Effect of a protein preload on food intake and satiety feelings in response to duodenal fat perfusions in healthy male subjects

Sibylle Oesch¹, Lukas Degen¹ and Christoph Beglinger¹

Authors’ appointments and work carried out at the:

¹Clinical Research Center, Department of Research and Division of Gastroenterology, University Hospital, CH-4031 Basel, Switzerland

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Correspondence address:
Christoph Beglinger; M.D.
Division of Gastroenterology
University Hospital
CH-4031 Basel, Switzerland
Phone +41 61 2655175
Fax +41 61 2653847
Abstract

The control of food intake and satiety requires a coordinated interplay. Oral protein and duodenal fat inhibit food intake and induce satiety, but their interactive potential is unclear. Our aim was therefore to investigate the interactions between an oral protein preload and intraduodenal (ID) fat on food intake and satiety feelings. Twenty healthy male volunteers, were studied in a randomized, double-blind, 4-period crossover design. On each study day, subjects underwent one of the following treatments: a) water preload plus ID saline perfusion; b) water preload plus ID fat perfusion; c) protein preload plus ID saline perfusion; d) protein preload plus ID fat perfusion. Subjects were free to eat and drink as much as they wished. An oral protein preload significantly reduced caloric intake (19%, p < 0.01). The simultaneous administration of an oral protein preload and ID fat did not result in a positive synergistic effect with respect to caloric consumption, rejecting the initial hypothesis that the two nutrients exert a positive synergistic effect on food intake. An oral protein preload but not ID fat altered the feelings of hunger and fullness. These data indicate that the satiety effect of an oral protein preload is not amplified by ID fat: indeed, the effect of a protein preload does not seem to be mediated by cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) or peptide YY (PYY). Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety.

Key words: eating behavior; gastrointestinal satiety signals
Introduction

Obesity and its associated complications are a significant health problem in industrialized countries. By current estimates, one-third of US adults are obese and another third are overweight (13). Similar trends can be seen worldwide and there is no sign that this trend is abating. Obesity is a major risk factor for various diseases such as type II diabetes, cardiovascular diseases, stroke and several types of cancer (breast cancer, colon cancer) (18). Several systems seem to be involved in the regulation of bodyweight; one of them is primarily concerned with short-term regulation of food intake, i.e. how often and how much is eaten on a given day. Over the past years numerous components of this regulatory network have been identified and the gastrointestinal (GI) tract has been found to be a major player. The close relationship between the GI system and the brain in regulating food intake and satiety requires a coordinated interplay. However, little is known about the interaction between different physiological signals and processes that control food intake and satiety in humans.

On the basis of animal experiments, it is assumed that food intake is suppressed by stimulation of specific receptors within the GI tract. Inspired by this hypothesis, Welch et al. observed some 20 years ago that a lipid emulsion infused into the ileum reduced food intake in healthy volunteers, but eating habits were not influenced by an intravenous administration of a similar fatty emulsion (23). In follow-up studies, we and others extended these observations by documenting a satiating effect of duodenal fat perfusions with the following key elements: 1) decreased food consumption, 2) decreased feelings of hunger, and 3) increased plasma cholecystokinin (CCK) release (17). Additional effects of duodenal lipid infusion include early fullness and a delay in gastric emptying (6). Other gastrointestinal
peptides that have been associated with nutrient stimulated inhibition of food intake include glucagon-like peptide-1 (GLP-1) and peptide YY (PYY3-36) (3, 11). Nevertheless, the interactions between different macronutrients in the regulation of appetite and food intake have hardly been explored. In general, protein as an oral preload is considered to be the most satiating component, followed by fat (8). In humans, the interactions between protein and fat have not been investigated.

The present study is thus designed to further understand the potential interaction between protein and fat in regulating food intake in humans. We were particularly interested to see whether GLP-1 and PYY are associated with this interaction. The aim of this study was to better understand the regulation of food intake in humans by exploring the interaction of the stomach and the small intestine. An oral protein preload was given to stimulate gastric signals, together with intraduodenal (ID) fat perfusion, which should trigger intestinal signals (CCK, GLP-1, PYY).

**Methods**

**Overview**

A randomized, double-blind, four-period, Latin square design was carried out in 20 healthy, paid male volunteers. Each participant underwent tests on four experimental days, separated by at least 1 week. On each experimental day, the intake of a standardized meal with related variables was measured. A continuous ID perfusion of either fat or saline (control) was given throughout the entire experiment. Forty minutes after starting the respective ID perfusion, an oral preload of either 400 ml of water or 400 ml of protein shake was given. After an additional 20 min, subjects were
invited to eat and drink as much as they wished. The experimental design is shown in Figure 1.

Subjects

Each subject gave written informed consent for the study. The protocol was approved by the Human Ethics Committee of the University Hospital in Basel. Before acceptance, each participant was required to complete a medical interview and received a full physical examination. Inclusion criteria were:

1) BMI within 15% of desirable weight for height
2) Age between 18-45 years
3) Non-smokers
4) No active medical problems
5) Taking no medication
6) No allergies including food allergies
7) No history of GI disorders or weight problems

Twenty male subjects completed the study (mean age 26.7 ± 4.9 years, range 21-43 years; BMI 22.2 ± 1.3 kg/ m², range 20.1-24.5 kg/ m²).

Experimental procedure

Four treatments, separated by at least 7 days, were randomly performed in each subject. Shortly before each experiment, a radiopaque polyvinyl feeding tube (external diameter: 8 French) with an opening at the tip of the tube was inserted through the nose into the duodenum. This procedure allowed subjects to eat and
drink with a minimum amount of discomfort from the tube. After placement, the position of the tube was located fluoroscopically and the tip of the tube was positioned 100 cm distally to the teeth. It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment.

On the day of the experiment, each subject ate a light breakfast (if this was his normal habit), but no snacks were allowed after 8 AM. At noon, after insertion of a catheter into a forearm vein for phlebotomy, the experiment was started with a first continuous perfusion. The treatments were identical in design except for the ID perfusions and the oral preloads.

The first treatment consisted of an ID perfusion of saline for the duration of the experiment. Forty minutes after starting the perfusion, an preload of 400 ml of water was given orally. After an additional twenty min, subjects were invited to eat and drink as much as they wished. The second treatment was similar: ID saline was given throughout the whole experiment, but 400 ml of an oral protein shake was given instead of water. The third and fourth experiments used ID fat (corn seed oil) throughout the entire experiment instead of saline, combined with either water or protein shake as respective preloads. A perfusion rate of 0.375 ml/min for a total of 120 min (load 41 g of fat; total energy content: 371 kcal) was chosen from previous experiments (5, 23).

The preload used in this study was based on experiments performed by Matzinger et al. (16). The shake was made of protein mixed with water to equal a total of 400 ml. The shake contained the following nutrients: 52.7 g milk protein, 0.29 g carbohydrates, 0.58 g fat, 0.05% aspartam and 1.3% vanilla flavor (total energy content: 218 kcal).

Twenty minutes after the preload, a standard meal was presented to the subjects, who were then invited to eat and drink as much as they wished for 60 min. The meal
consisted of 1) orange juice, 2) ham sandwiches (72 g wheat bread, 10 g butter, and 25 g ham) and 3) chocolate pudding. The composition of the test meal with its corresponding nutritive values is listed in Table 1. Non-sparkling water could be taken during the meal as a non-caloric beverage. The order of food intake had to follow the above schedule. To reduce the participants’ awareness of the amount of food eaten, food was presented in small samples and in excess. The ID fat perfusion solution was filled in a black syringe which made it indistinguishable in appearance from the control solution (saline), and the person in charge of the experiments was unaware of the respective treatment, thereby making it possible to deliver treatments in a double-blind fashion. The amount of food eaten, the volume of fluid imbibed, and the time for each subject to complete the meal were quantified. From these observations, the total calorie intake could be calculated. Before, during, and after the preload, blood was drawn at 20 min intervals for plasma CCK, GLP-1 and PYY determinations in EDTA-coated tubes (6 µmol/l) containing aprotinin (500 KIU/ml blood). Plasma samples were kept frozen at –20°C until analysis.

After the start of the perfusion, subjects scored their subjective feelings for hunger and fullness at 15 min intervals for the duration of each experiment. A visual analog scale (VAS) that ranged from 1 through 10 indicated their respective scores on a questionnaire. The scale and scores have previously been designed and described in detail by Drewe et al. (5) and Welch et al. (23). In brief, a score of zero for hunger indicated that the subject was not hungry at all, two indicated “slightly hungry”, five indicated “moderately hungry”, eight indicated “very hungry”, and 10 indicated “absolutely ravenous”. The score for fullness was similar. The study was finished 60 min after meal start.
Biochemical analysis

Plasma immunoreactive CCK concentrations were measured by a sensitive radioimmunoassay (RIA) based on an antiserum against CCK-8. It has a negligible cross-reactivity to gastrin. Plasma samples were extracted with ethanol. The detection limit of the assay was 0.3 pmol/l plasma using CCK-8 as a standard. Details of the assay have already been described (10). GLP-1 (bioactive form) immunoreactivity was measured as previously described (9). The antiserum is specific for GLP-1 and does not cross-react with any other members of the glucagon family of peptides. The detection limit of the assay was 3 pmol/l. Before the RIA, plasma samples were extracted with ethanol.

Total PYY concentrations were measured by a sensitive RIA based on an antiserum against PYY 1-36 and 3-36. The lowest level of PYY which could be detected by this assay was 10 pg/ml when using a 100 µl sample size. There is no cross-reactivity between the antiserum and other members of the glucagon family of peptides.

Statistical analysis

The power calculations of this study are based on previous studies. The expected reduction of food intake (kcal) by ID fat was assumed to be 12% compared to the control treatment (water preload and ID saline), whereas the expected reduction of food intake in response to a protein preload was assumed to be 20%. Accepting a significance level of 95% and a power of 80% the required sample size had to be at least 18 subjects.

The amount of food eaten (g) and the amount of fluid drunk (ml), including the corresponding energy intake (kcal), were compared between the treatments by analysis of variance (ANOVA). In case of significance, ANOVA was followed by
multiple paired t-tests with Bonferroni correction. Plasma hormone data were evaluated by calculating the area under the plasma concentration/time curve (AUC). AUC was calculated by a linear trapezoidal rule from T0 to 80 min. Hormone data were analyzed by ANOVA. If significant differences were detected, ANOVA was followed by a paired t-test with Bonferroni correction. Differences in scores for hunger and fullness were obtained by subtracting the feelings at 60 min from the baseline value. The differences between the treatments were compared using the same statistical procedures described above.

Results

All subjects tolerated the study procedures well. None of the volunteers experienced any side effects such as nausea.

Food Intake

The amount of food eaten and the corresponding caloric intake were both reduced after perfusion of fat into the duodenum (Table 2). Indeed, when compared to the control treatment, 15 of 20 subjects ate less and consumed fewer calories with fat perfusion, but these effects did not statistically differ from controls. When ID fat was given with a water preload, the reduction in the amount of food eaten was 13%, resulting in an 11% reduction in caloric intake compared to the control experiment (ID saline and water preload). Fluid intake was not affected by ID fat, but eating time was reduced by 12%. An oral protein shake given in combination with ID saline perfusion significantly reduced the amount of food eaten (20%), with a corresponding 19% reduced caloric intake compared to the control experiment (p < 0.01 and p < 0.01,
respectively). Fluid intake was not significantly affected, but eating time was reduced 
(p < 0.05). Finally, the administration of ID fat plus an oral protein shake preload 
resulted in the strongest reduction in the amount of food consumed (29%) and 
reduced caloric intake (27%). The reduction in food intake and caloric consumption 
was, however, neither significantly different from the combination protein shake 
preload plus saline ID perfusion nor from the combination water preload plus ID fat. 
Fluid intake was lowest with the combination of a protein preload plus ID fat, but the 
difference was only significant in comparison to the control treatment (water preload 
with ID saline) (p < 0.01). The decrease in food and fluid intake after a protein 
preload and ID fat was accompanied by a significantly reduced eating time (Table 2, 
p < 0.01).

To further analyze potential interactions, the following contrasts were calculated: 
water/saline – protein/saline – (water/fat – protein/fat). The data presented in Table 3 
clearly indicate that the disparity between water/ID saline and protein shake plus ID 
saline on the one hand, and that between water/ID fat and protein shake plus ID fat 
on the other, showed no significant difference either for the amount of food eaten and 
the resulting caloric intake or for the amount of fluid imbibed.

We also analyzed whether the potential interactions between the oral protein preload 
and ID fat were additive or positive/negative synergistic: the measured value from the 
combined treatment (the delta between protein preload plus ID fat and control) was 
compared to the calculated value of both treatments alone (the sum of the deltas 
between protein preload and control and ID fat and control). The data are presented 
in Table 4.
Eating behavior

The protein preload significantly influenced the mean VAS (Figures 2a and 2b). Subjects experienced a reduced degree of hunger and a concomitant increased feeling of fullness in the premeal period with administration of the protein preload. When we compared baseline scores with 60 min values, the difference reached statistical significance (Table 5). Subjects felt less hungry and fuller with the protein preload compared to ID saline or fat. Fat perfusion alone to the duodenum had no significant effect; furthermore, the combination of a protein preload plus ID fat was not more effective than protein preload plus ID saline. These data indicate that the protein preload was largely responsible for the observations.

Plasma Hormones

During the control treatment (water preload plus ID saline), plasma hormone responses (CCK, PYY and GLP-1) remained stable in the premeal period (Figures 4a and 4b, data for CCK not shown). The protein preload (400 ml) did not stimulate plasma CCK, PYY or GLP-1 concentrations. With the fat ID perfusion, PYY and GLP-1 concentrations slightly increased, but not significantly (Figures 4a and 4b). However, the ID fat perfusion did evoke a significant increase ($p < 0.05$) in plasma CCK levels (Figure 3).
Discussion

In the present study we have examined the interactions evoked by an oral protein preload with duodenal fat perfusion on food intake and appetite sensations in healthy male subjects.

The role of ID fat in initiating short-term satiation was first extensively explored in animals. On the basis of these observations, it was assumed that food intake is suppressed by stimulation of specific receptors within the GI tract. Inspired by this hypothesis, Welch et al. (23) observed that the infusion of a lipid emulsion into the ileum reduced food intake in healthy volunteers. Studies from our laboratory (15) have confirmed these findings as we could show that a fat perfusion to the duodenum significantly reduced food intake compared to an ID saline perfusion. In the same study, it could also be shown that the inhibition of food intake in response to intestinal lipid was mediated by CCK. In the present study, ID fat perfusion alone also reduced the amount of food eaten (13% compared to placebo) and the total caloric intake (11% compared to placebo), but the reduction did not reach statistical significance. Although the design of the present study was similar to previous studies with respect to fat dose, experimental design and duration of fat perfusion, the variability of the individual responses to ID fat was greater than in previous studies and the reduction of food intake did not reach statistical significance. Fifteen volunteers ate less when ID fat was perfused (water as preload) compared to the control treatment, but the five remaining volunteers ate less under placebo conditions compared to a water preload and ID fat perfusion. Due to these results it can be speculated that certain individuals have a reduced sensation to ID fat.

It is well-established that, among all macronutrients, protein is more satiating than carbohydrate or fat as oral preloads (12). Several short-term studies have been done
to examine the satiating effect of oral protein preloads in healthy human volunteers (4, 12, 19, 20, 22). These various studies compared a variety of nutrient preloads and examined the effect of amino acids given intraduodenally (2), but none has examined the interaction between an oral protein preload and ID fat. Our main interest was the investigation of potential interactions between gastric satiety signals induced by the protein shake and satiety signals induced by ID fat. Both macronutrients, when given alone, can reduce food intake and trigger satiety, but do they exert additive or synergistic effects when combined? We have previously seen that a nutrient-based preload interacts with ID fat (17), whereas gastric distension induced by a non-nutrient based distension with barostat did not produce such an effect (unpublished data). When we investigated the interaction of an oral carbohydrate-based preload in combination with ID fat, a synergistic inhibitory effect was observed. From these results we inferred that ID fat interacts with gastric signals to regulate food intake.

The results of the present study illustrate that an oral protein preload with ID fat or ID saline reduced the amount of food eaten and the total caloric intake to a similar extent compared to the control treatment. There was no statistically significant difference between the two experimental conditions protein preload/ID saline and protein preload/ID fat. Further analyzing the data, the measured value from the combined treatment (the delta between protein preload plus ID fat and control) did not differ from the calculated value of both treatments alone (the sum of the deltas between protein preload and control and ID fat and control). This result implies that the simultaneous administration of an oral protein preload and ID fat resulted in no synergistic reduction in caloric consumption, thereby rejecting the hypothesis that the two nutrients exert a positive synergistic effect on food intake. The potential interaction between an oral protein preload and ID fat seems to be additive. These observations have been made with one single dose of ID fat and oral protein.
Different doses of ID fat and/or different amounts of a protein preload could show differing results with respect to potential interactions.

Two observations were unexpected: 1) the fact that the reduction of food intake caused by ID fat did not reach statistical significance and 2) that the interaction between an oral protein preload and ID fat seems to be additive. There are several possible explanations for these unexpected observations, and we will consider them with their relative limitations. One potential limitation is the time interval between the preload and the test meal. Gastric emptying is a major determinant in the regulation of food intake (7, 8, 17). Perfusion of fat to the small intestine has been shown to retard gastric emptying (7, 8). The rate of gastric emptying of the oral preload could be relevant, if the satiety effects induced by the preload are mediated by intestinal rather than gastric mechanisms. The stomach would therefore be fuller after the protein-rich preload with fat perfusion compared to the treatment with water preload plus ID saline perfusion. On the other hand, if the oral protein preload activates intestinal mechanisms rather than gastric signals, a delay in gastric emptying would retard activation of the intestinal mechanisms. Our initial hypothesis was based on the assumption that the oral preload would stimulate gastric signals, which would be synergistic to the intestinal mechanisms induced by fat. The results clearly illustrate that this is not the case. Another potential problem is the difference in taste between the oral protein preload and the water preload. Orosensory differences can affect eating behavior. This could have been avoided by a direct intragastric infusion of the preload, but at the expense of additional discomfort for the volunteers as this would have required a second tube.

ID fat stimulates the secretion of a number of gastrointestinal hormones, some of which are associated with the regulation of food intake. In the present study we measured the increase in plasma concentrations of the satiety peptides, CCK, GLP-1
and PYY. All three peptides have been shown to modulate short-term control of food intake during a test meal intake. Here we observed a significant increase in plasma CCK after ID fat, confirming previous observations. On the other hand, no significant changes in plasma GLP-1 or plasma PYY concentrations occurred in the premeal period. GLP-1 is mainly stimulated by carbohydrates (14, 21) and less so by other macronutrients.

Feinle and coworkers (6, 7) have suggested that GLP-1 plasma levels rise after ID fat, but these findings could not be confirmed in the present study: a small, but non-significant, increase in GLP-1 secretion could be seen after 60 min of duodenal fat perfusion. It is conceivable that the 60 min perfusion of fat used in the present study was too short to induce a significant increase in GLP-1 release. On the other hand, the release of PYY is clearly dependent on the caloric load and on duodenal fat. The amount of fat delivered to the small intestine seems to be a crucial factor, as small loads of calories or fat are not associated with significant changes in plasma PYY concentrations (1). In the present study we could not detect any significant change in plasma PYY in response to the small duodenal fat load given. This finding concurs with previous observations investigating PYY responses to various types of nutrients (1). Indeed, the present study is one of the first attempts in humans investigating the effect of intraduodenal fat administration on the secretion of PYY. PYY is characteristically released in proportion to both the caloric content of a meal and its energy source composition. Increasing ingested amounts of an identical meal lead to proportionally increased plasma levels of PYY (1). With isocaloric meals consisting exclusively of either fat, carbohydrates, or proteins, the highest levels of plasma PYY were detected after the fat meal, followed by the carbohydrate meal, while very little PYY was noted with the protein meal (1). Under the present experimental conditions, neither ID fat perfusion nor the protein meal were able to stimulate significant
amounts of PYY, indicating that the caloric load of both macronutrients was too small to induce PYY release. Data from our laboratory suggest that mixed meals with less than 500 kcallories do not stimulate PYY secretion (C Beglinger and G Gamboni, unpublished observations). Taken together, the plasma hormone data imply that the inhibitory effects of the oral protein preload on food intake and appetite sensation are not mediated by changes in circulating plasma hormone concentrations. Furthermore, the effects of duodenal fat are not mediated by changes in plasma GLP-1 and/or plasma PYY levels, but largely dependent on CCK release. These observations are in agreement with our previous findings: the reduction of food intake induced by ID fat plus a liquid shake was reversed by administration of a CCK-A receptor antagonist (15, 17) suggesting that CCK is indeed the mediator of this effect.

To summarize the findings of the present study, we have observed that an oral protein preload significantly reduced caloric intake. The protein preload and ID fat in combination resulted in no additive reduction in calorie consumption, which means that protein and ID fat do not exhibit synergistic effects on food intake. As a consequence, the satiety effects of an oral protein preload are not amplified by ID fat. A protein preload triggers GI signals to induce satiety, but this effect does not seem to be mediated by CCK, GLP-1 or PYY. Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety.

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References


**Table 1**: Composition of test meal with corresponding nutritive values

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Carbohydrates (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice (100ml)</td>
<td>10</td>
<td>&lt;1</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Ham sandwich (100g)</td>
<td>36</td>
<td>5</td>
<td>9</td>
<td>274</td>
</tr>
<tr>
<td>Chocolate pudding (100g)</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>132</td>
</tr>
</tbody>
</table>
Table 2: Effect of ID saline or ID fat together with a preload of either water or a protein shake on eating behavior in 20 healthy male subjects

<table>
<thead>
<tr>
<th>Treatment: Preload/ID perfusion</th>
<th>Food Intake (g)</th>
<th>Calories (kcal)</th>
<th>Eating time (min)</th>
<th>Volume imbibed (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Water/saline</td>
<td>470±27</td>
<td>1243±74</td>
<td>26±2</td>
<td>361±44</td>
</tr>
<tr>
<td>b) Water/fat</td>
<td>408±30</td>
<td>1100±78</td>
<td>23±2</td>
<td>310±40</td>
</tr>
<tr>
<td>c) Protein/saline</td>
<td>376±39†</td>
<td>1013±112†</td>
<td>21±2*</td>
<td>332±50</td>
</tr>
<tr>
<td>d) Protein/fat</td>
<td>334±34‡</td>
<td>906±102‡</td>
<td>19±2†</td>
<td>263±31†</td>
</tr>
</tbody>
</table>

Data are means ± SE. ID, intraduodenal. * = p<0.05, † = p<0.01, ‡ = p<0.001, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.
**Table 3:** Effect of treatments on food parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Food Intake (g)</th>
<th>Calories (kcal)</th>
<th>Volume imbibed (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>20±36</td>
<td>35±81</td>
<td>-18±41</td>
</tr>
<tr>
<td>t-value</td>
<td>0.55</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>p-value</td>
<td>0.59</td>
<td>0.67</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Data are means ± SE. Intrasubject differences between treatments were calculated by the formula: water/ID saline – protein/ID saline – (water/ID fat – protein/ID fat). Differences were analyzed by the paired t-test.
Table 4: Comparison of the mean (± SE) difference in calorie intake from the combined treatment of protein preload and ID fat with the calculated values of both treatments when given alone.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Combined treatment</th>
<th>Treatments given alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories [kcal]</td>
<td>337±74</td>
<td>372±94</td>
</tr>
</tbody>
</table>

Data are mean ± SE. The difference between the measured value from the combined treatment and the calculated value of both treatments alone was calculated by the formula: \((\text{water/ID saline} – \text{protein/ID fat}) – [(\text{water/ID saline} – \text{protein/ID saline}) + (\text{water/ID saline} – \text{water/ID fat})]\).
Table 5: Baseline and 60 min scores after ID saline or fat with a preload of water or protein in 20 healthy male subjects.

<table>
<thead>
<tr>
<th></th>
<th>Water/saline</th>
<th>Water/fat</th>
<th>Protein/saline†</th>
<th>Protein/fat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.6±0.4</td>
<td>1.1±0.3</td>
<td>1.7±0.3</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>60min</td>
<td>2.2±0.5</td>
<td>2.5±0.4</td>
<td>4.4±0.5</td>
<td>3.5±0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Water/saline</th>
<th>Water/fat</th>
<th>Protein/saline*</th>
<th>Protein/fat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.5±0.5</td>
<td>8.0±0.3</td>
<td>7.8±0.3</td>
<td>8.2±0.3</td>
</tr>
<tr>
<td>60min</td>
<td>7.9±0.5</td>
<td>7.4±0.4</td>
<td>6.1±0.5</td>
<td>6.1±0.6</td>
</tr>
</tbody>
</table>

Data are mean ± SE. *p < 0.05, †p < 0.01, ‡p < 0.001, all vs. control (water/saline).

Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.
Figure 1: Experimental design of study. ID, intraduodenal; VAS, Visual Analog Scale.
Figure 2a: Subjective sensations for fullness experienced by 20 healthy male subjects before and after food ingestion during ID perfusion of saline (Sal) or fat. Twenty minutes before food consumption volunteers received a preload of either water or protein (400ml). Results are expressed as means +/- SE. * = p<0.01, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.
Figure 2b: Subjective sensations for hunger experienced by 20 healthy male subjects before and after food ingestion during ID perfusion of saline (Sal) or fat. Twenty minutes before food consumption volunteers received a preload of either water or protein (400ml). Results are expressed as means +/- SE. * = p<0.01, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.
**Figure 3**: Area under plasma concentration/time curve (AUC) plasma CCK responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means ± SE. * = p<0.05. Significant difference between control (water/saline) and ID fat plus water preload. Significant difference between control and ID fat plus protein preload and between ID saline plus protein preload and ID fat plus protein preload. Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.
Figure 4a: GLP-1 plasma responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means +/- SE.
Figure 4b: PYY plasma responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means +/- SE.