

# Examination of Protein Quantity and Protein Distribution across the Day on Ad Libitum Carbohydrate and Fat Intake in Overweight Women

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## Abstract

**Background:** The effects of meal-specific protein quantity and protein distribution throughout the day on daily food intake are relatively unknown.

**Objectives:** The aims were to test 1) whether the consumption of higher-protein (HP) compared with normal-protein (NP) meals consumed at each eating occasion reduce free-living, daily carbohydrate and fat intakes in overweight women during energy balance conditions and 2) whether the distribution of protein consumed throughout the day affects food intake outcomes.

**Methods:** Seventeen women [mean  $\pm$  SEM age: 33  $\pm$  1 y; body mass index (in kg/m<sup>2</sup>): 27.8  $\pm$  0.1] completed the following tightly controlled, crossover design study. Participants were provided with and randomly consumed three 6-d eucaloric diets containing NP or HP (15% or 25% of energy as protein, respectively). The protein content within the NP diet used an even distribution pattern (EVEN; 21  $\pm$  1 g protein/meal) throughout the day, whereas the protein contents within the HP diets used either EVEN (35  $\pm$  1 g protein/meal) or an uneven distribution pattern (UNEVEN; 19  $\pm$  1 g protein/breakfast, 26  $\pm$  1 g protein/lunch, 63 g protein/dinner). On day 7 of each diet, the participants were asked to consume the diet-specific absolute protein quantity (in grams) at each eating occasion but were provided with a surplus of carbohydrate- and fat-rich foods to consume, ad libitum, during each eating occasion.

**Results:** Eating more protein (HP compared with NP) or evenly distributing protein throughout the day (HP-EVEN compared with HP-UNEVEN) did not reduce the consumption of ad libitum fat- and carbohydrate-rich foods throughout the day (NP-EVEN: 2850  $\pm$  240 kcal/d; HP-EVEN: 2910  $\pm$  240 kcal/d; HP-UNEVEN: 3160  $\pm$  200 kcal/d). Despite the lack of differences in daily energy intake, the breakfast meal within the HP-EVEN diet led to lower ad libitum carbohydrate and fat intakes than the breakfast meals in the NP-EVEN and HP-UNEVEN diet conditions ( $P < 0.05$ ).

**Conclusion:** Providing 30 g protein/meal at each eating occasion throughout the day did not influence free-living, daily intake of highly palatable, carbohydrate- and fat-rich foods in overweight women. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02614729. *Curr Dev Nutr* 2017;1:e001933.

## Introduction

Over the past 10 y, many acute studies have identified the effects of consuming single, higher-protein (HP) compared with normal-protein (NP) meals on appetite control, satiety, and subsequent food intake (1, 2). The majority of studies detected postprandial increases in satiety, as shown through increased postprandial fullness and peptide YY (PYY) responses after the consumption of HP or NP meals (1, 2). Despite the consistent satiety effects, <20% of these



**Keywords:** high-protein diets, food choice, food intake, ad libitum, protein distribution, overweight, women

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Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://cdn.nutrition.org>.

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Abbreviations used: EVEN, even distribution pattern; HP, higher-protein; NP, normal-protein; UNEVEN, uneven distribution pattern.

studies reported reductions in energy intake at the next eating occasion after the HP or NP meals (1). The disconnect between the satiety and energy intake data lends further support that postprandial appetitive sensations do not serve as a proxy for subsequent food intake (3).

Alternatively, it has also been suggested that assessing subsequent food intake during a single ad libitum homogeneous test meal or assorted buffet within a laboratory setting is not generalizable to the habitual consumption of meals across the day in a free-living environment (4). However, limited data exist with respect to whether increased protein consumption at each eating occasion reduces ad libitum food intake across the entire day.

Another important factor to consider is the amount of protein provided within each eating occasion. A retrospective analysis from our laboratory suggests that the consumption of 30 g protein in a single meal elicits greater satiety than do quantities ranging from 15 to 25 g protein (5). Whether the consumption of 30 g protein consumed during single eating occasions reduces within-meal and end-of-meal food intake of other foods has not been examined in the current literature. Thus, the purpose of this study was to examine the effects of protein quantity and protein distribution, with a focus on 30 g protein provided during breakfast, lunch, and dinner, on free-living, ad libitum carbohydrate and fat intakes during energy balance conditions in healthy overweight women.

## Methods

### Experimental design

A randomized crossover design study was completed in overweight, sedentary, but otherwise healthy women. Seventeen participants completed 3 tightly controlled 6-d eucaloric diets containing NP or HP (15% or 25% of daily energy from protein, respectively) in 1 of 6 randomly assigned orders. The protein content within the NP diet used an even distribution pattern (EVEN) throughout the day, whereas the protein contents within the HP diets used either EVEN or an uneven distribution pattern (UNEVEN), with less protein consumed in the morning and greater quantities consumed at dinner. The UNEVEN protein content distribution was representative of typical protein intake patterns observed in the United States (6). On day 7, absolute protein consumption (in grams) was maintained within each respective diet, but carbohydrate- and fat-rich foods were provided, ad libitum, as “side dishes” and “desserts” within a free-living environment. Meal and daily energy and macronutrient contents were assessed. Each of the diets occurred during the follicular phase of the menstrual cycle; thus, there were 2- to 3-wk washout periods between diets. The diet randomization scheme was determined by using Research Randomizer ([www.researchrandomizer.org](http://www.researchrandomizer.org)) (7).

### Study participants

From January 2014 to May 2015, healthy, sedentary, but overweight women were recruited through advertisements, flyers, and e-mail listservs to participate in the study. Participants were eligible to participate if they met the following criteria: 1) female; 2) aged 18–52 y; 3) overweight [BMI (in kg/m<sup>2</sup>): 25–29.9]; 4) sedentary (<8000 steps/d); 5) normal menstrual cycles (between 26 and 32 d; 5 in

past 6 mo); 6) nonsmoking (for the past year); 7) no metabolic, hormonal, or neural conditions or diseases that influence metabolism, appetite, or cognition; 8) no past history of surgical interventions for the treatment of obesity; 9) no weight loss or gain ( $\geq 4.5$  kg in the past 6 mo); 10) no medication that would directly influence appetite or cognition; 11) no change in any medications (over the past 3 mo); 12) consuming  $\leq 800$  mg caffeine/d and of this,  $\leq 260$  mg caffeine consumed before lunch; 13) not pregnant (or planning to become pregnant); 14) not currently or previously following a specific diet, including high-protein, vegan, vegetarian, etc.; 15) conventional (typical) and consistent sleep patterns (i.e., awake hours between 0500 and 2300 with no afternoon naps; rating quality of sleep as fairly to very good on the Pittsburgh Sleep Quality Index); averaging  $\geq 6$  sleep hours/night over the past month; 16) not clinically diagnosed with an eating disorder; 17) having a score of  $< 4$  on the Three-Factor Eating Questionnaire; 18) having a Profile of Mood States, Second Edition–Depression-Dejection Scale score within 1.5 SDs of the age-, sex-, and race-specific normative mean (8, 9); 19) no allergies or aversions to the study foods; 20) no history of drug or alcohol abuse; 21) habitually consuming breakfast, lunch, and dinner; 22) willing to maintain current inactivity patterns throughout the study; and 23) generally healthy, as assessed from the medical history questionnaire.

Women were the target population in this study to maintain a single-sex population, because it has been shown that ingestive behavior differs among men and women (10, 11). In addition, women typically have a lower consumption of daily protein and consume less animal-based protein (12); thus, examining the effects of increased animal-based protein consumption in women is warranted.

Fifty women were screened for the study; 17 met the screening criteria and signed the study consent and 16 completed all study procedures. Participant characteristics of those who completed the study are presented in **Table 1**. In general, the women were healthy, sedentary, and overweight. The study purpose, procedures, and risks were explained before participants signed the consent forms. The University of Missouri institutional review board approved this study, and all procedures were followed in accordance with the ethical standards of the institutional review board. The participants were compensated for completing all study procedures. The study was registered at [clinicaltrials.gov](http://clinicaltrials.gov) (NCT02614729).

### Baseline

To establish energy needs, the participants reported to our facility over a 5-d period to complete the following procedures. During visit 1, the participants arrived in the morning in a fasted condition and height and weight were measured. A questionnaire documenting the previous night's sleep was completed, and they were given an accelerometer (BodyMedia Sense Wear) to wear on the nondominant arm over the next 3 consecutive days to estimate physical activity and sleep duration. The participant was then taken to a self-contained, comfortable and quiet, dimly lit room for the resting metabolic rate measures. The participants were placed in a reclined position and acclimated to room temperature, etc., for 30 min. The resting metabolic rate was then measured over a 30-min period by using an indirect calorimetry metabolic cart and metabolic hood

**TABLE 1** Characteristics of the women who completed the study<sup>1</sup>

	Value
Demographic information	
Sample size, <i>n</i>	16
Age, y	34 ± 2
Weight, kg	77.0 ± 1.8
Height, cm	167 ± 1
BMI, kg/m <sup>2</sup>	27.7 ± 0.4
Habitual activity, steps/d	6270 ± 2180
Weight-stable energy needs, kcal/d	1970 ± 120
Three-Factor Eating Questionnaire, AU <sup>2</sup>	2.6 ± 0.1
Habitual sleep, h/night	7.5 ± 0.7
Habitual dietary information <sup>3</sup>	
Total energy intake, kcal/d	2060 ± 140
Morning intake, % of total energy intake	20 ± 3
Midday intake, % of total energy intake	28 ± 2
Afternoon/evening intake, % of total energy intake	52 ± 3
Protein, g	80 ± 5
% of total energy intake	16 ± 0
Morning intake, % of total energy as protein	17 ± 3
Midday intake, % of total energy as protein	33 ± 3
Afternoon/evening intake, % of total energy as protein	50 ± 3
Carbohydrate, g	234 ± 17
% of total energy intake	46 ± 1
Fat, g	89 ± 7
% of total energy intake	39 ± 1

<sup>1</sup>Values are means ± SEMs unless otherwise indicated, *n* = 16.

<sup>2</sup>AU, arbitrary units.

<sup>3</sup>Intake was assessed from 3-d dietary recalls at baseline. Data were calculated by using Mifflin et al. (13), with an activity factor of 1.35.

(Parvo Medics). Over the next 2 d, the participants continued to wear the accelerometer. The resting energy expenditure and accelerometer data were used to estimate eucaloric daily intake (Table 1). The energy requirements were estimated to the nearest 100 kcal/d and used for the development of the diets. In addition, for 3 d (2 weekdays and 1 weekend day) the participants completed multipass dietary recalls to assess eating patterns and macronutrient contents of their habitual diets (Table 1).

### Dietary treatments

The participants completed the following tightly controlled feeding study in which 3 eucaloric diets were provided for 6 d/diet (Table 2). The NP diet contained 15% of daily energy as protein, 55% as carbohydrate, and 30% as dietary fat. The HP diets contained 25% of daily intake as protein, 45% as carbohydrate, and 30% as fat. The EVEN patterns included protein distribution of 27% protein within each meal and the remaining 17% within the evening snack. The UNEVEN pattern included protein distribution of 15% protein at breakfast, 20% at lunch, 50% at the dinner meal, and 15% during the evening snack. All of the diets included similar protein types, with 60% of protein within the diets as beef proteins and 40% from mixed plant proteins (primarily soy and gluten). Participants picked up the meals on the day before each 7-d testing period, and reheating instructions were provided. As a measure of compliance, the participants were required to complete meal- and snack-specific food inventory logs. The logs also provided instruction for when the meals and snack were to be consumed. In addition,

participants were instructed to consume only foods provided to them, document all deviations (i.e., foods not consumed or extra foods consumed), and return all wrappers and uneaten foods. For all study days, breakfast was consumed within 1 h after waking, lunch was consumed 4 h after breakfast, dinner was consumed 4 h after lunch, and the evening snack was consumed 2 h after dinner. Baseline dietary recall data indicated that all intermeal intervals for participants lasted 4 ± 1 h (mean ± SEM). Last, participants reported no concerns when adhering to the 4-h intermeal intervals during the 6-d acclimation periods of each treatment.

### Free-living, ad libitum feeding design

On day 7 of each pattern, each participant completed a free-living, ad libitum testing day. Within each eating occasion, participants were required to consume the same absolute protein quantity (in grams) provided on days 1–6 during each respective treatment (Table 2). In addition to consuming the required protein, participants were provided with an excess of carbohydrate- and fat-rich foods and were permitted to consume these, ad libitum, within each meal (see **Supplemental Table 1**). Participants were provided with meal- or snack-specific food inventory logs and instructed to consume the meals at the same times as in days 1–6. Within the instructions, the participants were required to only consume the respective foods within each meal time and then instructed to not eat those foods at any other time throughout the testing day (i.e., the breakfast skillet

**TABLE 2** Dietary characteristics of the study diets during days 1–6 in 16 women<sup>1</sup>

	NP-EVEN	HP-EVEN	HP-UNEVEN
Breakfast			
Energy content, kcal	560 ± 10	560 ± 10	550 ± 10
Total protein, g	21 ± 1	35 ± 1	19 ± 1
Carbohydrates, g	77 ± 1	63 ± 1	78 ± 1
Fat, g	19 ± 1	19 ± 1	19 ± 1
Lunch			
Energy content, kcal	560 ± 10	570 ± 10	560 ± 10
Total protein, g	21 ± 1	35 ± 1	26 ± 1
Carbohydrates, g	77 ± 1	63 ± 1	72 ± 1
Fat, g	19 ± 1	19 ± 1	19 ± 1
Dinner			
Energy content, kcal	560 ± 10	570 ± 10	570 ± 10
Total protein, g	22 ± 1	35 ± 1	63 ± 1
Carbohydrates, g	76 ± 1	63 ± 1	63 ± 1
Fat, g	19 ± 1	19 ± 1	19 ± 1
Evening snack			
Energy content, kcal	330 ± 10	320 ± 10	320 ± 10
Total protein, g	12 ± 1	20 ± 1	19 ± 1
Carbohydrates, g	44 ± 1	36 ± 1	36 ± 1
Fat, g	11 ± 1	11 ± 1	11 ± 1
Daily intake			
Energy content, kcal	2010 ± 10	2020 ± 10	2000 ± 10
Total protein, g	77 ± 1	125 ± 1	127 ± 1
Carbohydrates, g	274 ± 1	225 ± 1	221 ± 1
Fat, g	68 ± 1	68 ± 1	67 ± 1
Diet palatability, <sup>2</sup> mm	68 ± 4	62 ± 4	64 ± 4

<sup>1</sup>Values are means ± SEMs unless otherwise indicated, *n* = 16. EVEN, even distribution pattern; HP, higher-protein; NP, normal-protein; UNEVEN, uneven distribution pattern.

<sup>2</sup>A visual analog scale of 1–100 mm was included with end anchors of 1 = extremely dislike and 100 = extremely like.

was only allowed to be consumed at breakfast, etc.). Participants were asked to keep and return all uneaten foods and to not share their study foods. The foods and packaging were weighed before pack-out and re-weighed upon completion of day 7.

### Power, data, and statistical analyses

The differential response in daily energy intake from Weigle et al. (14) after the consumption of NP or HP control diets compared with an HP ad libitum diet was used to determine sample size estimates for the current study. The  $-472 \pm 90$  kcal reduction in daily intake after the HP ad libitum diet compared with the NP control diet led to an effect size ( $d$ ) of 1.17 and indicated that a sample size of  $n = 10$  would provide  $>80\%$  power to detect differences in daily intake. Furthermore, the reduction of  $-441 \pm 61$  kcal in daily intake after the HP ad libitum diet compared with the HP control diet led to an effect size ( $d$ ) of 1.08 and indicated that a sample size of  $n = 11$  would provide  $>80\%$  power to detect differences in daily intake. Thus, the inclusion of  $n = 16$  within the current study is sufficient to detect significant differences in our primary outcome of daily energy intake.

Summary statistics (sample means and SEMs) were computed for the following data: energy consumed within the required protein foods, ad libitum carbohydrate and fat foods, and total foods within each eating occasion and across the day. In addition, the ad libitum foods were categorized into “side dishes” consumed with the protein foods and “dessert” foods consumed at the end of the meal. A repeated-measures ANOVA was applied to identify main effects of protein quantity and protein distribution for all outcomes. When main effects were detected, pairwise comparisons were performed to identify differences between treatments. Analyses were conducted by using the Statistical Package for the Social Sciences (version 23.0; SPSS).  $P < 0.05$  was considered significant.

## Results

Daily intake for each single-day, ad libitum testing period (day 7) is shown in **Table 3**. By design, daily protein intake was greater with the HP-EVEN and HP-UNEVEN diets than with the NP-EVEN diet (both,  $P < 0.05$ ), with no differences between HP diets. However, eating more protein during each meal did not spontaneously reduce ad libitum energy consumed from fat- and carbohydrate-rich foods. In addition, protein distribution had no effect on daily ad libitum fat and carbohydrate intakes. When categorizing the fat- and carbohydrate-rich foods, no differences in the consumption of within-meal “side dishes” or end-of-meal “desserts” were detected between diets (data not shown.) Last, no differences in daily food intakes were detected between diets.

Specific meal comparisons were also performed and are shown in **Figure 1**. Again, by design, protein intake was greater at breakfast, lunch, and dinner after the HP-EVEN diet than after the NP-EVEN diet ( $P < 0.05$ ), whereas the HP-UNEVEN diet showed lower protein intake at breakfast and greater intake at dinner than did the HP-EVEN diet (both,  $P < 0.05$ ). Although no differences in ad libitum consumption of carbohydrate- and fat-rich foods were detected within the lunch and dinner meals of the study diets, the

**TABLE 3** Daily food intake throughout the free-living day 7 in 16 women<sup>1</sup>

	NP-EVEN	HP-EVEN	HP-UNEVEN
Required foods consumed			
Energy, kcal	509 ± 3 <sup>a</sup>	771 ± 10 <sup>b</sup>	744 ± 16 <sup>b</sup>
Protein, g	64 ± 1 <sup>a</sup>	102 ± 1 <sup>b</sup>	100 ± 2 <sup>b</sup>
Carbohydrate, g	21 ± 1 <sup>a</sup>	26 ± 1 <sup>b</sup>	23 ± 1 <sup>a</sup>
Fat, g	14 ± 1 <sup>a</sup>	26 ± 1 <sup>b</sup>	26 ± 1 <sup>b</sup>
Ad libitum foods consumed			
Energy, kcal	2340 ± 230 <sup>a</sup>	2140 ± 230 <sup>a</sup>	2410 ± 210 <sup>a</sup>
Protein, g	34 ± 3 <sup>a</sup>	31 ± 4 <sup>a</sup>	35 ± 3 <sup>a</sup>
Carbohydrate, g	338 ± 34 <sup>a</sup>	317 ± 34 <sup>a</sup>	349 ± 28 <sup>a</sup>
Fat, g	96 ± 10 <sup>a</sup>	85 ± 10 <sup>a</sup>	99 ± 10 <sup>a</sup>
Total foods consumed <sup>2</sup>			
Energy, kcal	2850 ± 240 <sup>a</sup>	2910 ± 240 <sup>a</sup>	3160 ± 200 <sup>a</sup>
Protein, g	98 ± 3 <sup>a</sup>	134 ± 4 <sup>b</sup>	135 ± 2 <sup>b</sup>
Carbohydrate, g	359 ± 34 <sup>a</sup>	343 ± 34 <sup>a</sup>	372 ± 28 <sup>a</sup>
Fat, g	111 ± 10 <sup>a</sup>	111 ± 10 <sup>a</sup>	125 ± 9 <sup>a</sup>

<sup>1</sup>Values are means ± SEMs unless otherwise indicated,  $n = 16$ . Data across columns were compared by using repeated-measures ANOVA with post hoc pairwise comparisons. Means without a common superscript letter differ,  $P < 0.05$ . EVEN, even distribution pattern; HP, higher-protein; NP, normal-protein; UNEVEN, uneven distribution pattern.

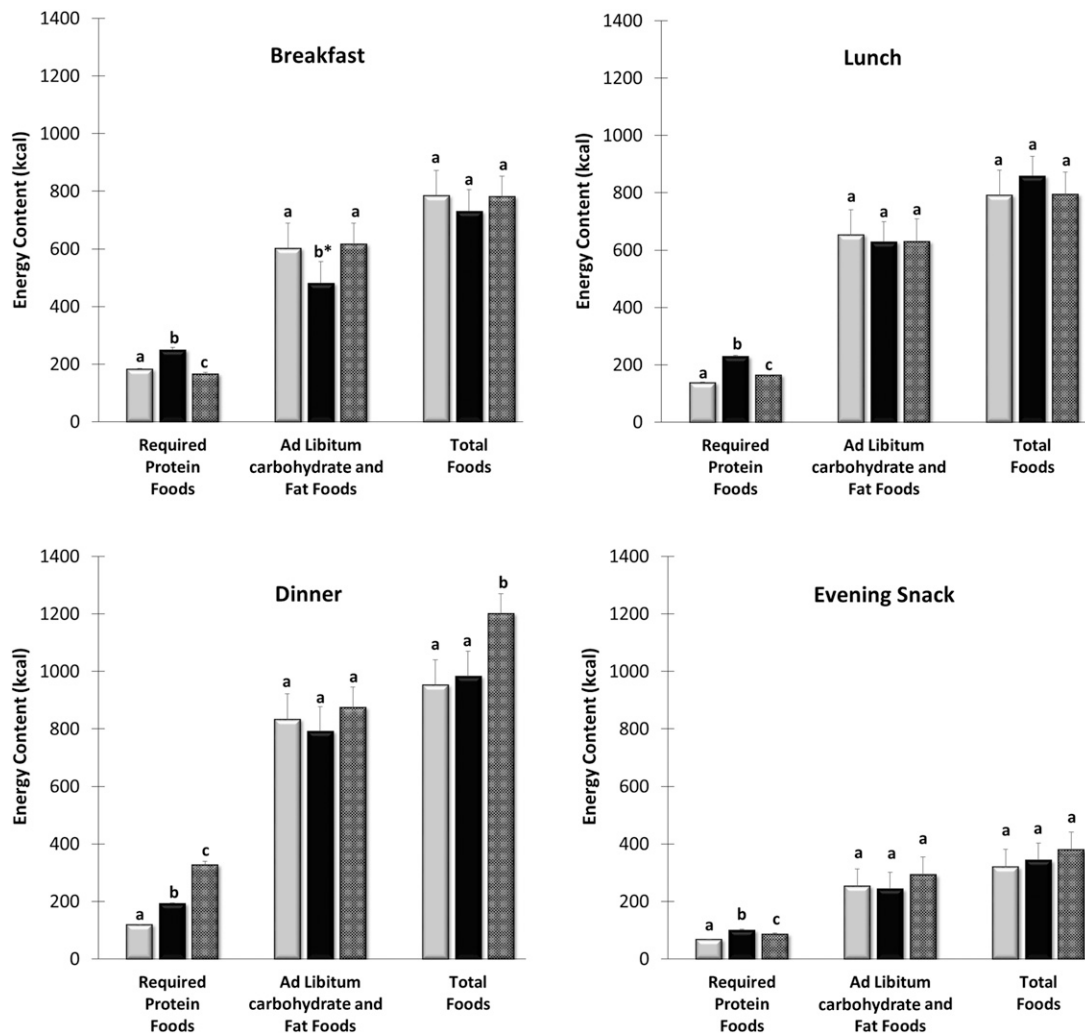
<sup>2</sup>Total foods consumed = required foods + ad libitum foods.

consumption of more protein at breakfast within the HP-EVEN diet led to lower carbohydrate and fat consumption at breakfast compared with the NP-EVEN diet ( $P$ -trend = 0.06) and HP-UNEVEN diet ( $P < 0.05$ ). Last, total energy at dinner was greater after the HP-UNEVEN diet than after the NP-EVEN and HP-EVEN diets (both,  $P < 0.05$ ). No other differences were detected.

## Discussion

Protein quantity and distribution across the day had no significant effect on daily consumption of ad libitum carbohydrate- and fat-rich foods during subchronic energy balance in overweight women. Although daily ad libitum carbohydrate- and fat-rich food consumption was not different between the diets, a modest reduction in carbohydrate- and fat-rich foods was observed within the HP breakfast meal compared with the NP breakfast meals, lending continued support for the benefits of consuming increased dietary protein during the first eating occasion of the day. Longer-term studies containing ad libitum assessments of within-meal “side dishes,” end-of-meal “desserts,” and between-meal “snack” comparisons are necessary to elucidate the effects of protein on reductions in daily food intake proposed as a potential mechanism to explain improved body-weight management, as has been reported for the consumption of HP diets (1).

A few studies have assessed the effects of increased dietary protein on ad libitum food intake across the day (14, 15); however, the experimental designs within these studies were quite different than that of the current study. As described in Skov et al. (15), 65 overweight and obese adults completed a randomized controlled trial in which they were prescribed a 6-mo diet with macronutrients set at specific percentages, although energy content of the diet varied. The HP diet consisted of 30% protein, 40% carbohydrate, and 30% fat, whereas the NP diet consisted of 12%



**FIGURE 1** Energy consumed at each eating occasion after the protein-specific requirements, depicted as NP-EVEN (light-gray bars), HP-EVEN (black bars), and HP-UNEVEN (dark-patterned bars). Repeated-measures ANOVA with post hoc pairwise comparisons was used. Bars without a common lowercase letter differ,  $P < 0.05$ . \*NP-EVEN compared with HP-EVEN,  $P = 0.06$ . EVEN, even distribution pattern; HP, higher-protein; NP, normal-protein; UNEVEN, uneven distribution pattern.

protein, 58% carbohydrate, and 30% fat. Because the participants were required to maintain specific macronutrient ratios, they were unable to select macronutrient-specific foods within a given meal. Thus, the ad libitum component included the ability to eat more (or less) of the “homogeneous” study foods to assess energy content changes while maintaining specific macronutrient ratios. The HP diet led to voluntary reductions in daily (energy) intake of  $\sim 450$  kcal/d compared with the NP diet (15). More recently, Weigle et al. (14) completed a prospective study in which 19 adults were prescribed an HP diet with a macronutrient composition of 30% protein, 50% carbohydrate, and 20% fat for 12 wk. Again, like Skov et al. (15), macronutrient composition was maintained over the study period; however, the participants were permitted to consume more (or less) of the study foods. Daily food intake decreased by  $441 \pm 63$  kcal after the HP diet.

The current study design did not lead to reductions in daily (energy) intake after the HP compared with after the NP diets. Potential reasons for the discrepant findings may be due to the

experimental design differences. First, the current study included a subchronic design with 6 d of controlled energy balance before each test day, unlike Skov et al. (15) and Weigle et al. (14) who used 6 mo and 12 wk, respectively. The shorter time frame of the current study may not have been long enough to elicit changes in ad libitum food intake in response to increased dietary protein. Although speculative, it is also plausible that the ability to choose highly palatable, high-fat or high-carbohydrate foods in an ad libitum fashion within the current study allowed the hedonic reward cues to override satiety to promote overeating (16). The high palatability and reward associated with carbohydrate- and fat-rich foods have been reported to be an underlying cause for nonhomeostatic overeating (17). More work is required to explore this concept.

Although increased protein consumption throughout the day did not reduce daily carbohydrate and fat intakes, modest reductions were detected within the HP breakfast meal. These data are in line with our previous work that showed greater postprandial satiety after HP breakfast meals than when protein was consumed at

lunch or dinner (6). The lack of reduction in daily intake in the current study might have been due to the absence of snacking opportunities, which has been shown to be reduced after the habitual consumption of HP breakfasts (18, 19).

Along these lines, there has been an increased focus on the distribution of protein across the day, because most Americans consume well below the 30-g protein recommendation at the breakfast meal and well above this amount at dinner (20). Protein consumption also tracks total energy consumed at each meal, with greater energy consumed at the dinner meal and less consumed at breakfast (20). The women in the current study also showed an UNEVEN dietary pattern at baseline, with greater energy consumed at dinner and lower energy at breakfast (Table 1). Protein distribution patterns at baseline were also similar to that of the UNEVEN pattern, with ~48% of protein consumed at dinner and ~12% at breakfast. Due to the satiety effects of 30 g protein (5), we hypothesized that the HP-EVEN pattern would reduce ad libitum intake compared with the HP-UNEVEN pattern. However, no differences in ad libitum carbohydrate and fat intakes or daily intake were detected between the HP distribution patterns. Interestingly, regardless of protein distribution, the participants voluntarily ate more food at dinner than at breakfast, with lunch energy intake being intermediate. Thus, the addition and redistribution of protein was not an effective strategy to alter habitual UNEVEN eating patterns. Instead of targeting an EVEN distribution pattern, there is some, albeit limited, data supporting an UNEVEN pattern that is skewed toward the consumption of more energy in the morning and less in the evening for greater weight loss compared with the UNEVEN pattern in the current study (21). Future studies that include this type of distribution in addition to increased dietary protein are needed to explore these strategies for weight management.

### Limitations

The current study included intervention periods of 6 d/diet. Thus, it is unclear whether the habitual consumption of increased dietary protein, evenly consumed across the day, would elicit improvements in daily food intake over the long term.

Our experimental design did not allow for the examination of intermeal ad libitum snacking, because the ad libitum carbohydrate- and fat-rich foods were provided within each meal and at the very end of the meal. All of the participants within this study showed snacking behavior at baseline, contributing to an average of  $16.5\% \pm 9.2\%$  of total daily energy intake as snacks. The satiety effects of protein typically remain as long as 4 h after a meal; thus, the inclusion of ad libitum snacking occasions between meals may have yielded different findings between the NP and HP diets.

In addition, the participants consumed ~900 more calories during day 7 than what they habitually consume. It is well documented that overeating occurs when the variety of the food provided is increased (22). Specifically, dopamine-stimulated food motivation is increased in response to novel food exposure and contributes to the increases in energy intake when a variety of new foods are present (22). Thus, it is possible that the novel foods presented within the ad libitum testing day 7 increased daily energy intake.

Postprandial appetite and satiety assessments were not completed during the free-living, ad libitum testing day for the following reasons. On the basis of the impact of external or environmental cues on ingestive behavior (23), it is possible that the continual acknowledgment of one's perceived hunger or fullness state (every 10–30 min throughout the day) might alter subsequent food intake. Thus, we chose to reduce any effects of appetitive cues and to assimilate the free-living environment typically void of these types of questions.

Finally, although power for this study was appropriately calculated on the basis of published evidence (14), the experimental design of the referenced study was of longer duration (i.e., 12 wk). Although similar reductions in daily intake were also detected within 24 h of following the HP ad libitum diet in the referenced study, it is possible that the current study was underpowered to detect differences in daily intake.

### Conclusions

These data suggest that protein quantity and protein distribution across the day have little impact on free-living, single-day, ad libitum food intake of highly palatable, carbohydrate- and fat-rich foods in overweight women. Longer-term studies examining the effects of protein quantity and distribution that assess within-meal, end-of-meal, and between-meal eating behaviors and food preferences are needed to identify the protein-related mechanisms of improved weight management observed with HP diets.

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