The role of the stomach in the control of appetite and the secretion of satiation peptides

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Steinert RE, Meyer-Gerspach AC, Beglinger C. The role of the stomach in the control of appetite and the secretion of satiation peptides. Am J Physiol Endocrinol Metab 302: E666–E673, 2012. First published January 3, 2012; doi:10.1152/ajpendo.00457.2011.—It is widely accepted that gastric parameters such as gastric distention provide a direct negative feedback signal to inhibit eating; moreover, gastric and intestinal signals have been reported to synergize to promote satiation. However, there are few human data exploring the potential interaction effects of gastric and intestinal signals in the short-term control of appetite and the secretion of satiation peptides. We performed experiments in healthy subjects receiving either a rapid intragastric load or a continuous intraduodenal infusion of glucose or a mixed liquid meal. Intraduodenal infusions (3 kcal/min) were at rates comparable with the duodenal delivery of these nutrients under physiological conditions. Intraduodenal infusions of glucose elicited only weak effects on appetite and the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). In contrast, identical amounts of glucose delivered intragastrically markedly suppressed appetite ($P < 0.05$) paralleled by greatly increased plasma levels of GLP-1 and PYY ($\Delta$3-fold, $P < 0.05$). Administration of the mixed liquid meal showed a comparable phenomenon. In contrast to GLP-1 and PYY, plasma ghrelin was suppressed to a similar degree with both intragastric and intraduodenal nutrients. Our data confirm that the stomach is an important element in the short-term control of appetite and suggest that gastric and intestinal signals interact to mediate early fullness and satiation potentially by increased GLP-1 and PYY secretions.

Small intestine; humans; glucagon-like peptide-1; gut; interactions

EATING IS ORGANIZED INTO DISCRETE MEALS and determined by meal size and meal frequency. Multiple regulatory pathways seem to promote or inhibit eating and thus regulate energy balance. Gastrointestinal (GI) satiation signals such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), or peptide YY (PYY) as examples are secreted from the small and large intestine in response to food ingestion and signal to the brain via neural or endocrine pathways to inhibit eating (8). Their plasma levels are low in the fasting state and rise during a meal, which is consistent with the satiating effect observed when peripherally infused in rats and humans (1, 19, 26). In contrast, the peptide ghrelin is secreted from the stomach during fasting and plasma level fall shortly after meals consistent with a hunger-inducing action observed in rats and humans (47, 53).

Gastric parameters such as stomach distention are another important source of negative feedback (37). Feelings of hunger and satiety have long been associated with gastric motor and sensory functions, and more importantly, gastric and intestinal

signals have been reported to interact (14, 18, 42). We and other groups could show that in humans oral preloads combined with (exogenously administered or endogenously stimulated) CCK or GLP-1 synergistically increase satiation and reduce food intake (9, 29, 33). Furthermore, Feinle et al. (12) demonstrated that sensory responses to gastric distension are altered by duodenal infusions of nutrients, with more “meallike” sensations of fullness after gastric distension plus intraduodenal nutrients. Animal studies support these concepts (24, 31, 45, 52) and suggest that a combination of gastric signals and intestinal nutrient stimulation is necessary to elicit optimal satiation and adequate control of eating. In humans, however, little information is available on the mechanisms of interaction between gastric and intestinal signals to modulate appetite and the secretion of satiation peptides. Therefore, we sought to investigate potential interaction effects by using a paradigm in which subjects received either an intragastric (IG) load or an intraduodenal (ID) infusion of glucose or a mixed liquid meal. Direct nutrient infusions into different areas of the GI tract bypass cognitive cues and thus provide information on the isolated properties of nutrients and the relative role of a specific GI segment in the secretion of satiation peptides and the short-term control of appetite.

SUBJECTS AND METHODS

Overall Study Design

The study was conducted as a randomized, double-blind, placebo-controlled, parallel-designed trial. The protocol was submitted and approved by the State Ethics Committee of Basel, Switzerland, and the study was carried out in accordance with the principles of the Declaration of Helsinki. Each subject gave written informed consent for the study. The criteria for exclusion were smoking, substance abuse, regular intake of medications (except for oral contraceptives), medical or psychiatric illness, and any abnormalities detected upon physical examination or laboratory screening. None of the subjects had a history of GI disorders, food allergies, or dietary restrictions. Subjects were instructed to abstain from alcohol, caffeine, and strenuous exercise for 24 h before each treatment. The day before each study day, subjects consumed a restricted, simple-carbohydrate standard dinner before 8 PM and fasted from 10 PM. On each study day, subjects were admitted to the Phase 1 Research Unit of the University Hospital of Basel at ~7 AM. Subjects swallowed a radiopaque polyvinyl feeding tube (external diameter 8 French) that was positioned in either the stomach or duodenum. The IG position was confirmed by rapid injection of 10 ml of air and auscultation of the upper abdomen. The ID position was verified by fluoroscopy. Because of radiation exposure during fluoroscopy, female subjects were excluded from ID experiments. The feeding tubes were firmly attached behind the ear to prevent further progression during the experiments. An antecubital vein catheter was inserted into a forearm vein for blood collection.

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Experimental Procedure

Part A: IG vs. ID administration of glucose. Part A included 34 healthy, normal-weight male and female volunteers [mean age: 23.4 ± 0.4 yr, range 19–30 yr; mean body mass index (BMI): 22.3 ± 0.3 kg/m², range 19.0–25.0 kg/m²]. Twenty-four subjects (13 females, 11 males) received an IG load of 75 g of glucose (Hänself, Herisau, Switzerland) dissolved in 300 ml of tap water (total caloric load 300 kcal) within 2 min (t = 0–2 min). Ten male subjects received an ID infusion of glucose at a rate of 3.0 kcal/min and 2.5 ml/min for 120 min. With this infusion rate, the total caloric load at 100 min was equal to the IG glucose load (300 kcal). The test solutions were freshly prepared each morning of the study and were at room temperature when administered.

Part B: IG vs. ID administration of a mixed liquid meal. Part B included 26 healthy, normal-weight male and female volunteers (mean age: 24.0 ± 0.4 yr, range 20–28 yr; mean BMI: 22.0 ± 0.3 kg/m², range 19.1–24.9 kg/m²). Sixteen subjects (8 females, 8 males) received an IG load of 500 ml of a mixed liquid meal (Ensure, 17% protein, 30% fat, and 53% carbohydrates, total caloric load 600 kcal; Abbott, Baar, Switzerland) and 10 male subjects an ID infusion of the mixed liquid meal at a rate of 3.0 kcal/min and 2.5 ml/min for 120 min. With this infusion rate the total caloric load at 100 min was one-half of the IG load (300 vs. 600 kcal). The test solutions were freshly prepared each morning of the study and were at room temperature when administered.

Blood Sample Collection

At regular time intervals, blood samples were collected on ice into tubes containing EDTA (6 µmol/l), aprotinin (500 KIU/ml), and a dipeptidylpeptidase IV inhibitor (50 µmol/l). The tubes were centrifuged at 4°C at 3,000 rpm for 10 min. After centrifugation, the plasma samples were processed into different aliquots and stored at −70°C until analysis.

Appetite Measurements

Appetite perceptions (feelings of hunger, fullness, and satiety) were assessed immediately after each blood collection using visual analog scales (VAS). VAS consisted of a horizontal, unstructured, 100-mm line with words anchored at each end describing the extremes of a unipolar question (most positive and most negative rating). Subjects assign a vertical mark across the line to index the magnitude of their unipolar question (most positive and most negative rating). Subjects were instructed to take enough time to rate their appetite sensation as precisely as possible. Quantifications of the measurements were made by measuring the distance from the left end of the line to the mark. The scales and scores have been designed and described previously in more detail (2).

Laboratory Analyses

Active GLP-1 was measured with a commercially available ELISA kit (Millipore, Billerica, MA). The intra- and inter-assay coefficients of variation were <9.0% and <13.0%, respectively. Active PYY, total ghrelin, and insulin were measured with commercially available radioimmunoassay kits (Millipore; Linco Research, St. Charles, MO; and Cisbio International, Bagnols, France). The intra-assay coefficients of variation were <15% for active PYY, <10.0% for total ghrelin, and <12.2% for insulin. The interassay coefficients of variation were <11% for active PYY, <4.7% for total ghrelin, and <9.0% for insulin. The methods have been described recently in more detail (35, 39, 46). Plasma glucose concentration was measured using the glucose oxidase method (Bayer Consumer Care, Basel, Switzerland).

Assessment of Gastric Emptying

In a subset of 16 subjects (part A: 4 females and 4 males, mean BMI 21.7 ± 0.6 kg/m², mean age 22.5 ± 0.7 yr; part B: 5 females and 3 males, mean BMI 21.7 ± 0.4 kg/m², mean age 24.3 ± 0.8 yr), gastric emptying rates were assessed using the [13C]sodium acetate breath test. This test is an accurate, noninvasive, simple method without radiation exposure and represents a reliable alternative to scintigraphy, the gold standard for measuring gastric emptying (3, 16). The test solutions were labeled with 50 mg [13C]sodium acetate; the substrate is rapidly absorbed in the proximal small intestine and metabolized in the liver with the production of [13CO2], which is exhaled rapidly, thus reflecting gastric emptying of nutrients (3, 16). Subjects were asked to exhale through a mouthpiece to collect an end-expiratory breath sample into a 100-ml foil bag at certain time intervals. The [13CO2] breath content was then determined by nondispersive infrared spectroscopy using an isotope ratio mass spectrometer (Wagner Analysen Technik, Bremen, Germany). [13C] abundance in breath was expressed as relative difference (%e) from the universal reference standard (carbon from Pee Dee Belemnite limestone). [13C] enrichment was defined as the difference between preprandial [13C] abundance in breath and [13C] abundance at the defined time points postprandially and was given in δ over basal (DOB, %e). On the basis of these values, time to reach maximal emptied speed and areas under the curve (AUCs) of the responses were calculated.

Statistical Analysis

Descriptive statistics were used for demographic variables such as age, weight, height, and BMI. Hormone and glucose profiles were analyzed by calculating AUCs. To test for significant differences between the treatments, AUCs were compared using Student’s unpaired t-test. VAS ratings were analyzed by calculating AUCs from baseline. Student’s unpaired t-tests were used to test for differences between treatments. All parameters were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov test methods. All statistical analysis was done using the statistical software package SPSS for Windows Version 14.0 (SPSS, Chicago, IL). Values were reported as means ± SE. Differences were considered statistically significant with P < 0.05.

RESULTS

Part A: IG Vs. ID Administration of Glucose

Plasma glucose and insulin. Blood glucose level increased more rapidly in response to IG-administered glucose in the early postprandial phase. In the later postprandial phase, blood glucose levels were clearly higher with ID-perfused glucose (P < 0.05; Fig. 1A), which also resulted in significantly greater insulin secretions (P < 0.05; Fig. 1B). Total blood glucose and insulin excursion over 100 min were significantly higher with ID-perfused glucose (P < 0.05; Fig. 1, A and B).

Plasma GLP-1, PYY, and ghrelin. IG administration of glucose resulted in markedly higher active GLP-1 and active PYY releases than equicaloric ID glucose infusions. AUCs were significantly greater, particularly in the early postprandial phase (0–60 min), for both hormones after IG-administered glucose (P < 0.05; Fig. 2, A and B). In contrast, comparable amounts of plasma ghrelin were suppressed by both IG- and ID-administered glucose (Fig. 2C).

Appetite measurements. IG administration of glucose clearly increased fullness and satiety feelings and reduced hunger; in contrast, equicaloric ID glucose infusions over 100 min resulted in only small effects on appetite. AUCs were significantly higher, particularly in the early postprandial phase.
No sex differences were observed in the IG part for any of the measurements.

**Gastric Emptying of Glucose and the Mixed Liquid Meal**

The mixed liquid meal emptied more slowly than glucose. Time to maximum emptying speed was delayed significantly (glucose 63.8 ± 3.8, mixed liquid meal 116.3 ± 11.9, \( P < 0.05 \)) and the AUC of the 120-min responses reduced significantly compared with the glucose meal (\( P < 0.05 \); Fig. 7, A and B).

**DISCUSSION**

The GI tract plays a major role in the control of appetite and food intake, and it is accepted that the stomach participates in this process by conveying satiation signals to the brain. However, there are only few human data exploring the potential interactions between the stomach and the small intestine in appetite control and the secretion of satiation peptides. We...
performed a randomized, double-blind, parallel-designed study in healthy subjects receiving either a rapid IG load or a continuous ID infusion of glucose or a mixed liquid meal. ID infusions (3 kcal/min) were at rates comparable with the mean duodenal delivery of these nutrients under physiological conditions (4, 21, 44), and in the glucose experiments, the total caloric load at 100 min was equal after both ID and IG administration (300 kcal). To examine the interaction effects between the stomach and the small intestine, we compared early postprandial appetite and hormone responses of subjects having received the rapid IG loads (stomach distention/stomach nutrient stimulation) vs. subjects having received the direct ID infusions (intestinal nutrient stimulation only). We found that infusions of glucose directly into the small intestine elicit only weak effects on appetite and the secretion of GLP-1 and PYY. In contrast, identical amounts of glucose delivered into the stomach markedly suppressed appetite paralleled by significantly greater plasma levels of GLP-1 and PYY. Administration of the mixed liquid meal (IG vs. ID) showed a comparable phenomenon with even augmented effects on appetite and hormone secretion after IG administration. However, interpretation of these data is limited given the different caloric loads (600 vs. 300 kcal). Finally, and in contrast to GLP-1 and PYY secretions, we found that plasma ghrelin levels were suppressed to a similar degree with both IG and ID nutrient administration. There are several explanations for these observations, and we will consider them in the following in the context of the available literature.

**IG Nutrient Administration is More Potent in Suppressing Appetite than ID Nutrient Infusions**

Studies in animals and humans have documented that gastric distension makes an important contribution to early satiation. These observations are based on experimental procedures such as balloon distension, pyloric occlusion studies, or gastric fistulae experiments (8, 14, 22, 23). Gastric satiation has been found to be volumetric, with the stomach monitoring meal volumes based on mechanoreceptors innervated by multiple vagal branches (40, 42, 43). In addition, in the 1970s and 1980s, Gibbs et al. (17), Liebling et al. (25), and Welch and colleagues (49, 50) coined the term “intestinal satiety,” which was based on their observation that satiation could be elicited by infusion of food to the duodenum. In contrast to gastric satiation, intestinal satiation is largely nutrient dependent and mediated by neural and endocrine signals. The gut hormones CCK, GLP-1, or PYY₃₋₃₆ were found to be associated with the nutrient-stimulated inhibition of food intake (1, 19, 29, 42).
Interactions between gastric and intestinal signals in the control of short-term satiation have been a focus of work in many laboratories. In studies performed in rhesus monkeys and rats, Wirth and McHugh (52) and Kaplan et al. (24) combined feeding experiments with the subsequent withdrawal of food from the stomach. The experiments document the importance of gastric distension for short-term satiation in the presence of postpyloric nutrient administration. In humans, most of the studies have used traditional preload paradigms to demonstrate that the potency of the signal to stop eating is increased when intestinal stimulation is combined with gastric distention (5, 29, 35). Interaction effects between gastric and intestinal signals have also been demonstrated for CCK and GLP-1 when exogenously administered in combination with a preload in both animals and humans (9, 31, 33, 45, 48).

Our data are in line with the body of the literature and support that gastric distention makes a potent contribution to short-term satiation. Moreover, our data are complementary to earlier reports by Cecil et al. (6) and French and Cecil (13), who found that direct infusion of a soup into the small intestine had no significant effect on hunger, desire to eat, or fullness, but the same soup fed orally or IG significantly suppressed appetite over time. We extend these findings by showing that IG nutrient administration (compared with ID-administered nutrients) exerts significantly greater effects on appetite, which is paralleled by a significantly higher secretion of satiation peptides.

**IG Nutrient Administration is More Potent in Stimulating the Secretion of GLP-1 and PYY But Not Ghrelin than ID Nutrient Infusions**

The augmented hormone responses after IG administration compared with ID infusions may suggest the existence of...
interaction effects between gastric and intestinal signals in stimulating the secretion of GLP-1 and PYY. Neural links between the stomach and the small intestine could potentiate peptide responses and thereby influence appetite. Such a notion is also supported by data from Schirra et al. (44). They also found that oral administration of glucose results in markedly higher GLP-1 but not glucose-dependent insulinocept peptide release compared with identical duodenal glucose infusions.

However, we and others have shown that pure mechanical gastric distension by itself or during ID nutrient infusions does not cause a further rise in plasma PYY or CCK (11, 36); thus, presently no data supporting the existence of a “mechanistic” gastric phase of gut peptide secretion are available. However, to the best of our knowledge, no studies have examined the isolated effects of “nutrient” gastric distention with concomitant ID nutrient infusions. An earlier study by Deutsch (10) has proposed that gastric satiation is at least in part nutritive, but such a hypothesis still awaits further proof. The results of this study have been criticized by many, and the large body of experimental evidence points to gastric satiation being triggered by mechanical distention rather than nutrient or chemical stimulation [for review, see Powley and Phillips (42)].

An alternative explanation for the different effects of IG and ID nutrients on the secretion of GLP-1 and PYY could be a different initial rate of duodenal delivery of nutrients. It has been well documented that the secretion of gut peptides such as GLP-1 or PYY depends critically on nutrient entry into the small intestine (7, 20, 34, 41).

Under physiological conditions, gastric emptying of glucose and other nutrient liquids is closely regulated (22, 27, 30); in humans, emptying approximates an overall rate of 1–3 kcal/min (4, 21, 44). The early phase (gastric emptying during gastric fill) is usually more rapid, presumably because of the time for nutrients to initiate the intestinal inhibitory feedback (23, 27, 32). A subsequent linear rate of nutrient delivery has been reported by Brener et al. (4) and others (21, 23). In contrast, Schirra et al. (44) found that gastric emptying of glucose is not constant but declines exponentially over time.

Here, we selected a duodenal infusion rate, which most likely mirrored the reported physiological ranges. Moreover, to compare the rate of ID infusions (3 kcal/min) with gastric emptying under physiological conditions (IG infusions), we measured gastric emptying using the [13C]acetate breath technique. Consistent with the literature, the data show that the mixed liquid meal empties more slowly than glucose, presumably relating to the different material properties, macronutrient composition, and subsequent gut receptor responses. However, we could not directly compare the speed of gastric emptying (following IG infusions) with the ID infusion rates since the [13C]sodium acetate method is only an indirect measurement and not applicable for such calculations. However, the fact that we found that blood glucose levels increased more rapidly in response to IG glucose suggests that gastric emptying slightly exceeded duodenal glucose infusions in the early postprandial phase (Fig. 1A). The initial, more rapid rate of duodenal delivery after IG infusions may thus account for the accelerated secretion of GLP-1 and PYY. This would be in line with data on the impact of changes in the rate of glucose entry into the small intestine on the secretion of incretins, insulin, and blood glucose (7, 20, 28, 34, 41). Horowitz et al. (20) found that in normal subjects gastric emptying accounts for about 34% of the variance in peak plasma glucose after a 75-g oral glucose load. In addition, they performed elegant experiments in which subjects received an ID glucose infusion for 120 min; on one day the infusion rate was variable with a rapid initial rate (between 3 and 6 kcal/min) for -15 min and a slower rate of 1 kcal/min subsequently. On the other day, the infusion rate was constant at 1 kcal/min (7, 34, 41). They found that an initial, more rapid small intestinal glucose delivery increases glycemic, insulinocep, and incretin responses, supporting the notion that the rate of duodenal delivery of nutrients may be critical for peptide secretion.

Finally, we also found that IG and ID nutrients suppressed comparable amounts of plasma ghrelin, which is in line with earlier studies (38, 51). We infer from these observations that, in contrast to GLP-1 and PYY, only intestinal stimulation is responsible for ghrelin suppression and that this effect is independent of the rate of duodenal delivery. However, we did not measure active ghrelin and thus may have missed significant differences in the active form.

Conclusion

Our experiments confirm that the stomach is essential in the short-term control of appetite and suggest that gastric and intestinal signals interact to mediate early fullness and satiation potentially by increased GLP-1 and PYY secretions. However, further research is required to determine whether gastric and intestinal signals truly interact to modulate the secretion of satiation peptides. When extrapolating our findings to normal physiological eating conditions, one also has to consider that cognitive and sensory factors may influence the outcome.
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DISCLOSURES

All authors disclose that they do not have any financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

AUTHOR CONTRIBUTIONS

R.E.S., A.C.M.-G., and C.B. edited and revised the manuscript; R.E.S., A.C.M.-G., and C.B. prepared the figures; R.E.S. and C.B. drafted the manuscript; R.E.S., A.C.M.-G., and C.B. interpreted the results of the experiments; R.E.S. and A.C.M.-G. performed the experiments; R.E.S. analyzed the data; R.E.S., A.C.M.-G., and C.B. approved the final version of the manuscript.

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