Comparative effects of intraduodenal protein and lipid on ghrelin, peptide YY, and leptin release in healthy men

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Abstract

Dietary nutrients stimulate the release of many gut hormones, which are released in response to nutrient ingestion in humans, especially carbohydrates and, to a lesser extent, fat (26, 27). The acute effect of protein on leptin release is not known. The nutrient-driven release of gut hormones is associated with a suppression of subsequent energy intake. When ingested orally, high-protein meals appear to be the most satiating (4, 7, 24), and there is evidence that fat may be a more potent appetite suppressant than carbohydrate, at least in normal-weight subjects (7). The effect of nutrients on appetite have also been attributed to the potent release of gastrointestinal (GI) hormones, particularly CCK (7, 30) and PYY (4). Indeed, in a pooled analysis of studies from our laboratory using intraduodenal (ID) infusion of nutrients (to bypass the stomach and associated interindividual variations in gastric emptying) (30), we identified the magnitude of stimulation of both plasma CCK and pyloric pressures (the latter control the rate of gastric emptying) as independent determinants of subsequent energy intake in response to ID lipid and carbohydrate.

Since, as discussed, both lipid and protein suppress energy intake (4, 7, 24), we recently evaluated the hypothesis that the two nutrients would also have comparable effects on upper GI motor and hormone functions (29). Surprisingly, despite the anticipated effects on energy intake, ID lipid and protein differed substantially in their effects on these functions. While lipid potently stimulated plasma CCK and GLP-1, as well as pyloric pressures, the effects of protein were much less. In contrast, protein potently stimulated insulin and glucagon, while lipid had negligible effects, suggesting that the mechanisms underlying the effects of lipid and protein on energy intake vary substantially.

Since PYY (3) and ghrelin (36) also play key roles in the regulation of energy intake, and leptin serves as a long-term control of energy balance, but may also be involved in the short-term modulation of energy intake (18), we have now analyzed the remaining plasma samples from our recent study (29) to evaluate the comparative effects of ID lipid and protein on ghrelin, PYY, and leptin.
METHODS

Subjects

Of the 20 lean, healthy men that participated in a previous study (29), sufficient plasma was available in 13 of these [age: 22.2 ± 0.7 (range: 18–30) yr; body mass index: 22.1 ± 0.4 (range 19.3–24.8) kg/m²; waist circumference: 79 ± 1 cm] for further hormone analyses. Inclusion/exclusion criteria have been described previously (29). In brief, they were all unrestrained eaters (33), weight stable, not on any diet to lose or gain weight, and had no history of GI disease or symptoms. The trial was registered at the Australia and New Zealand Clinical Trial Registry (http://www.anzctr.org.au, no 12609000949280) and approved by the Royal Adelaide Hospital Research Ethics Committee, and the subjects provided written, informed consent.

Study Outline

In the original study (29), each subject received five treatments (3 kcal/min lipid, 2 kcal/min lipid + 1 kcal/min protein, 1 kcal/min lipid + 2 kcal/min protein, 3 kcal/min protein, and saline control) in a double-blind, cross-over fashion. For the purpose of this report, samples were analyzed only for the following treatments: 1) lipid at 3 kcal/min, 2) protein at 3 kcal/min, and 3) saline control. Treatments were infused ID for 90 min, and subjects were presented with a buffet-style meal immediately afterward. Blood samples for measurement of plasma concentrations of ghrelin, PYY, and leptin were taken at regular intervals throughout the infusions and after the meal.

Subjects arrived at the laboratory at 0830 after an overnight fast. They were intubated with a small-diameter manometric catheter to measure antpyloroduodenal motility [reported previously (29)] and to administer the ID infusions. An intravenous cannula was placed in a right forearm vein for blood sampling. At t = 0 min, a baseline blood sample was taken, infusion of one of the three treatments commenced, and blood samples were then obtained at 15-min intervals. The lipid infusion was a soy-based triglyceride emulsion containing linoleic, oleic, and palmitic acid (Intralipid 300 mOsomol/L; Baxter Healthcare, Severna Park, MD), and the protein infusion consisted of whey protein [hydrolyzed whey protein isolate, DH17 Ultra (18.5% hydrolysate); MyoPure; Muscle Brand, Petersham, NSW, Australia]. We have used the lipid emulsion extensively in our previous studies, and whey protein (from dairy) is a major component of protein in the Australian diet. At the end of the infusion (t = 90 min), the catheter was removed, and subjects were presented with a standardized cold, buffet-style meal, which they consumed freely for up to 30 min, until comfortably full. After the meal (t = 120 min), a final blood sample was obtained, the cannula was then removed, and the subject was allowed to leave the laboratory.

Plasma Hormone Measurements

Blood samples were collected into ice-chilled, EDTA-coated tubes. Plasma was obtained by centrifugation for 15 min at 3,200 rpm at 4°C and stored at −70°C for further analysis. Plasma total ghrelin (pg/ml) was measured using a radioimmunoassay (RIA) without protein extraction (RediTech Pharmaceuticals, Mountain View, CA) (20). No cross-reactivities with any relevant molecule have been reported. Intra-assay and interassay coefficients of variation (CVs) were 5.0 and 15.0%, respectively. The detection limit was 44 pg/ml. Plasma total PYY (pg/ml) was measured using a RIA (Linco Research, St. Charles, MO) (31). No cross-reactivities with other relevant antibodies have been found. Intra- and interassay CVs were 5.3 and 7.0%, respectively. The detection limit was 10 pg/ml. Plasma leptin (ng/ml) was also determined using a RIA (Biotrend Chemikalien, Cologne, Germany). Intra-assay and interassay CVs were 5.0 and 4.5%, respectively. The detection limit was 0.44 ng/ml.

Statistical Analysis

Data analysis was performed using SPSS software (version 20; SPSS, IBM, Chicago, IL). Data were analyzed by two-way repeated-measures ANOVA with time (t = 0–90 min) and treatment as factors. Post hoc comparisons, adjusted for multiple comparisons using Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Premeal (t = 90 min) and postmeal (t = 120 min) concentrations were compared using paired t-test. Statistical significance was accepted at P < 0.05, and data are presented as means ± SE.

RESULTS

Plasma Ghrelin

Effect of infusion. Baseline ghrelin concentrations did not differ between study days (Fig. 1A). There was a treatment by time interaction (P < 0.001) for plasma ghrelin. The suppression of ghrelin by lipid and protein was evident from 30 min; both lipid and protein suppressed plasma ghrelin, compared with control, between t = 30–90 min (P < 0.05 for all), with no differences between them.

Effect of meal. On the control day, there was a reduction (P < 0.001) in plasma ghrelin after the meal (t = 120 min) compared with the premeal concentration (t = 90 min). Moreover, ghrelin was lower after the meal following both lipid and protein, compared with the respective premeal concentration, so that ghrelin concentrations remained lower following lipid and protein compared with control (P < 0.001).

Plasma PYY

Effect of infusion. Baseline PYY concentrations did not differ between study days (Fig. 1B). There was a treatment by time interaction (P < 0.001) for plasma PYY. Both lipid (P < 0.001) and protein (P < 0.05) stimulated PYY compared with control; however, the stimulation of PYY by lipid was substantially greater compared with protein, between 30 and 90 min (P < 0.001). The increase in PYY by lipid was apparent at t = 15 min, while the response to protein remained low throughout the infusion.

Effect of meal. On the control day, there was an increase (P < 0.001) in plasma PYY after the meal compared with the premeal concentration. PYY also increased further (P < 0.001) after the meal following protein, but not lipid, compared with the respective premeal concentration. PYY concentrations after the meal remained much higher following lipid, compared with control and protein (P < 0.001).

Plasma Leptin

Effect of infusion. Baseline leptin concentrations did not differ between study days (Fig. 1C). Moreover, there was no effect of treatment on plasma leptin concentrations.

Effect of meal. There was no difference in plasma leptin after the meal when compared with the premeal concentration.

DISCUSSION

Our study demonstrated that, in healthy humans, both protein and lipid, when infused directly into the duodenum in isocaloric amounts, reflecting the normal rate of gastric emptying, potently, and comparably, suppressed ghrelin; while...
both stimulated PYY, lipid was about 4 or 5 times more potent than protein. Neither lipid nor protein stimulated leptin.

Our recent study investigated the hypothesis that, since both lipid and protein reduce energy intake, they would activate the same GI mechanisms to mediate this effect (29). In contrast to our hypothesis, lipid, but not protein, potently stimulated CCK and GLP-1 release, while protein, but not lipid, marked an increase in insulin and glucagon (29). Thus, the effects of lipid and protein on energy intake are likely to be mediated by different mechanisms. Lipid may act predominantly through GI mechanisms, including the stimulation of CCK and GLP-1, and motor activity, particularly pyloric pressures, while protein has much weaker effects on these mechanisms. Instead, protein stimulated glucagon and insulin, and besides their effects on glycemia, glucagon may have energy intake-suppressant effects (16). It is very likely that lipid and protein may also use other GI hormones, including ghrelin and PYY, to mediate their energy intake-suppressant effects, and our data establish that isocaloric ID infusions of lipid and protein both potently suppress ghrelin, while ID lipid infusions, in particular, potently stimulate PYY. ID infusions of both lipid and protein have been shown to suppress ghrelin (11, 28); however, their relative effects have hitherto been unknown. The outcome of oral studies (1, 7, 15) suggests that high-protein meals may have stronger ghrelin-suppressant effects than high-fat meals. However, lipid is known to have more potent stimulatory effects on pyloric motility than protein (29), thus slowing gastric emptying and associated delivery of nutrients to the small intestine. To ensure a standardized rate of nutrient delivery to the small intestine, we used direct ID nutrient infusion and, using this paradigm, found that ghrelin was suppressed markedly, and comparably, by lipid and protein. Previous studies have provided evidence that small intestinal exposure to nutrient (glucose) was sufficient for ghrelin suppression (22), and while our current observations extend this to the effects of lipid and protein, since both ID lipid and protein had very potent ghrelin-suppressant effects, our data also show that ghrelin suppression by ID nutrients was not maximal, but that there was a further reduction in response to the meal, suggesting that intragastric, and potentially oral, mechanisms also play a role.

Some recent studies have suggested a major role for PYY in protein-mediated satiation (4), since plasma PYY concentrations were reported to be much higher after high-protein, when compared with high-fat and high-carbohydrate, meals. However, these findings were not replicated in a subsequent study from our laboratory using a meal with a more moderate protein content (7). We demonstrated previously that ID lipid potently stimulates the release of PYY in a dose-related fashion (23), while ID infusion of protein, albeit for only 60 min, appeared to have a much smaller impact on PYY (28). The current study

![Graph A: Ghrelin](image1)

![Graph B: PYY](image2)

![Graph C: Leptin](image3)

**Fig. 1.** Plasma concentrations for ghrelin (A), PYY (B), and leptin (C) during 90-min intraduodenal infusion of either lipid or protein (at 3 kcal/min), or saline control, and after an ad libitum test meal (t = 120 min). Data are expressed as means ± SE; n = 13 for ghrelin and PYY, and n = 12 for leptin (n = 1 had to be excluded due to technical problems with the assay). Repeated-measures two-way ANOVAs with time and treatment as factors were used to determine differences in plasma hormone concentrations. In case of significant differences, post hoc comparisons, using Bonferroni’s correction to adjust for multiple comparisons, were used to assess differences between treatments. Paired t-tests were used to compare pre- (t = 90 min) and post- (t = 120 min) meal values. A: treatment by time interaction (P < 0.001). #Significantly different from control (P < 0.05 for all values for both lipid and protein). §Significantly different from premeal (t = 90 min) concentration (P < 0.001). B: treatment by time interaction (P < 0.001). §Significantly different from control (lipid: P < 0.001, protein: P < 0.05); *Significantly different from protein (P < 0.001). §Significantly different from premeal concentration (P < 0.001). C: no treatment or time effects.
establishes that while both lipid and protein stimulated PYY compared with control, lipid was much more potent. Thus, it appears that while PYY is likely to be involved in lipid-induced reduction of energy intake, it may not be a main regulator of protein-mediated intake suppression. However, studies using receptor antagonists are required to determine a definitive role for endogenous hormones in energy intake regulation, although these are not currently available for use in humans. The plasma PYY response to lipid in the current study was maximal, since there was no further increase in response to the meal, suggesting that small intestinal exposure to lipid was sufficient for maximum PYY release. In contrast, the magnitude of the rise in plasma PYY after protein was greater after the meal, relative to the effect of the ID infusion, suggesting an important role for oral and/or gastric nutrient exposure for protein-induced PYY release, although the possibility of a contribution of other macronutrients contained in the meal cannot be excluded. These factors warrant further investigation.

Leptin is a long-term signal for energy stores and reflects the nutritional status, i.e., it increases with increasing body weight and decreases during food restriction (18, 34). There is evidence, however, that leptin may play a role in short-term regulation of energy intake. In rodents, acute injection of both leptin and CCK-8, at doses that by themselves did not affect intake, reduced subsequent food intake, indicating that leptin may increase the sensitivity to short-term signals, including CCK, in the brain (2, 35). Moreover, there is limited evidence that plasma leptin may increase after a high-fat meal (17) and may be stimulated more after a high-carbohydrate, compared with a high-fat, meal in healthy subjects (27). In these studies, changes in leptin were determined over periods of 8 (17) to 9 (27) h, and other studies did not find any acute changes after a 3-h period (19). Our study establishes that lipid and protein, when administered ID at a rate reflecting postprandial gastric emptying, do not stimulate leptin in the immediate postprandial hours, although we cannot exclude that a longer duration of nutrient exposure may be required. In addition, any potential contribution of gastric mechanisms following oral nutrient ingestion warrants evaluation.

Other mechanisms through which protein may reduce energy intake include direct effects on specific areas in the brain, including the hypothalamus and the brain stem, mediated by direct central effects of certain amino acids. For example, L-leucine, which is abundant in whey protein, activates the mammalian target of rapamycin (mTOR) in the hypothalamus, which influences energy homeostasis, resulting in a reduction in energy intake and body weight in rats (8), and also triggers signals in the caudomedial nucleus of the solitary tract in the brain stem, which is also involved in intake regulation (6). Furthermore, we recently reported that energy intake in response to an ID L-tryptophan infusion is correlated inversely with plasma L-tryptophan concentrations (31), indicative of a contribution of central effects of L-tryptophan, or its metabolites, and potentially other amino acids.

Some limitations of our study warrant recognition. Only men were included, as they are more sensitive to dietary manipulation than females (25). Furthermore, the effects of lipid on GI hormones are dependent on fatty acid chain length, so that fatty acids with ≥12 carbon atoms in the chain have more potent effects on gut hormones, than those with <12 carbon atoms in the chain (12, 13). Therefore, the effects on hormones determined in this study may differ when other sources of protein, e.g., casein, are ingested, or in response to different amino acids. We administered our infusions at only one caloric load, thus, cannot draw any conclusions on the comparative dose-response effects of the infusions.

**Perspectives and Significance**

The current data provide additional evidence that ID lipid and protein have discrepant effects on the release of gut hormones. Thus, while both suppressed ghrelin comparably, lipid had much more potent effects to stimulate PYY release, while neither stimulated leptin. Our findings may have implications for the targeted use of nutrients to modulate energy intake. For example, studies utilizing their combined administration to enhance their effects on intraintestinal and extraintestinal appetite-regulatory mechanisms, and, thus, their energy intake-suppressant effects, are warranted.

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