Beneficial effects of a higher-protein breakfast on the appetitive, hormonal, and neural signals controlling energy intake regulation in overweight/obese, “breakfast-skipping,” late-adolescent girls1–3

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ABSTRACT
Background: Breakfast skipping is a common dietary habit practiced among adolescents and is strongly associated with obesity.
Objective: The objective was to examine whether a high-protein (HP) compared with a normal-protein (NP) breakfast leads to daily improvements in appetite, satiety, food motivation and reward, and evening snacking in overweight or obese breakfast-skipping girls.
Design: A randomized crossover design was incorporated in which 20 girls [mean ± SEM age: 19 ± 1 y; body mass index (in kg/m²): 28.6 ± 0.7] consumed 350-kcal NP (13 g protein) cereal-based breakfasts, consumed 350-kcal HP egg- and beef-rich (35 g protein) breakfasts, or continued breakfast skipping (BS) for 6 d. On day 7, a 10-h testing day was completed that included appetite and satiety questionnaires, blood sampling, predinner food cue–stimulated functional magnetic resonance imaging brain scans, ad libitum dinner, and evening snacking.
Results: The consumption of breakfast reduced daily hunger compared with BS with no differences between meals. Breakfast increased daily fullness compared with BS, with the HP breakfast eliciting greater increases than did the NP breakfast. HP, but not NP, reduced daily ghrelin and increased daily peptide YY concentrations compared with BS. Both meals reduced predinner amygdala, hippocampal, and midfrontal corticolimbic activation compared with BS. HP led to additional reductions in hippocampal and parahippocampal activation compared with NP. HP, but not NP, reduced evening snacking of high-fat foods compared with BS.
Conclusions: Breakfast led to beneficial alterations in the appetitive, hormonal, and neural signals that control food intake regulation. Only the HP breakfast led to further alterations in these signals and reduced evening snacking compared with BS, although no differences in daily energy intake were observed. These data suggest that the addition of breakfast, particularly one rich in protein, might be a useful strategy to improve satiety, reduce food motivation and reward, and improve diet quality in overweight or obese teenage girls. This trial was registered at clinicaltrials.gov as NCT01192100. Am J Clin Nutr 2013;97:677–88.

INTRODUCTION
Obesity continues to adversely influence the lives of American young people, with the current prevalence remaining at 33% (1). Recent evidence has isolated several key factors that play a critical role in the etiology of this disease (1, 2). One in particular is the common, unhealthy dietary habit of breakfast skipping (BS)4, which is strongly associated with an increased prevalence of weight gain, increased BMI, and obesity (2–4). In addition, breakfast skippers have poor diet quality (2) and make poor food choices (eg, snacking on nutrient-poor, high-fat, and/or high-sugar foods and beverages) compared with breakfast consumers (5–7). These data lend support for the addition of breakfast to combat obesity in young people.

Breakfast studies over the past 10 y have primarily examined the effects of ready-to-eat cereal (RTEC) consumption on childhood and adolescent obesity and its associated risk factors (8). In general, increased RTEC consumption is associated with lower BMI, lower percentage body fat, and a decreased prevalence of overweight/obesity (8). In a 10-y longitudinal observational study, girls who frequently ate RTEC during childhood exhibited a lower percentage body fat compared with those who skipped breakfast (8). Although there is clear evidence showing the benefits of an RTEC breakfast, limited data exist in comparing RTEC with other breakfast foods.

A diet rich in high-quality protein is gaining scientific support as a successful strategy to promote weight loss and/or prevent weight gain or regain in adults [see review by Westerterp-Plantenga et al (9)]. One key factor in the effectiveness of higher protein meals/diets includes the improvement in appetite control and satiety (10–14). In our pilot studies (15, 16), we showed that a protein-rich breakfast meal led to decreased appetite and increased satiety throughout the morning compared with skipping breakfast or consuming a normal-protein (NP) breakfast. We also showed that a protein-rich breakfast reduces prelunch neural activation in brain regions that control food motivation/reward compared with skipping breakfast or consuming an NP RTEC breakfast. Last, when assessing energy content consumed at an ad libitum lunch, we found that the consumption of a protein-rich breakfast led to fewer kilocalories consumed at lunch compared with BS or after the NP breakfast. Collectively,

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4 Abbreviations used: BS, breakfast skipping; ES, effect size; HP, high protein; MU-BIC, University of Missouri’s Brain Imaging Center; NP, normal protein; PYY, peptide YY; RTEC, ready-to-eat cereal.

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these data support the role for the daily consumption of protein-rich breakfast meals in young people who skip breakfast.

The current study extends the previous findings by examining the previous responses over the course of an entire day, not just throughout the morning. We also incorporated a novel approach of assessing evening energy intake through an ad libitum snack assessment. The objective of the current study was to examine whether a high-protein (HP) breakfast leads to daily improvements in appetite control, satiety, food motivation/reward, and evening snacking compared with NP RTEC breakfast meals in overweight/obese BS teenage girls.

SUBJECTS AND METHODS

Experimental design

Twenty overweight or obese BS teenage girls participated in this randomized crossover-design study. The participants randomly completed the following breakfast patterns at home for 6 d: 1) BS, 2) consumption of NP breakfast meals, or 3) consumption of HP breakfast meals. On the seventh day of each pattern, the participants reported to the University of Missouri’s Brain Imaging Center (MU-BIC) in the morning to complete the respective 10-h testing day (Figure 1). The participants began the day by either skipping breakfast or consuming their respective breakfast meal. Blood samples and assessments of perceived appetite and satiety were completed at specific times throughout the day. A standardized NP lunch was provided 4 h after breakfast. Before dinner, a brain scan was completed by using fMRI to identify brain activation patterns in response to food stimuli. After the fMRI, an ad libitum dinner was provided. The participants were then given evening snacks to consume ad libitum, at home, throughout the remainder of the day. There was a washout period of at least 7 d between each pattern.

Study participants

From October 2010 to May 2011, late-adolescent girls were recruited from the Columbia, MO, area through advertisements, flyers, and e-mail listservs to participate in the study. Eligibility was determined through the following inclusion criteria: 1) age range of 15–20 y; 2) overweight to obese [BMI (in kg/m²): 25–34.9]; 3) no metabolic or neurologic diseases or other health complications; 4) taking no medications that would influence food intake regulation, appetite, or metabolism; 5) not clinically diagnosed with an eating disorder; 6) not currently or previously (within the past 6 mo) on a weight-loss or other special diet; 7) not pregnant; 8) infrequently eating breakfast (ie, ≤2 breakfast occasions/wk as assessed from a 7-d screening breakfast questionnaire); and 9) right-handed (for consistency with the fMRI neural responses).

One hundred and forty-seven teens were initially interested in participating in the study. Twenty-two participants met the screening criteria, had 3 available Saturdays to complete the 10-h testing days, and began the study. Twenty of the participants completed all study procedures (August 2011). Of those who did not complete the study, one dropped out due to mild claustrophobia that developed during the MRI and one was excluded due to noncompliance to the testing day procedures.

Participant characteristics of those who completed the study are presented in Table 1. All participants and their parents were informed of the study purpose, procedures, and risks and signed the consent/assent forms. The study was approved by the University of Missouri Health Sciences Institutional Review Board, and all procedures were followed in accordance with the ethical standards of the institutional review board. The participants received a total of $450 ($150/testing day) for completing all study procedures.

Breakfast treatments

The participants completed each of the 3 breakfast treatments for 7 consecutive days. For BS, the participants continued to follow their habitual practice of skipping breakfast and completed the day 7 testing day accordingly. For NP and HP breakfasts, the participants were provided with specific breakfast meals and asked to consume these at home (before school) between 0700 and 0930 for 6 d. Throughout this period, the participants were permitted to eat ad libitum throughout the remainder of each day. On day 7, they completed the respective testing day.

There was a washout period of at least 7 d between each of the breakfast patterns in which the participants returned to their previous BS behavior.

![FIGURE 1. Diagram of the 10-h testing day procedures. MU, University of Missouri; NP, normal protein.](image-url)
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**TABLE 1**
Characteristics of the study participants (n = 20)

| Value |
|--------|

| Participant characteristics | Value |
|-----------------------------|--------|
| Age (y)                     | 19 ± 1^1 |
| Height (cm)                 | 167 ± 1 |
| Weight (kg)                 | 79.6 ± 2.1 |
| BMI (kg/m²)                 | 28.6 ± 0.7 |
| Skips breakfast (no. of times/wk) | 6 ± 1 |
| First eating or drinking occasion of the day (time of day) | 1230 ± 0015 |

| Reasons for skipping breakfast (%) | Value |
|-------------------------------------|--------|
| Not enough time in the morning (would rather sleep) | 90 |
| Not hungry | 30 |
| Nothing to eat | 10 |

^1 Mean ± SEM (all such values).

**Breakfast meals**

The energy content of the breakfast meals comprised 18% of daily energy intake (~350 kcal) estimated from the energy expenditure equations specific for adolescents (17). The macronutrient composition of the NP breakfast contained 15% protein (13 g protein), 65% carbohydrate, and 20% fat, whereas the HP breakfast contained 40% protein (35 g protein), 40% carbohydrate, and 20% fat. Although plant-based and dairy protein sources were included in both breakfast meals, egg and beef proteins were included as the predominant protein sources in the HP breakfast due to their high protein-carbohydrate ratio, high protein quality, food form (ie, solid food compared with liquid), and/or versatility (eg, egg-based waffles, egg-based burrito, beef sausage). In addition to being matched for fat content, the breakfast meals were similar in energy density, dietary fiber, sugar content, dairy and plant protein quantities, and palatability (see Table 2).

Adherence to the treatments was assessed during the 6 acclimation days. For the BS treatment, the participants were asked to complete daily check-off logs indicating that they did not eat or drink anything besides water before 1000 h. For the NP and HP treatments, the participants were asked to complete daily check-off logs indicating that they consumed the study breakfast meal at the designated time. They were also required to return any uneaten food and all food containers. There was 100% compliance for all testing days and 99% compliance during the acclimation days for all breakfast treatments.

**Specific testing day procedures**

On day 7 of each breakfast pattern, the participants reported to the MU-BIC' between 0600 and 0800 after an overnight fast to complete the 10-h testing day. Each participant was seated in a reclining chair and, for the next 30 min, acclimated to the room and familiarized herself with the testing day procedures. A catheter was then inserted into the antecubital vein of the non-dominant arm and kept patent by saline drip throughout the remainder of the day. At time −15 min, a baseline (fasting) blood sample was drawn, and a set of computerized questionnaires were completed. At time +0 min, the respective breakfast meal including 240 mL water was provided during the NP and HP days and only water was provided during BS. The participants consumed the meal and/or water within 30 min. Blood sampling was performed, and questionnaires were completed throughout the next 8 h. At +240 min (ie, 4 h postbreakfast), an NP standardized lunch meal was provided. The meal contained 500 kcal (15% protein, 65% carbohydrates, 20% fat) and consisted of a turkey sandwich, pudding, pretzels, and fruit. The participants were also provided with 240 mL of water. The participants consumed this meal within 30 min. At time +495 min (predinner), the catheter was removed and an fMRI brain scan was performed. After this procedure (+540 min), the participants were given dinner to eat ad libitum. After dinner, they were given a cooler containing various snacks to consume ad libitum, at home, throughout the remainder of the evening.

**Questionnaires**

Computerized questionnaires, which assessed perceived sensations (ie, hunger, fullness, desire to eat, prospective food consumption), were completed throughout each of the testing days (Figure 1). In addition, at the end of each breakfast meal, a palatability (ie, “Overall, how much do you like this meal”) questionnaire was given. The questionnaires contained visual analog scales incorporating a 100-mm horizontal line rating scale for each response. The questions are worded as “How strong is your feeling of” with anchors of “not at all” to “extremely.” The Adaptive Visual Analog Scale Software was used for data collection (Neurobehavioral Research Laboratory and Clinic).

**Repeated blood sampling and hormonal analyses**

Twenty blood samples (4 mL/sample, 80 mL testing day) were collected throughout the testing day (Figure 1). The samples were collected in test tubes containing EDTA and protease inhibitors [4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (CenterChem Inc) and dipetidyl peptidase-4 inhibitor (Millipore)] to reduce protein degradation. Within 10 min of collection, the samples were centrifuged at 4°C for 10 min. The plasma was separated and stored in microcentrifuge tubes at −80°C for future analysis. Plasma active ghrelin and total peptide YY (PYY) were measured by using the Milliplex MAP magnetic bead–
based multianalyte, metabolic panel, 4-plex assay (Millipore) and Magpix Luminex technologies (Luminex Corporation).

**fMRI to assess brain activation**

Brain activation responses were assessed before dinner on each of the 3 testing days. During the fMRI brain scan procedure, the participants were placed in a supine position on the sliding MRI table. A structural scan was performed to identify anatomic structures. This scan lasted ~10 min. During the fMRI phase, the participants focused on a set of photographs that were projected onto a screen and easily viewed through a mirror. The fMRI paradigm, used in our previous study (16), incorporated stimuli from 3 categories of pictures, including food, nonfood (animals), and blurred baseline images. The pictures from each category were presented in blocks of images. Ten photographs (of the same type of stimuli) were presented per block. The scan involved 3 repetitions of each block of stimulus-producing images (ie, food, animals), alternated with blocks of randomized blurred images. Each photograph was projected for 2.5 s, with an interstimulus interval of 0.5 s. There was a total of 13 blocks of stimuli presented. Individual pictures were randomly assigned to appropriate blocks and were never repeated. Animal pictures were used to control for visual richness and general interest (ie, appealing but not appetizing). The functional scan lasted ~7 min and was performed in duplicate. Scanning was performed at the MU-BIC on a 3-Tesla Siemens Trio scanner (Siemens Medical Solutions).

**Ad libitum dinner**

The ad libitum dinner occurred 5 h after lunch and consisted of microwavable chicken parmesan pizza pockets. A single pocket contained 290 kcal (14% protein, 64% carbohydrate, and 22% fat). The participants were presented with 4 pockets cut into sections to blind them to the quantity being consumed. They were instructed to eat as much as they desired until feeling “comfortably full” within a 30-min timeframe. Additional pocket sections were provided as needed. All contents were separately weighed before the meal, and any remains were weighed after the meal to determine the amount consumed. Total dinner energy intake and macronutrient composition were then determined.

**Ad libitum evening snacks**

After dinner was completed, the participants were provided with a cooler containing the following snack foods to consume ad libitum, at home, throughout the evening: cookies, cakes, granola bars, candy (hard, chocolate, gummy), nacho chips, popcorn, crackers, pretzels, microwavable macaroni and cheese, string cheese, apple slices, grapes, carrots, snack-size ice cream, beef jerky, yogurt, and microwavable pizza pockets. The cooler contained a total of 4000 kcal. All food items were initially recorded and weighed. The participants were instructed to return all uneaten foods as well as all wrappers and containers from the snacks that were consumed. Any partially eaten, returned items were weighed accordingly. The foods were then retrospectively grouped according to the following food categories: desserts, candy, salty foods, chocolate, high fat (ie, >5 g/serving), and healthy (ie, fruit, vegetables, and low-fat dairy and meat).

**Data and statistical analysis**

Power analyses were performed before the start of the study to identify appropriate sample size. The effect size (ES) of the protein-related changes after the breakfast treatments from our pilot studies (15, 16) were determined for the following outcomes: postbreakfast perceived hunger (ES = 1.48), postbreakfast PYY (ES = 1.50), subsequent meal energy intake (ES = 0.58), and prelunch neural activation in the amygdala (ES = 0.91). These ESs indicated that a sample size of n = 20, α = 0.05, would provide >80% power to detect differences between breakfast treatments.

To assess the perceived appetite, satiety, and hormonal responses over a total of 8 h, AUC was calculated from the fasting (baseline) time point and the postbreakfast time points for each outcome. We further divided the testing days into 4 time segments to represent early and late morning (ie, 0–120 min postbreakfast and 120–240 min, respectively) as well as early and later afternoon (ie, 240–360 min postbreakfast and 360–480 min postbreakfast, respectively). With all AUC measurements, the trapezoidal rule was used (18). With regard to the ad libitum evening snacks, total energy content, macronutrient composition, and types of foods consumed were determined.

A repeated-measures ANOVA was then used to compare main effects of treatment, time, and treatment × time interactions for the perceived sensations and hormonal responses. When main effects were detected, pairwise comparisons using the least significant difference test were applied to compare differences between treatments, time segments, and interactions. A repeated-measures ANOVA was used to compare main effects of treatment of the energy content, macronutrient composition, and/or food categories from the ad libitum dinner and evening snacking. When main effects were detected pairwise comparisons using the least significant difference test were applied.

The brain activation responses were analyzed by using the Brain Voyager QX (version 2.2) statistical package and random effects (Brain Innovation). Preprocessing steps included trilinear three-dimensional motion correction, sync-interpolated slice scan-time correction, two-dimensional spatial smoothing with 4-mm Gaussian filter, and high-pass filter temporal smoothing. Functional images were realigned to the anatomic images obtained within each session and standardized by using Brain Voyager Talairach transformation, which conforms to the space defined by the Talairach and Tournoux’s stereotaxic atlas (19). Functional scans were discarded if head movement was >3 mm along any axis (x, y, or z). To determine the effects of breakfast on neural activity associated with food motivation, a repeated-measures ANOVA was performed on the brain activation maps within the Brain Voyager software with the use of stimulus [food (ie, appetizing and appealing) compared with nonfood (ie, animal, nonappetizing but appealing) × breakfast (BS compared with NP; BS compared with HP)] comparisons. To identify whether the macronutrient composition of the breakfast meal would differentially affect the neural responses, a repeated-measures ANOVA was again performed with the use of a stimulus (ie, food compared with nonfood) × breakfast (NP compared with HP) comparison. Variables representing the experimental conditions were modeled with a hemodynamic response filter and entered into the model with the use of random effects. Contrast between conditions was assessed with t-statistics with the use of random
effects. On the basis of previous research with this paradigm, a priori regions of interest included the amygdala, hippocampal formation (hippocampus and parahippocampal cortex), cingulate, insula, striatum, orbitofrontal cortex, and prefrontal cortex (16, 20, 21). To identify significant activations in a priori regions, a cluster-level statistical threshold was applied to correct for multiple comparisons (22, 23). By using this approach, significance was set at $P = 0.01$, with a cluster-level false-positive rate of $\alpha = 0.05$.

With regard to the regions-of-interest data analysis, follow-up analyses of a priori regions of interest were conducted in regions noted above that achieved significance in the breakfast-pattern analyses.

Current data, albeit limited, suggest that menstrual cycle phase influences appetite control and food intake regulation (24–27). Although we were unable to schedule all testing days during the follicular phase of each participant’s menstrual cycle, we completed the following procedures to explore potential effects. Menstrual cycle day for each testing day for each participant was first documented and subsequently included as a potential covariate by using a mixed-factor ANOVA. However, our analyses indicated that menstrual cycle phase was not found to act as a covariate with study outcomes; thus, the data are reported as unadjusted means.

Analyses were conducted by using the Statistical Package for the Social Sciences (version 19.0; SPSS Inc). $P < 0.05$ was considered to be significant. All data are reported as means ± SEMs.

RESULTS

Perceived hunger and fullness (satiety)

The perceived hunger and fullness responses completed every 30 min throughout each of the 3 breakfast patterns are depicted in the line graphs in Figure 2, whereas the bar graphs depict the AUC analyses for key periods across the day and total daily response.

Repeated-measures ANOVA showed significant main effects of treatment ($P < 0.001$), time ($P < 0.001$), and a treatment × time interaction ($P < 0.001$) on perceived hunger responses. Post hoc pairwise comparisons showed that the NP and HP breakfast meals led to a 60% reduction in daily hunger (ie, total AUC) compared with BS ($P < 0.001$). No differences in total hunger AUC were observed between breakfast meals. Regardless of treatment (ie, time effect), perceived hunger progressively increased throughout the morning (early compared with late morning; $P < 0.05$), declined throughout the afternoon (early compared with late afternoon; $P < 0.05$), and remained lower in the afternoon than in the morning ($P < 0.01$). In addition, the greatest increase in hunger was observed during late morning compared with other times (all comparisons; $P < 0.05$). When comparing treatment differences across specific time segments (ie, treatment × time interactions), early morning, late morning, and early afternoon hunger were significantly lower after the NP and HP breakfast meals compared with BS (each segment; $P < 0.005$). No differences were observed between the breakfast meals for any of the time segments.

Repeated-measures ANOVA showed significant main effects of treatment ($P < 0.01$), time ($P < 0.001$), and a treatment × time interaction ($P < 0.001$) on perceived fullness responses. Post hoc pairwise comparisons showed that both NP and HP breakfast meals led to increased fullness throughout the day (ie, total AUC) compared with BS ($P < 0.001$). When comparing meals, HP led to a greater increase in total fullness (30% increase) compared with NP (10% increase; $P < 0.03$). With regard to the time effect, although no differences in early compared with late morning or early compared with late afternoon fullness were detected, fullness was lower in the morning than in the afternoon ($P < 0.001$). When examining treatment differences across specific time segments (ie, treatment × time interactions), early and late morning fullness was significantly higher after the NP and HP breakfast meals than after BS (both segments; $P < 0.005$). The HP breakfast, but not the NP breakfast, led to higher early afternoon fullness compared with BS ($P < 0.005$). When comparing meals, the HP breakfast led to greater early and late afternoon fullness compared with the NP breakfast ($P < 0.005$).

Desire to eat and prospective food consumption

The perceived desire to eat and prospective food consumption responses completed every 30 min throughout each of the 3 breakfast patterns are shown in the line graphs in Figure 3, whereas the bar graphs depict the AUC analyses for key periods across the day and total daily response.

Repeated-measures ANOVA showed significant main effects of treatment ($P < 0.001$), time ($P < 0.001$), and a treatment × time interaction ($P < 0.005$) on desire to eat and prospective food consumption. Post hoc pairwise comparisons showed that the NP and HP breakfast meals led to a 30% reduction in daily desire to eat and prospective food consumption (ie, total AUC) compared with BS ($P < 0.003$). No differences in total desire to eat or prospective food consumption AUC were observed between breakfast meals. Regardless of treatment (ie, time effect), desire to eat and prospective food consumption progressively increased throughout the morning (early compared with late morning; $P < 0.05$), declined throughout the afternoon (early compared with late morning; $P < 0.05$), and remained lower in the afternoon than in the morning ($P < 0.01$). In addition, the greatest increase in desire to eat and prospective food consumption was observed during late morning compared with other times (all comparisons; $P < 0.05$). When comparing treatment differences across specific time segments (ie, treatment × time interactions), early and late morning desire to eat and prospective food consumption were significantly lower after the NP and HP breakfast meals compared with BS (both segments; $P < 0.05$). The HP breakfast, but not the NP breakfast, led to lower early and late afternoon desire to eat and prospective food consumption compared with BS (both segments; $P < 0.05$). No differences were observed between the breakfast meals.

Hormonal responses

The ghrelin and PYY responses completed every 30 min throughout each of the 3 breakfast patterns are depicted in the line graphs in Figure 4, whereas the bar graphs depict the AUC analyses for key periods across the day and total daily response.

Repeated-measures ANOVA showed significant main effects of treatment ($P < 0.05$), time ($P < 0.001$), and a treatment × time interaction ($P < 0.05$) with plasma ghrelin concentrations. Post
FIGURE 2. Perceived hunger (A) and fullness (B) responses throughout the testing days in 20 adolescent girls. The line graph displays the time course of change throughout the 10-h days in the BS (□), NP (△), and HP (●) patterns; the bar graphs depict total and specific time segment AUCs across the day. Post hoc pairwise comparison analyses were performed when main effects and interactions were detected. Different lowercase letters denote significance ($P < 0.05$) between testing days. The △ on the $x$-axis denotes the breakfast meal; the ▲ on the $x$-axis denotes the lunch meal. BS, breakfast skipping; HP, high protein; NP, normal protein.
FIGURE 3. Desire to eat (A) and preoccupation with thoughts of food (B) throughout the testing days in 20 adolescent girls. The line graph displays the time course of change throughout the 10-h days in the BS (■), NP (▲), and HP (●) patterns; the bar graphs depict total and specific time segment AUCs across the day. Post hoc pairwise comparison analyses were performed when main effects and interactions were detected. Different lowercase letters denote significance between testing days (P < 0.05). The △ on the x-axis denotes the breakfast meal; the ▲ on the x-axis denotes the lunch meal. BS, breakfast skipping; HP, high protein; NP, normal protein; PFC, prospective food consumption.
hoe analyses showed that the HP breakfast, but not the NP breakfast, led to a 20% suppression in the daily ghrelin response (ie, total AUC) compared with BS (P < 0.05). However, no difference in total ghrelin AUC was observed between breakfast meals. Regardless of treatment (ie, time effect), plasma ghrelin concentrations progressively increased throughout the morning (early compared with late morning; P < 0.05), declined in the early afternoon (late morning compared with early afternoon; P < 0.001), and increased throughout the remainder of the day (early compared with late afternoon; P < 0.005). In addition, the lowest ghrelin response was observed during early morning compared with other times (all comparisons; P < 0.05). When comparing treatment differences across specific time segments (ie, treatment × time interactions), early morning ghrelin was lower after the NP and HP meals than after BS (P < 0.05). The HP meal, but not the NP meal, also led to lower early and late afternoon ghrelin compared with BS (both segments; P < 0.05). Between breakfast meals, early afternoon ghrelin was lower after HP than after NP (P < 0.05).

Repeated-measures ANOVA tended to show main effects of treatment (P = 0.11), time (P < 0.005), and a treatment × time interaction (P = 0.083) on PYY concentrations. Post hoc pairwise comparisons showed that the HP breakfast, but not the NP breakfast, led to a 250% elevation in total AUC for PYY compared with BS (P < 0.05). No differences in total AUC for PYY were observed between the breakfast meals. Regardless of treatment (ie, time effect), PYY concentrations progressively increased throughout the day, with the highest concentrations in the late afternoon compared with all other time segments (all comparisons; P < 0.05). When comparing treatment differences across specific time segments (ie, treatment × time interactions), the HP breakfast, but not the NP breakfast, led to early morning (P < 0.05), late morning (P = 0.08), early afternoon (P = 0.09), and late afternoon (P = 0.07) increases in PYY compared with BS. When comparing meals, the early morning and late afternoon segments after the HP breakfast were higher compared with the NP breakfast meal (both segments; P < 0.005).

**Brain activation before dinner**

As shown in Figure 5, contrast maps of the brain activations reaching significance when contrasting food greater than non-food when comparing BS with breakfast (panel A) or NP compared with HP (panel B). Predinner brain activity in response to food stimuli was greater in the amygdala, hippocampus, and midfrontal gyrus regions when breakfast was skipped compared with consuming either of the breakfast meals (Figure 5A, all contrasts; P < 0.01). When comparing the predinner responses between NP and HP breakfast meals, greater activations were observed in the hippocampus and parahippocampus regions before dinner with the NP compared with the HP breakfast meals (Figure 5B, all contrasts; P < 0.01).

**Energy intake assessments**

Energy intake across the day is shown in Figure 6. The breakfast and lunch meals were held constant at 350 and 500 kcal, respectively, whereas dinner and snacks were consumed ad libitum. No significant difference in energy content of the ad libitum dinner meal was observed between treatments (HP: 787 ± 50 kcal; NP: 820 ± 71 kcal; BS: 845 ± 60 kcal). However, with ad libitum snacking, BS and NP led to greater evening snacking (656 ± 108 and 621 ± 110 kcal, respectively) compared with HP (486 ± 84 kcal; both P < 0.05). The difference in snacking was primarily due to fewer high-fat snacks consumed after the HP meal (17.4 ± 3.6 g fat) compared with BS (25.0 ± 4.1 g fat; P < 0.05) or the NP meal (23.8 ± 4.4 g fat; P < 0.05) (see Supplemental Table 1 under “Supplemental data” in the online issue).

With regard to daily intake, BS led to similar daily intake (2002 ± 111 kcal) compared with HP (2123 ± 71 kcal). However, NP led to greater daily intake (2292 ± 115 kcal) compared with BS (P < 0.003) and HP (P < 0.05). When expressed as dietary compensation, 65% of the energy content of the HP breakfast was compensated for throughout the day, whereas only 17% of the NP meal was compensated for throughout the day.

**DISCUSSION**

The consumption of 350-kcal breakfast meals led to daily reductions in perceived hunger, desire to eat, and prospective food consumption; daily increases in perceived fullness; and reduced dinnertime neural activation in select corticolimbic brain regions that control food motivation/reward in overweight/obese BS teens. Additional benefits were observed with the consumption of the HP beef- and egg-based breakfast compared with the NP cereal-based version. Specifically, the HP breakfast led to greater increases in daily perceived fullness and greater reductions in corticolimbic activation compared with the NP breakfast. Furthermore, only the HP breakfast led to daily reductions in the hunger-stimulating hormone ghrelin, increases in the satiety hormone PYY, and reductions in evening snacking, particularly of high fat foods, compared with skipping breakfast. Thus, the addition of breakfast, particularly one rich in protein, might be an important dietary strategy to improve satiety, reduce food motivation/reward, and improve diet quality in overweight/obese teen girls.

One of the key contributors to the adolescent obesity epidemic is the evidence showing that overweight/obese teens are highly sensitive to the modern food environment, which provides overexposure and easy access to highly palatable, energy-dense foods (28–30). This sensitivity is supported by the fact that young people consume nearly half of their daily calories between 1600 and 2400 h; the snack foods often craved and consumed consist of highly palatable, but calorically dense foods with little nutritional value (eg, desserts, candy, chips) (31). These habits contribute substantially to the shift away from eating according to physiologic need toward reward-driven eating, the latter of which leads to positive energy balance and obesity (28). Skipping breakfast exacerbates the desire to snack. Adolescents who skip breakfast typically snack on more desserts, high-fat salty foods, and sodas compared with breakfast consumers (5–7). Data from the current study support these findings by showing that skipping breakfast compared with eating breakfast leads to greater evening snacking on high-fat foods. However, the reduction in unhealthy snacking was evident only after the HP breakfast meal.

The consumption of dietary protein appears to modulate key gastrointestinal hormones, which, in turn, provide signals to the central, homeostatic, neuronal pathways of the nucleus tractus solitarius of the brainstem and the arcuate nucleus of the hypothalamus to alter appetite, satiety, and ultimately regulate
FIGURE 4. Ghrelin (A) and PYY (B) responses throughout the testing days in 20 adolescent girls. The line graph displays the time course of change throughout the 10-h days in the BS (○), NP (▲), and HP (●) patterns; the bar graphs depict total and specific time segment AUCs across the day. Post hoc pairwise comparison analyses were performed when main effects and interactions were detected. Different lowercase letters denote significance between testing days ($P < 0.05$). The △ on the x-axis denotes the breakfast meal; the ▲ on the x-axis denotes the lunch meal. BS, breakfast skipping; HP, high protein; NP, normal protein; PYY, peptide YY.
Data from our laboratory and others indicate that the consumption of HP meals (containing 28–92 g of protein) leads to postmeal reductions in the hunger-stimulating hormone ghrelin and/or increases in the satiety-stimulating hormone PYY, which are accompanied by reductions in perceived hunger and increases in satiety compared with NP meals (10, 11, 13, 15, 33–38). Unfortunately, many of these studies incorporate a breakfast preload design, which only assesses 4-h postmeal responses. The current study design allowed us to examine sustained effects over the course of an 8-h day. By using this approach, we found that both breakfast meals equivalently led to reductions in perceived hunger, desire to eat, prospective food consumption, and plasma ghrelin along with increases in perceived fullness throughout the early and/or late morning periods. However, only the HP breakfast led to sustained alterations in perceived desire to eat, prospective food consumption, fullness, and plasma ghrelin into the afternoon periods. Last, plasma PYY was elevated throughout the morning and afternoon periods but only after the HP breakfast. These data support the role of increased dietary protein at the morning meal to provide immediate and/or sustained improvements in the appetitive and hormonal signals that control food intake regulation.

Limitations

Despite the appetitive, hormonal, and neural alterations with the addition of a 350-kcal HP breakfast, daily intake, albeit nonsignificant, was greater (~120 kcal) compared with when breakfast was skipped. Thus, the HP breakfast was only partially compensated for by the end of the day. Because this was an acute study with only 6 acclimation days, it is plausible that habitual consumption of an HP breakfast might lead to complete compensation or overcompensation of the breakfast meal. Over the longer term, this may lead to reductions in daily energy intake (see reference 32 and references 10, 13, and 15).

![Brain activation contrast maps before dinner after the BS, NP, and HP testing days in 20 adolescent girls; all contrasts represent food greater than nonfood (animal) fMRI scans. Repeated-measures ANOVA examining the main effect of treatment with post hoc pairwise comparison analyses were performed when a main effect was detected. P < 0.01 represented significance. BS, breakfast skipping; COR, coronal view; HP, high protein; NP, normal protein.](image-url)
intake and weight loss. This hypothesis is supported by cross-sectional data showing that BS is strongly associated with overeating, weight gain, and obesity (2, 3, 40, 41). Furthermore, although daily intake was not reduced after the HP breakfast, fewer high-fat evening snacks were consumed. These data suggest that eating an HP breakfast might improve diet quality by replacing unhealthy foods (consumed in the evening) with healthier, nutrient-rich foods at breakfast. Further work to identify chronic effects, feasibility, and the effectiveness of a long-term breakfast intervention is warranted.

Although differences in the neural responses to the breakfast treatments were detected in the current study, several activation clusters were much smaller (ie, 10–140 voxels) compared with those in our previous study (ie, 32–260 voxels) (16). These differences are likely due to the time differential from when the fMRI was performed (ie, 8 h postbreakfast in our current study compared with 4 h postbreakfast in our previous study).

A key factor that may have partially blunted the protein and/or breakfast responses includes the incorporation of a high-carbohydrate/low-protein lunch meal provided during each testing day. Although we found improvements in satiety throughout the day, along with reduced reward-driven eating behavior and snacking in the evening, some of the protein effects of breakfast may have been blunted due to the lunch meal. Thus, a potential next step is to assess whether an HP breakfast, alone or in combination with an HP lunch, leads to synergistic and more robust effects.

Conclusions

Compared with skipping breakfast or consuming an NP cereal-based breakfast, an HP breakfast, containing 35 g of high-quality beef and egg protein, beneficially altered key physiologic (energy-driven) and nonphysiologic (reward-driven) signals that control food intake regulation. Although daily intake was not reduced after the HP breakfast, fewer high-fat evening snacks were consumed. Collectively, these data suggest that the daily addition of breakfast, particularly one rich in protein, appears to be an appropriate strategy to improve satiety, reduce food motivation/reward, and improve diet quality by replacing unhealthy evening snacking with nutrient-rich foods at breakfast in overweight/obese teen girls.

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The authors’ responsibilities were as follows—HJL: designed the research, wrote the manuscript, and had primary responsibility for the final content; and HJL, LCO, SMD, and HAH: conducted the research and analyzed the data. All of the authors substantially contributed to the completion of the manuscript and read and approved the final version. The Beef Checkoff and the Egg Nutrition Center/American Egg Board supplied the funds to complete the study but were not involved in the design, implementation, analysis, or interpretation of data. None of the authors had any conflicts of interests.

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