

# Higher Protein Intake Preserves Lean Mass and Satiety with Weight Loss in Pre-obese and Obese Women

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## Abstract

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**Objective:** To examine the effects of dietary protein and obesity classification on energy-restriction-induced changes in weight, body composition, appetite, mood, and cardiovascular and kidney health.

**Research Methods and Procedures:** Forty-six women, ages 28 to 80, BMI 26 to 37 kg/m<sup>2</sup>, followed a 12-week 750-kcal/d energy-deficit diet containing higher protein (HP, 30% protein) or normal protein (NP, 18% protein) and were retrospectively subgrouped according to obesity classification [pre-obese (POB), BMI = 26 to 29.9 kg/m<sup>2</sup>; obese (OB), BMI = 30 to 37 kg/m<sup>2</sup>].

**Results:** All subjects lost weight, fat mass, and lean body mass (LBM;  $p < 0.001$ ). With comparable weight loss, LBM losses were less in HP vs. NP ( $-1.5 \pm 0.3$  vs.  $-2.8 \pm 0.5$  kg;  $p < 0.05$ ) and POB vs. OB ( $-1.2 \pm 0.3$  vs.  $-2.9 \pm 0.4$  kg;  $p < 0.005$ ). The main effects of protein and obesity on LBM changes were independent and additive; POB-HP lost less LBM vs. OB-NP ( $p < 0.05$ ). The energy-restriction-induced decline in satiety was less pronounced in HP vs. NP ( $p < 0.005$ ). Perceived pleasure increased with HP and decreased with NP ( $p < 0.05$ ). Lipid–lipoprotein profile and blood pressure improved and kidney function mini-

mally changed with energy restriction ( $p < 0.05$ ), independently of protein intake.

**Discussion:** Consuming a higher-protein diet and accomplishing weight loss before becoming obese help women preserve LBM. Use of a higher-protein diet also improves perceptions of satiety and pleasure during energy restriction.

**Key words:** cardiovascular function, kidney function, hunger and satiety, pleasure, energy restriction

## Introduction

Overweight and obesity are serious public health concerns because of their high prevalence [ $>116$  million adult Americans (61%) exhibiting a BMI  $\geq 25$  kg/m<sup>2</sup> and 31% of  $\geq 30$  kg/m<sup>2</sup>] (1,2) and elevation of associated risks for a myriad of diseases, including hypertension, dyslipidemia, type 2 diabetes, and coronary heart disease (3,4). Thus, it is essential to examine potential dietary strategies that will lead to a reduction in these risk factors.

There is no single effective dietary intervention for weight loss (5). However, higher-protein diets continue to be a popular approach (5). They reportedly lead to a greater loss of body weight (5–8), fat mass (5–8), and preservation of lean body mass (LBM)<sup>1</sup> (9) compared with normal-protein diets. This is attributed, in part, to an increased thermogenic response to higher-protein meals (5,10,11). Higher-protein diets also lead to decreased perceived hunger and desire to eat (12,13) and increased postmeal, daily, and long-term satiety (5,11,14).

Higher-protein diets, specifically those with increased quantities of saturated fat, may adversely effect cardiovas-

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<sup>1</sup> Nonstandard abbreviations: LBM, lean body mass; HP, higher protein; NP, normal protein; POB, pre-obese; OB, obese; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; GFR, glomerular filtration rate; BUN, blood urea nitrogen.

**Table 1.** Subject characteristics of HP ( $n = 21$ ) vs. NP ( $n = 25$ ) groups

Subject characteristics*	Baseline		Post-intervention		Change	
	NP	HP	NP	HP	NP	HP
Age (years)	53 ± 3	46 ± 2	N/A	N/A	N/A	N/A
Height (cm)	165 ± 2	163 ± 2	N/A	N/A	N/A	N/A
Weight† (kg)	83.4 ± 2.2	82.6 ± 3.4	74.5 ± 2.1	74.5 ± 3.3	-9.5 ± 1.0	-8.1 ± 0.4
BMI† (kg/m <sup>2</sup> )	30.5 ± 0.6	30.7 ± 0.9	27.1 ± 0.6	27.7 ± 0.9	-3.4 ± 0.3	-3.0 ± 0.1
Body fat† (%)	44.6 ± 0.6	44.2 ± 0.9	41.2 ± 1.0	39.8 ± 1.4	-3.4 ± 0.5	-4.4 ± 0.6
Fat mass† (kg)	37.4 ± 1.3	37.0 ± 2.1	31.0 ± 1.5	30.4 ± 2.2	-6.6 ± 0.6	-6.6 ± 0.4
LBM†‡ (kg)	43.3 ± 1.0	43.0 ± 1.4	40.8 ± 0.8	41.5 ± 1.3	-2.8 ± 0.5	-1.5 ± 0.3

Repeated-measures ANOVA: ( $p < 0.05$ ).

\* Data expressed as mean ± SE.

Main effect: † time (pre to post).

‡ Time × group interaction.

N/A, not applicable.

cular and kidney function (5,15), whereas those with lower saturated fat lead to beneficial effects on cardiovascular risk factors (8,9,14,16).

The majority of the previously mentioned weight loss studies (6–9,13,14,16) included both overweight and obese subjects with broad BMI criteria of 25 to 40 kg/m<sup>2</sup>. It was assumed that these subjects would respond in the same manner, regardless of their classification of overweight status. However, it is currently well documented that, unlike normal-weight or overweight individuals, obese individuals display numerous metabolic, appetitive, and energy-regulating hormonal abnormalities that impair mechanisms controlling energy balance (4). Because of these abnormalities, it is possible that obese individuals may respond differently to a higher-protein weight loss diet compared with normal-weight or modestly overweight individuals.

The primary aim of this study was to examine the effects of a higher-protein (HP) vs. normal-protein (NP) energy restriction diet, matched for total fat intake, on body weight, body composition, appetite, mood state, and markers of cardiovascular and kidney functions. A secondary (retrospective) aim of this study was to determine whether obesity status, specifically pre-obese (POB) vs. obese (OB) classification, defined by the World Health Organization as a BMI of 25.0 to 29.9 kg/m<sup>2</sup> for POB and a BMI of 30.0 to 39.9 kg/m<sup>2</sup> for OB (Classes I and II) (17), differentially affects the energy restriction-induced changes in the parameters listed above.

## Research Methods and Procedures

### Subjects

Potential participants were recruited from newspaper advertisements and posted flyers. Eligibility was based on the

following criteria: 1) women  $\geq 21$  years of age; 2) BMI between 25 and 37 kg/m<sup>2</sup>; 3) not dieting and no weight loss or gain ( $>4.5$  kg) within the last 6 months; 4) nonsmoking; 5) habitual/stable activity pattern within the last 3 months; 6) clinically normal blood profiles (specifically, normal liver and kidney functions; fasting blood glucose  $< 110$  mg/dL); and 7) nondiabetic. Fifty-four women began the intervention and eight women dropped out after week 1 (14.8%). Specifically, six women dropped out because of scheduling conflicts and two for reoccurring health issues related to constipation and upset stomach. Thus, 46 women completed the study (age:  $50 \pm 2$  years; BMI:  $30.6 \pm 0.5$  kg/m<sup>2</sup>; Table 1). All study procedures were approved by the Purdue University Biomedical Institutional Review Board, and all subjects were informed of the purpose, procedures, and potential risks of the study before signing the informed consent document. Each subject received a monetary stipend for participating in the study.

### Experimental Design

This was a 12-week longitudinal, controlled-feeding study. Testing was performed before (baseline) and at the end of the intervention (post) periods. This included two fasting blood samples, measurements of body composition, and 3 days of 24-hour energy intake, appetite, and mood state ratings. The subjects were randomly assigned to one of two groups and consumed either a HP (30% of total energy intake from protein;  $n = 21$ ) or NP (18% protein;  $n = 25$ ) energy-restricted diet for 12 weeks. Body weight was measured two times each week, and subjects completed daily food check-off logs. The subjects in the HP and NP groups were retrospectively subgrouped according to BMI (POB: 25.0 to 29.9 kg/m<sup>2</sup>; OB: 30.0 to 37.0 kg/m<sup>2</sup>), which resulted

in the following groups: HP-POB ( $n = 9$ ); NP-POB ( $n = 11$ ); HP-OB ( $n = 12$ ); and NP-OB ( $n = 14$ ).

### **Diet**

At baseline, each subject's energy requirement to maintain a stable body weight was estimated to be 1.5 times the subject's resting energy expenditure calculated using the Harris-Benedict equation for women (18). During the 12-week energy-restricted period, all subjects were counseled by a research dietitian to consume a diet that was 750 kcal/d less than their estimated (weight stable) energy intake and contained either the Recommended Dietary Allowance of 0.8 g protein/kg per day (NP group) or 175% of the Recommended Dietary Allowance (1.4 g protein/kg per day; HP group) for protein. The macronutrient distribution of the HP diet was 30% of energy from protein (with 40% of the protein from pork), 45% carbohydrate, and 25% fat. For the NP diet, the macronutrient distribution was 18% protein (with no-striated tissue consumed), 57% carbohydrate, and 25% fat. Each day of the study, the subjects consumed a multivitamin/mineral tablet (Centrum; Wyeth Consumer Healthcare, Madison, NJ) and two calcium citrate tablets (400 mg calcium/tablet, one consumed in the morning and one in the evening; total 800 mg calcium/d).

The subjects were provided with 7-day menus with specified quantities of typical, and brand-specific food items to purchase and consume. In addition, the HP group was provided with portioned quantities [ $6.4 \pm 0.1$  oz/d ( $181 \pm 3$  g/d)] of cooked pork (loin, ham, or Canadian bacon) products comprising 40% of their protein intake, whereas the NP group was given portioned quantities of milk [ $10.0 \pm 3.8$  oz/d ( $296 \pm 112$  mL/d)] comprising 13% of their protein intake. This study was not intended or designed to compare sources of high-quality proteins (pork vs. milk). The milk was provided to the NP group as a convenient means of matching contact time with the subjects in the HP group. The subjects were given copies of the 7-day menus, instructed by a dietitian on effective ways to follow the dietary plan, and provided with shopping lists to help with food purchases. To document adherence to the diet, the subjects completed daily food check-off logs. Specifically, these food logs included a list of the food items and quantities to be consumed each day based on their prescribed energy intake. Subjects were instructed to eat all of the food items on the daily log and mark those that were consumed, and any uneaten food was unmarked and later subtracted from the prescribed intake total. Eating food not provided by the study was highly discouraged. If this occurred, extra food was recorded on the food log, and energy and macronutrient composition were calculated using Nutritionist Pro Version 2.0 (First Data Bank, San Bruno, CA). The 750-kcal/d energy-deficit diet (mean energy deficit:  $-33 \pm 1\%$  of weight maintenance energy intake at baseline) theoretically would result in an  $\sim 8.2$  kg weight loss over 12 weeks.

### **Body Weight and Body Composition**

Fasted-state body weight was measured twice weekly using an electronic platform scale (ES200L; Mettler, Toledo, OH). Whole body and regional body composition were determined at baseline and intervention Week 12 using DXA (GE LUNAR Prodigy with EnCORE software version 5.60, Madison, WI).

### **Appetite**

Appetite questionnaires, assessing appetitive sensations (i.e., hunger, fullness, desire to eat) were loaded onto a handheld personal digital assistant (Palm-Pilot M100; Palm Computing, Sunnyvale, CA). The subjects completed the questionnaires every waking hour for 3 nonconsecutive days during both the pre- and post-intervention periods and were instructed to record all meal times during the 3 days. A visual analog scale incorporating a 100-point rating scale was used to assess each sensation (19). Twenty-four-hour and meal-related (i.e., 60-minute pre- and postprandial) appetite sensations were averaged over the 3 days for the pre- and poststudy measures.

### **Mood State**

Concurrent with the appetite questionnaires, the subjects completed mood state assessments of arousal and pleasure (20). An affect grid incorporating various emotional states (i.e., stress, depression, excitement, relaxation, arousal, and sleepiness) was used to obtain arousal scores, ranging from 1 to 9 (arousal to sleepiness), and pleasure scores, ranging from 1 to 9 (pleasure to displeasure) (20). Twenty-four-hour and meal-related (i.e., 60-minute pre- and postprandial) mood states were averaged over the 3 days for the pre- and poststudy measures.

### **Analytical Measurements**

*Cardiovascular, Metabolic, and Kidney Disease Risk Factors.* On 2 separate days during baseline and intervention Week 12, fasting blood samples were taken from an antecubital vein. Blood samples were collected in serum-separator tubes and processed to obtain serum for subsequent analysis of total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triacylglycerol, glucose, and creatinine. These compounds were measured by photometric assays (Chemistry Immuno Analyzer AU5700; Olympus, Center Valley, PA) performed by MidAmerica Clinical Laboratories (Indianapolis, IN). Low-density lipoprotein-cholesterol (LDL-C was estimated using the following equation:

$$\text{LDL-C} = \frac{\text{total cholesterol} - \text{HDL-C} - \text{triacylglycerol}}{5} \quad (1)$$

To assess kidney function, glomerular filtration rate (GFR) was estimated using the following Modification of Diet in Renal Disease equation (21):

**Table 2.** Dietary characteristics

	Energy intake during intervention week 1*		Energy intake during intervention week 12*	
	NP	HP	NP	HP
Total† (kcal/24 h)	1530 ± 40	1560 ± 60	1500 ± 40	1540 ± 60
Protein†‡§ (g/d)	68 ± 3	116 ± 41	67 ± 3	115 ± 4
Carbohydrate†‡§ (g/d)	218 ± 6	174 ± 6	214 ± 6	172 ± 6
Fat† (g/d)	43 ± 1	45 ± 2	36 ± 2	42 ± 2

Data expressed as mean ± standard error.

Independent sample *t* test: (*p* < 0.05).

Repeated-measures ANOVA (*p* < 0.05).

\* Estimated from the daily food check-off logs.

Main effect: † time (pre to post).

‡ NP vs. HP group.

§ Group (NP vs. HP).

GFR (mL/min/1.73 m<sup>2</sup>) = 186

× [serum creatinine (mg/dL)]<sup>-1.154</sup>

× [age(years)]<sup>-0.203</sup> × (0.742 if female)

× (1.210 if African American) (conventional units) (2)

Reclining and sitting systolic and diastolic blood pressures were measured twice during the baseline and poststudy periods by the same research technician using a sphygmomanometer.

**Total Protein Intake.** To document group-specific differences in total protein intake, blood urea nitrogen (BUN) was determined from fasting blood samples taken during baseline and poststudy and measured using a photometric assay (Chemistry Immuno Analyzer AU5700; Olympus) performed by MidAmerica Clinical Laboratories. The BUN concentration, which changes with altered dietary protein intake (22), was used as a crude index of total protein intake and dietary compliance.

**Data and Statistical Analyses**

All measurements are reported as mean ± SE. Baseline measurements (age, height, body weight, BMI, fat mass, LBM, 24-hour energy intake, meal-related appetite and mood state ratings, and markers of cardiovascular and kidney functions) were examined using an independent Student's *t* test comparing subjects according to protein intake. A repeated-measures ANOVA examining main effects of time, protein intake, and/or BMI status and interactions were performed to examine the changes in these variables (pre- to poststudy). Post hoc analyses were performed using Student's *t* tests to detect differences within (pre vs. post)

and between (NP vs. HP) groups, whereas least significant difference procedures were used to detect specific differences between the combined protein and obesity groups (POB-HP, POB-NP, OB-HP, OB-NP). *p* < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS (Version 14.0; SPSS, Chicago, IL).

**Results**

**Subject Characteristics**

There were no differences in age, height, weight, BMI, or body composition between the HP and NP groups at baseline (Table 1).

**Dietary Characteristics and Protein Intake**

Table 2 lists the dietary characteristics of the prescribed 750-kcal/d energy-restricted diet compared with the energy intake estimated from the daily food check-off logs throughout the 12-week intervention. At the start of the intervention, total energy and fat intakes were not different between groups. By design, protein intake was greater [30% of total intake from protein (1.41 ± 0.02 g/kg per day)] in the HP group compared with the NP group [18% of total intake from protein (0.82 ± 0.02 g/kg per day), whereas carbohydrate intake was lower in the HP group (45% of total intake from carbohydrate) compared with the NP group (57% of total intake from carbohydrate; Table 2). Regardless of group, at the end of the 12 weeks, actual total energy, protein, carbohydrate, and fat intakes were slightly lower than prescribed (Table 2). In comparing the differences in protein intake between groups, the HP group consumed significantly more protein at the end of the study [Week 12; 29.5 ± 0.1% of total intake from protein (1.52 ± 0.02 g/kg

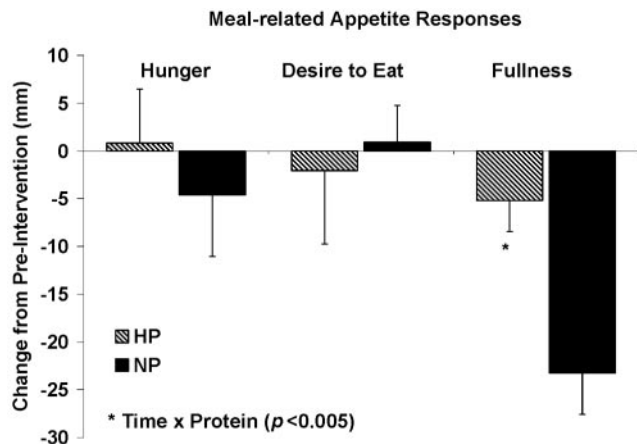


Figure 1: Change in appetite after the 12-week energy restriction in the HP ( $n = 21$ ) vs. NP ( $n = 25$ ) groups. Repeated-measures ANOVA examining the main effects of time and protein and the time  $\times$  protein interaction.

per day)] compared with the NP group [ $18.2 \pm 0.1\%$  of total intake from protein ( $0.92 \pm 0.02$  g/kg per day);  $p < 0.001$ ].

A time by protein interaction ( $p < 0.001$ ) was observed with the BUN measurement such that the HP diet led to a significant increase in BUN ( $+1.5 \pm 0.8$  mg/dL), whereas those consuming the NP diet had a reduction in BUN ( $-2.3 \pm 0.5$  mg/dL).

**Body Weight and Body Composition Changes**

A gradual loss of body weight (overall average rate of  $-0.39 \pm 0.02$  kg/wk) was observed in all subjects regardless of protein intake. Furthermore, at the end of the 12-week intervention, weight loss was not different between the HP and NP groups (Table 1). All groups lost significant amounts of fat mass (main effect of time:  $p < 0.001$ ) and LBM (main effect of time:  $p < 0.001$ ) throughout the 12-week intervention (Table 1). The HP had greater preservation of LBM compared with NP (time by protein interaction:  $p < 0.05$ ).

**Appetite and Mood State Responses**

While increases in hunger and desire to eat ratings were anticipated during energy restriction, no significant differences in the 24-hour (data not shown) or meal-related (Figure 1) responses occurred from pre to post or between groups. No difference in 24-hour feelings of fullness occurred from pre to post or between groups (data not shown). Average meal-related feelings of fullness (measured 60 minutes after the consumption of each meal over 3 days) were reduced after the 12-week energy restriction (main effect of time:  $p < 0.05$ ). However, the reduction in postprandial feelings of fullness were less pronounced in HP vs. NP (Figure 1). Specifically, the postprandial feeling of

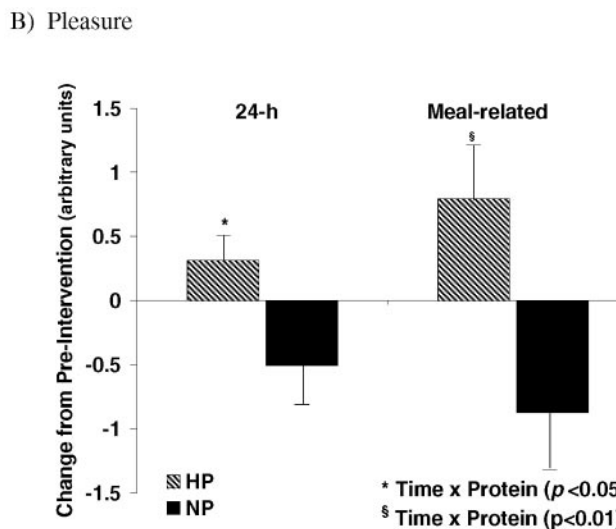
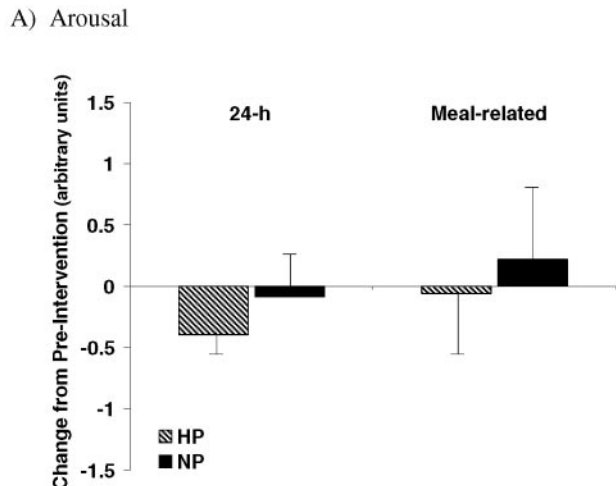


Figure 2: Change in mood state (A, arousal; B, pleasure) after the 12-week energy restriction in the HP ( $n = 21$ ) vs. NP ( $n = 25$ ) groups. Repeated-measures ANOVA examining the main effects of time and protein and the time  $\times$  protein interaction.

fullness was reduced by 27% in the NP group, whereas the HP group only had a reduction of 10% (time by protein interaction:  $p < 0.005$ ).

Twenty-four hour and meal-related arousal remained relatively unchanged in both groups over the 12-week intervention (Figure 2A). Differential responses in 24-hour and meal-related global pleasure were observed between groups (Figure 2B). The NP group had a  $6 \pm 5\%$  reduced feeling of 24-hour global pleasure, whereas the HP group had a  $6 \pm 4\%$  increase in global pleasure despite the energy restriction (time by protein interaction:  $p < 0.05$ ). Similarly, the NP group had a  $10 \pm 6\%$  reduction in meal-related feelings of global pleasure, whereas the HP group had a  $22 \pm 13\%$  increase (time by protein interaction:  $p < 0.01$ ).

**Table 3.** Cardiovascular, metabolic, and kidney disease risk factors before and after energy restriction

Fasting measures	Baseline		Postintervention		Difference	
	NP	HP	NP	HP	NP	HP
Glucose*†‡§ (mg/dL)	96 ± 2	86 ± 2	89 ± 2	87 ± 1	-7 ± 1	0.8 ± 2
Creatinine*‡§ (mg/dL)	0.87 ± 0.02	0.78 ± 0.02	0.83 ± 0.02	0.80 ± 0.01	-0.05 ± 0.02	0.02 ± 0.01
GFR*‡§ (mL/min)	74 ± 3	86 ± 2	78 ± 2	84 ± 2	4 ± 2	-2 ± 2
Total cholesterol†‡ (mg/dL)	206 ± 6	190 ± 6	176 ± 5	158 ± 5	-29 ± 5	-32 ± 6
HDL-C† (mg/dL)	63 ± 3	65 ± 2	57 ± 3	56 ± 2	-6 ± 2	-9 ± 1
LDL-C*†‡ (mg/dL)	118 ± 4	103 ± 6	96 ± 4	85 ± 4	-22 ± 4	-18 ± 5
Triacylglycerol† (mg/dL)	122 ± 10	108 ± 12	110 ± 9	85 ± 11	-12 ± 8	-22 ± 10
Blood pressure						
Supine						
Systolic† (mm Hg)	114 ± 2	109 ± 3	110 ± 2	104 ± 2	-4 ± 2	-6 ± 3
Diastolic† (mm Hg)	72 ± 2	68 ± 1	64 ± 1	65 ± 1	-6 ± 1	-3 ± 1
Sitting						
Systolic† (mm Hg)	113 ± 2	109 ± 3	110 ± 2	104 ± 2	-3 ± 2	-5 ± 2
Diastolic† (mm Hg)	73 ± 2	69 ± 1	65 ± 2	66 ± 1	-6 ± 2	-4 ± 1

Data expressed as mean ± SE.

Independent sample *t* test: ( $p < 0.05$ ).

Repeated-measures ANOVA ( $p < 0.05$ ).

\* NP vs. HP group at preintervention.

Main effect: † time (pre to post).

‡ Group (NP vs. HP).

§ Time × group interaction.

### Cardiovascular, Metabolic, and Kidney Disease Risk Factors

The impact of protein intake during weight loss on selected markers of cardiovascular, metabolic, and kidney health are shown in Table 3. In comparing these risk factors at preintervention, despite random assignment of subjects to a group, glucose, creatinine, and LDL-C were lower and GFR was higher in the HP group vs. the NP group ( $p < 0.05$ ). A main effect of time was observed with fasting glucose, total cholesterol, HDL-C, LDL-C, and triacylglycerol, whereas the main effect of group was observed with fasting glucose, creatinine, GFR, total cholesterol, and LDL-C (Table 3). Time by protein interactions were also found with fasting glucose, creatinine, and GFR (Table 3). Post hoc analyses using independent sample Student's *t* tests show no differences in the glucose, creatinine, GFR, HDL-C, LDL-C, and triacylglycerol responses between the NP and HP groups at postintervention. Furthermore, post hoc paired-sample Student's *t* tests in the NP group comparing pre to post measures showed significant reductions ( $p < 0.001$ ) in fasting glucose, total cholesterol, HDL-C, and LDL-C, with no significant change in creatinine and GFR. The HP group experienced significant reductions

(post hoc paired sample Student's *t* tests:  $p < 0.001$ ) in total cholesterol, HDL-C, LDL-C, and triacylglycerol, with no significant change in fasting glucose, creatinine, and GFR from pre to post.

Supine and sitting systolic and diastolic blood pressures decreased after the energy restriction (main effect of time:  $p < 0.05$ ), with no differences in responses between groups.

### Obesity Classification

A retrospective analysis was performed at the completion of the study to identify whether obesity status differentially affected the primary outcomes during energy restriction. When grouped according to BMI status, the OB in both the HP and NP groups (mean BMI:  $33.2 \pm 0.4$  kg/m<sup>2</sup>) had significantly ( $p < 0.001$ ) higher preintervention body weight, fat mass, and LBM compared with the POB-HP and NP groups (mean BMI:  $27.3 \pm 0.3$  kg/m<sup>2</sup>).

All subjects, regardless of obesity status, lost body weight, fat mass, and LBM (main effect of time:  $p < 0.001$ ; Figure 3). No differences in body weight ( $p = 0.148$ ) and fat mass ( $p = 0.977$ ) changes occurred between POB and OB (Figure 3). However, a differential response in the amount of LBM loss was identified according to obesity

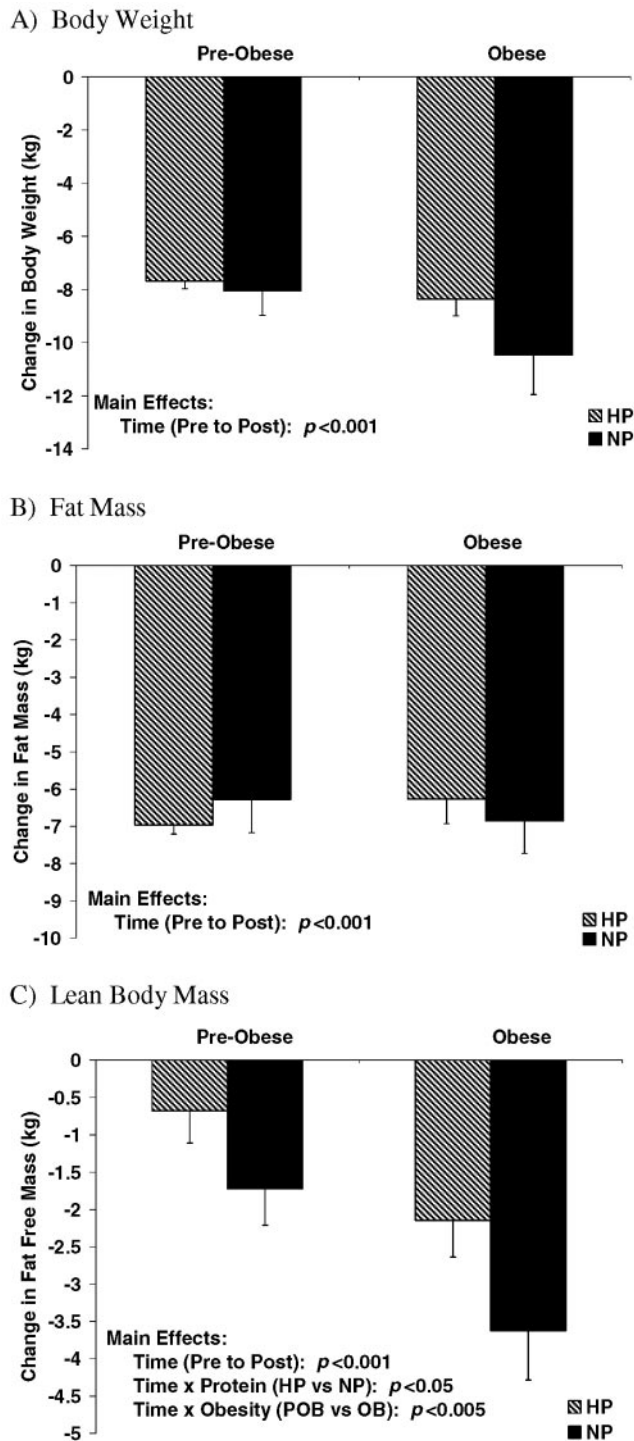


Figure 3: Changes in body weight (A) and body composition (B and C) in response to protein intake and BMI status (HP and NP POB;  $n = 9$  and  $11$ , respectively; HP and NP OB,  $n = 12$  and  $14$ , respectively). Repeated-measures ANOVA examining the main effects of time, protein, and BMI and the time  $\times$  protein  $\times$  BMI interaction.

status such that the POB had greater preservation of LBM compared with the OB ( $-1.2 \pm 0.3$  vs.  $-2.9 \pm 0.4$  kg, respectively; main effect of obesity status:  $p < 0.005$ ), whereas body weight and body fat loss were not different (Figure 3). However, whereas the main effects of protein intake and obesity status were significant, no time by protein by obesity interaction was observed ( $p = 0.693$ ). Thus, the effects on the preservation of LBM seem to be independent and additive. Specifically, the NP-OB exhibited the greatest loss of LBM ( $-3.6 \pm 0.6$  kg) followed by HP-OB ( $-2.1 \pm 0.4$  kg), NP-POB ( $-1.7 \pm 0.5$  kg), and HP-POB ( $-0.7 \pm 0.3$  kg).

While dietary protein seemed to affect satiety and mood state, no differences between POB and OB subjects were observed within each protein group (data not shown). Likewise, hunger and desire to eat were unaffected by obesity status.

When examining the markers of cardiovascular, metabolic, and kidney functions with obesity classification, no differences were observed at baseline in any of the risk factors. Regardless of obesity status, all subjects had improved total cholesterol, LDL-C, triacylglycerol, and systolic and diastolic blood pressure at the completion of the energy restriction. No differences existed between POB and OB subjects within or between HP and NP groups (data not shown).

### Discussion

While the Recommended Dietary Allowance for protein intake is 0.8 grams protein/kg per day (23), we found that women who consumed an HP ( $\approx 1.4$  g protein/kg per day) energy-restricted weight loss diet experienced a greater preservation of LBM, smaller reduction in satiety, and increased global pleasure while losing body weight and body fat compared with women who consumed a similar energy deficit with NP intake ( $\approx 0.8$  g protein/kg per day). Furthermore, we identified a differential response to an HP diet based on obesity classification such that POB women, especially those who consumed the HP diet, exhibited greater preservation of LBM compared with the OB women who consumed the typical Recommended Dietary Allowance for protein.

The dietary interventions are described in terms of protein intake (i.e., HP and NP) that required a reduction of another macronutrient to ensure the treatments were isocaloric. We chose to keep fat intake constant, to omit alcohol intake, and to offset the HP intake with a decrease in carbohydrate consumption. This permitted holding energy density constant and posed a limited shift in carbohydrate ( $< 20\%$ ) relative to the change of protein (72%). Nevertheless, there are potential interactions among the macronutrients that warrant consideration in interpretation of the data.

Previous studies with similar experimental designs to that of this study have found similar changes in body weight, body composition, or satiety. Specifically, Farnsworth et al.

(9) examined the effects of an HP (27%) vs. NP (16%), 4-week, energy-restricted diet (1400 to 1500 kcal/d) on body weight and composition changes in 57 overweight and obese men and women. No differences in body weight and fat mass existed between diets, but the higher protein diet led to a greater preservation of LBM. In a longer study (4 months) in which 48 overweight and obese women were asked to consume either an HP (30%) or NP (18%), energy-restricted diet (1700 kcal/d), Layman et al. (8) reported a greater loss of body weight ( $-8.7 \pm 0.7$  kg) in the HP group vs. the NP group ( $-7.8 \pm 0$  kg), as well as fat mass. There was also a greater preservation of LBM after the HP diet ( $-2.0 \pm 1.0$  vs.  $-2.7 \pm 0.1$  kg).

HP diets are associated with reduced hunger and/or increased satiety (5,11,14,24). However, the majority of the longitudinal dietary intervention studies have only indirectly measured these parameters by examining dropout rates. Johnston et al. (14) examined the effects of an HP diet for 6 weeks and observed an HP diet led to greater satisfaction and less hunger after Month 1 of the trial, with no differences occurring by the end of the study. While we found no differences in hunger and desire to eat during the HP and NP restriction diets, satiety was better preserved in those subjects consuming the HP diet. This effect was noted at the end of the study, suggesting that the effect is maintained throughout a period of energy restriction. The subjects in the HP group also experienced increased global pleasure from pre- to poststudy, whereas the NP group had reduced feelings of global pleasure. It may be speculated that the increased pleasure state was observed in the HP women because of their increased satiety during weight loss. Further work in this area of research is needed to substantiate these speculations.

Whereas HP diets promote beneficial changes in body composition and appetite, they have been linked with increased risks of cardiovascular and kidney diseases. Numerous studies examining changes in glycemic control and lipid concentrations with HP energy restriction diets observed either lower glycemic meal responses along with a greater reduction in triacylglycerol and/or free fatty acid concentrations compared with a NP energy-restricted diet or no difference between groups (8,9,16,25,26). Creatinine concentration and creatinine clearance have also been examined with energy restriction and remained unchanged or slightly increased with HP diets (14,16,27). Significant reductions in fasting total cholesterol, HDL-C, LDL-C, and triacylglycerol concentrations were observed with no change in glucose with increased dietary protein during energy restriction. Of the 46 subjects in our study, 27 had abnormally high lipid profiles at baseline; however, at the end of the 12-week energy restriction, only 12 subjects had abnormal lipid profiles. We also measured serum creatinine concentrations and GFR to examine the effects of HP diets on kidney function and found no significant change from pre to

post intervention in the HP group. Further study is required with direct measures of kidney function. Taken together, increased protein during energy restriction seems to be as safe and effective as normal protein in reducing body weight, improving cardiovascular health, and maintaining normal kidney function during energy restriction but leads to beneficial changes in body composition.

Previous studies comparing obese and lean men and/or women lend support for differences in physiological responsiveness to HP diets because of their metabolic perturbations in obese individuals. Specifically, Labayen et al. (28) examined the effect of acute dietary protein intake and fat mass status on substrate oxidation rates and found that obese individuals have higher fasting lipid oxidation compared with lean subjects and greater postprandial fat use after a HP meal compared with lean subjects. Alternatively, Marques-Lopes et al. (29) reported that overweight and obese men had lower fat oxidation after the consumption of a high carbohydrate meal compared with lean men. Thus, there seems to be significant metabolic differences in how lean and overweight/obese individuals use macronutrients. However, whether POB individuals exhibit a similar metabolic profile compared with those who are already obese has not been examined.

The combined effects of increased protein intake and lower obesity status led to greater preservation of LBM with similar losses in body weight and body fat. The differential response in LBM may be reflective of metabolic differences between the groups. This was a retrospective analysis, and whereas we had adequate power (0.85) to detect differences in LBM between protein and obesity groups, we may have been underpowered to detect a difference in body weight and fat mass. However, when separately examining these parameters according to protein groups or obesity classification, no differences were observed. While speculative, our data suggest that obese individuals may be more susceptible to the negative effects of consuming a normal protein diet during energy restriction and should potentially incorporate increased dietary protein into a weight loss program.

In conclusion, the consumption of an HP energy-restricted diet led to a better preservation of LBM while losing body weight and body fat along with a smaller reduction in satiety and increased global pleasure. Furthermore, the preservation of lean tissue seems to be additive when consuming an HP diet in a POB state.

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