motility parameters showed that only basal pyloric pressure was associated with the amount eaten at the buffet meal ($\beta = -0.35$, $P < 0.05$). The AUC for plasma insulin ($\beta = 0.54$, $P < 0.01$) and values at $t = 120$ min for plasma insulin ($\beta = -29.33$, $P < 0.01$) and CCK ($\beta = -437.79$, $P < 0.01$) were predictors of energy intake.

DISCUSSION

This study provides novel insights into the effects of administration of glucose directly into the small intestine at loads lower than (1 kcal/min), comparable with (2 kcal/min), and higher than (4 kcal/min) the rate at which gastric emptying normally occurs on blood glucose and plasma insulin, GLP-1, GIP and CCK concentrations, antropyloroduodenal motility, and energy intake in healthy males. Of particular note are the following. 1) While there was a rise in blood glucose in response to all glucose infusions, there was no difference in the overall response to 2 and 4 kcal/min. 2) While a progressive

Fig. 2. Basal pyloric pressures (A) and isolated pyloric pressure waves (IPPWs, B) in response to 120-min intraduodenal glucose (25%, 1,390 mosmol/l) infusions at G1, G2, or G4 or saline (4.2%, 1,390 mosmol/l) control in 10 healthy males. Data are means ± SE. A: G4 stimulated basal pyloric pressure between $t = 30$ and 120 min compared with control, G1, and G2. *$P < 0.05$ vs. control. §§$P < 0.05$ vs. G1. §$P < 0.01$ vs. G2. B: G4 increased the number of IPPWs compared with control, G1, and G2. *$P < 0.05$ vs. control. §§$P < 0.05$ vs. G1. §$P < 0.01$ vs. G2.
Table 4. Amount eaten, energy intake, and %macronutrient distribution at the buffet meal (i.e., between t = 120 and 150 min) following intraduodenal infusions of 25% glucose (at G1, G2, or G4) or 4.2% saline

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Amount, g</th>
<th>Energy, kJ</th>
<th>%kJ from fat</th>
<th>%kJ from CHO</th>
<th>%kJ from protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (Control)</td>
<td>1,568±142</td>
<td>4,444±492</td>
<td>28±2</td>
<td>53±3</td>
<td>20±2</td>
</tr>
<tr>
<td>G1</td>
<td>1,461±126</td>
<td>4,999±255</td>
<td>29±2</td>
<td>50±3</td>
<td>20±1</td>
</tr>
<tr>
<td>G2</td>
<td>1,395±132</td>
<td>5,020±364</td>
<td>30±4</td>
<td>50±2</td>
<td>20±1</td>
</tr>
<tr>
<td>G4</td>
<td>1,157±166†‡</td>
<td>3,935±480†‡</td>
<td>27±2</td>
<td>57±4</td>
<td>19±1</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10. Intraduodenal infusions: 25% (1,390 mosmol/l) glucose or 4.2% (1,390 mosmol/l) saline. CHO, carbohydrate. Significant differences: from control (*), from G1 (†), and from G2 (‡).

rise in plasma insulin occurred in response to all glucose infusions, there was no difference between 1 and 2 kcal/min and a substantially greater response to 4 kcal/min. 7) There was a transient modest rise in plasma GLP-1 in response to intraduodenal glucose, but a sustained elevation was only evident with 4 kcal/min, which was progressive from ~t = 45 min, and a “meal-related” rise in GLP-1 occurred following the 1 and 2 kcal/min, but not the 4 kcal/min, infusion. 4) There was a load-dependent stimulation of GIP and CCK with a subsequent plateau and a meal-related increase only after control and 1 kcal/min for GIP, and after control and the 1- and 2-kcal/min infusions for CCK. 5) Antral pressures were suppressed by all glucose infusions, whereas the stimulation of basal pyloric pressure and suppression of duodenal PWs only occurred during the 4-kcal/min infusion. 6) A reduction in food intake was evident after the 2- and 4-kcal/min, but not the 1-kcal/min, infusion. And 7) there were significant relationships between blood glucose, plasma insulin, GLP-1, GIP, and CCK and antrypyloroduodenal motility, energy intake, and the amount eaten and between pyloric pressures and the amount eaten.

Our study establishes that there are substantial variations in the effect of different duodenal glucose loads on glycemia, insulinemia, and incretin hormones. While, predictably, all the effect of different duodenal glucose loads on glycemia, eaten and between pyloric pressures and the amount eaten.

GLP-1 is released from L cells, the density of which is greatest in the distal small intestine (12). Hence, a gradual progressive rise in GLP-1 may have been anticipated. Schirra et al. (51) have suggested that GLP-1 secretion requires a threshold of glucose delivery of ~1.8 kcal/min to be exceeded. However, the present study, as well as two other studies (7, 41), has established the capacity of loads as low as 1 kcal/min to cause an early transient stimulation of plasma GLP-1, albeit modest in magnitude. While the latter may reflect the presence of L cells in the duodenum (56), a recent study suggests that GLP-1 is only released in response to glucose when >60 cm of small intestine are exposed (35). Hence, the “early” transient GLP-1 response may be accounted for, initially, by relatively greater rapid small intestinal transit and subsequent slowing. Studies in rodents also indicate that GLP-1 may be released through a neuroendocrine loop to the distal small intestine, whereby the release of GIP from duodenal K cells in response to glucose acts through vagal afferent pathways to simulate the L cell indirectly (46, 47). Accordingly, the mechanism(s) underlying the initial increase in plasma GLP-1 warrants further investigation. The GLP-1 response to the 4-kcal/min infusion was substantially greater than to the other glucose loads and progressive, and this likely reflects the spread of glucose over a longer length of small intestine; the enteral glucose load resulting in a maximum GLP-1 response remains to be determined. Given the comparable GIP, but vastly different GLP-1, responses to the 2- and 4-kcal/min infusions, it seems reasonable to speculate that the stimulation of GLP-1 was primarily responsible for the increased insulin response. In contrast, GIP may be responsible for the insulin response to the lower duodenal glucose loads, although there are clear limitations in attempting to estimate the relative contributions of GLP-1 and GIP to postprandial insulin release on a molar plasma level basis (52). Clearly, our observations in healthy subjects should not be extrapolated directly to type 2 diabetes, which is characterized by a delay in insulin release (41), impaired secretion of GLP-1 (57), an impaired insulinotropic effect of GIP but not GLP-1 (23), and a high prevalence of disordered gastric emptying and gastroduodenal motility (26). However, it will be important to determine the effects of different small intestinal glucose loads in this group. CCK is released from L cells, which are confined to the proximal small intestine (43); therefore, as expected with GIP, all glucose infusions stimulated plasma CCK concentrations in a load-dependent fashion.

The tight regulation of glucose entry into the small intestine in health reflects the integration of motor activity in the proximal stomach, antrum, pylorus, and duodenum (11, 21);
the stimulation of PWs localized to the pylorus may be the most important mechanism (58). All glucose treatments, as well as the control, stimulated IPPWs during the first 30 min of infusion, followed by a decline. The response to control is not surprising, given that it would have provided an osmotic stimulus known to slow gastric emptying (1), and the highest glucose load (4 kcal/min) stimulated IPPWs more, for some 45 min. Consistent with our observations, Edelbroek et al. (11) reported in healthy men that intraduodenal glucose infusion at 2.4 kcal/min for 120 min initially stimulated IPPWs and basal pyloric pressure, but these responses were not sustained, suggesting that there may be adaptation of the pylorus to the presence of glucose in the small intestine. The present study also confirms previous data, showing sustained suppression of antral PWs during intraduodenal glucose infusion (11, 21, 44). In our recent study (35), suppression of antral motility was evident when glucose was infused at 3.5 kcal/min for 60 min into both the duodenum and distal small intestine (i.e., allowed access to the entire small intestine) and not when it was confined to the proximal 60 cm. Hence, the observed suppression of antral PWs by the 1-kcal/min load is perhaps surprising, as one would not expect this load to reach beyond the proximal 60 cm of the small intestine before being absorbed, but this may reflect the longer duration of glucose infusion in this study. The effects of intraduodenal glucose on motility may also be secondary to the consequent elevation in blood glucose (2, 15, 19), although this cannot explain the discordant duodenal and pyloric responses to the 2- and 4-kcal/min infusions, given that the glycemic responses were comparable. That the magnitude of the rise in blood glucose was related to the suppression of antral PWs is not surprising, given that blood glucose concentrations as low as ~8 mmol/l can inhibit antral pressures (2) and slow gastric emptying compared with euglycemia (~4–6 mmol/l) (2). The effects of hyperglycemia on motility do not appear to be mediated by hyperinsulinemia (30).

There is a close relationship between the initial rise in blood glucose after oral carbohydrate and gastric emptying (25). Accordingly, interventions that result in a slowing of gastric emptying, and are associated with a pattern of antropyloroduodenal motility that favors this, may reduce postprandial glycemic excursions (17). In this study, there were significant relationships between rises in blood glucose, plasma insulin, GLP-1, GIP, and CCK and the stimulation of basal pyloric tone and the suppression of duodenal PWs, which may account for slowing of gastric emptying. The observed relationship between plasma GIP and the suppression of antral PWs is surprising, given that GLP-1, not GIP, appears to be important in the regulation of gastric emptying (39, 50). Although there was a significant relationship between both plasma GLP-1 and CCK concentrations and the glucose load, this was not reflected in the AUCs and should, accordingly, be viewed circumspectly.

Energy intake was only suppressed after the highest glucose load (4 kcal/min; total 480 kcal), although the amount (in g) of food consumed was also reduced by the 2-kcal/min infusion. That a decrease in energy intake was only evident after 4 kcal/min is not surprising, as previous studies employing glucose loads of 2 kcal/min for 90 min (180 kcal) (44) and 2.86 kcal/min for 120 min (343 kcal) (8, 38) failed to show any reduction in energy intake in healthy young males. The only study, to date, that has determined a reduction in energy intake during intraduodenal glucose infusion, compared with saline, used a 3.2-kcal/min infusion of glucose for 90 min, which approximates to 288 kcal (32). Interestingly, this load (288 kcal) is less than that in studies providing 2.86 kcal for 120 min (8, 38), in which no reduction in energy intake was observed. This may indicate that the rate of glucose infusion or, similarly, the length of small intestinal exposure to glucose, rather than the duration of infusion per se, is more important in the regulation of energy intake, since digestion and absorption are likely to be completed over a shorter length of intestine during slower infusions. The effect of exposing greater lengths of the small intestine (e.g., to the midjejunum) warrants further investigation. There is evidence that interplays exist among gastrointestinal hormones, motility, insulin release, and energy intake. Our study suggests that basal pyloric tone and CCK and insulin were independent predictors of food and energy intake, respectively. Both exogenous and endogenous CCK slow gastric emptying/motility and decrease energy intake but have no effect on insulin secretion (3, 5, 16, 29, 54). Exogenous and endogenous GLP-1 modulate gastric motility, stimulate insulin secretion, and decrease energy intake (36, 50, 52). The substantial increase in GLP-1 concentrations during the 4-kcal/min infusion may have contributed to the observed decrease in energy intake by stimulating insulin release (60). It has recently been suggested that the stimulation of pyloric motility may, per se, diminish energy intake (5, 61). In a recent study, intravenous CCK stimulated pyloric pressures and decreased energy intake, whereas GLP-1, at least in the dose used, failed to stimulate pyloric pressures and did not decrease energy intake (5).

In conclusion, this study has established that variations in the delivery of glucose into the small intestine have differential effects on blood glucose, insulin, incretin, and CCK responses, gastrointestinal motility, and energy intake in healthy subjects. These observations have implications for an understanding of the regulation of postprandial glycemia and energy intake in type 2 diabetes.

GRANTS

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