

# Adults Who Are Overweight or Obese and Consuming an Energy-Restricted Healthy US-Style Eating Pattern at Either the Recommended or a Higher Protein Quantity Perceive a Shift from "Poor" to "Good" Sleep: A Randomized Controlled Trial

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## ABSTRACT

**Background:** Limited evidence suggests that consuming a higher-protein diet during weight loss improves subjective indices of sleep in overweight and obese adults.

**Objective:** We sought to a priori assess the effects of consuming the recommended versus a higher protein Healthy US-Style Eating Pattern during energy-restriction on sleep quality indices.

**Design:** Using a randomized, parallel study design, 51 adults (mean  $\pm$  SEM age: 47  $\pm$  1 y; BMI: 32.6  $\pm$  0.5 kg/m<sup>2</sup>) consumed a controlled USDA Healthy US-Style Eating Pattern containing 750 kcal/d less than their estimated energy requirement for 12 wk. Participants were randomly assigned to consume either 5 or 12.5 oz-equivalent (eq)/d of protein foods. The additional 7.5 oz-eq/d came from animal-based protein sources and displaced primarily grains. Objective (wristworn actigraphy) and subjective (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale) sleep quality indices were measured at baseline, week 6, and week 12.

**Results:** Among all participants, body mass decreased ( $-6.2 \pm 0.4$  kg). Dietary protein intake did not affect any objective or subjective sleep quality outcomes measured (repeated measures ANOVA). Over time, objective measures of time spent in bed, time spent sleeping, sleep onset latency, and time awake after sleep onset did not change; however, sleep efficiency improved ( $1 \pm 1\%$ ; P = 0.027). Subjectively, global sleep scores [GSS:  $-2.7 \pm 0.4$  arbitrary units (au)] and daytime sleepiness scores ( $-3.8 \pm 0.4$  au; both P < 0.001) improved over time. The GSS improvement transitioned the participants from being categorized with "poor" to "good" sleep (GSS: >5 compared with  $\leq 5$  au of a 0–21 au scale; baseline 7.6  $\pm$  0.4 au, week 12: 4.8  $\pm$  0.4 au).

**Conclusions:** Although objective sleep quality may not improve, adults who are overweight or obese and poor sleepers may become good sleepers while consuming either the recommended or a higher-protein energy-restricted Healthy US-Style Eating Pattern. This trial was registered at clinicaltrials.gov as NCT03174769. *J Nutr* 2020;150:3216–3223.

Keywords: weight-loss, heart-health, cardiovascular, sleep quality, melatonin secretion

# Introduction

Most adults require between 7 and 9 h of sleep. In 2001, only 13% of adults, during the weeknight, were sleeping  $\leq 6$  h (1); 8 y later,  $\sim 20\%$  of adults were sleeping  $\leq 6$  h; nearly 30% of American adults were chronically undersleeping by 2018. During this time, adults increasingly reported they had difficulty either initiating or maintaining sleep (1). Short sleep durations, poor sleep quality, and disordered sleep patterns are related to both obesity and obesity-related comorbidities, such as type 2 diabetes, cardiovascular disease, hypertension, poor

lipid–lipoprotein status, and premature death (2-7). These outcomes are typically associated with chronic unhealthy Western-style eating patterns (8-12). Improvements in obesity-related disease risk and sleep quality may be incurred by improving eating pattern quality.

As a tool to aid professionals in educating Americans to follow healthy eating patterns, the Dietary Guidelines for Americans were designed to prevent the development of diet-related chronic diseases (13). Poor or insufficient sleep was reviewed among the list of chronic diseases by the 2015–2020 Dietary Guidelines Advisory Committee (14). This committee

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acknowledged that research was emerging indicating relations between sleep patterns, dietary intakes, and obesity risk; however, the potential impact of diet on sleep-related outcomes did not warrant a conclusion due to insufficient research (15). The available research had assessed the influence of sleep (16) on dietary choices (17-19); however, less attention had been paid to the reverse relation-how diet influences sleep. In general, dietinduced weight loss is considered to improve sleep quality and increase sleep duration (20-22). In randomized controlled trials of dietary energy restriction, emerging but inconsistent evidence suggested indices of sleep could be influenced by macronutrient distribution (23–25). In particular, dietary protein intake may influence sleep because the amino acids tryptophan and tyrosine are precursors of the sleep-related neurotransmitters melatonin and dopamine (26, 27). Although both diet and sleep are associated with obesity and chronic diseases, limited research exists documenting the potential relations between both energy status and macronutrients distribution and indices of sleep (26).

The primary aim of this randomized controlled-feeding study was to assess the effects on sleep quality indices of consuming a USDA Healthy US-Style Eating Pattern with either the recommended or higher amounts of animal-based proteinrich foods during energy restriction. Changes in sleep quality were objectively measured using wrist-worn actigraphs and subjectively measured using questionnaires. We hypothesized that among adults who are overweight or obese, objectively and subjectively measured indices of sleep quality would improve over time when a higher-protein, but not the recommended protein, energy-restricted Healthy US-Style Eating Pattern was followed.

# Methods

#### **Experimental design**

This investigation utilized data from a 14-wk free-living study that included a 2-wk baseline period followed by a 12-wk randomized,

controlled-feeding, energy-restricted intervention. Participants were randomly allocated to consume a Healthy US-Style Eating Pattern with either the recommended protein quantity (RP) or high protein (HP). Measurements were taken at baseline and intervention weeks 6 and 12. The clinical laboratory manager, who was not involved in data collection or analysis, generated the random allocation sequence using the first generation on randomization.com and assigned participants to the intervention in a 1:1 allocation ratio. The participants could not foresee which group they were allocated to and were blinded during the intervention. All prespecified outcomes are reported.

#### **Participants**

Sixty-nine adults classified with poor sleep (global sleep score:  $\geq 5$ ) and a BMI (kg/m<sup>2</sup>) indicating overweight or obesity were recruited from the greater Lafayette, IN, community between January 2017 and August 2019. Inclusion criteria were the following: age 30-69 y, BMI 25.0-39.0, weight stable ( $\pm$  3 kg during the previous 3 mo), not following an exercise program, nonsmoking, not diabetic, no acute illness, not pregnant or lactating, willing and able to travel to testing facilities, natural waist circumference >102 cm for men and >88 cm for women, fasting glucose <10 mg/dL, systolic and diastolic blood pressures <140/90 mmHg, serum total cholesterol <260 mg/dL, LDL cholesterol <160 mg/dL, triacylglycerol <400 mg/dL, global sleep score  $\geq$ 5, clinically normal serum albumin and prealbumin concentrations, and not diagnosed with severe sleep apnea or insomnia. Each participant signed a study consent form prior to enrollment and received a monetary stipend for participation. This study was approved by the Purdue University Biomedical Institutional Review Board and is registered at clinicaltrials.gov as NCT03174769.

#### **Dietary intervention**

During the 2-wk baseline period, participants continued to consume their habitual, self-selected, diet. During the 12-wk intervention, all participants consumed an energy-restricted diet consistent with a Healthy US-Style Eating Pattern ranging between 1400 and 2600 kcal/d (Table 1) (13). The energy restriction was achieved by providing  $\sim$ 750 kcal/d less than the estimated energy requirement for each participant; this was calculated using the sex-specific equations for adults who are overweight or obese with a low activity level (physical activity coefficients were 1.12 for men and 1.16 for women) (28). The study dietitian, using ProNutra software (version 3.4.0.0, Viocare Inc.), created the 7-d rotating menus for each group in 200 kcal increments from 1400 to 2600 kcal/d (Supplemental Table 1). Each participant was randomly assigned to consume either 5 oz-equivalent (eq)/d from the Dietary Guidelines for Americans (29) Protein Foods group (RP) or 12.5 oz-eq/d (HP). The additional 7.5 oz-eq came from increasing the number of servings of animal-based protein foods (meats, eggs, poultry, and seafood) within the protein foods group. The dietary energy from the additional protein was compensated for by reducing quantities of select items from non-protein food groups (Table 2). Herbal seasoning of food, water, and caffeine-containing, non-energy containing beverages were allowed ad libitum during the intervention.

During baseline, each participant's habitual, self-selected, diet was assessed using a 3-d dietary recall method (Nutrition Data System for Research software, version 2014). During the intervention, we placed an online order through a local supermarket to provide each participant with all study foods. Either the gram or cup quantities of each food to be consumed was listed in the menu under 5 eating occasions, breakfast, lunch, afternoon snack, dinner, and evening snack. For each eating occasion, we provided instructions on how to prepare and cook the foods for consumption. Participants were not instructed to consume each meal at a specific hour of the day; however, they were instructed to consume all foods and beverages provided for the day within that day; foods and beverages (except water) not provided by us were not permitted. To aid in measuring food portions, we provided participants with a digital food scale and measuring cups. Dietary compliance was encouraged by study staff in person, by e-mail, and by phone; we assessed compliance using both daily menu checklists and weekly body weight weigh-ins. We used a digital platform scale (model ES200L; Ohaus Corporation) to measure body weight once weekly during the

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Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Supplemental Table 1, Table 2, and Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.

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Abbreviations used: au, arbitrary unit; eq, equivalent; ESS, Epworth Sleepiness Scale; GSS, global sleep score; HP, high-protein group consuming 6 ozeq/d more protein foods than recommended as part of an energy-restricted Healthy US-Style Eating Pattern; PSQI, Pittsburgh Sleep Quality Index; RP, recommended-protein group consuming the recommended quantity of protein foods in an energy-restricted Healthy US-Style Eating Pattern.

	1400 kcal/d		2600 kcal/d	
	RP	HP	RP	HP
Vegetable, c-eq/d	1.8	1.9	4.5	4.3
Dark-green vegetables, c-eq/wk	2.7	4.2	5.3	5.8
Red and orange vegetables, c-eq/wk	2.9	2.5	10.2	9.5
Legumes (beans and peas), c-eq/wk	0.5	0.4	1.1	1.0
Starchy vegetables, c-eq/wk	3.4	2.5	5.7	4.5
Other vegetables, c-eq/wk	2.9	3.9	9.2	9.2
Fruits, c-eq/d	1.8	1.8	2.2	2.3
Grains, oz-eq/d	4.1	2.4	9.5	7.7
Whole grains, oz-eq/d	1.6	1.2	3.5	3.1
Refined grains, oz-eq/d	2.5	1.2	6.0	4.6
Dairy, c-eq/d	2.2	1.9	2.7	2.9
Protein foods, oz-eq/d	5	12.5	5	12.5
Seafood, oz-eq/wk	6	14	6	14
Meats, poultry, eggs, oz-eq/wk	26	72	26	72
Nuts seeds, soy products, oz-eq/wk	3	2	3	2
Fats				
Oils, g	29	29	63	142
Solid fats, g	104	0	82	20
Limit on calories for other uses, kcal, % of calories	11	206	93	80

**TABLE 1** The prescribed daily and weekly food group servings for the RP and HP groups at the range of energy intakes during the 12-wk weight loss intervention<sup>1</sup>

<sup>1</sup>c, cup, equal to 236.59 mL; eq, equivalent; HP, group consuming 7.5 oz-eq/d more protein foods than recommended as part of an energy-restricted Healthy US-Style Eating Pattern; RP, group consuming the recommended quantity of protein foods (5 oz-eq/d) in an energy-restricted Healthy US-Style Eating Pattern.

12-wk intervention period. All diet-related activities and assessments were performed in conjunction with the Indiana Clinical Research Center Bionutrition Facility at Purdue University.

#### Indices of sleep quality

Objective variables of sleep [sleep duration, onset latency, sleep efficiency, and total number of minutes awake after sleep onset (WASO)] were measured as described (30) for 7 consecutive days during baseline, week 6, and week 12 testing with wrist-worn actigraphs (Actiwatch 2; Respironics, Phillips). For each participant, the average of the usable days from the 7 d were used. Actigraphy measurements were taken every 30 s and scored using the manufacturer's algorithm set at medium sensitivity (Actiwatch Software version 6.0.9). The rest intervals were determined using the sleep and wake times 1) indicated by the participants pressing the log button on the Actiwatch; 2) indicated in the sleep questionnaires filled out by the participants each morning and night; and 3) predicted by the Actiwatch software. Six of 153

**TABLE 2**Nutritional information of the prescribed eatingpatterns during the 12-wk weight loss intervention1

Nutritional information	RP	HP	
Energy, kcal/d	1773 ± 323	1667 ± 292	
Total fat, g/d	57 $\pm$ 11	$47~\pm~10$	
Total fat, % energy	$29 \pm 0$	$25 \pm 1$	
Total carbohydrate, g/d	$245~\pm~50$	$184~\pm~45$	
Total carbohydrate, % energy	55 $\pm$ 1	$44~\pm~3$	
Protein, g/d	87 ± 7	$134 \pm 9$	
Protein, % energy	$20 \pm 1$	$33 \pm 3$	
Protein, g·kg <sup>-1</sup> ·d <sup>-1</sup>	$0.9~\pm~0.08$	$1.5~\pm~0.13$	

<sup>1</sup>Values are means  $\pm$  SDs; n = 30 for RP, and n = 21 for HP. HP, group consuming 7.5 oz-eq/d more protein foods than recommended as part of an energy-restricted Healthy US-Style Eating Pattern; oz-eq, ounce-equivalent; prescribed, the nutritional information of the eating pattern planned for the participants based on the outputs from Nutrition Data System for Research software; RP, group consuming the recommended quantity of protein foods (5 oz-eq/d) in an energy-restricted Healthy US-Style Eating Pattern.

participants' actigraphy data (51 participants × 3 time points) were excluded from analyses due to non-study-related circadian shifts (n = 1) or Actiwatch malfunction at >1 time point (n = 5). We measured 1069 nights of actigraphy in 51 participants, but 118 (11%) nights were not usable due to Actiwatch malfunction or non-study-related activities that interfered with the participants' regular sleep schedule. Analyses were performed on all participants (n = 51) and all usable data were an average of  $\geq 3$  nights.

The time of the onset of melatonin secretion was used to measure shifts in circadian phase position. Participants collected saliva samples hourly at home on a single day at baseline, week 6, and week 12 beginning 5 h before usual sleep time and ending 1 h after. Participants were instructed to store the saliva samples in their freezer until bringing them to the laboratory on ice at their next weekly bodyweight weigh-in. Saliva samples were then transferred to a -80°C freezer until shipped on dry ice for melatonin concentration analysis. The collection tubes were translucent, and participants were not instructed to keep the samples under cover. Salivary melatonin concentrations were measured in duplicate using a commercially available immunoassay (Salimetrics assay #1-3402; intraassay CV%: 5.42%; interassay CV%: 8.90%) by a commercial analytical laboratory (Salimetrics, LLC). Circadian phase position was determined by linear interpolation between the time points before and after the melatonin concentration increased and stayed above 4 pg/mL. Samples for circadian phase position were only available for a subset of participants, due to missing samples and inadequate sample volume or integrity.

Subjective variables of sleep were assessed using validated questionnaires. Participants completed the Pittsburgh Sleep Quality Index (PSQI) questionnaire regarding the previous 4 wk of their sleep. A higher global sleep score [0–21 arbitrary units (au)] indicates poorer sleep; a global sleep score  $\geq 5$  is classified as "poor sleep" (31). Participants also filled out the Epworth Sleepiness Scale (ESS) to measure subjective daytime sleepiness (32). The total ESS score is the sum of 8 subscores and can range between 0 and 24. A higher score indicates more daytime sleepiness.

#### **Statistical analysis**

Power calculations were completed using the G\*Power software (version 3.1.4, Heinrich-Heine-Universität). In a previous 12-wk study

TABLE 3	Baseline characteristics of participants in the RP and HP groups <sup>1</sup>

	RI	þ	HP		
Characteristic	Females ( $n = 24$ )	Males $(n = 6)$	Females ( $n = 19$ )	Males ( <i>n</i> = 2)	
Race, n					
White/Caucasian	22 (91.6)	6 (100)	17 (89.5)	2 (100)	
Black/African American	1 (4.2)	0 (0)	0 (0)	0 (0)	
Other	1 (4.2)	0 (0)	2 (10.5)	0 (0)	
Ethnicity					
Not Hispanic/Latino	24 (100)	6 (100)	19 (100)	2 (100)	
Hispanic/Latino	0 (0)	0 (0)	0 (0)	0 (0)	
Age, y	48 ± 11	$38 \pm 4$	50 $\pm$ 8	$39~\pm~6$	
Body weight, kg	87 ± 12	$100 \pm 14$	87 ± 10	102 $\pm$ 25	
Height, cm	166 $\pm$ 5	$176 \pm 6$	$167 \pm 6$	177 ± 11	
BMI, kg/m <sup>2</sup>	$32 \pm 4$	$32 \pm 4$	$31 \pm 3$	$32 \pm 4$	
Fat mass, kg	$40 \pm 9$	38 ± 7	$39 \pm 7$	46 ± 3	
Lean mass, kg	47 ± 7	62 ± 8	$48 \pm 5$	$56 \pm 22$	

<sup>1</sup>Values are means ± SDs or frequencies (%). HP, group consuming 7.5 oz-eq/d more protein foods than recommended as part of an energy-restricted Healthy US-Style Eating Pattern; oz-eq, ounce-equivalent; RP, group consuming the recommended quantity of protein foods (5 oz-eq/d) in an energy-restricted Healthy US-Style Eating Pattern.

(33), the changes in global sleep score during energy restriction were 0.4  $\pm$  2.1 (mean  $\pm$  SD) and  $-1.4 \pm 2.1$  for their RP and HP groups, respectively. An a priori power calculation showed that n = 23/group would provide >80% power to detect a differential response between our RP and HP groups ( $\alpha = 0.05$ , 2 tails, effect size = 0.86).

Analyses were completed using data from the 51 participants who finished the intervention. All results are presented as LSmeans  $\pm$  SEs of the LSmean, unless otherwise stated, because both age and sex were covariates. The main effect of time and the group-by-time interaction for each of the indices of sleep were assessed using a 2  $\times$  3 factor repeated-measures ANOVA (MIXED procedure; group: RP, HP; time: baseline, week 6, and week 12). Group-by-time interactions were the effect of dietary protein quantity (group) from baseline to week 6 to week 12 (time). Outliers were removed when standardized residuals were <-3 or >3. The MIXED procedure with a compound symmetry covariance structure is robust to violations of normally distributed data and sphericity (34). An unpaired, 2-tailed t-test (TTEST procedure) was used to test for group differences in baseline characteristics. Statistical significance for main effects and interaction effects were considered when P < 0.05. Statistical analysis was performed using SAS software (version 9.3; SAS Institute).

## Results

#### **Participants**

Sixty-nine participants completed all baseline testing and started the intervention between June 2017 and August 2019. Recruitment was stopped after reaching the target number of participants to account for dropouts. Eighteen participants left the study during the intervention period (RP, n = 4; HP, n = 14) and were higher in the HP group (n = 14) than the RP group (n = 4). Fifty-one participants (RP, n = 30, 6 males and 24 females; HP, n = 21, 2 males and 19 females) completed all study procedures and their data were analyzed (**Supplemental Figure 1**). Baseline demographics are presented in Table 3. There were no differences between groups for any of the measured outcomes at baseline.

#### **Dietary intervention**

The nutritional information for the habitual, self-selected diets consumed by participants during baseline is presented in **Supplemental Table 2**. There were no differences in dietary intakes assessed between groups prior to enrollment

in the study. Among all participants, body mass decreased  $(-6.2 \pm 0.4 \text{ kg})$  and the response over time was not different between groups (P < 0.05), which is consistent with consuming an energy-restricted diet.

#### **Sleep indices**

Dietary protein intake did not affect any of the objective (actigraphy and circadian phase position) or subjective [global sleep score (GSS) and ESS] sleep quality outcomes measured (**Table 4**). Over time, objective measures of time spent in bed, time spent sleeping, sleep onset latency, and time awake after sleep onset did not change; however, sleep efficiency improved ( $1 \pm 1\%$ ; P < 0.027). Subjective measures of GSS ( $-3.8 \pm 0.4$  au) and daytime sleepiness score ( $-3.8 \pm 0.4$  au; both P < 0.001) improved over time. The GSS improvement transitioned the group of participants from being categorized with a poor to a good sleep condition (GSS >5 compared with  $\leq 5$  au of a 0-21 au scale; baseline:  $7.9 \pm 0.5$  au; week 12:  $4.0 \pm 0.6$  au).

## Discussion

Similarly to the light-dark cycle, food choices may influence peripheral tissues circadian rhythms (35). Disruptions in circadian clocks can negatively affect the expression of genes related to both the endocrine system and metabolism (35). Indeed, metabolic syndrome has been linked to persons with dysregulated or disrupted sleep (35); more research is needed to elucidate how the reverse relation of eating patterns causally effects sleep quality (23-25). This study was the first to assess the effect of consuming higher amounts of dietary protein, achieved using animal-based protein food group items (meats, eggs, poultry, and seafood), in an energy-restricted Healthy US-Style Eating Pattern on changes in sleep quality indices. Among adults who lost weight and consumed a healthy dietary pattern, most objective sleep quality measures, measured using actigraphy, did not change; however, objective sleep efficiency and both perceived sleep quality and daytime sleepiness improved (subjective). These results do not support our hypothesis that consuming a higher protein diet compared to a recommended protein diet, will lead to relatively greater

TABLE 4	Changes in sleep quality in the RP and HP groups after consuming an energy-restricted Healthy US-Style Eating Pattern for
12 wk <sup>1</sup>	

				Change from pre- to postintervention			
Parameters				Within	Group	<i>P</i> value	
	Baseline	Week 6	Week 12	group	independent	Time	$\operatorname{Group} \times \operatorname{time}$
Actigraphy							
Time in bed, <sup>2</sup> min					$2 \pm 8$	0.891	0.862
RP	$423\pm10$	$426~\pm~10$	$421~\pm~10$	$-2 \pm 9$			
HP	$413\pm12$	416 $\pm$ 13	$418\pm12$	$5\pm10$			
Time sleeping, <sup>2</sup> min					$5\pm 6$	0.421	0.922
RP	$384~\pm~10$	$394 \pm 10$	$389\pm11$	$5\pm7$			
HP	$374\pm13$	$379\pm13$	$378\pm12$	$4 \pm 9$			
Sleep efficiency, <sup>2</sup> %					1 ± 1	0.026	0.691
RP	$84 \pm 1$	86 ± 1	86 ± 1	$2 \pm 1$			
HP	$83 \pm 2$	$84 \pm 2$	$84 \pm 2$	$1 \pm 1$			
WASO, <sup>2</sup> min					$-2 \pm 1$	0.071	0.294
RP	$37 \pm 3$	$33 \pm 3$	$33 \pm 3$	$-4 \pm 2$			
HP	$39 \pm 4$	$37 \pm 4$	39 ± 4	0 ± 2			
Onset latency, <sup>3</sup> min					$-4 \pm 2$	0.139	0.441
RP	$19 \pm 3$	17 ± 3	$13 \pm 3$	$-7 \pm 3$			
HP	16 ± 4	$14 \pm 4$	$14 \pm 4$	$-2 \pm 3$			
Snooze time, <sup>4</sup> min					$-1 \pm 1$	0.537	0.303
RP	$16 \pm 2$	$14 \pm 2$	$13 \pm 2$	$1 \pm 2$			
HP	$16 \pm 3$	$15 \pm 3$	$15 \pm 2$	$-3 \pm 2$			
Midsleep variance, <sup>5</sup> min					$3 \pm 3$	0.610	0.971
RP	$35 \pm 4$	$37 \pm 4$	38 ± 4	4 ± 4			
HP	41 ± 4	42 ± 4	43 ± 4	$2\pm5$			
Bed time, <sup>6</sup> h:min					$-00:07 \pm 00:08$	0.477	0.307
RP	$23:52 \pm 00:15$	23:32 ± 00:15	23:33 ± 00:15	$-00:20 \pm 00:11$			
HP	$23:28 \pm 00:18$	$23:28 \pm 00:18$	$23:33 \pm 00:18$	$00:05 \pm 00:13$			
Wake time, <sup>7</sup> h:min	20120 1 00110				$-00:07 \pm 00:07$	0.363	0.157
RP	06:52 ± 00:14	06:31 ± 00:14	06:31 ± 00:14	$-00:21 \pm 00:10$		0.000	0.107
HP	$06:32 \pm 00:14$ $06:32 \pm 00:16$	$06:32 \pm 00:17$	$06:39 \pm 00:17$	$00:27 \pm 00:10$ $00:07 \pm 00:12$			
Circadian phase position, <sup>8</sup> h:m		00.02 ± 00.17	00.00 ± 00.17	00.07 ± 00.12	00:09 ± 00:18	0.307	0.599
RP	22:19 ± 00:25	22:05 ± 00:26	22:15 ± 00:26	$-00:04 \pm 00:23$	00.00 ± 00.10	0.007	0.000
HP	$22:05 \pm 00:25$ $22:06 \pm 00:32$	$21:43 \pm 00:32$	$22:28 \pm 00:32$	$00:22 \pm 00:23$			
Questionnaires	22.00 ± 00.02	21.43 ± 00.32	22.20 ± 00.32	00.22 ± 00.27			
Global sleep score, <sup>9</sup> au					$-2.7 \pm 0.4$	<0.001	0.697
RP	$7.5 \pm 0.4$	$3.7 \pm 0.4$	$4.5 \pm 0.4$	$-3.0 \pm 0.5$	- 2.7 _ 0.4	<0.001	0.037
HP	$7.5 \pm 0.4$ $7.6 \pm 0.5$	$3.7 \pm 0.4$ $4.5 \pm 0.5$	$4.3 \pm 0.4$ 5.1 ± 0.5	$-3.0 \pm 0.3$ $-2.5 \pm 0.6$			
ESS, <sup>10</sup> au	7.0 ± 0.0	4.J ± 0.J	J.I _ U.J	-2.J _ U.U	$-1.8 \pm 0.4$	<0.001	0.806
RP	9.6 ± 0.7	$6.5 \pm 0.7$	$7.5 \pm 0.7$	$-2.1 \pm 0.6$	$-1.0 \pm 0.4$	< 0.001	0.000
HP	$9.6 \pm 0.7$ 10.4 ± 0.8	$6.5 \pm 0.7$ $7.6 \pm 0.8$	$7.5 \pm 0.7$ $8.9 \pm 0.8$	$-2.1 \pm 0.6$ $-1.9 \pm 0.7$			

<sup>1</sup>Values are LSmeans ± SEs unless otherwise indicated. A repeated-measure ANOVA (MIXED procedure, SAS version 9.3; SAS Institute) was used to test for main effects of time and group-by-time interactions with age and sex as covariates. Outliers were removed when standardized residuals were <-3 and >3. au, arbitrary units; ESS, Epworth Sleepiness Scale; HP, group consuming 7.5 oz-eq/d more protein foods than recommended as part of an energy-restricted Healthy US-Style Eating Pattern; Mid-sleep time; oz-eq, ounce-equivalent; RP, group consuming the recommended quantity of protein foods (5 oz-eq/d) in an energy-restricted Healthy US-Style Eating Pattern; WASO, wake after sleep onset.

<sup>2</sup>Baseline: n = 30 (RP) and 20 (HP), missing = 1 (HP); week 6: n = 29 (RP) and 17 (HP), missing = 1 (RP) and 3 (HP), outlier = 1 (HP); week 12: n = 27 (RP) and 21 (HP), missing = 2 (RP), outlier = 1 (RP).

<sup>3</sup>Baseline: *n* = 30 (RP) and 19 (HP), missing = 1 (HP), outlier = 1 (HP); week 6: *n* = 29 (RP) and 17 (HP), missing = 1 (RP) and 3 (HP), outlier = 1 (HP); week 12: *n* = 27 (RP) and 20 (HP), missing = 2 (RP), outlier = 1 (RP) and 1 (HP).

<sup>4</sup>Baseline: *n* = 30 (RP) and 20 (HP), missing = 1 (HP); week 6: *n* = 29 (RP) and 18 (HP), missing = 1 (RP) and 3 (HP); week 12: *n* = 27 (RP) and 20 (HP), missing = 2 (RP), outlier = 1 (RP) and 1 (HP).

<sup>5</sup>Baseline: *n* = 30 (RP) and 20 (HP), missing = 1 (HP); week 6: *n* = 29 (RP) and 18 (HP), missing = 1 (RP) and 3 (HP); week 12: *n* = 28 (RP) and 21 (HP), missing = 2 (RP). <sup>6</sup>Baseline: *n* = 30 (RP) and 19 (HP), missing = 1 (HP), outlier = 1 (HP); week 6: *n* = 29 (RP) and 17 (HP), missing = 1 (RP) and 3 (HP), outlier = 1 (HP); week 12: *n* = 28 (RP) and 21 (HP), missing = 2 (RP).

<sup>7</sup>Baseline: *n* = 30 (RP) and 20 (HP), missing = 1 (HP); week 6: *n* = 28 (RP) and 18 (HP), missing = 1 (RP) and 3 (HP), outlier = 1 (RP); week 12: *n* = 28 (RP) and 21 (HP), missing = 2 (RP).

<sup>8</sup>Baseline: *n* = 12 (RP) and 8 (HP), missing = 18 (RP) and 13 (HP); week 6: *n* = 11 (RP) and 13 (HP), missing = 19 (RP) and 8 (HP); week 12: *n* = 11 (RP) and 8 (HP), missing = 18 (RP) and 13 (HP), outlier = 1 (NP).

<sup>9</sup>Baseline: n = 29 (RP) and 20 (HP), outlier = 1 (RP) and 1 (HP); week 6: n = 30 (RP) and 21 (HP); week 12: n = 30 (RP) and 21 (HP).

 $^{10}n = 30$  (RP) and 21 (HP) at each time point.

improvements in objective and subjective sleep quality indices. Our results suggest that consuming an energy-restricted Healthy US-Style Eating Pattern containing either 5 or 12.5 oz-eq/d of protein foods can improve both perceived sleep quality and daytime sleepiness.

We measured both objective (actigraphy) and subjective (PSQI and ESS) indices of sleep. Although perceived sleep quality improved among all participants, most objective sleep quality indices did not change over time. Objective measurements can distinguish between sleep and wake time motor activities to assess sleep duration, efficiency, and disturbances (36). Although actigraphs cannot measure sleep phases, there is  $\sim 90\%$  agreement between actigraphy and polysomnography—the gold standard for measuring sleep (37). Salivary melatonin concentrations were measured prior to and after usual bedtime to assess possible shifts in physiological sleep onset; however, similar to the actigraph outcomes, those remained unchanged over time. Subjective evaluations of sleep identify physical and psychological perceptions of sleep quality and well-being. Although we did not perform psychological evaluations, previous studies performed with weight loss have shown improvement in anxiety and depression scores (38, 39). Furthermore, interventions designed to improve both the attitudes and perceptions toward sleep successfully improve participant's sleep quality (40). Perhaps participants in the current study experienced improvements in both their overall mood and attitudes toward sleep by losing weight as part of a "sleep"-related study; this could be reflected in their subjective sleep quality evaluations. However, we would expect this manifest in the objective assessments, also.

Contrary to the results reported in the current study, results from 2 previous studies (both presented in the same publication) (33) showed decreased global sleep scores (improved perceived sleep) with higher protein intakes; however, the designs of these studies differ from the design of ours in several ways. First, the previous studies (33) tested a wider range of protein intakes. Participants in study 1 (33) consumed either 10%, 20%, or 30% of their energy as protein; in study 2 (33), they consumed either 0.8 or 1.5 g protein/kg body mass. Participants in both lower protein groups (10% or 0.8 g·kg<sup>-1</sup>·d<sup>-1</sup>) did not experience improvements in perceived sleep (33); perceived sleep only improved in the groups prescribed 20%, 30%, and 1.5  $g \cdot kg^{-1} \cdot d^{-1}$  (33). The recommended (lowest) protein group in the current study was prescribed  $20 \pm 1\%$  of energy as protein  $(0.97 \pm 0.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1})$ . In study 1 (33), this amount of protein consumption is comparable to the "middle" protein group ( $\sim 20\%$ ); they also perceived an improvement in sleep.

A "higher" protein diet may provide a higher concentration of circulating tryptophan. Higher tryptophan concentrations should promote flux across the blood-brain barrier to induce serotonin and melatonin synthesis-both are thought to improve sleep (41, 26, 42, 43). However, increasing circulating tryptophan concentrations by consuming a higher-protein diet may also promote higher concentrations of large neutral amino acids; these amino acids compete with tryptophan for transport across the blood-brain barrier. These phenomena may explain why perceived sleep quality improves with graded intakes of dietary protein but has no effect after it hits a "saturable-dose" limit. At marginal (<20% of energy as protein) intakes of protein, increasing protein consumption may increase tryptophan concentrations without interference from the increasingly large neutral amino acid concentrations. At a saturable-dose limit (e.g., ~20%), higher protein intakes may provide more tryptophan; however, the concomitant increase in large neutral amino acid intake may be in sufficient concentrations to interfere with tryptophan's transport across the blood-brain barrier. This conjecture is speculative in nature and requires follow-up studies to validate.

Second, although the previous 2 studies (33) were also conducted during energy restriction, participants in those studies were not specifically consuming a healthy eating pattern; participants in study 2 consumed a Western-style eating pattern specifically designed to be unhealthy (33). Unhealthy eating patterns are generally carbohydrate rich. Higher carbohydrate intakes increase insulin secretion. Insulin mediates muscle uptake of the large neutral amino acids competing for transport across the blood-brain barrier with tryptophan. An increase in tryptophan crossing the blood-brain barrier should promote serotonin and melatonin synthesis for sleep promotion. Therefore, consuming an "unhealthy" eating pattern that induces higher insulin concentrations may promote better sleep quality. However, a meta-analysis of 4 randomized controlled trials reported that "poor" sleepers (GSS >5 au) tended to consume more carbohydrate than "good" sleepers (GSS  $\leq 5$  au) (44). Notably, the authors were not able to document the glycemic index of the carbohydrates. Results from 2 previous studies suggest that the glycemic index may influence sleep quality: Higher glycemic index carbohydrate consumption was associated with longer sleep durations (45) and improved sleep latency (46). It is also likely that dietary carbohydrate and protein work synergistically to influence circadian patterns and sleep quality (47). However, currently there is inconsistent and limited evidence to support that the quantity or glycemic index of carbohydrates consumed influence sleep quality indices because improvements in perceived sleep were observed in groups consuming "higher" protein "healthy" (33) and "unhealthy" (current study) eating pattern, albeit during weight loss.

This study has several limitations. First, improvements in objective sleep quality could have occurred for several reasons: consuming an energy-restricted diet, consuming a Healthy US-Style Eating Pattern, random effect over time, and regression toward the mean. In a separate analysis, we included body mass as a covariate; however, the results remained unaffected. Another consideration is that participants knew prior to enrollment that sleep was being assessed. Thus, participants could have anticipated that their sleep would improve with time. Therefore, we cannot make a causal claim; however, we can say the participants, who consumed a healthy energy-restricted diet at 2 levels of protein intake, perceived their sleep to improve. Second, the relative imbalance between males and females could have influenced the finding. Females tend to require more sleep than males, have a shorter circadian cycle, and recover more quickly from sleep deprivation. Third, while the higher attrition rates in the HP group are primarily the result of participants removing themselves for non-study-related reasons (i.e., lack of time, medical event), it is not without possibility that data loss could explain the null results. Fourth, as with all longitudinal human clinical research outside of a metabolic ward, we cannot be certain only the study foods and beverages provided to the participants were consumed. However, participants reported high dietary adherence based on the returned menu checklists; they also lost body mass in accordance to the prescribed dietary intervention. This indicates that participants, by and large, followed the prescribed dietary intervention. We also assessed sleep quality using several modalities (subjective and objective measures) to measure a variety of sleep quality components (sleep duration, wake after sleep onset, sleep latency, sleep

efficiency). This comprehensive assessment of sleep allowed us to investigate the sleep quality, architecture, and timing that are important components of sleep (48).

In conclusion, although objective sleep quality may not improve, consuming either a recommended or a higher-protein Healthy US-Style Eating Pattern during weight loss is associated with improvements in perceived sleep quality and daytime sleepiness among adults who are overweight or obese.

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