Effect of small intestinal glucose load on plasma ghrelin in healthy men

Kimberly Cukier, Amelia N. Pilichiewicz, Reawika Chaikomin, Ixchel M. Brennan, Judith M. Wishart, Christopher K. Rayner, Karen L. Jones, Michael Horowitz, and Christine Feinle-Bisset

University of Adelaide Discipline of Medicine, Royal Adelaide Hospital, Adelaide, and National Health and Medical Research Council of Australia Centre of Clinical Research Excellence in Nutritional Physiology, Interventions and Outcomes, Adelaide, South Australia, Australia

Submitted 5 March 2008; accepted in final form 8 June 2008

Cukier K, Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Horowitz M, Feinle-Bisset C. Effect of small intestinal glucose load on plasma ghrelin in healthy men. Am J Physiol Regul Integr Comp Physiol 295: R459–R462, 2008. First published June 11, 2008; doi:10.1152/ajpregu.00169.2008.—Postprandial ghrelin suppression arises from the interaction of meal contents with the small intestine and may relate to elevations in blood glucose and/or plasma insulin. We sought to determine whether the suppression of ghrelin by small intestinal glucose is dependent on the glucose load and can be accounted for by changes in blood glucose and/or plasma insulin. Blood glucose, plasma insulin, and plasma ghrelin levels were measured in 10 healthy males (aged 32 ± 4 yr; body mass index: 25.1 ± 0.4 kg/m²) during intraduodenal glucose infusions at 1 kcal/min (G1), 2 kcal/min (G2), and 4 kcal/min (G4), as well as intraduodenal hypertonic saline (control) for 120 min. There was a progressive decrease in ghrelin in all treatments, control at 45 min and between 90 and 120 min (P < 0.05) and G1 (P < 0.05), G2 (P < 0.0001), and G4 (P < 0.0001) between 30 and 120 min to reach a plateau at ~90 min. There was no difference in plasma ghrelin between G1, G2, or G4. Control suppressed ghrelin to a lesser extent than intraduodenal glucose (P < 0.05). The suppression of ghrelin was not related to rises in blood glucose or plasma insulin. Suppression of ghrelin by intraduodenal glucose in healthy males is apparently independent of the glucose load and unrelated to blood glucose or insulin levels.

intraduodenal glucose loads; glycemia; plasma insulin

GHRELIN, A PEPTIDE PRODUCED predominantly by the stomach, is an endogenous regulator of energy homeostasis (6). Ghrelin levels increase with fasting and fall postprandially (6); the magnitude of this suppression is dependent on meal composition (7) and is postgastric, arising from the interaction of nutrients with the small intestine (21). For example, the suppression of ghrelin by identical intragastric and intraduodenal glucose loads is comparable, whereas intragastric water has no effect on ghrelin (17). In addition, both insulin and glucose modulate ghrelin secretion (5, 8, 19).

Nutrient-mediated feedback from the small intestine plays a major role in the regulation of both gastric emptying (4) and appetite (11), and the magnitude of this feedback is known to be load-dependent, reflecting the length of small intestine exposed to nutrient (12). The small intestine is also sensitive to the osmotic properties of a meal, so that hypertonic, non-nutrient liquids empty more slowly from the stomach than isotonic liquids (14).

There is evidence that the suppression of ghrelin by enteral glucose may also be load-dependent (13). We have recently reported the effects of intraduodenal hypertonic saline and different glucose loads (i.e., 1 kcal/min, 2 kcal/min, 4 kcal/min) on glycemia and insulinemia in healthy subjects (18). Although all glucose loads increased blood glucose, there was little difference in the responses to the 2 kcal/min and 4 kcal/min loads, which was attributable to a substantially greater insulin response to the latter. By measuring ghrelin on stored plasma samples from this study, we have aimed to determine whether 1) the suppression of ghrelin by small intestinal glucose is load-dependent, 2) hypertonic saline influences ghrelin, and 3) the suppression of ghrelin by small intestinal glucose can be accounted for by changes in plasma insulin and/or glucose.

MATERIALS AND METHODS

Subjects. Ten healthy males (aged 32 ± 4 yr; body mass index: 25.1 ± 0.4 kg/m²) were studied (18). No subject had a history of gastrointestinal disease or was taking medication known to affect gastrointestinal motility or appetite. 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. The number of subjects included was based on power calculations derived from our previous work (13, 17).

Experimental design. The study protocol has been described previously (18). 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 mOsm/l) solution, at 1) 1 kcal/min (G1), 2) 2 kcal/min (G2), or 3) 4 kcal/min (G4), or 4) intraduodenal hypertonic (4.2%, 1,390 mOsm/l) saline (control), all for 120 min (18). The intraduodenal 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. All infusions were given at a rate of 4 ml/min.

Each subject attended the Discipline of Medicine at 0830 after an overnight fast (14 h for solids, 12 h for liquids). A catheter (Dentsleeve International, Mutu Scientific, Ontario, Canada) was inserted into the stomach via an anesthetized nostril and then allowed to pass into the duodenum by peristalsis. The catheter incorporated a channel used for intraduodenal infusion at ~14.5 cm from the pylorus. The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference as described previously (10). An intravenous cannula was placed in a forearm vein for blood sampling. At t = 0 min, the intraduodenal infusion commenced and was continued for 120 min (18). Blood samples were taken at 15-min intervals between t = −15–60 min, and then at 30-min intervals between t = 60–120 min. At t = 120 min, the infusion was terminated and the catheter was removed (18).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Fasting (baseline) concentrations of blood glucose, plasma insulin, and ghrelin

<table>
<thead>
<tr>
<th></th>
<th>Saline (Control)</th>
<th>G1</th>
<th>G2</th>
<th>G4</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<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>Ghrelin, pg/l</td>
<td>1,128±123</td>
<td>1,246±160*</td>
<td>1,170±110</td>
<td>1,184±139</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10. Fasting concentrations (baseline) are concentrations before commencement of intraduodenal infusions of 25% (1,390 mOsmol/l) glucose at 1 (G1), 2 (G2), or 4 (G4) kcal/min, or 4.2% (1,390 mOsmol/l) saline. *Significantly different vs. control; P < 0.05.

**RESULTS**

The studies were well tolerated by all but one subject, who experienced nausea during the control infusion. That study was completed, and all data were included in the analysis.

**Blood glucose.** There was no difference in baseline blood glucose concentrations (Table 1). As reported (18), blood glucose concentrations were higher during all glucose infusions between t = 15–120 min when compared with control (P < 0.05; Fig. 1A). The increase in blood glucose was greater during G2 between t = 30–60 min, and G4 between t = 15–60 min, when compared with G1 (P < 0.01 for both). There was no difference between G2 and G4, except at t = 30 min (P < 0.01). After ~t = 60 min, blood glucose fell progressively (P < 0.01) during G2 and G4, to concentrations close to baseline by t = 120 min (P = 0.07 for both).

**Plasma insulin.** There was no difference in baseline plasma insulin concentrations (Table 1). There was a rise in insulin following all glucose infusions when compared with control (P < 0.05; Fig. 1B), with no difference between G1 and G2. Plasma insulin was substantially greater during G4 when compared with G1 and G2 between t = 30–120 min and t = 45–120 min, respectively (P < 0.01 for both).

**Plasma ghrelin.** There were differences in the baseline values for plasma ghrelin (Table 1). There was a progressive decrease in ghrelin with all treatments (Fig. 1C); control at t = 45 min and between t = 90–120 min (P < 0.05) and G1 (P < 0.05), G2 (P < 0.0001), and G4 (P < 0.0001) between t = 30–120 min. The suppression of ghrelin was greater than control during both G1 (P < 0.05) and G2 (P < 0.01) between t = 60–120 min, and G4 (P < 0.05) between t = 30–120 min. There was no difference between G1, G2, and G4 throughout the infusion period. There were no significant relationships...
between the suppression of plasma ghrelin with increases in blood glucose or plasma insulin.

DISCUSSION

This study has evaluated the effects of intraduodenal glucose loads, which approximate the lower limit (1 kcal/min), average (2 kcal/min) and upper limit (4 kcal/min) of the rate at which glucose normally empties from the stomach (4), and intraduodenal hypertonic saline, on plasma ghrelin in healthy subjects. We have established that 1) the suppression of ghrelin by intraduodenal glucose is not load-dependent, 2) intraduodenal hypertonic saline suppresses ghrelin, but to a lesser extent than glucose, and 3) the suppression of plasma ghrelin by intraduodenal glucose is not dependent on the magnitude of the concomitant elevations in blood glucose or insulin.

In healthy subjects, glucose solutions empty from the stomach in an overall linear pattern, after an initial phase (5–10 min) that may be more rapid (4). This load-dependent, concentration-independent phenomenon is accounted for by inhibitory feedback arising from the small intestine (4, 10), the magnitude of which is dependent on the length of small intestine exposed to glucose (12). The effects of small intestinal glucose to suppress appetite (11) and stimulate glucose-dependent insulino-tropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and CCK (13) are also load-dependent. Hence, the apparent load independency of ghrelin suppression within the normal range of gastric emptying is perhaps surprising, although, presumably, a minimum load would be required to elicit an effect, and this load remains to be determined. Therefore, intraduodenal glucose loads of less than 1 kcal/min may be required to determine the “threshold” at which ghrelin is suppressed. In contrast, it appears that an intraduodenal glucose load of 1 kcal/min for 120 min appears sufficient to elicit maximum suppression. Our observations indicate that ghrelin suppression is, at least in part, concentration-dependent, as is known to be the case for the stimulation of canine pancreatic bicarbonate secretion by fat in the small intestine (15). We have reported that intragastric water has no effect on ghrelin (17), whereas in the current study hypertonic saline induced a modest suppression. While additional studies to evaluate the effect of intraduodenal osmolality on ghrelin would be of interest, as the four intraduodenal infusions were iso-osmotic, osmolality cannot account for the substantially greater suppression induced by glucose.

Despite the fact that insulin and glucose may share a temporal association with ghrelin, a functional relationship has not been clearly established, and there are a number of conflicting observations (5, 8, 19). As reported, while all the glucose infusions increased glycemia and insulinemia, there were substantial differences in the responses (18). Despite these, ghrelin was uniformly suppressed, apparently to a “threshold” level, by each of the intraduodenal glucose loads. This suggests that neither blood glucose nor insulin play a substantial role in the suppression of ghrelin by intraduodenal glucose. This is consistent with the findings of Caixas et al. (5), who suggested that ghrelin suppression is mediated by the presence of nutrients in the gastrointestinal tract and is not influenced by insulin levels. Ghrelin suppression is macronutrient dependent, with carbohydrate and fat ingestion reducing, and protein possibly increasing, ghrelin levels (7, 9). Peripherally released CCK, GIP, and glucagon have been implicated in the suppression of ghrelin (1, 3). Central nervous system mechanisms may also be important (16).

Perspectives and Significance

In interpreting our observations, a number of potential limitations should be recognized. We only studied healthy males and, therefore, cannot exclude gender-specific effects on ghrelin (9). Venous blood glucose measurements were measured, and the precision of blood glucose could have been improved using arterial glucose levels; however, the differences in the 1 kcal/min infusion and both the 2 kcal/min and 4 kcal/min infusion were substantial. Although ghrelin levels appeared to plateau after 90 min of each of the intraduodenal glucose loads, we cannot be certain that maximum suppression was achieved; further studies employing more prolonged intraduodenal glucose loads are required. We also did not quantify small intestinal motility or transit, which may be a confounding factor, particularly given that motility is influenced by glycemia (13) and ghrelin (20).

In conclusion, this study has established that the suppression of ghrelin by intraduodenal glucose in healthy males is independent of the glucose load and apparently unrelated to blood glucose or insulin levels.

REFERENCES


