Liquid meal composition, postprandial satiety hormones, and perceived appetite and satiety in obese women during acute caloric restriction

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Abstract
Objective: The purpose of this study was to compare postprandial satiety regulating hormone responses (pancreatic polypeptide (PP) and peptide tyrosine tyrosine (PYY)) and visual analog scale- (VAS) assessed perceived appetite and satiety between liquid high-protein (HP) and high-carbohydrate (HC) meals in obese women during acute (24-h) caloric restriction.

Design: Eleven obese premenopausal women completed two conditions in random order in which they consumed 1500 calories as six 250-calorie HP meals or six 250-calorie HC meals over a 12-h period. Blood samples were taken at baseline and every 20 min thereafter and analyzed for PP and PYY concentrations. At these same points, perceived hunger and fullness were assessed with a VAS. The incremental area under the curve (ΔAUC) was used to compare postprandial responses.

Results: The 12-h PP and PYY ΔAUC were greater (P<0.05) during the HP condition (PP: 4727 ± 1306 pg/ml·12 h, PYY: 1373 ± 357 pg/ml·12 h) compared with the HC condition (PP: 2300 ± 528 pg/ml·12 h, PYY: 754 ± 246 pg/ml·12 h). Perceived hunger and fullness were not different between conditions (P>0.05). The greatest changes in PYY and perceived fullness occurred after the morning meals during both conditions.

Conclusions: These data suggest that in obese women during acute caloric restriction before weight loss, i) liquid HP meals, compared with HC meals, result in greater postprandial PP and PYY concentrations, an effect not associated with differential appetite or satiety responses, and ii) meal-induced changes in PYY and satiety are greatest during the morning period, regardless of dietary macronutrient composition.

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Introduction
Pancreatic polypeptide (PP) and peptide tyrosine tyrosine (PYY) belong to the gastrointestinal PP-fold family of proteins (1), and experimental studies demonstrate that both peptides are acute satiety signals (2, 3, 4). PP is secreted from the pancreatic islet F cells via a vagal cholinergic mechanism in response to a meal (5). I.v. infusions of PP in healthy individuals (4), individuals with Prader–Willi syndrome (6), and mice (7) cause a reduction in food intake and the peptide effects are mediated through Y4 receptor signaling (8). On the other hand, PYY is secreted from the L cells of the intestine in response to a meal (9) and circulates as either PYY1-36 or PYY3-36 (10). PYY3-36 is the truncated form and is the main circulating form of the peptide (10). Exogenous administration of PYY reduces food intake in humans (2, 11), rats (11), and mice (11) while PYY3-36 effects are mediated through Y2 receptor signaling (8, 11). Collectively, these observations suggest that postprandial PP and PYY secretion constitute an important mechanism in the control of appetite and satiety and that modulation of these hormones might aid in the treatment or prevention of obesity.

Caloric restriction is often the first-line therapy to help treat obesity. Lower calorie diets induce weight loss, but increased hunger and reduced satiety can reduce the compliance of a low-calorie diet. However, altering the composition of the diet may be helpful. Although limited, some studies suggest that chronic higher protein (HP)
Materials and methods

Study population

The University of Missouri Institutional Review Board reviewed this study and found it ethical to conduct. All participants provided written informed consent before participating. Obese premenopausal women were recruited for this study using email list serves, fliers posted around the community, and word of mouth. Inclusion criteria included a BMI ≥ 30 kg/m²; body fat percent > 30% (assessed via the BOD POD), between 30 and 50 years of age; sedentary (engaged in exercise < 1 time per week); no previous history of sleep apnea; heart, lung, kidney, endocrine, or liver disease; normal fasting blood glucose concentrations (< 100 mg/dl), not on hormone replacement therapy or taking any medications known to alter appetite or satiety; non-tobacco users; and not pregnant or lactating.

Study design

In a crossover design, the participants completed two conditions in random order in which they consumed 1500 liquid calories as six 250-calorie HP meals or six 250-calorie HP meals over a 12-h period during 24 h of acute caloric restriction (12-h fast followed by 12 h of feeding every 2 h). The participants were given 1500 calories because a 1200–1500 calorie energy-restricted diet has been shown to be effective for weight loss in obese individuals. The meals were administered once every 2 h during the 12-h testing period, and this higher meal frequency was chosen because it has been reported that women in Western cultures consume frequent meals per day, with an average eating occasion of five per day. In addition, the women in our study reported consuming between three and six meals per day, and this varied from day to day. Each experimental condition was separated by at least 1 month, occurred during the follicular phase of the menstrual cycle, and the participants were instructed to maintain their normal dietary and physical activity habits while participating in this study. Each participant consumed an energy-balanced diet and kept a dietary record during the 3 days before each 12-h testing day.

Testing day

All participants arrived at the laboratory at 0700 h following a 12-h overnight fast. Upon arrival, a registered nurse inserted a venous catheter into the antecubital vein of the forearm of the participant, which was kept patent with a saline drip. During each condition, venous blood samples were taken at baseline and every 20 min thereafter for 12 h. The participant’s perceived hunger and fullness were assessed with a 100 mm linear visual analog scale (VAS) at baseline and every 20 min thereafter. The participants marked
a single vertical line through the horizontal line of each scale between the extremes (i.e. ‘not at all hungry’ to ‘as hungry as I have ever felt’ and ‘not at all full’ to ‘as full as I have ever felt’) to indicate their perceived hunger and satiety at that time. The VAS is a valid and reliable scale used to assess appetite and satiety (33, 34). During testing, the participants were sedentary and were either sitting or lying down in a bed. The only physical activity they performed was getting up to use the bathroom.

During the HC condition, the meal was a canned Nutritional Drink (Walgreens Co., Deerfield, IL, USA) containing 250 calories (8 fl oz) as 9 g protein, 40 g carbohydrate, 6 g fat, and 0 g fiber (energy consisting of 14.4% protein, 64% carbohydrate, and 21.6% fat). The glycemic index of the HC meal was 115 (determined by Nutrition Data System for Research (NDSR), University of Minnesota, Minneapolis, MN, USA). During the HP condition, the meal was made by mixing one half of a can of the Nutritional Drink with 24.5 g of a commercially available protein supplement (Pro Complex, Optimum Nutrition, Inc., Sunrise, FL, USA), 3 g olive oil (Great Value, Walmart Co., Bentonville, AR, USA), and 4 fl oz of water so that the drink contained 250 calories (8 fl oz) as 29 g protein, 20 g carbohydrate, 6 g fat, and 0 g fiber (energy consisting of 46.4% protein, 32% carbohydrate, and 21.6% fat). The glycemic index was 114 for the HP meal. The energy density was identical between the HP and HC meals and was 4.54 cal/g. The shakes were provided to the participants either as a chocolate or vanilla flavor, depending on the participant’s preference.

**Body composition**

The BOD POD (Life Measurements, Inc.) was used to assess the body composition of the participants during the screening to determine whether the participants met the inclusion criteria. The participants fasted for 3 h before being tested and wore skintight clothing during the test. Lung volume was measured and body fat % was predicted by the computer program using the Siri equation.

**Energy balance**

The Mifflin resting energy expenditure (REE) prediction equation (35) was used to calculate the 24-h REE of all the participants as this equation has been shown to be most accurate in obese individuals (36). The participants performed little physical activity during the testing day, thus the REE calculated was multiplied by an activity factor of 1.2 to get the predicted 24-h energy expenditure during the testing day. The average predicted 24-h REE was 1975 ± 50 calories, thus the participants were calorically restricted by 475 ± 50 calories (calculated as REE − 1500 calories consumed).

**Dietary assessment**

To control for the effect that diet could have on PP, PYY, or perceived appetite and satiety responses, the participants kept detailed dietary records during the 3 days before each condition. A photocopy of this dietary record was given to the participants and they were asked to precisely replicate the dietary records before the subsequent visit. Dietary records were analyzed for total energy, macro/micronutrient content, and glycemic index using the NDSR.

**Blood handling and analysis**

Venous blood samples (~3 ml) were collected by syringes, transferred immediately into serum separator tubes, and separated by centrifugation at 1409 g for 15 min at 4 °C. The serum was stored in cryogenic vials at −80 °C until analysis. The hormone concentrations of PP and PYY (total) were determined from the same sample in duplicate using a MILLIPLEX magnetic bead-based quantitative immunoassay with the MAGPIX instrumentation (Millipore, Billerica, MA, USA). The intra-assay coefficients of variability (CV) among the samples was 10% for PP and 9% for PYY while the interassay CV was 11% for PP and 14% for PYY.

**Statistical analysis**

The Statistical Package for the Social Sciences statistical software, version 18.0 (IBM SPSS Statistics), was used to perform the statistical tests. For the primary analysis, the 12-h postprandial PP, PYY, hunger, and fullness responses were quantified using the incremental area under the curve (iAUC represents change from baseline), which was calculated using the trapezoidal method (37). A paired samples t-test was used to compare the 12-h iAUC between the HP and HC conditions. For the secondary analysis, the 4-h iAUC was calculated for the morning (time 0700–1100 h), afternoon (1100–1500 h), and evening (1500–1900 h) and a repeated measures ANOVA was performed with follow-up Bonferroni-adjusted t-tests to identify statistically significant differences between the 4-h iAUC during the different times of day within each condition. Statistical significance was set at P ≤ 0.05 and values are reported as means ± s.e.m.

**Results**

**Participant characteristics and diet**

Eleven obese premenopausal women participated in this study. The baseline characteristics of the women are listed in Table 1. The participants remained weight stable while participating in this study (defined as weight gain/loss < 2 kg). There were no differences
in dietary energy consumed, macronutrient composition, or glycemic index during the 3 days before each condition.

**Fasting and postprandial hormonal responses**

Figure 1 shows the PP responses during both conditions. Fasting PP concentrations were not significantly different between conditions (Fig. 1B, \( P > 0.05 \)). The 12-h PP \( i \text{AUC} \) was significantly greater (\( P = 0.02 \)) during the HP condition compared with the HC condition (Fig. 1C). The 4-h \( i \text{AUC} \) was not significantly different between the morning, afternoon, and evening within each condition (Fig. 1A, \( P > 0.05 \)).

Figure 2 shows the PYY responses during both conditions. Fasting PYY concentrations were not significantly different between conditions (Fig. 2B, \( P > 0.05 \)). The 12-h PYY \( i \text{AUC} \) was significantly greater (\( P = 0.05 \)) during the HP condition compared with the HC condition (Fig. 2C). During the HP condition, the rise in PYY concentrations in response to the meals (4-h \( i \text{AUC} \)) was significantly greater during the morning compared with the afternoon (\( P < 0.03 \)) but not the evening (Fig. 2A, \( P > 0.05 \)). Similarly, during the HC condition, perceived fullness increased more in response to the meals during the morning compared with the afternoon and evening (Fig. 2A, \( P = 0.04 \)).

**Fasting and postprandial hunger and fullness responses**

Figure 3 shows the perceived hunger responses during both conditions. Baseline hunger ratings were not significantly different between conditions (Fig. 3B, \( P > 0.05 \)). The 12-h hunger \( i \text{AUC} \) was not significantly different between conditions (Fig. 3C, \( P > 0.05 \)). During the HP condition, perceived hunger declined more in response to the meals (4-h hunger \( i \text{AUC} \)) in the morning compared with the afternoon (\( P = 0.03 \)) but not the evening (Fig. 3A, \( P < 0.05 \)). There were no significant differences in perceived hunger during the different times of day during the HC condition (Fig. 3A, \( P > 0.05 \)).

Figure 4 shows the perceived fullness responses during both conditions. Baseline fullness ratings were not significantly different between conditions, but there was a trend for baseline fullness to be greater during the HP condition (Fig. 4B, \( P = 0.055 \)). The 12-h fullness \( i \text{AUC} \) was not significantly different between conditions (Fig. 4C, \( P > 0.05 \)). During the HP condition, perceived fullness increased more in response to the meals during the morning compared with the afternoon and evening (Fig. 4A, \( P = 0.04 \)). Similarly, during the HC condition, perceived fullness increased more in response to the meals during the morning compared with the afternoon and evening (Fig. 4A, \( P < 0.01 \)).

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**Table 1** Participant characteristics. Values are means ± S.E.M.

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| Age (years) | 40.4 ± 2.0 |
| Height (m) | 1.7 ± 1.6 |
| Weight (kg) | 97.2 ± 5.4 |
| BMI (kg/m²) | 35.4 ± 1.2 |
| Body fat percent | 47.1 ± 1.7 |
| Waist circumference (cm) | 103.4 ± 3.7 |
| Hip circumference (cm) | 121.5 ± 3.5 |
| Fasting blood glucose (mg/dl) | 81.5 ± 2.4 |
| Fasting insulin (µIU/l) | 9.4 ± 1.4 |
| QUICKI | 0.35 ± 0.01 |
| HOMA-IR | 2.06 ± 0.22 |

QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostatic model of insulin resistance.
Discussion

The effect of liquid meal composition on the acute satiety signals PP and PYY and perceived appetite and satiety, in obese women during acute caloric restriction, has not been studied. The findings from this study show, for the first time, that during acute caloric restriction liquid HP meals stimulate greater postprandial concentrations of the satiety peptides PP and PYY compared with liquid HC meals throughout an entire day of eating in obese women, a group that is likely to use a liquid diet to induce weight loss. However, the greater postprandial concentrations of the satiety peptides PP and PYY induced by the liquid HP meals were not associated with greater satiation compared with liquid HC meals. In addition, the greatest increase in PYY and increase in perceived fullness occurred during the morning period during both conditions, and these responses were much less after subsequent meals. These data suggest that in obese women during acute caloric restriction before weight loss, i) liquid HP meals, compared with HC meals, result in greater postprandial PP and PYY concentrations, an effect not associated with altered appetite or satiety, and ii) meal-induced changes in PYY and satiety are greatest during the morning period, regardless of dietary macronutrient composition.

Postprandial PP concentrations remain elevated for up to 6 h after meal ingestion (38) and the current line of evidence indicates that PP is an acute satiety signal (4, 6, 7). To our knowledge, only one study has shown that a HP meal induces greater postprandial PP

Figure 2 Peptide tyrosine tyrosine (PYY) time course and incremental area under the curve (iAUC). (A) The 4-h PYY iAUC for the morning, afternoon, and evening periods. (B) Time course of postprandial PYY. (C) The 12-h PYY iAUC. Values are represented as means ± S.E.M. † Meal was eaten. aP < 0.05 compared with morning 4-h iAUC within high-protein (HP) condition. bP < 0.05 compared with morning 4-h iAUC within high-carbohydrate condition. cP < 0.05 compared with HP condition.

Figure 3 Perceived hunger time course and incremental area under the curve (iAUC). (A) The 4-h hunger iAUC for the morning, afternoon, and evening periods. (B) Time course of postprandial hunger. (C) The 12-h hunger iAUC. Values are represented as means ± S.E.M. † Meal was eaten. aP < 0.05 compared with morning 4-h iAUC within high-protein condition.
Despite greater concentrations of the satiety peptides PP and PYY with HP meals in this study, appetite and satiety were not different between liquid HP and HC meals. This response is contrary to most of the published work in this area. The majority of studies that have compared appetite and satiety between liquid HP and HC meals have used liquid preloads before ad libitum feeding. Most studies demonstrate that liquid HP preloads decrease energy intake (22, 23, 24, 25, 26, 27) and increase satiety or reduce appetite (22, 25, 26, 27, 39) more so than liquid HC preloads. This response is not universal as another study demonstrated that liquid HP preloads did not alter satiety or energy intake differently compared with a liquid HC preload (28). In the context of chronic caloric restriction, studies have shown that a HP diet consisting of solid food is associated with greater satiation compared with a lower protein diet (12, 13, 14). Our data with liquid meals do not support these previous studies and show that during acute caloric restriction, liquid HP meals do not increase perceived fullness or reduce perceived hunger more than liquid HC meals over the course of an entire day of eating. The difference in responses between studies may be because of differences in study designs, test populations, and different test meals. Given the limited research examining the impact of liquid meal macro-nutrient composition during acute or chronic caloric restriction on appetite and satiety, more research is warranted in obese individuals, a group that is most likely to use a liquid diet for weight loss.

This study has some potential limitations that should be considered when interpreting the findings. First, we examined hormonal and perceived appetite and satiety responses in obese women before weight loss and during an acute (24-h) period of caloric restriction. It is possible that perceived hunger, satiety, and hormonal responses may change over time during or after weight loss when a liquid diet is consumed, although this has not been examined. Previous work that has used solid meals demonstrated that when individuals were fed a HC diet, postprandial perceived fullness declined ~24% after 12 weeks of caloric restriction compared with baseline, whereas the decline was attenuated with a HP diet (declined 5%). However, the change in perceived hunger and desire to eat changed minimally (<5%) from pre- to post-weight loss and was not different between HP or HC meals (13). It is possible that the lack of difference between HP and HC meals in the current study may have been that the participants consumed the liquid diet for only one day, but it may be that a longer duration would be required to observe differences in satiety between liquid HP and HC meals. Another limitation of this study is that we did not measure the palatability of the meals. Palatability could have an effect on appetite and satiety ratings (40), which could have influenced the results of our study. In addition to palatability, meal frequency could potentially alter appetite and satiety responses, which could limit the translation of these

concentrations compared with a lower protein meal (15), and this may be one mechanism by which HP meals induce greater satiation compared with lower protein meals. In line with this study, our data show that liquid HP meals induce greater postprandial PP concentrations compared with liquid HC meals in obese women over the course of 12 h of eating. The PP response to each meal during both conditions was not significantly different between the different times of day and was highly variable. In addition to PP, the gut hormone PYY is an acute satiety signal and involved in the regulation of bodyweight (2, 11). Our data show greater postprandial PYY concentrations in response to HP meals, compared with HC meals, and this finding is in agreement with previous work (16). Furthermore, the PYY response to each meal, during both conditions, was greatest in the morning compared with the afternoon and evening.

Figure 4 Perceived fullness time course and incremental area under the curve (AUC). (A) The 4-h fullness AUC for the morning, afternoon, and evening periods. (B) Time course of postprandial fullness. (C) The 12-h fullness AUC. Values are represented as means ± S.E.M. † Meal was eaten. *P < 0.05 compared with morning 4-h AUC within high-protein condition. †*P < 0.05 compared with morning 4-h AUC within high-carbohydrate condition.

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findings to a lower meal frequency (i.e. three meals per day). However, no published study has assessed how liquid meal frequency alters perceived appetite and satiety in obese women during acute caloric restriction. Unpublished observations from our laboratory suggest that liquid meal frequency does not alter perceived appetite or satiety over the course of an entire day of eating solid food in overweight or obese men consuming an energy-balanced diet (41). Lastly, we used Caucasian, obese premenopausal women; thus, extrapolation of these findings to other populations of different sex and age is limited.

In conclusion, these data suggest that liquid HP meals, compared with liquid HC meals, result in greater postprandial PP and PYY concentrations, an effect not associated with altered appetite or satiety, in obese women during an acute period of caloric restriction before weight loss. In addition, meal-induced changes in PYY and satiety are greatest during the morning period compared with the afternoon and evening. The clinical implications of these findings are that a liquid HP diet may not offer any benefit in terms of appetite control before weight loss. In addition, meal-induced changes in PYY and satiety are greatest during the morning period compared with the afternoon and evening. The clinical implications of these findings are that a liquid HP diet may not offer any benefit in terms of appetite control, energy regulation, and body composition in all populations.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
T D Hedden was involved in data collection and analysis, data interpretation, and manuscript preparation. Y Liu and L Sims were involved in data collection and blood analysis. M L Kearney was involved in data collection and analysis and manuscript preparation. A T Whaley-Connell, A Chockalingam, and K C Dellsperger were involved in data interpretation and manuscript preparation. T J Fairchild was involved in study design, data interpretation, and manuscript preparation. A Kanaley was involved in study design, data interpretation, data collection, and analysis, and manuscript preparation.

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