Original Article
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Obesity

Normal vs. High-Protein Weight Loss Diets in Men: Effects on Body Composition and Indices of Metabolic Syndrome

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Objective: This study assessed the effectiveness of a prescribed weight-loss diet with 0.8 versus 1.4 g protein·kg^{-1} day^{-1} on changes in weight, body composition, indices of metabolic syndrome, and resting energy expenditure (REE) in overweight and obese men.

Design and Methods: Men were randomized to groups that consumed diets containing 750 kcal day^{-1} less than daily energy needs for weight maintenance with either normal protein (NP, $n = 21$) or higher protein (HP, $n = 22$) content for 12 weeks. The macronutrient distributions of the NP and HP diets were 25:60:15, and 25:50:25 percent energy from fat, carbohydrate, and protein, respectively. Assessments were made pre and post intervention. The subjects were retrospectively subgrouped into overweight and obese groups.

Results and Conclusion: Both diet groups lost comparable body weight and fat. The HP group lost less lean body mass than the NP group (-1.9 ± 0.3 vs. -3.0 ± 0.4 kg). The effects of protein and BMI status on lean body mass loss were additive. The reductions in total cholesterol, HDL-C, triacylglycerol, glucose, and insulin, along with LDL-C, total cholesterol-to-HDL-C ratio, and HOMA-IR, were not statistically different between NP and HP. Likewise, macronutrient distributions of the diet did not affect the reductions in REE, and blood pressure. In conclusion, energy restriction effectively improves multiple clinical indicators of cardiovascular health and glucose control, and consumption of a higher-protein diet and accomplishing weight loss when overweight versus obese help men preserve lean body mass over a short period of time.

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Introduction

The prevalence of overweight (BMI between 25 and 29.9 kg m^{-2}) and obesity (BMI $\geq$ 30 kg m^{-2}) among US adults has increased dramatically over the past few decades with currently 34 and 68% of the population, respectively, either obese or overweight/obese (1). Overweight and obesity are associated with high prevalence of metabolic syndrome, a group of risk factors including hypertension, hyperlipidemia, hyperglycemia, and central obesity (2). Dietary energy restriction is an effective means to lose weight (3). Among myriad energy-restricted diets, higher-protein diets (>25% of energy from protein) are recommended and promoted because they may suppress appetite (4-6) and help preserve lean body mass (7-9), which may lead to a corresponding retention of resting energy expenditure (REE) (10). REE retention is essential for weight maintenance after weight loss (11). An effective weight-loss diet should focus on weight reduction and successfully reducing indices of metabolic syndrome. There are still debates on the optimal weight-loss diet to treat overweight and obesity-related complications. Higher-protein diets with protein replacing carbohydrate are reported in some studies to reduce insulin resistance (8) and blood pressure (12), but not others (13,14). Higher-protein diets with protein from animal sources are often high in saturated fat which may have a tendency to increase low-density-lipoprotein-cholesterol (LDL-C). In addition, consuming a higher-protein diet may compromise renal function and worsen insulin resistance in individuals predisposed to kidney diseases (15). In the current study, we hypothesized that compared with a normal-protein diet, overweight and obese men consuming a higher-protein, energy-restricted diet would successfully lose weight and improve metabolic health while retaining lean body mass and without impairing renal function over 12 weeks of controlled feeding.

Overweight and obese adults are often included in weight loss studies without regard to their clinical weight status, presumably based on the assumption that their responses would not differ. However, evidence suggests that obese individuals are at higher risk of metabolic syndrome (16) and it is possible that BMI classification

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(overweight vs. obese) may influence the changes in body composition and metabolic health parameters after weight loss. In a previous study, we observed that when overweight and obese women consumed normal or higher protein diets, comparable to those consumed by the men in the current study, for 12 weeks, the group of women who were overweight at the start of the intervention lost less lean body mass than the women who were obese (17). In regards to body composition changes, the main effects of normal vs. higher protein intake and overweight vs. obese classification were independent and additive. The current study extends these findings with comparable analyses in men.

The primary purpose of this study was to assess the effectiveness of a prescribed weight-loss diet with 0.8 versus 1.4 g kg^{-1} day^{-1} of protein on energy-restriction-induced changes in body weight, body composition, metabolic syndrome-related indices, REE, and kidney function in overweight and obese men. The secondary purpose was to assess if BMI classification (overweight vs. obese) would affect the outcomes listed above.

Methods and Procedures

Subjects

Potential subjects were recruited via local newspaper advertisement and campus mail. The Purdue University Biomedical Institutional Review Board approved the study protocol and each subject signed an informed-consent form before enrollment (clinicaltrial.gov registration ID: NCT00812162). Inclusion criteria were as follows: (1) male 21 years and older; (2) BMI between 25.0 and 39.9 kg m^{-2}; (3) weight stable (<4.5 kg weight change within last 6 months); (4) nonsmoking; (5) constant habitual activity patterns within last 3 months; (6) clinically normal blood profiles (specifically, normal liver and kidney functions; fasting blood glucose <110 mg dL^{-1}). Each subject received a monetary stipend for participating in the study. Fifty-five men met the entry requirements and started the intervention. Forty-five subjects completed the study and two were excluded from the final analyses due to noncompliance with testing procedures and dietary control. Thus, data from 43 subjects were analyzed and reported [42 Caucasian (98%), 1 African American (2%)].

Study design

This 13-week protocol included 1 week of baseline and 12 weeks of controlled-feeding intervention with energy restriction. Testing at baseline and intervention week 12 included two fasting blood samples on separate days, one body composition measurement, and dietary intakes assessment on 3 nonconsecutive days. Subjects consumed their usual, unrestricted diets ad libitum during baseline and were randomly assigned to consume either a normal-protein (NP; 15% of total energy from protein; $n = 21$), or higher-protein (HP; 25% of total energy from protein; $n = 22$) energy-restricted diet for 12 weeks. Fat content was kept constant at 25% for both diets, while carbohydrate content was 60% for the NP diet and 50% for the HP diet. The authors recognize that the changes in outcomes might be attributable to increased protein or decreased carbohydrate. For descriptive purposes, the dietary interventions are described with respect to protein (NP vs. HP) because the relative difference between groups for protein intake is greater than for carbohydrate (60% vs. 21%, respectively). Subjects were also retrospectively subgrouped according to their BMI status [overweight, BMI = 25.0-29.9 kg m^{-2}; obese, BMI = 30-39.9 kg m^{-2}], which resulted in 17 overweight and 27 obese subjects.

Dietary intervention

The energy content of each subject's diet was 750 kcal day^{-1} less than their energy requirement, which was estimated using the Harris-Benedict equation (18) with an activity factor of 1.5. The NP diet contained the recommended dietary allowance (RDA) of 0.8 g protein·kg^{-1} day^{-1} while the HP diet contained 1.4 g protein kg^{-1} day^{-1}, which equaled ~15 and ~25% of total energy from protein, respectively, at the start of the intervention period. Subjects consumed one multivitamin/mineral supplement (Centrum; Wyeth Consumer Healthcare, Madison, NJ) and two calcium citrate tablets (400 mg calcium/tablet; total 800 mg calcium/day) daily.

Subjects were counseled to follow 7-day menus with specified quantities of typical and brand-specific food items to purchase and consume. Before starting the diet, each subject met with the study dietitian and was instructed on effective ways to follow and adhere to the diet plan. The NP group menus were void of animal flesh foods (i.e., striated tissues) and egg products; they were given portioned quantities of milk comprising 13% of their total protein intake, while the HP group was provided with portioned quantities of cooked lean pork (loin, ham, or Canadian bacon) and egg products comprising 40% of their total protein intake (25% from pork and 15% from eggs). Thus, the NP diet was lacto-vegetarian and the HP diet was omnivorous. This study was not designed to compare sources of protein (dairy vs. meat/egg). Milk was given to the NP group to equalize the interactions and contact time with the study coordinator that the HP group received. To document adherence to the diet, subjects were asked to complete daily food check-offs and return them to the research laboratory on weekly basis. The food check-off contained a list of each food item and its quantity the subject needed to consume on that day. Subjects were asked to check the consumed food items and to note any foods or beverages not consumed. Extra foods or beverages consumed (highly discouraged) were also recorded on the food logs by the subjects. Subjects' actual dietary intakes were analyzed using the food check-offs with adjustments made for any documented deviations from the prescribed menus accounted for. Energy and macronutrient composition were calculated using ProNutra (Release 3.2, Viocare Technologies, Princeton, NJ).

Clinical measurements

Body weight and composition. During the intervention, each subject's body weight was recorded twice weekly using an electronic platform scale (ES200L; Mettler, Toledo, OH). Fasting state fat mass (including android and gynoid fat mass) and lean body mass were measured using dual-energy X-ray absorptiometry (DXA; GE Medical Systems/LUNAR Prodigy^{TM}) at baseline and postintervention.

Blood pressure. On two separate days at both baseline and postintervention, reclining and sitting systolic and diastolic blood pressures were measured in duplicate using a sphygmomanometer. Subjects rested for 15 min before blood pressures were taken.

Resting energy expenditure and substrate oxidation. Indirect calorimetry (MedGraphics Cardiopulmonary Diagnostics Systems, MedGraphics Corporation, St. Paul, MN) was used to measure CO_2 production and O_2 consumption, which were used to estimate REE

TABLE 1 Subject characteristics at baseline and postintervention[a]

Parameter	Group	Baseline	Post	Change
Age[b], y	NP	44.8 ± 3.6 (24, 43, 75)	–	–
	HP	51.0 ± 2.6 (24, 52, 69)	–	–
Height, cm	NP	179.4 ± 1.4	–	–
	HP	179.6 ± 1.4	–	–
Weight[b], kg	NP	103.2 ± 2.8	92.6 ± 2.6	−10.6 ± 0.6
	HP	101.2 ± 2.7	92.1 ± 2.4	−9.1 ± 0.7
BMI[b], kg m^{-2}	NP	32.0 ± 0.7	28.7 ± 0.7	−3.3 ± 0.2
	HP	31.4 ± 0.7	28.6 ± 0.6	−2.8 ± 0.2
Fat mass[c], kg	NP	36.6 ± 1.5	29.0 ± 1.5	−7.6 ± 0.4
	HP	35.2 ± 1.7	28.0 ± 1.5	−7.2 ± 0.5
Lean body mass[d], kg	NP	63.2 ± 1.5	60.2 ± 1.4	−3.0 ± 0.4
	HP	62.4 ± 1.6	61.0 ± 1.5	−1.9 ± 0.3
Resting energy expenditure[c], kcal d^{-1}	NP	1911 ± 66	1740 ± 64	−171 ± 71
	HP	1948 ± 81	1844 ± 65	−104 ± 77

[a]Mean ± SE, NP: normal-protein group ($n = 21$), HP: higher-protein group ($n = 22$).
[b]Age (y) is presented as mean ± SE (min, median, max).
[c]Significant change over time, $P < 0.05$.
[d]Significant group-by-time interaction, $P < 0.05$.

in a fasting state. Three-hour urine collections were made on the REE testing days while the subject was in a fasting state and urinary urea nitrogen excretion was measured. Substrate oxidation was estimated using the following equations (19):

Protein oxidation (g min^{-1}) = 6.25 × urinary urea nitrogen excretion (N, g min^{-1})
Carbohydrate oxidation (g min^{-1}) = 4.55VCO_2 (L min^{-1}) − 3.21VO_2 (L min^{-1}) − 2.87N
Fat oxidation (g min^{-1}) = 1.67VO_2 (L min^{-1}) − 1.67VCO_2 (L min^{-1}) − 1.92N

Blood collections

Fasting state blood samples were collected on 2 days at baseline and post-intervention, into serum-separator tubes, centrifuged for 10 min at 4,000*g* and 4°C to obtain serum, and stored at −80°C until analysis.

Biochemical analyses and calculations

Cardiovascular, metabolic status. Total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triacylglycerol, glucose, and creatinine were measured by photometric assays (Chemistry Immuno Analyzer AU5700; Olympus, Center Valley, PA) at MidAmerica Clinical Laboratories (Indianapolis, IN). Serum insulin concentration was measured by an electrochemiluminescence immunoassay method on an Elecsys 2010 analyzer (Roche Diagnostic Systems) and insulin resistance (HOMA-IR) was calculated using: glucose (mg dL^{-1}) × insulin (μU mL^{-1})/405 (20). LDL-C was estimated using the following equation:

LDL-C = total cholesterol − HDL-C − (triacylglycerol/5) (21). The total cholesterol to HDL-C ratio was also calculated.

Kidney disease risk factor. Glomerular filtration rate (GFR) was estimated to assess kidney function using the following formula (22):

GFR (mL/min/1.73 m^2) = 186 × [serum creatinine (mg/dL)]$^{-1.154}$ × [age (years)]$^{-0.203}$ × (0.742 if female) × (1.210 if African American)

Total protein intake. Blood urea nitrogen (BUN) was measured using a photometric assay (Chemistry Immuno Analyzer AU5700; Olympus) performed by MidAmerica Clinical Laboratories. BUN was used to crudely evaluate differences in protein consumption between groups and subjects' dietary compliance (23).

Statistical analysis

Statistical analyses were performed using SAS (version 9.2.1; SAS Institute, Cary, NC). Group data are presented as mean ± SE. Baseline parameters were compared using independent Student's *t* test between the NP and HP groups. Repeated measures ANOVA were used to evaluate the main effects of time, protein intake, and BMI status and their interactions on the dependent variables. Baseline values between groups were included in the model as covariates. Post hoc analyses used Student's *t* test to compare the changes overtime between groups. $P < 0.05$ was considered statistically significant.

Results

Subject characteristics

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

Dietary intake and compliance. As designed, the prescribed NP diet contained less protein and more carbohydrate ($P < 0.001$) compared with the HP diet and fat contents were comparable in both diets (Table 2). Table 2 also shows the total energy and macronutrients the groups of subjects actually consumed at intervention weeks 1 and 12. Subjects' macronutrient intakes did not change

TABLE 2 Dietary energy and macronutrients prescribed and consumed during the first and last week of intervention[a]

Parameter	Group	Prescribed	Week 1	Week 12
Energy, kcal d^{-1}	NP	2,320 ± 75	2,263 ± 75	2,273 ± 78
	HP	2,269 ± 70	2,234 ± 70	2,254 ± 75
Protein, g d^{-1}	NP	84 ± 2	82 ± 2	82 ± 2
	HP	143 ± 4	141 ± 4	143 ± 4
Fat, g d^{-1}	NP	66 ± 2	64 ± 2	65 ± 2
	HP	64 ± 2	62 ± 2	63 ± 2
Carbohydrate, g d^{-1}	NP	362 ± 13	354 ± 12	355 ± 13
	HP	286 ± 10	282 ± 10	284 ± 11

[a]Mean ± SE; NP: normal-protein group ($n = 21$), HP: higher-protein group ($n = 22$).

from week 1 to 12 of the intervention (Table 2). At week 1, the NP and the HP groups consumed 0.80 ± 0.02 g kg^{-1} day^{-1} and 1.40 ± 0.02 g kg^{-1} day^{-1} of protein, respectively ($P < 0.001$). At week 12, the NP group consumed 0.88 g kg^{-1} day^{-1} and the HP group consumed 1.55 g kg^{-1} day^{-1} of protein as subjects lost weight ($P < 0.001$). The apparent increase in protein intake from week 1 to 12 was due to reduced body weight among subjects; the quantity of protein each subject consumed remained constant over time.

BUN concentration, which reflects the amount of dietary protein consumed, was not statistically different between the NP (16 ± 1 mg dL^{-1}) and HP (17 ± 1 mg dL^{-1}) groups at baseline. At the end of the study, BUN decreased in the NP group (−1.7 ± 0.7 mg dL^{-1}) and increased in the HP group (+1.7 ± 0.8 mg dL^{-1}), consistent with different dietary protein intakes during the intervention (group-by-time interaction $P < 0.01$).

Body weight and body composition changes. Subjects lost weight and body fat ($P < 0.0001$), independent of dietary protein (Table 1). The HP group lost less lean body mass compared with the NP group (Table 1, group-by-time interaction $P < 0.05$). Android/gynoid fat ratio decreased in both NP (−0.05 ± 0.02) and HP (−0.08 ± 0.01) groups ($P < 0.05$).

Resting energy expenditure and substrate oxidation. Resting energy expenditure (REE) decreased ($P < 0.05$) from baseline to postintervention, independent of dietary protein. There was a trend of linear relation between the changes of lean body mass (kg) and REE (kcal, REE = 15.0 + 67.1 × lean body mass, $P = 0.06$). Protein oxidation decreased in the NP group from baseline to post-intervention (0.062 ± 0.008 vs. 0.037 ± 0.004 g min^{-1}, $P < 0.05$) but did not change in the HP group (0.066 ± 0.006 vs. 0.055 ± 0.006 g min^{-1}). Fat oxidation increased overtime in NP (0.032 ± 0.005 vs. 0.049 ± 0.006 g min^{-1}, $P < 0.05$) but not in HP group (0.035 ± 0.006 vs. 0.040 ± 0.004 g min^{-1}). Carbohydrate oxidation did not change in either NP (0.187 ± 0.021 vs. 0.160 ± 0.015 g min^{-1}) or HP (0.178 ± 0.011 vs. 0.179 ± 0.010 g min^{-1}) groups.

Cardiovascular, metabolic, and kidney disease risk factors. At baseline, no statistically significant differences between groups were observed for parameters of metabolic syndrome and kidney health (Table 3). Blood lipids (total cholesterol, HDL-C, LDL-C, and triacylglycerol) decreased over time ($P < 0.001$), independent of dietary protein. Although HDL-C decreased, cholesterol/HDL also significantly decreased. The insulin and glucose concentrations decreased and insulin resistance (HOMA-IR) improved in both the NP and HP groups ($P < 0.05$). In addition, blood pressure decreased over time ($P < 0.01$), independent of dietary protein.

Serum creatinine decreased in the NP group only (group-by-time interaction: $P < 0.05$). Glomerular filtration rate (GFR) remained unchanged over time in the HP group and increased in the NP group (Table 4, group-by-time interaction: $P < 0.05$).

BMI classification. Subjects were retrospectively grouped according to their baseline BMI as overweight (BMI = 25.0-29.9 kg m^{-2}) or obese (BMI ≥ 30.0 kg m^{-2}). The overweight group ($n = 17$, 40% of subjects) had a lower BMI (overweight: 28.4 ± 0.4 vs. obese: 33.9 ± 0.5 kg m^{-2}), body weight (overweight: 91.4 ± 2.1 vs. obese 109.2 ± 1.9 kg), fat mass (overweight: 29.5 ± 1.2 vs. obese: 40.0 ± 1.1 kg) and lean body mass (overweight: 58.5 ± 1.4 vs. obese: 65.6 ± 1.3 kg) than the obese group ($n = 26$, 60% of subjects) at baseline ($P < 0.001$). After weight loss, the obese group lost more body weight (−10.8 ± 0.6 kg vs. −8.5 ± 0.7 kg) and fat mass (−8.0 ± 0.4 kg vs. −6.5 ± 0.5) than the overweight group, and dietary protein intake did not influence these responses (Table 4). The effects of protein and BMI status on lean body mass loss were additive: the HP group lost less lean body mass than the NP group and the overweight group lost less lean body mass than the obese group. The effect of BMI status on the changes in body composition was not statistically confirmed when the responses were expressed as a % of total body weight loss or % loss of baseline tissue mass (Table 4).

Triacylglycerol concentration was higher in the obese group (245 ± 36 mg dL^{-1}) compared with the overweight group (133 ± 14 mg dL^{-1}, $P < 0.05$) at baseline. After weight loss, the obese group had a greater triacylglycerol decrease than the overweight group (−105 ± 22 vs. −32 ± 29 mg dL^{-1}, respectively, $P < 0.05$). BMI status did not affect other cardiovascular/metabolic markers, HOMA-IR, kidney function, blood pressures, REE or substrate oxidation (data not shown).

TABLE 3 Cardiovascular, metabolic, and kidney disease risk factors at baseline and post-intervention[a]

Parameter	Group	Baseline	Postintervention	Change
Total cholesterol[b], mg dL^{-1}	NP	191 ± 9	157 ± 8	−34 ± 6
	HP	201 ± 14	157 ± 8	−44 ± 8
HDL-C[b], mg dL^{-1}	NP	45 ± 3	41 ± 3	−4 ± 1
	HP	43 ± 2	40 ± 2	−4 ± 1
Cholesterol/HDL-C[b]	NP	4.58 ± 0.33	4.14 ± 0.32	−0.44 ± 0.17
	HP	4.85 ± 0.35	4.09 ± 0.21	−0.76 ± 0.18
LDL-C[b], mg dL^{-1}	NP	108 ± 7	90 ± 7	−20 ± 5
	HP	110 ± 6	93 ± 5	−22 ± 4
Triacylglycerol[b], mg dL^{-1}	NP	184 ± 21	130 ± 14	−54 ± 13
	HP	217 ± 42	126 ± 12	−91 ± 33
Glucose[b], mg dL^{-1}	NP	92 ± 1	91 ± 2	−1 ± 2
	HP	96 ± 2	93 ± 2	−3 ± 1
Insulin[b], μU mL^{-1}	NP	13.2 ± 1.6	9.5 ± 1.4	−3.7 ± 1.4
	HP	14.2 ± 1.6	8.8 ± 0.8	−5.4 ± 1.2
HOMA-IR[b]	NP	3.03 ± 0.39	2.15 ± 0.33	−0.88 ± 0.06
	HP	3.44 ± 0.42	2.06 ± 0.20	−1.38 ± 0.23
Sitting systolic BP[b], mm Hg	NP	133 ± 2	122 ± 2	−11 ± 2
	HP	134 ± 3	123 ± 2	−11 ± 2
Sitting diastolic BP[b], mm Hg	NP	84 ± 2	79 ± 1	−5 ± 1
	HP	83 ± 2	77 ± 2	−6 ± 2
Reclining systolic BP[b], mm Hg	NP	130 ± 2	123 ± 2	−7 ± 2
	HP	134 ± 3	122 ± 2	−12 ± 2
Reclining diastolic BP[b], mm Hg	NP	82 ± 2	75 ± 1	−7 ± 2
	HP	82 ± 2	74 ± 1	−8 ± 1
Creatinine[c], mg dL^{-1}	NP	1.07 ± 0.03	0.98 ± 0.02	−0.09 ± 0.03
	HP	1.07 ± 0.03	1.07 ± 0.03	0.00 ± 0.03
Glomerular filtration rate[c], mL min^{-1}	NP	83 ± 3	91 ± 3	9 ± 2
	HP	79 ± 2	80 ± 2	0 ± 2

[a]Mean ± SE. NP: normal-protein group ($n = 21$), HP: higher-protein group ($n = 22$).
[b]Significant change over time, $P < 0.001$.
[c]Significant group-by-time interaction, $P < 0.05$.

TABLE 4 Body composition changes in overweight and obese groups[a]

Parameter	Group	Overweight (kg)	Obese (kg)	Overweight (% loss from total body weight loss)	Obese (% loss from total body weight loss)	Overweight (% loss of baseline tissue)	Obese (% loss of baseline tissue)
Body weight[b]	NP	−9.5 ± 1.6	−11.0 ± 0.6	_	_	10 ± 1	10 ± 1
	HP	−7.4 ± 1.0	−10.7 ± 0.9	_	_	8 ± 1	10 ± 1
Fat mass[b]	NP	−7.3 ± 0.8	−7.7 ± 0.5	81 ± 7	70 ± 3	24 ± 2	20 ± 2
	HP	−6.0 ± 0.7	−8.4 ± 0.7	82 ± 4	79 ± 3	21 ± 3	20 ± 1
Lean body mass[c],[d]	NP	−2.2 ± 0.6	−3.3 ± 0.4	19 ± 7	30 ± 3	4 ± 2	5 ± 1
	HP	−1.4 ± 0.5	−2.3 ± 0.5	17 ± 4	21 ± 3	2 ± 1	3 ± 1

[a]Mean ± SE. Overweight $n = 17$ (NP = 6, HP = 11), Obese $n = 26$ (NP = 15, HP = 11).
[b]Significant effect of BMI status on changes in kg: Obese lost more than overweight group, without differential response between the NP and HP groups ($P < 0.001$).
[c]Main effects of BMI status and diet groups on changes in kg ($P < 0.05$).
[d]Main effects of diet groups on % loss from total body weight loss ($P = 0.06$) and % loss of baseline tissue ($P < 0.05$).

Discussion

Debates continue regarding the optimal macronutrient distribution to achieve weight loss. In the current study, both the NP and HP groups lost comparable body weight. This finding is consistent with the previous study conducted by our group in overweight and obese women using a similar experimental design (17) and other studies with similar intervention length (7,8,24). However, results from longer-term studies are inconclusive. Some studies found that an energy-restricted diet with higher-protein content resulted in greater weight loss (25,26), while others found no effect of dietary protein on weight loss (27,28). A 2-year weight-loss study showed that the macronutrient distribution of the diets did not influence weight loss (29). That study (29) had a large sample size, a high retention rate and a diverse population, which helped minimize non-nutritional influences on the results. One recent, controlled-feeding study comparing protein quantity and sources on weight loss found neither quantity nor source of protein was associated with weight or fat loss in adults (12). Collectively, it appears that when energy intake is controlled, macronutrient distribution does not affect the amount of weight loss that can be achieved.

Primary goals of weight loss trials are to maximize body fat reduction and to reduce the indices of metabolic syndrome (2). However, energy restriction-induced weight loss also results in the loss of lean body mass. Lean body mass, primarily muscle, is essential for performing physical functions (30). Results from different weight-loss trials showed that consuming more protein during weight loss can help attenuate lean body mass loss (7,17,31). Specifically, consuming a higher-protein diet (1.6 g kg^{-1} day^{-1}) for 10 weeks lead to 0.9 kg less lean body mass loss compared to consuming a normal-protein diet (0.8 g kg^{-1} day^{-1}) (7). Overweight and obese men in the current study who consumed 1.4 vs. 0.8 g kg^{-1} day^{-1} of protein for 12 weeks lost 1.1 kg less lean body mass. We found a comparable lean body mass preservation (1.3 kg less loss) with a higher protein diet in overweight and obese women with similar study design (17). Alternatively, results from a 12-week weight-loss study (8) showed the reduction of lean mass loss with higher protein diet was observed in women, but not in men. The authors speculated that the higher-protein diet was not sufficient enough to preserve lean body mass in men (1.1 g kg^{-1} day^{-1} in men vs. 1.4 g kg^{-1} day^{-1} of protein in women) (8). A meta-regression (9) showed that lean body mass retention increases with each quartile of protein intake: a significant difference existed between the upper two quartiles (>1.05 g kg^{-1} day^{-1}) and the first quartile (<0.7 g kg^{-1} day^{-1}). Thus, the difference of protein intakes between the higher- and lower-protein diets could also contribute to the observed difference in lean body mass retention. Consuming a weight-loss diet with protein at the RDA level may not be sufficient to preserve lean body mass but the evaluation of the optimal protein intake during energy restriction in terms of lean body mass retention still needs to be done.

REE comprises 60-70% of total energy expenditure (2) and lean body mass is a predominant contributor to REE (32,33). Thus, a dietary regimen that would help preserve, or attenuate the loss of lean body mass after weight loss is desirable for subsequent weight maintenance. Some research found that a higher-protein diet (10) lead to a less REE reduction after weight loss while others did not (34). In the current study, REE decreased over time among all subjects independent of dietary protein, with a trend of linear relation between the changes of REE and lean body mass. A recent systematic review found that lean body mass was not associated with the changes of REE and may not be suitable to predict REE with energy-restriction-induced weight loss (35).

Protein from animal sources usually contain higher amounts of cholesterol and saturated fat, which may increase blood lipid concentration and the risk of cardiovascular diseases (17). In the current study, total cholesterol HDL-C, LDL-C and triacylglycerol all decreased, independent of dietary protein, which was consistent with our previous research in women (17) and research by others (7,28). Collectively, these findings suggest weight loss is a stronger factor in lowering blood lipids than quantity and source of protein consumed during the period of energy restriction.

There is little evidence to support that higher-protein diets may impact renal function in healthy individuals (36). The current study showed that GFR in the subjects from the HP group did not change over time, which was consistent with other studies (7,17). It appears that subjects from the NP group had improved kidney functions with an increase in GFR. However, the only variable in GFR formula is serum creatinine concentration and the NP group decreased creatinine concentration overtime. It is likely that the decrease in creatinine in the NP group was attributable to a reduction of the creatine pool due to consuming a meat-free diet during the intervention, compared to the subjects' habitual omnivorous diets.

The obese subjects lost more body weight and fat mass than the overweight subjects in kg, indicating the amount of weight loss may be affected by the degree of energy restriction as well as obesity status. In terms of lean body mass loss, the overweight group had a greater preservation of lean body mass (kg) compared with the obese group, which was consistent with our previous study in women (17). However, subjects had similar reductions in percent loss which suggest BMI status did not affect the magnitude of body composition changes. These findings are contrary to the findings of our previous study in women which showed that the effect of BMI status maintained for percentage comparisons. Whether there is a gender difference still needs further investigation.

Both subjective and objective measures were used to assess compliance in this study. A 750 kcal daily energy deficit should lead to ~9.2 kg weight loss over 12 weeks in theory, assuming 25% loss is lean body mass and 75% is fat mass (37). Subjects' average weight loss was 9.8 ± 0.49 kg, among which 7.38 ± 0.35 kg (75%) was fat mass. Subjects were required to come to the laboratory twice weekly to record their body weight and return the daily food check-off lists. These strategies were very effective in monitoring subjects' weight change and dietary adherence. The findings that BUN increased in the HP group and decreased in the NP group also supports the subjects' compliance, and that habitual dietary protein intakes in these subjects were between 0.8 (RDA) and 1.4 g kg^{-1} day^{-1}. The decrease in total protein intake from baseline for the NP group is consistent with what would occur when a person decreases the quantity but does not change the macronutrient distribution of their usual diet to achieve weight loss. This reduced protein intake might have influenced the body composition responses with weight loss, but another ''control'' group that maintained their total protein intake while losing weight would be required to evaluate this possibility. Because the protein contents of the NP and HP diets were different not only in quantity, but in food sources and quality (animal vs. plant protein), it is not possible to attribute the differential

responses between diet groups specifically to one of these factors. In addition, different fat composition between the two diets due to different protein sources (animal vs. plant) may also impact the outcome measures.

In conclusion, overweight and obese men who consumed a 750 kcal energy deficient diet for 12 weeks successfully lost weight and improved indices of metabolic syndrome. Although the amount of weight loss does not depend on macronutrient distribution, consumption of a higher-protein, energy-restricted diet can attenuate lean body mass loss during weight loss compared with an isocaloric normal-protein diet. The metabolic and physiological impacts of retaining more lean body mass are not evident with respect to REE and improvements in clinical health parameters that comprise metabolic syndrome. O

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