REVIEW
Role of protein and carbohydrate sources on acute appetite responses in lean and overweight men

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Abstract
Dietary protein induces greater satiety compared with carbohydrate in lean subjects, which may involve appetite-regulatory gut hormones. Little is known about the duration of effect, influence of protein and carbohydrate source and relevance to non-lean individuals. We compared the effect of various dietary proteins and carbohydrates on post-prandial appetite ratings, *ad libitum* energy intake (EI) and appetite hormones in lean and overweight men. Three randomised double-blinded cross-over studies examined appetite response (appetite ratings, ghrelin, glucagon-like peptide-1 (GLP-1) and cholecystokinin) to liquid preloads over three to four hours followed by a buffet meal to assess *ad libitum* EI. The 1-MJ preloads contained ~55 g of protein (whey, casein, soy and gluten), carbohydrate (glucose, lactose and fructose) or combined whey/fructose. EI was 10% higher following glucose preloads compared with protein preloads, observed at three hours but not four hours. Protein ingestion was followed by prolonged elevation of cholecystokinin and GLP-1 (two hours) and suppression of ghrelin (three to four hours) compared with glucose and independent of protein type. Replacing some whey with fructose attenuated the effect of protein on these hormones. Treatment effects on EI and appetite hormones were independent of bodyweight status, despite higher GLP-1 and lower ghrelin in overweight subjects. Protein-rich liquid preloads reduce EI over three hours in overweight men compared with glucose. These findings suggest a potential application for protein-rich drinks and/or foods to facilitate reduced EI. Future studies should explore additional dietary manipulations that may enhance this relationship, and confirm these effects within the context of energy-restricted dietary patterns.

Key words: appetite, carbohydrate, ghrelin, GLP-1, overweight, protein.

INTRODUCTION
Overconsumption of energy from food and beverages is an important precursor to the increase in obesity prevalence worldwide. The potential role of appetite regulation in managing overweight and obesity warrants investigation, and dietary factors that may modulate appetite are of interest.

Dietary patterns with an increased proportion of energy from protein are associated with greater weight loss compared with higher carbohydrate intakes. Acute studies also show that dietary protein lowers subsequent appetite and energy intake (EI) compared with carbohydrate and fat. Little is known about the contribution of gastrointestinal derived appetite hormones (such as ghrelin, a ‘hunger signal’, and satiety signals such as cholecystokinin and glucagon-like peptide-1, GLP-1) to this relationship. Low-glycemic-index carbohydrates are also proposed to extend satiety; however, there is substantial disagreement about this relationship and its influence in a mixed diet.

Although dietary factors that affect appetite have potential applications in treating overweight and obesity, most appetite studies have been performed in lean subjects. Such findings may not directly translate to the overweight population.

This review will summarise the key findings of three previously published studies, with an overall objective to investigate the effect of dietary protein and carbohydrate sources on acute changes in gastrointestinal derived appetite hormones, subjective appetite ratings and *ad libitum* EI in lean and overweight/obese men. These studies were funded by the National Centre of Excellence in Functional Foods.

The aims of the three studies were: study 1: to compare the effects of proteins which differ in rate of gastric emptying (whey, fast; casein, slow) with carbohydrates which differ in glycemic index (glucose, high; lactose, low) on appetite responses and rate of gastric emptying over three hours in...
overweight/obese men (n = 19, body mass index, BMI = 32.1 ± 0.9 kg/m²); study 2: to compare the effects of protein type (soy, whey and gluten) on appetite responses over three hours compared with glucose, and to investigate the effect of bodyweight status on these outcomes in lean and overweight/obese men (n = 72, BMI range 20.6–39.9 kg/m²); study 3: to describe the effect of whey protein on appetite responses over four hours, and to investigate the effect of combining whey and fructose on these outcomes compared with either dietary component consumed alone in overweight/obese men (n = 28, BMI = 32.2 ± 0.6 kg/m²).

**METHODS**

A detailed description of the methods for each study has been previously described.18–20 Briefly, healthy men aged between 20 and 65 years were recruited by public advertisement. All studies were approved by the Commonwealth Scientific Industrial Research Organisation Human Ethics Committee. Subjects gave informed, written consent to participate.

**Experimental protocol**

Subjects attended the clinic on four occasions with seven days between visits. Upon arrival at the clinic, fasting subjects’ weight and height were measured in light clothing. Fasting blood samples were collected (~15 minutes), after which subjects completed a questionnaire to assess subjective appetite ratings (visual analogue scale, VAS).21

The preloads were consumed at 09:00 hours (time 0) (treatment order randomised). Subsequent blood samples and VAS were collected 15, 30, 45, 60, 90, 120 and 180 minutes after time 0 (+240 minutes in study 3). Subjects were then given a buffet-style lunch which was consumed ad libitum.

**Dietary protocol**

Preloads (1 MJ) were made from ~50 g of test ingredient, water (100 g), skim milk (studies 1 and 3; 200 g) and flavour (Table 1). The buffet lunch foods were weighed before and after consumption to calculate EI.

**Biochemistry**

Serum and plasma were isolated and stored at −80°C. Plasma ghrelin, cholecystokinin-8 (CCK-8) (following ethanol extraction), GLP-1 (studies 2 and 3), glucose, paracetamol (an indirect marker of gastric emptying (study 1)), and serum insulin were measured using commercially available kits and reagents.18–20

**Statistical analysis**

Results are expressed as means ± SEM. In study 1, there were no differential effects between whey and casein

<table>
<thead>
<tr>
<th>Preload</th>
<th>Energy (kJ)</th>
<th>Protein (g) (% of energy)</th>
<th>Fat (g) (% of energy)</th>
<th>Carbohydrate (g) (% of energy)</th>
<th>Energy density (kJ/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1025</td>
<td>7.2 (12)</td>
<td>0.2 (1)</td>
<td>56.0 (87)</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>1025</td>
<td>7.2 (11)</td>
<td>0.2 (1)</td>
<td>56.0 (88)</td>
<td>2.5</td>
</tr>
<tr>
<td>Whey</td>
<td>1069</td>
<td>52.2 (83)</td>
<td>0.5 (2)</td>
<td>10.3 (15)</td>
<td>2.7</td>
</tr>
<tr>
<td>Casein</td>
<td>1090</td>
<td>52.4 (83)</td>
<td>1 (3)</td>
<td>10.2 (15)</td>
<td>2.7</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1158</td>
<td>1 (1.5)</td>
<td>3.6 (11)</td>
<td>63.0 (87)</td>
<td>2.5</td>
</tr>
<tr>
<td>Whey</td>
<td>1216</td>
<td>51 (71)</td>
<td>3.6 (11)</td>
<td>13.5 (18)</td>
<td>2.6</td>
</tr>
<tr>
<td>Soy</td>
<td>1199</td>
<td>50 (71)</td>
<td>3.6 (11)</td>
<td>13.5 (18)</td>
<td>2.6</td>
</tr>
<tr>
<td>Gluten</td>
<td>1227</td>
<td>51 (71)</td>
<td>3.9 (12)</td>
<td>13.5 (17)</td>
<td>2.7</td>
</tr>
<tr>
<td>Study 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1097</td>
<td>7 (11)</td>
<td>0.5 (1.0)</td>
<td>60 (88)</td>
<td>2.64</td>
</tr>
<tr>
<td>Fructose</td>
<td>1097</td>
<td>7 (11)</td>
<td>0.5 (1.0)</td>
<td>60 (88)</td>
<td>2.64</td>
</tr>
<tr>
<td>Whey</td>
<td>1147</td>
<td>57 (84.5)</td>
<td>0.5 (1.5)</td>
<td>10 (14)</td>
<td>2.76</td>
</tr>
<tr>
<td>Whey/Fructose</td>
<td>1122</td>
<td>32 (48.5)</td>
<td>0.5 (1.5)</td>
<td>35 (50)</td>
<td>2.70</td>
</tr>
</tbody>
</table>

(a) Dietary composition based on data from Australian Food Composition Tables22 and ingredient manufacturers.
(b) Glucose (Ace Chemical Company, Adelaide, Australia).
(c) Lactose (Ace Chemical Company).
(d) Whey protein isolate (Murray Goulburn Nutritional, Melbourne, Australia).
(e) Calcium caseinate (Murray Goulburn Nutritional).
(f) Isolated soy protein (The Solae Company, West Chatswood, Australia).
(g) Gemtec (Manildra Group, Auburn, Australia).
(h) Fructose (Ace Chemical, Adelaide, Australia).
identified for any parameters, so data for both preloads are presented as a mean called ‘protein’.

The distance between the left end of the VAS scale and each mark was measured (mm) and the change in rating from baseline was calculated.

ANOVA with repeated measures was used to determine the effects of the treatment and time (minutes) with the treatment order, BMI, and fasting insulin and glucose included as covariates. One-way ANOVA was performed to determine treatment effect on EI. For all statistical analyses, where a significant main effects was determined, a Tukey’s post hoc test was performed to determine differences between group means. Statistical analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL, USA). Differences are considered significant if $P < 0.05$.

**RESULTS**

**Study 1**

EI was $10 \pm 3\%$ higher after the glucose preload compared with lactose and protein preloads ($P < 0.05$, Table 2). The summed appetite rating was higher after the glucose preload compared with the protein and lactose ($P < 0.05$; Figure 1).

The glucose preload produced a higher peak glucose compared with other preloads (time by treatment effect $P < 0.01$; Figure 1).

The trough in ghrelin remained stable for one hour before and after the protein and lactose preloads. In contrast, ghrelin returned to baseline levels within one hour of the trough after the glucose preload and then exceeded this level in the next hour ($P < 0.05$; Figure 1).

**Table 2** *Ad libitum* energy intake (EI) and post-prandial responses after consuming preloads (a)

<table>
<thead>
<tr>
<th>Preload</th>
<th>EI (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1 (b)</td>
<td></td>
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<tr>
<td>Glucose</td>
<td>4772 ± 264</td>
</tr>
<tr>
<td>Lactose</td>
<td>4231 ± 247</td>
</tr>
<tr>
<td>Protein</td>
<td>4279 ± 207</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>3546 ± 168</td>
</tr>
<tr>
<td>Whey</td>
<td>3219 ± 147</td>
</tr>
<tr>
<td>Soy</td>
<td>3209 ± 160</td>
</tr>
<tr>
<td>Gluten</td>
<td>3006 ± 147</td>
</tr>
<tr>
<td>Study 3 (c)</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4704 ± 274</td>
</tr>
<tr>
<td>Fructose</td>
<td>4942 ± 280</td>
</tr>
<tr>
<td>Whey</td>
<td>4623 ± 290</td>
</tr>
<tr>
<td>Whey/Fructose</td>
<td>4761 ± 277</td>
</tr>
</tbody>
</table>

(a) x ± SEM.
(b) Mean of whey and casein treatments.
(c) Glucose greater than protein and lactose ($P < 0.05$, one-way ANOVA with Tukey’s post hoc test).
(d) Glucose greater than gluten ($P < 0.05$, one-way ANOVA with Tukey’s post hoc test).
(e) No treatment effect ($P = 0.121$, one-way ANOVA with Tukey’s post hoc test).

Cholecystokinin (CCK) was higher 90 minutes after the protein preloads compared with glucose and lactose ($P < 0.05$, Figure 1). Plasma paracetamol levels were lower 90 minutes after the protein preloads compared with both carbohydrates ($P < 0.01$; Figure 1).

**Study 2**

EI was ~0.5 MJ higher after the glucose treatment compared with gluten ($P < 0.05$, Table 2). A similar, non-significant trend was observed with lower intake after the soy and whey treatments. Appetite ratings were independent of treatment ($P > 0.05$, data not shown).

Post-prandial plasma glucose and insulin were higher after glucose compared with all protein treatments (glucose $P < 0.0005$; insulin $P < 0.01$, Figure 2).

All protein loads prolonged the suppression of ghrelin ($P < 0.01$) and elevation of GLP-1 ($P < 0.01$) and cholecystokinin ($P < 0.05$), compared with glucose, with no difference between proteins (Figure 2).

Post-prandial responses were compared in lean (n = 18, BMI = 23.2 ± 0.3 kg/m²) and overweight (n = 20, BMI = 31.4 ± 0.8 kg/m²) subjects. There was no effect of BMI status on EI (lean 3371 ± 141 kJ; overweight 3310 ± 105 kJ; $P > 0.05$) and appetite ratings ($P > 0.05$; data not shown).

Fasting GLP-1 (overweight, 17.5 ± 1.3 pg/mL; lean, 14.7 ± 0.1 pg/mL; $P < 0.001$) and the post-prandial responses ($P < 0.038$; Figure 3) were higher in overweight subjects. Fasting ghrelin (lean, 493 ± 13 pg/mL; overweight, 684 ± 34 pg/mL; $P < 0.001$) and the post-prandial response ($P < 0.003$; Figure 3) were lower in overweight compared with lean subjects. The macronutrient-specific differences in ghrelin and GLP-1 described above were observed in both lean and overweight subjects, despite differences in concentration (Figure 3).

**Study 3**

EI after four hours was independent of preload type ($P = 0.121$, Table 2).

Plasma glucose was highest after the glucose treatment ($P < 0.0005$; Figure 4). Fructose produced an insulin response that was significantly lower than all other treatments ($P < 0.0005$; Figure 4).

Fasting ghrelin was lower after whey compared with the glucose treatment and, at 240 minutes, it was significantly lower than the glucose treatment ($P < 0.001$; Figure 4).

All beverages produced a rapid increase in GLP-1, after which concentration declined following all treatments except whey ($P = 0.002$; Figure 4).

Cholecystokinin response to whey was greater than whey/fructose, which is also greater than the response to fructose and glucose ($P = 0.009$; Figure 4).

**DISCUSSION**

Although it is known that protein consumption produces lower appetite and EI compared with carbohydrate and fat,
this has only been demonstrated in lean subjects. In the studies reviewed here, it has been demonstrated that protein is also more satisfying in overweight subjects. Further, we provide preliminary data indicating that the duration of satiety after protein may depend on the protein dose and that gastrointestinal derived hormones are likely to be involved in macronutrient-specific appetite responses.

The first study demonstrated that whey and casein protein preloads similarly prolonged the suppression of ghrelin and elevation of cholecystokinin, which corresponded with lower appetite and gastric emptying, respectively, compared with glucose.

The second study confirmed that protein prolongs ghrelin suppression and cholecystokinin elevation, independent of protein source. The post-prandial elevation in GLP-1 was also prolonged after all protein treatments, compared with glucose. Additionally, the present study demonstrated for the first time that fasting and post-prandial GLP-1 is increased in overweight compared with lean subjects. The macronutrient-specific differences in ghrelin and GLP-1 were observed in lean and overweight subjects, despite differences in overall concentration.

EI was also independent of bodyweight, indicating that adiposity may confer a reduced sensitivity to lower ghrelin and higher GLP-1. Indeed the previously described lower fasting ghrelin level in overweight subjects has not been associated with reduced hunger. Overweight and lean subjects consumed the buffet lunch together to replicate the

![Figure 1](image_url)
The effect of four liquid preloads (1 MJ) containing ~70% of energy from (⊙) soy, (△) whey, (●) gluten or (□) glucose on post-prandial plasma (a) glucose, (b) insulin, (c) ghrelin, (d) glucagon-like peptide-1 and (e) cholecystokinin in men (n = 38). Data are expressed as mean ± SEM. There are significant time by treatment effects for glucose (P < 0.0005), insulin (P < 0.01), ghrelin (P < 0.01), glucagon-like peptide 1 (P < 0.01) and cholecystokinin (P < 0.05) (repeated-measure ANOVA with Tukey’s post hoc test). First published in Bowen J, Noakes M, Clifton PM. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. J Clin Endocrinol Metab 2006; 91: 2913–19. Copyright 2006, The Endocrine Society.

The third study provided preliminary data on the interaction between dietary protein and carbohydrate and to extend the previous observations to four hours post preload. Fructose was combined with whey to avoid the reduction in plasma glucose below baseline that had previously coincided with an increased ghrelin.

The response to the combined treatment suggested that the effect of protein on ghrelin may be dose-dependent. Even though the effect of ghrelin persisted at four hours, EI intake at this time was independent of treatment.

Overall, these studies show a consistent ‘satiating’ profile of gut hormones (prolonged elevation of GLP-1 and cholecystokinin, then suppression of ghrelin) after dietary protein consumption. In contrast, the spike in ghrelin after glucose preloads coincides with higher appetite ratings and EI, supporting a likely role for ghrelin in meal initiation. This increase in ghrelin occurred in parallel with a small decrease in plasma glucose below fasting concentration, suggesting that the two events may be related. Factors that limit a decrease in glucose below fasting concentration warrant investigation. It would also be valuable to compare the timing of spontaneous requests for food with peak/trough concentrations in ghrelin, cholecystokinin and GLP-1.

EI was ~0.5 MJ lower after the protein preloads, indicating that approximately half of the energy consumed in the preload was compensated for at the buffet lunch three hours later, compared with glucose. This time frame represents the typical duration between a mid-meal snack and a main meal in the free-living setting (e.g., mid-morning to lunch or mid-afternoon to dinner). Therefore, in a dietary pattern where ‘between meals’ snacks are usually consumed, manipulating the protein content of a snack could help reduce subsequent intake.

An alternative approach to reducing overall EI is to extend the duration of satiety arising from a meal and abstain from ‘between meal’ snacking. In the third study, the 50-g protein liquid preload (1 MJ) did not have an advantage over carbohydrate-based preloads at meals consumed four hours later. Increasing the amount of protein above 50 g may prolong satiety and in turn reduce EI four hours later. However, there is a practical limit to the level of protein added to a meal or food/beverage. Alternative strategies should be investigated, such as ‘protein rich’ preloads that are also high-fibre or in a solid form. Solid foods empty from the stomach more slowly, and this is associated with higher satiation, compared with liquids.

The overall reduction of EI after protein preloads was relatively small. To maintain a negative energy balance in the free-living setting, it is likely that protein-rich foods and beverages would need to be combined with an intention to reduce EI. In this way, such foods may assist in weight loss by facilitating compliance with restricted food intake.
The subjects in all studies were limited to men; therefore, these findings require confirmation in women, which may differ because of an influence of menstrual cycle on eating behaviour.30

In conclusion, these studies have shown that dietary proteins consumed as liquids provide greater satiety and reduce ad libitum food intake compared with carbohydrate in overweight and obese men after three hours. This relationship may be partly explained by post-prandial changes in appetite-related hormones, which were independent of protein source and bodyweight status.

These findings have a potential application for functional food development. Higher-protein beverages and/or foods may facilitate compliance with energy-restricted diets for weight loss. Future research needs to explore the optimum dose of protein in single foods to achieve appetite reduction. Dietary manipulations that extend the duration of satiety, such as serving protein in solid foods, combining protein with slowly digested starches, fat and fibre should be investigated as additional strategies to extend or increase satiety. Finally, the contribution of these acute observations to longer-term energy balance requires demonstration.

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CONFLICT OF INTEREST

No conflict of interest has been declared by J. Bowen, M. Noakes or P. Clifton.

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Figure 4 The effect of beverages (1.1 MJ) containing ~85% of energy from (---O---) glucose, (-●--) fructose, (-△--) whey protein or (-▲--) combined whey/fructose on post-prandial plasma concentration of (a) glucose, (b) insulin, (c) ghrelin, (d) glucagon-like peptide-1 (GLP-1) and (e) cholecystokinin in obese men (n=28). Data are expressed as mean ± SEM and compared using repeated-measure ANOVA with Tukey’s post hoc test. Plasma glucose is significantly higher after glucose treatment compared with fructose, and the fructose response is greater than whey and whey/fructose (P<0.0005). Plasma insulin is significantly lower after fructose compared with all other treatments (P<0.0005). Plasma ghrelin after whey is different to the glucose treatment (P<0.0005). Plasma GLP-1 is significantly higher after whey compared with all other treatments (P=0.002). Cholecystokinin response to whey is greater than whey/fructose which is also greater than the response to fructose and glucose (P=0.009). First published in Bowen J. Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. Int J Obes (Lond) 2007; 31: 1696–703

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