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High Protein Intake Stimulates Postprandial GLP1 and PYY Release

Agatha A. van der Klaauw[1], **Julia M. Keogh**[1], **Elana Henning**[1], **Victoria M. Trowse**[1], **Waljit S. Dhillo**[2], **Mohammad A. Ghatei**[2], and **I. Sadaf Farooqi**[1]

[1]Department of Clinical Biochemistry, University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK

[2]Section of Investigative Medicine, Division of Diabetes, Endocrinology and Metabolism, Imperial College, London, UK

Abstract

Objective—Meals high in protein induce greater intermeal satiety than meals high in fat and carbohydrates. We studied the gut hormone response and subsequent food intake after breakfasts high in protein, carbohydrate or high in fat controlled for volume, calories and appearance.

Design and Methods—Eight healthy volunteers participated in this randomized three-way crossover study. Study breakfasts were calculated to provide 20% of daily energy requirements and provided either 60% of energy from protein, fat or carbohydrate. Blood was drawn half-hourly for 4 h; energy intake at a subsequent *ad libitum* meal was measured.

Results—Total ghrelin decreased after food intake equally with the three breakfasts. PYY levels were highest after the high protein breakfast ($P = 0.005$). Indeed, PYY at 240 min was highest after the high protein breakfast compared to the high fat breakfast and to the high carbohydrate breakfast ($P = 0.011$ and $P = 0.012$, respectively). GLP-1 levels were highest after the high protein breakfast ($P = 0.041$) at 120 min and remained higher throughout the study. These differences in gut hormones did not translate into differences in food intake (1023 ± 390 kcal after high protein, 1016 ± 388 kcal after high fat and 1158 ± 433 kcal after high carbohydrate).

Conclusion—We conclude that a high protein meal increases circulating concentrations of the gut hormones PYY and GLP-1, but when meals are matched for volume, appearance and caloric value, these gut hormone changes do not translate into a reduction in *ad libitum* food intake.

Introduction

Food ingestion triggers the release of several gastrointestinal hormones including ghrelin which is secreted by the stomach and glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) which are produced primarily by the L-cells in the distal small intestine and colon (1). Recently, several studies have suggested that changes in the macronutrient composition of meals can influence gut hormone release, with differences in the time course and peak

Correspondence: IS Farooqi (isf20@cam.ac.uk).

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concentrations of ghrelin and PYY reported (2–4) (Table 1). However, such manipulations often also affect the energy content, energy density and volume of food delivered, which may have independent effects on gut hormone secretion and may influence nutrient absorption and satiation. We performed a randomized three-way crossover study in which volunteers were given a test breakfast in which 60% of total energy content was derived from protein/fat/carbohydrate, with 20% derived from each of the other two macronutrients (fat and carbohydrate). All three test meals were matched for volume and total energy content and effects on hunger and satiety, postprandial gut hormone levels and subsequent food intake at an *ad libitum* meal were measured.

Methods

Eight healthy volunteers, who were weight stable, participated in this randomized three-way crossover study (five females and three males, mean age of 32 years, range 23-55 years). Exclusion criteria were use of any medication and presence of any medical illnesses or food allergies. Mean BMI of the subjects was 24.5 ± 0.9 kg m^{-2} (BMI range 19.8-27.5 kg m^{-2}). Written informed consent was obtained prior to the study and approval was obtained from the Local Regional Ethics Committee in Cambridge, UK. Each study occasion was separated by at least 1 week. Subjects were fasted from 22:00 h the night before the study and were admitted to the clinical research facility at 7:00 h. An intravenous indwelling cannula was inserted and volunteers rested for ~30 min. Blood was drawn and visual analogue scores to assess hunger and fullness were completed half-hourly from 7:30 h onwards ending at 12:00 h. Isocaloric, isovolaemic test breakfasts were given at 8:00 h and subjects were instructed to finish within 25 min. The calories given per breakfast were standardized for each participant to match 20% of the individually calculated energy requirements with the Schofield formula (5).

All three breakfasts consisted of pancakes with trimmings to give 60% of the energy content as protein/fat/carbohydrate with 20% provided by the other two macronutrients in each case. Specific attention was given to ensure subjects could not tell the nature of the manipulation by avoiding foods that are well recognized to be high in particular macronutrients (e.g. sausages which are known to be high in protein). Carbohydrates made up 60% of the energy content of the high-carbohydrate breakfast, leaving 20% for protein and 20% for fat. The high-carbohydrate breakfast consisted of buckwheat pancakes served with bacon and maple syrup. The energy content of the high-protein breakfast consisted of 60% of protein, 20% of fat and 20% of carbohydrate. The pancakes were made with high-protein pancake mix (Avidlite pancake mix) and served with no-sugar maple syrup and full-fat Greek yoghurt. Fat made up 60% of the energy content of the high-fat breakfast with 20% for carbohydrate and 20% for protein. The wholemeal pancakes were served with bacon and grated cheddar cheese (Table 2). We matched the total carbohydrate to sugar ratio for the three test breakfasts. A survey after the study was completed confirmed participants did not realize the breakfasts were designed to be high in one macronutrient. Water was added to match all test meals for volume. We note that previous work has shown that water intake included in the food itself versus water intake as a beverage in a glass may have differing effects on food intake (6). An *ad-libitum* lunch was served at 12:15 h. The macronutrient composition of the lunch was 50% carbohydrate, 30% fat and 20% protein providing a total of 20 MJ.

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Blood was collected in EDTA tubes containing 100 μL of aprotinin (ghrelin, PYY and GLP-1), lithium heparin tubes (insulin) and fluoride oxalate tubes (glucose). Plasma samples were centrifuged immediately at 4° C and stored at −80°C until assays were performed. Plasma glucose was assayed on the same day by using the glucose oxidase method. Insulin was quantified using a commercially available immunoassay (AutoDELFIA Insulin Kit; Perkin Elmer, Wellesley, MA), which has an intra-assay CV of 3.5-4.5%. Plasma PYY and total GLP-1 were assessed using an established in-house radio-immunoassay (RIA) described previously (7,8). The detection limit of the PYY and GLP-1 assays was 2.5 and 7.5 pmol l^{-1} with an intra-assay coefficient variation of 5.8 and 5.4%, respectively. The ghrelin assay crossreacted fully with both acylated and des-acylated ghrelin and did not crossreact with any other known gastrointestinal or pancreatic hormones (9). The antiserum, SC-10368 (Santa Cruz Biotechnology, CA), was used at a final dilution of 1:50,000. ^{125}I ghrelin was prepared using Bolton & Hunter reagent (Amersham International, UK) and purified by high-pressure liquid chromatography (HPLC). The assay detected changes of 25 pmol l^{-1} with 95% confidence limit. The intra-assay coefficient of variation (CV) was 5.5%.

Data are presented as mean ± standard error of the mean and analyzed using SPSS for Windows version 17.0. ANOVA analysis with repeated measures was used to test for within-subjects changes and between breakfasts differences using an interaction term for time and study breakfasts. Comparisons at specific sampling time points were made using ANOVA with post-hoc comparisons using Tukey's best to correct for multiple testing. A P value of 0.05 was considered significant.

Results

Hunger scores decreased after food intake (Figure 1a) and fullness scores increased. We did not find any differences in hunger scores (P = 0.777) or fullness scores (P = 0.888) nor in the area under the curve (AUC) (hunger scores P = 0.634 and fullness scores P = 0.461) between the different macronutrient manipulations.

We did not find any differences in hunger scores or gut hormones at fasting between the three different study occasions. Glucose concentrations peaked after 30 min for the high protein and high fat breakfasts (5.3 ± 0.9 mmol l^{-1} and 5.1 ± 0.8 mmol l^{-1}) and after 1 h for the high carbohydrate breakfast (5.2 ± 1.3 mmol l^{-1}). The maximal plasma insulin increase was more than twofold greater after the high carbohydrate breakfast (206.5 ± 148.6 pmol l^{-1}) than the high protein breakfast (97.7 ± 41.4 pmol l^{-1}) and 55% higher than the high fat breakfast (163.3 ± 57.3 pmol l^{-1}, P = 0.016).

Ghrelin levels decreased after food intake equally with the three breakfasts (Figure 1b). PYY levels were highest after the high protein breakfast (P = 0.005, Figure 1c). Indeed, after 180 min, PYY levels were highest after the high protein breakfast compared to the high fat breakfast and to the carbohydrate breakfast (P = 0.011 and P = 0.012, respectively). GLP-1 levels were also highest after the high protein breakfast (P = 0.041, Figure 1d) and remained higher throughout the study compared to the high carbohydrate and high fat breakfasts.

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There were no differences in ad libitum energy intake (1023 ± 390 kcal after high protein, 1016 ± 388 kcal after high fat, and 1158 ± 433 kcal after high carbohydrate) with the three test meals. It is possible that, had the timing of the *ad libitum* test meal not been fixed, differences in the onset of voluntary consumption (intermeal interval) may have emerged.

Discussion

In this study, we compared the response to three isovolaemic and isocaloric test breakfasts that were high in one macronutrient in healthy volunteers. We designed our study specifically controlling for total energy content, volume and appearance as visual cues can trigger expectations of hunger and satiety and may affect ghrelin responses (10). We found that a high protein meal resulted in increased secretion of PYY and GLP-1. However, this postprandial increase did not translate into feeling less hungry or more full or a reduction in food intake. We did not standardize food intake and meal timing on the day before the manipulation of the meal with different macronutrient composition; however, baseline values for hunger, glucose, insulin, PYY, GLP-I and ghrelin did not differ between the three occasions suggesting that the participants were in comparable metabolic states when studied.

Increased circulating levels of PYY after a high protein meal have been demonstrated previously (2,4) (Summarized in Table 1). In addition, for the first time, we found that GLP-1 levels were also elevated after a high protein meal. In previous studies, there were no differences in GLP-1 response after meals or drinks high in protein or carbohydrate/fat (4,11) or when meals with 10% and 25% protein contents were compared (12). Differences in the absorption of nutrients in liquids (11) and relatively low protein concentrations used in previous studies (12) could explain these differences. The similar response in ghrelin after high protein/carbohydrate/fat found in our study has been documented previously (4).

Postprandial changes in gut hormone secretion reflect the ability of dietary proteins to directly stimulate enteroendocrine cells after being hydrolyzed to peptides and amino acids. Proposed mechanisms have all addressed the fact that nutrients trigger enteroendocrine cell excitability and hence hormone release [reviewed by (13)]. Glutamine was recently found to increase intracellular calcium and cAMP in L cells ex vivo revealing a pathway for GLP-1 secretion (14). The unravelling of further mechanisms by which various proteins or their constituent amino acids stimulate gut hormone secretion will improve understanding of the differential response to the protein/fat and carbohydrate content of meals.

In contrast to previous studies [reviewed by (15)], we did not find that a high protein meal reduced subsequent food intake. Timing, amount of protein and the volume of food ingested have been found to be key factors in studying satiation, satiety and subsequent food intake. Very few studies have tailored the amount of the meal/preload to daily energy requirements as we did; this may account for some differences in the outcome of studies (15).

Gut hormones signal satiety via their paracrine actions on vagal afferents which project to the brainstem and hypothalamus and via endocrine mechanisms directly modulating activity in brain areas associated with reward and learning (16). The lack of correlation between changes in gut hormone levels and subsequent food intake illustrates that food intake is

mediated by many factors including central pathways which may modulate the response to peripheral gut-derived signals and nutrient availability.

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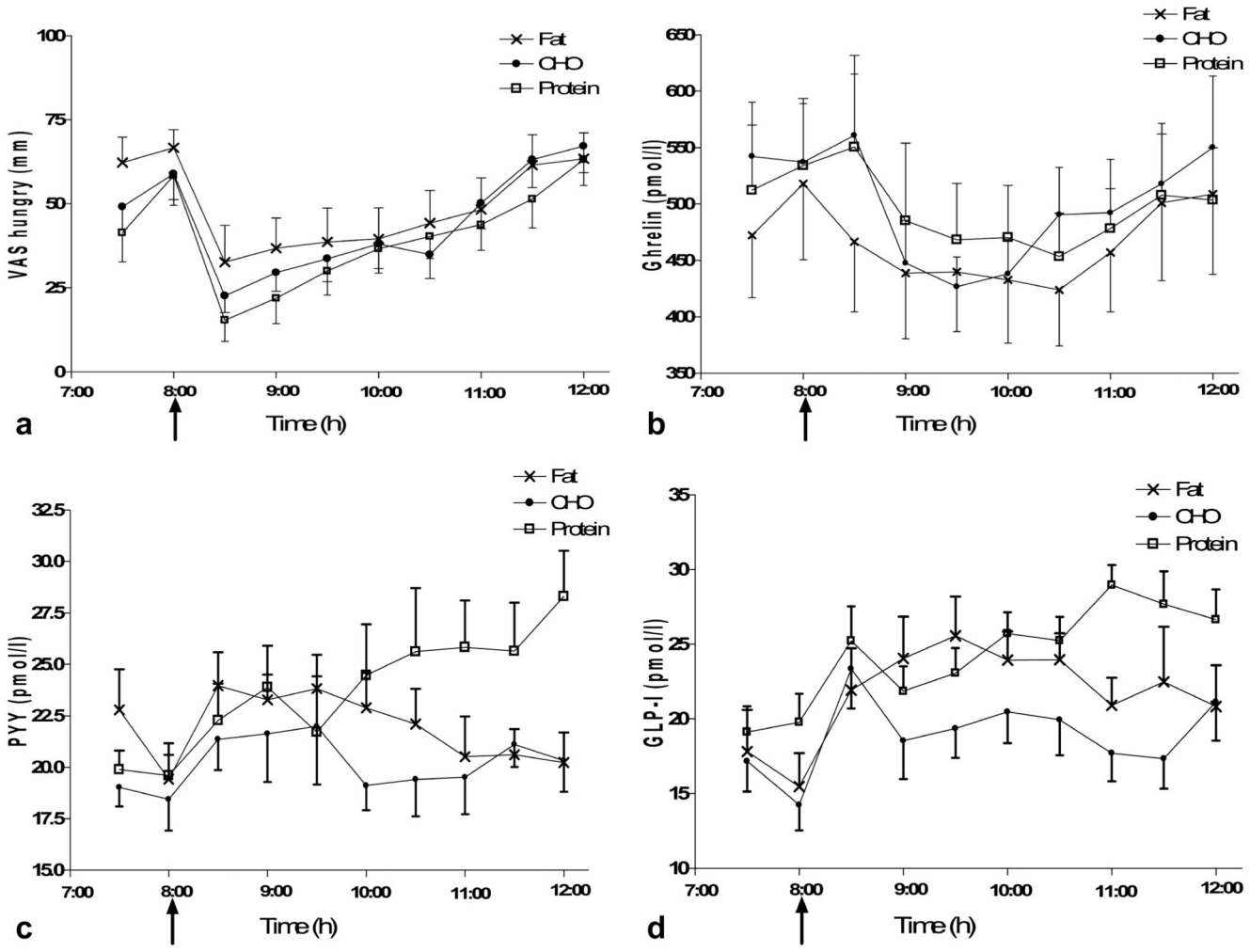

Figure 1.

Changes in hunger, satiety and gut hormones after meals differing in macronutrient composition. Mean ± standard error of the mean for VAS scores (**a**), ghrelin (**b**), PYY (**c**), and GLP-1 (**d**). ANOVA analysis with repeated measures was used. PYY and GLP-1 levels were significantly higher after the high protein breakfast compared to the high carbohydrate and high fat breakfasts.

Table 1

Summary of studies investigating the gut hormone response to macronutrient manipulations

Author	Subjects	Design	Control for	Outcomes
Erdmann et al.	10 healthy volunteers	Crossover design with 6 occasions	Volume: no	Ghrelin levels increased after high protein meal
Regulatory Peptides 2003		HF: 85%	Calories: no	Late ghrelin suppression less in HCHO compared to HF
		HP: 99% HCHO: 62% Fruits: 93% CHO 75 g glucose in 300 ml water sham feeding: gastric distension by guar Gut hormones: total ghrelin	Appearance: no Subsequent meal: no	
Batterham et al.	10 normal weight and 9 obese volunteers	Crossover design with three occasions	Volume: not mentioned	HP greatest increment in total plasma PYY and integrated PYY levels in normal and obese subjects.
Cell metabolism 2006		HF 66	Calories: yes, fixed	Active ghrelin, GLP-1 no differential responses to meals
		HP 65 HCHO 65 Gut hormones: PYY, active ghrelin, active GLP-I	Appearance: yes Subsequent meal: no	
Foster-Schubert et al. *Journal of Clinical Endocrinology and Metabolism* 2008	16 healthy volunteers	Crossover design with 3 occasions	Volume: yes	Suppression of acyl and total ghrelin protein during HP was greater than HCHO and suppression during HCHO was greater than HF
		HF & HP & HCHO: 80%	Calories: calculated to 20% energy requirements	
		Gut hormones: acyl and total grhelin	Appearance: yes	
			Subsequent meal: no	
Eller et al.	10 healthy men	Crossover design with 3 occasions	Volume: not mentioned	GLP-I no differences
Clinical Endocrinology 2007		HF 80%	Calories: yes, fixed (720 kcal/70 kg)	Greater des-acyl ghrelin decrease after HCHO (45%) than after HF (17%)
		HCHO 80% prolonged fast Gut hormones: des-acyl ghrelin, GLP-I	Appearance: no Subsequent meal: no	
Maffeis et al.	10 prepubertal obese boys	Crossover design with 3 occasions	Volume: no	PYY, CCK, and ghrelin concentrations not different
I*ntegrative Physiology* 2009		HF 52%	Calories: Calculated to 25% of energy requirements	GLP-1 higher after HF than MF
		Medium fat 27% HCHO 61% Gut Hormones: PYY, CCK, total	Appearance: no Subsequent meal: no	

Author	Subjects	Design	Control for	Outcomes
		ghrelin and total GLP-I		
Erdmann et al.	14 healthy volunteers	Crossover design with 5 occasions	Volume: no	Intake testmeal HF 244 g, HP 293 g, HCHO 321 g
Journal of Clinical Endocrinology and Metabolism 2004		HF: 86%	Calories: instructed to eat until satiation, at least 50% more provided.	After HCHO ghrelin decreased, with all the other meals ghrelin increased
		HP: 83% HCHO: 80% Fruits: 93% Vegetables: 75% Gut Hormones: total ghrelin	Appearance: no Subsequent meal: yes	
Al Awar et al.	11 healthy women	Crossover design with 3 occasions	Volume: yes	Ghrelin persisted at significantly lower levels for a longer duration after the HP meal compared to HF and balanced meal
Clinical Science 2005		Balanced 45% CHO, 45% F, 10% P HP 35% HF 45% Hormones: acylated ghrelin	Calories: Calculated to 30% energy requirements Appearance: yes Subsequent meal: no	
Monteleone et al.	14 healthy women	Crossover with 2 occasions	Volume: not mentioned	Nadir ghrelin lower after HCHO then after HF meal
Journal of Clinical Endocrinology and Metabolism 2003		HCHO: 77%	Calories: yes, fixed	
		HF: 75% Gut hormones: plasma ghrelin	Appearance: no Subsequent meal: no	
Lomenick et al.	13 normal weight and 19 obese children	Crossover with 3 occasions	Volume: not mentioned	AUC for ghrelin not different between meals
Journal of Clinical Endocrinology and Metabolism 2009		HCHO: 88%	Calories: yes, fixed	PYY significantly higher after HP compared to HF and HCHO in obese children not in normal weight children
		HP 44%: HF: 81% Gut hormones: total ghrelin, total PYY	Appearance: no Subsequent meal: no	

HCHO: high carbohydrate, HP: high protein, HF: high fat

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Table 2
Details of the composition of the test breakfasts

Macronutrient composition	60% protein/20% fat/20% carbohydrates	60% carbohydrate/20% fat/20% protein	60% fat/20% carbohydrate/20% protein
Ingredients	Pancakes (Avidlite pancake mix)	Pancakes (made with buckwheat, whole milk, rapeseed oil and 2 eggs)	Pancakes (Allinson whole meal flour, whole milk, rapeseed oil, sugar and 1 egg)
	Total full fat greek yoghurt	Unsmoked bacon	Unsmoked bacon
	Sugar free maple syrup	Canadian maple syrup no 1	Grated cheddar
CHO:sugar ratio	3.25	3.25	3.25

Percentages are energy of single macronutrient as percentage of total energy. The total energy provided was calculated to provide 20% of daily estimated energy requirements.