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Effect of Dietary Protein Content on Weight Gain, Energy Expenditure, and Body Composition During Overeating

A Randomized Controlled Trial

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OBESITY HAS BECOME A MAJOR public health concern with more than 60% of adults in the United States categorized as overweight and more than 30% as obese.^{1,2} People who become obese have been in a positive energy balance for an extended period. Swinburn et al³ have argued that this reflects an increase in food intake, but Church et al⁴ have presented data showing that reduced occupational activity might account for the positive energy balance. Although a majority of people in the United States are overweight or obese, there is a significant number of people with normal weights who do not become overweight or obese. As obesity develops, a number of metabolic changes occur, which may not completely reverse when weight is lost.⁵ These differences may re-

Context The role of diet composition in response to overeating and energy dissipation in humans is unclear.

Objective To evaluate the effects of overconsumption of low, normal, and high protein diets on weight gain, energy expenditure, and body composition.

Design, Setting, and Participants A single-blind, randomized controlled trial of 25 US healthy, weight-stable male and female volunteers, aged 18 to 35 years with a body mass index between 19 and 30. The first participant was admitted to the inpatient metabolic unit in June 2005 and the last in October 2007.

Intervention After consuming a weight-stabilizing diet for 13 to 25 days, participants were randomized to diets containing 5% of energy from protein (low protein), 15% (normal protein), or 25% (high protein), which they were overfed during the last 8 weeks of their 10- to 12-week stay in the inpatient metabolic unit. Compared with energy intake during the weight stabilization period, the protein diets provided approximately 40% more energy intake, which corresponds to 954 kcal/d (95% CI, 884-1022 kcal/d).

Main Outcome Measures Body composition was measured by dual-energy x-ray absorptiometry biweekly, resting energy expenditure was measured weekly by ventilated hood, and total energy expenditure by doubly labeled water prior to the overeating and weight stabilization periods and at weeks 7 to 8.

Results Overeating produced significantly less weight gain in the low protein diet group (3.16 kg; 95% CI, 1.88-4.44 kg) compared with the normal protein diet group (6.05 kg; 95% CI, 4.84-7.26 kg) or the high protein diet group (6.51 kg; 95% CI, 5.23-7.79 kg) ($P=.002$). Body fat increased similarly in all 3 protein diet groups and represented 50% to more than 90% of the excess stored calories. Resting energy expenditure, total energy expenditure, and body protein did not increase during overfeeding with the low protein diet. In contrast, resting energy expenditure (normal protein diet: 160 kcal/d [95% CI, 102-218 kcal/d]; high protein diet: 227 kcal/d [95% CI, 165-289 kcal/d]) and body protein (lean body mass) (normal protein diet: 2.87 kg [95% CI, 2.11-3.62 kg]; high protein diet: 3.18 kg [95% CI, 2.37-3.98 kg]) increased significantly with the normal and high protein diets.

Conclusions Among persons living in a controlled setting, calories alone account for the increase in fat; protein affected energy expenditure and storage of lean body mass, but not body fat storage.

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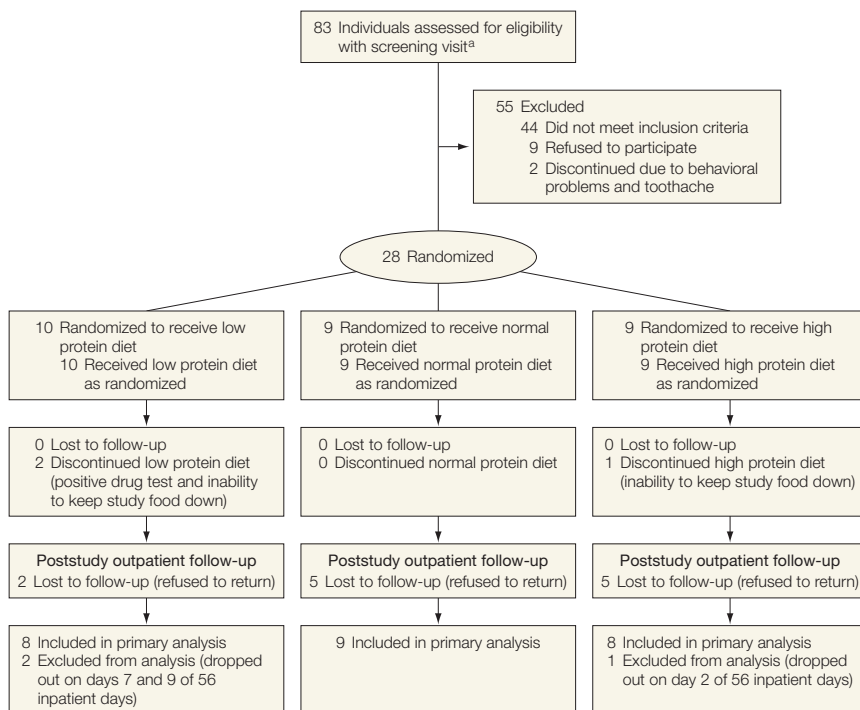
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flect differences in the way individuals handle the food they eat each day both during weight gain and weight loss.

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Figure 1. Flow Diagram Describing Recruitment, Study Flow, and Follow-up of the Participants

^aThere were 399 people who telephoned in response to the marketing plan, but only 83 were considered appropriate for further screening.

The concept that when people overeat the amount of weight gain is highly individual has intrigued medical science for a century.⁶⁻⁹ In a critical review of macronutrient composition and response to overfeeding, Stock¹⁰ cites 12 studies in human beings to support the view that when people overeat a diet that contains either high or low protein, they are less “metabolically efficient” than diets of average protein intake.¹⁰⁻¹⁹ This concept is appealing from an evolutionary perspective because the ability to waste “excess” calories when eating an unbalanced diet would ensure an adequate supply of nutrients while avoiding risks to survival as a result of excess weight gain.^{20,21} In Stock’s analysis,¹⁰ the greatest metabolic efficiency of weight gain during overfeeding was found when protein intake was 10% to 15% of the energy consumed. Conversely, the metabolic inefficiency or “wasting” of calories during overfeeding appeared when diets con-

tained low or high amounts of energy from protein.²² Overeating a diet low or high in dietary protein may maintain body weight through metabolic inefficiency because of the energy cost involved in sparing lean body mass with a low protein diet but expanding lean body mass with a high protein diet.²⁰

This study was designed to determine whether the level of dietary protein differentially affected body composition, weight gain, or energy expenditure under tightly controlled conditions in a randomized controlled trial.

METHODS

Participants

Twenty-five healthy, weight-stable males (n=16) and females (n=9) aged 18 to 35 years, with a body mass index (calculated as weight in kilograms divided by height in meters squared) between 19 and 30, were recruited from the Baton Rouge, Louisiana, commu-

nity using newspaper advertisements approved by an institutional review board. Details about the recruitment, randomization, and follow-up of this study appear in FIGURE 1. Participants were compensated based on their length of time spent in the metabolic unit. Race was self-reported (7 non-Hispanic whites, 16 blacks, and 2 Asians). All food was provided and participants resided in a metabolic unit for 10 to 12 weeks with no prescribed or regular exercise program. Alcohol and caffeine were prohibited throughout the study and smokers were excluded.

The study was reviewed prior to approval by a 3-member external advisory committee, reviewed after 1 year by another external advisory committee, and approved by the Pennington Biomedical Research Center institutional review board and the US Department of Agriculture. Safety was monitored by Donna Ryan, MD (associate director of clinical research, Pennington Biomedical Research Center, Louisiana State University). Participants provided written informed consent. The first participant was admitted in June 2005 and the last in October 2007. Additional information is provided in the eSupplement at <http://www.jama.com>.

Protocol

This was a randomized, parallel-group, inpatient study with the participants living in the metabolic unit from the beginning of the run-in period through the end of the overeating period (FIGURE 2). Neither participants nor inpatient staff was told the protein diet assignment, which was known only to the kitchen staff who supervised participants while they were eating. Participants were randomized to diets containing 5% of energy from protein (low protein), 15% (normal protein), or 25% (high protein), which they were overfed during the last 8 weeks of their 10- to 12-week stay in the inpatient unit. Compared with energy intake during the weight stabilization period, the diets provided 39.4% (95% CI, 37.3%-41.5%) more intake, which cor-

responds to 954 kcal/d (95% CI, 884-1022 kcal/d). Vital signs and body weights were measured daily. After the final day of overfeeding, participants remained in the unit for 1 day during which their diets were returned to baseline energy levels and diet compositions (15% from protein, 35% from fat, and 60% from carbohydrates).

Baseline Weight Stabilization

Energy requirements for weight maintenance were established over 13 to 25 days while consuming an isocaloric diet with 15% of energy from protein, 25% from fat, and 60% from carbohydrates (Figure 2). The initial energy content of the weight stabilizing diet was determined from 24-hour energy expenditure measured in the metabolic chamber on day 3 of the run-in multiplied by an activity factor of 1.15.¹⁸ On day 4, participants commenced a 9-day doubly labeled water study as well as weight

stabilization. Weight stability was a change in body weight of less than 1 kg during a 10-day period without changes in energy intake (Figure 2).

Diets

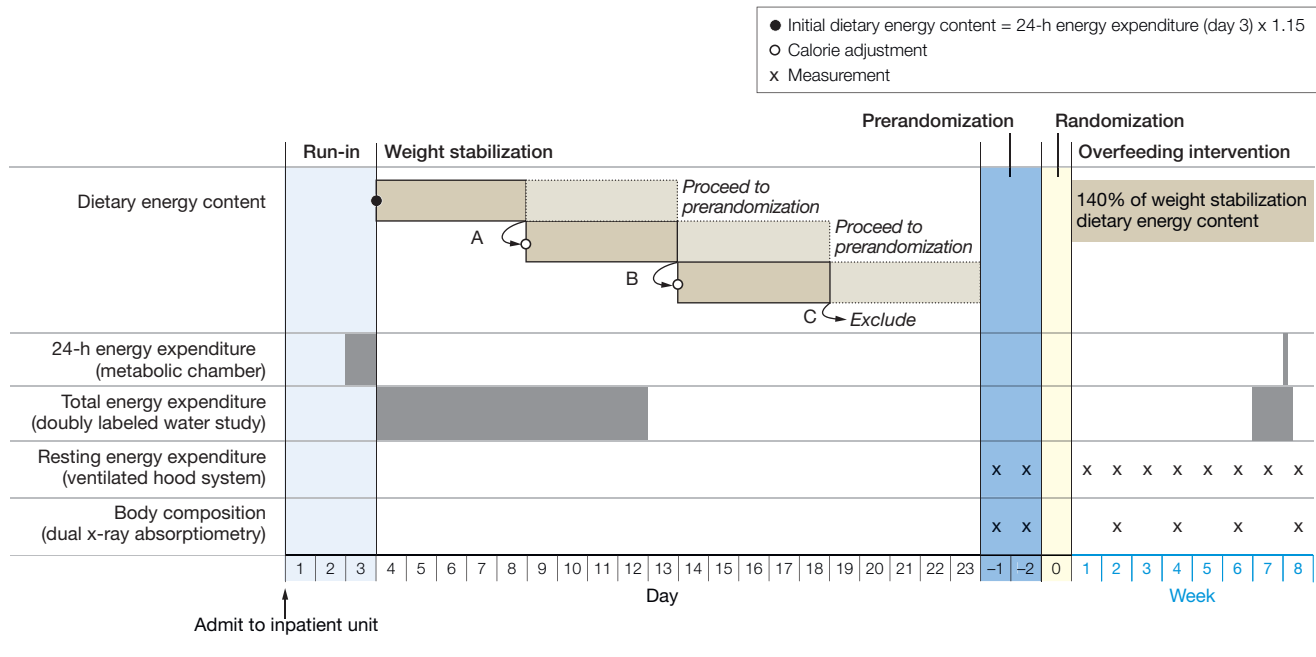
Diets were prepared by the metabolic kitchen and meals were provided in a 5-day rotation with overfeeding calories in proportion to run-in energy requirements. A 5-day diet for each participant was prepared in duplicate, composited, and frozen before shipping to Covance Laboratories for analysis of fat, protein, and carbohydrate. Absolute carbohydrate intake was kept constant throughout the study. The experimental diets varied by chemical analysis in the ratio of protein to fat. The low protein diet had 6% of energy from protein, 52% from fat, and 42% from carbohydrates. The normal protein diet had 15% of energy from protein, 44% from fat, and 41% from carbohy-

drates. The high protein diet had 26% of energy from protein, 33% from fat, and 41% from carbohydrates. There was 47 g/d (95% CI, 42.7-50.5 g/d) of protein in the in the low protein diet group, 139 g/d (95% CI, 117-162 g/d) in the normal protein diet group, and 228 g/d (95% CI, 188-268 g/d) in the high protein diet group compared with 90.2 g/d (95% CI, 83.6-96.9 g/d) during the weight stabilization period. Meal times were supervised by the dietary staff to ensure that all foods were eaten. The foods included in the 5-day dietary rotation and the quantity of food for each protein diet group at the level of calorie intake are listed in the eTable at <http://www.jama.com>

Resting Energy Expenditure

Resting energy expenditure was measured for 30 minutes each week with a ventilated hood system (Deltatrac II Metabolic Monitor, Datex-Ohmeda).²³

Figure 2. Study Design Showing Study Days Before Overfeeding and Weekly Events Thereafter



If a participant's body weight was stable during the first 5 days of weight stabilization (study days 4-8, body weight ± 1 kg), energy content was maintained for an additional 5 days. If body weight fluctuated by more than 1 kg during days 4 and 8, energy content was adjusted (A, open circle) and weight stabilization was restarted. If a participant's body weight was stable during the second 5-day period of weight stabilization (days 9-13), energy content was maintained for an additional 5 days. If body weight fluctuated by more than 1 kg between days 9 and 13, energy content was adjusted (B, open circle) and weight stabilization was restarted. If a participant's body weight was stable during the third period of weight stabilization (days 14-18), energy content was maintained for an additional 5 days. If a participant's body weight fluctuated by more than 1 kg between days 14 and 18, the participant was excluded (C).

Box. Equations Used in Protein Diet Study

Total energy expenditure (kcal/d)
= 22.4 rate of carbon dioxide
(3.9/respiratory quotient + 1.10)

Physical activity level = total energy
expenditure/resting energy expenditure

Non-resting energy expenditure = total
energy expenditure - (resting energy
expenditure + $0.1 \times$ total energy
expenditure)

Total Daily Energy Expenditure by Doubly Labeled Water

Total daily energy expenditure was measured during baseline and weeks 7 to 8 by the doubly labeled water method.²⁴ After 2 baseline urine samples were collected, participants drank a solution with 0.2 g/kg of total body water of $^1\text{H}_2^{18}\text{O}$ (Cambridge Isotopes) and 0.115 g/kg of total body water of $^2\text{H}_2\text{O}$ (Isotec Inc). After the initial void, 2 urine samples were obtained after 4.5 hours and 6 hours and then daily for the next 9 days. The ^2H and ^{18}O isotope elimination rates (k_{H} and k_{O}) were calculated using linear regression based on the isotopic enrichment. The rate of carbon dioxide production was calculated from the equations created by Schoeller,²⁵ as modified by Racette et al.²⁶ Total energy expenditure was calculated by multiplying the rate of carbon dioxide by the energy equivalent of carbon dioxide based on the measured respiratory quotient in the metabolic chamber at baseline and week 8 (BOX).

Body Composition

Body composition was measured at baseline and then biweekly during overfeeding using dual x-ray absorptiometry (Hologics QDR 4500A whole-body scanner). The scans were analyzed with QDR software version 11.1 (Hologics).

Statistical Analysis

Randomization was performed by Project Statistician, using the minimiza-

tion allocation method,²⁷ and included stratification for sex and body mass index (dichotomized as $\leq 19 < 26$, and $\leq 26 - \leq 30$).²⁸ When a participant was to be randomized, the statistician or associate was contacted for the participant's diet assignment. The randomization included a planned interim analysis after 30 participants had been enrolled.

Based on the point estimates of Stock,¹⁰ at the end of the overfeeding period, the normal protein group will increase their body weight by 6.6 kg, the high protein group by 4.2 kg, and the low protein group by 3.0 kg. A common dispersion estimate for weight gain ($\sigma_{\text{a}}=3.01$ kg) was used. A type I error rate (α level) of .05 was used with a 1-tailed alternative hypothesis that weight gain for the normal protein diet group exceeds that of the high protein diet group by 2.4 kg (6.6 kg - 4.2 kg). A single interim analysis was taken into consideration.

An initial power analysis testing for a 2.4 kg difference in weight gain between the low protein (5% of energy) and high protein (25% of energy) diets suggested that 20 participants in each treatment group (total of 60 participants) would provide 80% power to reject the null hypothesis. The difference in weight of 2.89 kg between the 5% and 15% protein groups and 3.35 kg between the 5% and 25% protein groups ($P=.002$) when the interim analysis was completed (after 28 patients were randomized and 25 completed the study) exceeded our predetermined end point, and the study was terminated by agreement between the statistician and the investigators. A retrospective power analysis with the 25 participants who completed the study and the observed weight changes provided a β of 0.87.

Baseline data are expressed as mean (standard deviation). One-way analysis of variance was used to compare baseline data. Changes from baseline are expressed as mean (95% confidence interval) and are adjusted for the corresponding baseline covariates, age, and sex. Differences between

groups were compared using the Tukey-Kramer method on the least squares means.

Predicted resting and total energy expenditures at 8 weeks were calculated from the baseline formula relating energy expenditure to lean body mass and body fat and inserting 8-week values for lean body mass and fat mass.²⁹ The measured (observed) minus predicted values were then tested against the null hypothesis that the observed minus predicted value was 0 to determine whether the observed values differed significantly from the predicted ones. Energy stored in protein was calculated as 1000 kcal/kg and energy in fat as 9300 kcal/kg.²⁵ Effects of treatment on change in body composition and energy expenditure were evaluated using simple regression analysis and again after adjusting for baseline covariates, age, and sex. The α level was set at .05 or less and statistical tests were 2-tailed. Analyses were performed using the JMP-7 statistics package (SAS Institute Inc).

RESULTS**Baseline Characteristics**

Twenty-seven participants were randomized, but 2 dropped out before the final measures of energy expenditure were obtained and are not included in the analysis. Sex was balanced across groups but blacks predominated in the normal protein diet group (TABLE). The mean (SD) body mass index was 25.1 (3.0) and ranged from 19.7 to 29.5. Body weight, lean body mass, fat mass, total daily energy expenditure, and resting metabolic rate at baseline were not significantly different between the 3 protein diet groups (Table).

Energy intake required for weight maintenance during the 10-day weight stabilization period that followed the 3-day run-in period was not different between the 3 diet groups ($P=.57$; Table), and was slightly but not significantly higher than baseline total energy expenditure (125 kcal/d [95% CI, 63-312 kcal/d]; $P=.18$). The target for excess energy was 40% above weight maintenance energy requirements and

was chosen to make this study similar to other studies in the literature.¹⁰⁻¹⁹ The actual excess energy reached 40% (95% CI, 36%-44%) based on nutrient tables and 40% (95% CI, 32%-48%) based on chemical analysis of the study menus. This provided an extra 954 kcal/d (95% CI, 885-1022 kcal/d) and was at the lower end of the range (890-2000 kcal/d) used in the studies reported by Stock.¹⁰⁻¹⁹

Changes in Body Composition

During the 56 days of overeating, the participants consumed an excess of 50 729 kcal/d (95% CI, 47 065-54 393 kcal; $P=.13$ between groups); they all gained weight and there were no differences by sex ($P=.12$) or by race ($P=.19$; FIGURE 3). The weight gain in the low protein diet group was 3.16 kg (95% CI, 1.88-4.44 kg), about half that of the other 2 groups (normal protein diet: 6.05 kg [95% CI, 4.84-7.26 kg]; high protein diet: 6.51 kg [95% CI, 5.23-7.79 kg]; $P=.002$) (Table). The rate of weight gain in the low protein diet group was significantly less than in the other 2 groups ($P<.001$). The

failure to increase lean body mass in the low protein group accounted for their smaller weight gain.

Lean body mass decreased during the overeating period by -0.70 kg (95% CI, -1.50 to 0.10 kg) in the low protein diet group compared with a gain of 2.87 kg (95% CI, 2.11 to 3.62 kg) in the normal protein diet group and 3.18 kg (95% CI, 2.37 to 3.98 kg) in the high protein diet group ($P<.001$). The overall increase in fat mass for all 3 groups was 3.51 kg (95% CI, 3.06 to 3.96 kg) from baseline and was not significantly different between the 3 groups ($P=.89$), although the low protein group added on average more than 200 g of fat (about 2000 kcal).

Changes in Energy Expenditure

Overeating led to a significant increase in resting energy expenditure in both the normal and high protein groups. This increase occurred mainly in the first 2 to 4 weeks and the slopes of the regression lines were not significantly different from each other (FIGURE 4). In contrast, resting energy expenditure in the low protein

group did not change significantly with overfeeding, and the slope of the regression line was not different from 0, but was significantly less than the other 2 groups ($P<.001$; Figure 4). Resting energy expenditure in the low protein group was significantly lower during the 8 weeks of overeating than the other 2 protein groups ($P<.001$), which were not different from each other (Figure 4). This increase in resting energy expenditure was strongly related to protein intake ($r=0.75$, $P<.001$).

In response to overfeeding, total energy expenditure (measured by doubly labeled water) increased significantly in the normal and high protein groups compared with the low protein group ($P=.007$), which was not significantly different from baseline (171 kcal [95% CI, -178 to 262 kcal]; $P=.15$; FIGURE 5). The change in total energy expenditure in the low protein group was significantly less than in the normal protein group (Tukey-Kramer $P<.05$; Figure 5). There also was a positive correlation between the change in total energy expenditure and protein

Table. Body Composition and Energy Metabolism at Baseline and Changes During 8 Weeks of Overfeeding

	Baseline				Change from Baseline ^a			
	Protein Diet Group, Mean (SD) ^b			P Value ^c	Protein Diet Group, Mean (95% CI)			P Value ^c
	Low: 5%	Normal: 15%	High: 25%		Low: 5%	Normal: 15%	High: 25%	
Age, y	22.9 (2.75)	22.9 (5.49)	26.8 (1.98)	.08				
Sex, No.								
Male	5	6	5					
Female	3	3	3					
Race, No.								
White	4	0	3					
Black	3	8	5					
Asian	1	1	0					
Weight, kg	69.1 (11.6)	77.6 (13.0)	76.0 (15.4)	.40	3.16 (1.88 to 4.44)	6.05 (4.84 to 7.26)	6.51 (5.23 to 7.79)	.002
Fat mass, kg	16.6 (5.07)	18.3 (7.40)	19.6 (6.38)	.66	3.66 (2.82 to 4.49)	3.45 (2.67 to 4.24)	3.44 (2.60 to 4.27)	.91
Lean body mass, kg	53.2 (10.3)	59.9 (12.5)	57.1 (13.5)	.53	-0.70 (-1.50 to 0.10)	2.87 (2.11 to 3.62)	3.18 (2.37 to 3.98)	<.001
Energy expenditure, kJ/d								
Total	9334 (1505)	9096 (2207)	8736 (1893)	.82	176 (-740 to 1095)	2186 (1321 to 3051)	1898 (974 to 2813)	.007
Resting	6475 (1413)	6207 (1069)	6153 (1187)	.85	-86.1 (-343 to 171)	669 (426 to 911)	949 (690 to 1204)	<.001
Physical activity level ^d	1.48 (0.31)	1.48 (0.29)	1.43 (0.26)	.94	0.021 (-0.13 to 0.18)	0.16 (0.019 to 0.31)	0.079 (-0.078 to 0.23)	.38
Non-resting energy expenditure, kJ/d	1924 (1473)	1979 (1761)	1706 (1558)	.12	245 (-668 to 1158)	1296 (435 to 2157)	756 (-156 to 1669)	.24
Protein, g/d	85.2 (9.2)	92.0 (18.0)	93.3 (19.7)	.57	-38.6 (-51.9 to -25.2)	47.6 (34.9 to 60.2)	135 (121 to 148)	<.001
Energy intake, kJ/d	9457 (1047)	10 357 (2139)	10 395 (2194)	.53	3866 (209)	4088 (209)	3783 (222)	.13

^aData for change from baseline were adjusted for age, sex, and baseline values.

^bUnless otherwise indicated.

^cCalculated using analysis of variance.

^dCalculated as total energy expenditure divided by resting energy expenditure.

intake ($r=0.56$, $P=.004$). Baseline physical activity level (calculated as total energy expenditure divided by resting energy expenditure) was low (mean [SD], 1.46 [0.28]) and did not change during the overeating period ($P=.38$; Figure 5). Non-resting energy expenditure was about one-

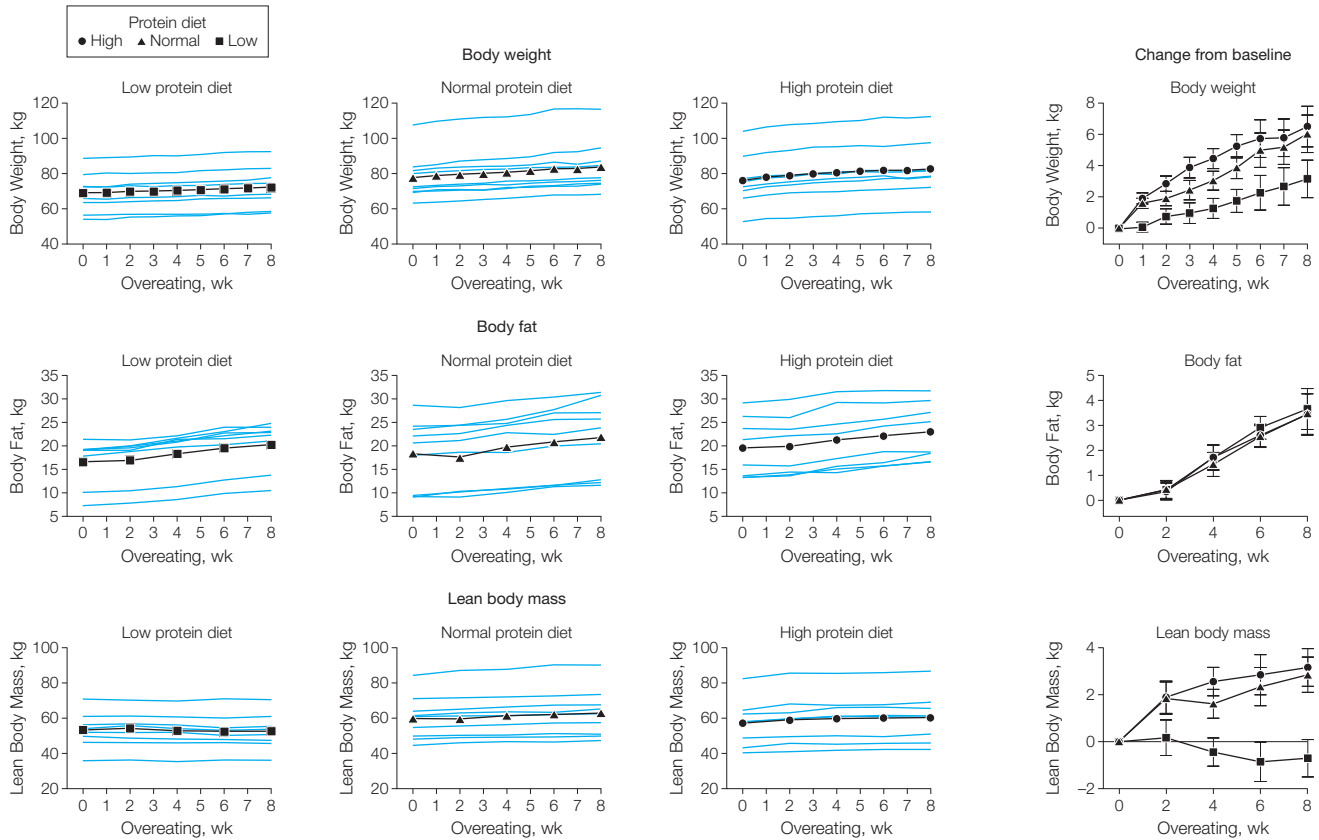
third of the total energy expenditure at baseline and did not change significantly with overeating.

Relation of Body Composition to Changes in Energy Expenditure

The metabolic efficiency of weight gain (defined as the excess energy

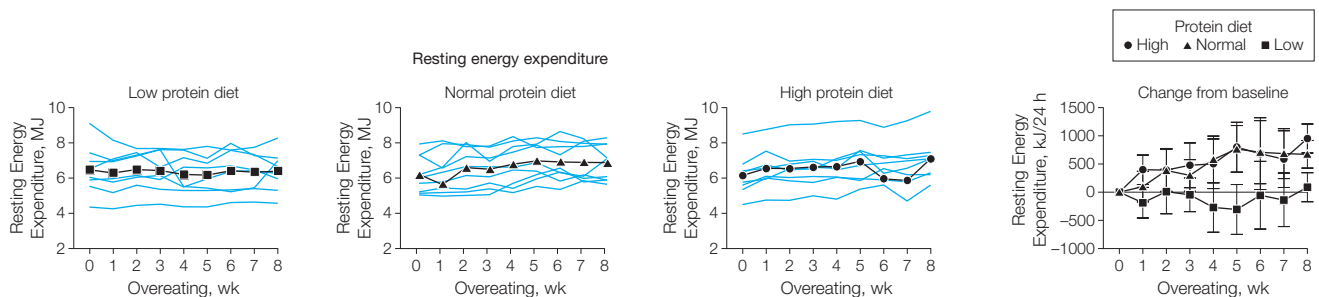
intake divided by weight gain⁵) was significantly higher in the low protein group (75.1 MJ/kg [95% CI, 54.1-96.0 MJ/kg]) than in the high protein group (38.0 MJ/kg [95% CI, 18.6-60.5 MJ/kg]; $P=.04$). The metabolic efficiency of weight gain in the normal protein group was 45.5

Figure 3. Changes in Body Weight, Body Fat, and Lean Body Mass During 8 Weeks of Overeating



The blue horizontal lines indicate individual participants. The error bars in the change from baseline graphs indicate 95% confidence intervals.

Figure 4. Changes in Resting Energy Expenditure During 8 Weeks of Overeating



The blue horizontal lines indicate individual participants. The error bars in the change from baseline graph indicate 95% confidence intervals.

MJ/kg (95% CI, 25.5-65.0 MJ/kg). When adjusted for age, sex, and baseline weight, there was no longer a treatment effect ($P=.15$). The limitations of this approach are clear when changes in body composition are taken into consideration. Extra energy intake predicted both the increase in lean body mass and body fat (FIGURE 6). In contrast, protein intake predicted the increase in lean body mass, but not the change in fat storage.

To examine further whether the change in energy expenditure could be explained by changes in body composition, we predicted 8-week values for energy expenditure from the baseline relation of energy expenditure to lean body mass and fat mass. The measured minus predicted energy expenditure for the low protein group was not significantly different from zero, indicating that the changes in body composition provided an adequate explanation for the changes in energy expenditure. For the normal and high protein groups, the measured energy expenditure after 8 weeks of overeating was significantly higher than predicted from the changes in body composition. From the regression line of protein intake and change in lean body mass, the protein intake required to prevent loss of lean body mass was 77.8 g/d (95% CI, 54.4-101.2 g/d), which is 30 g/d higher than the protein level that was provided by the low protein diet (47.0 g/d; 95% CI, 42.7-50.4 g/d).

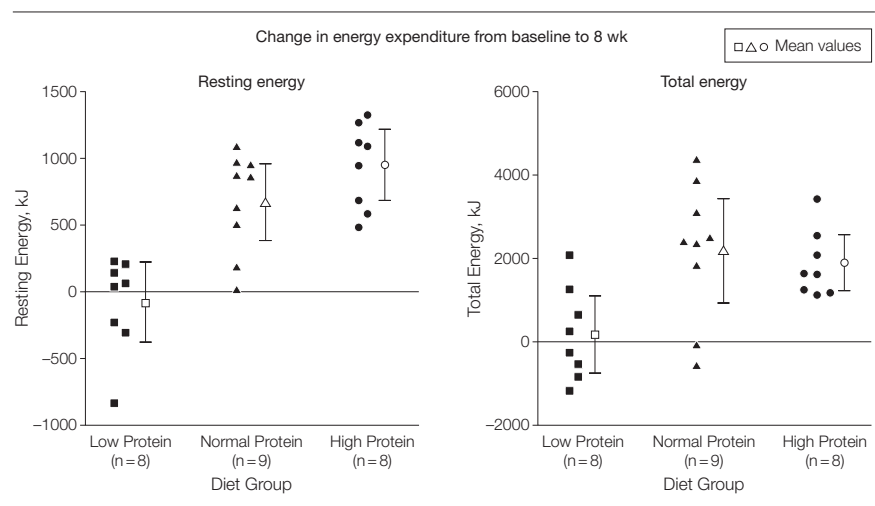
COMMENT

The key finding of this study is that calories are more important than protein while consuming excess amounts of energy with respect to increases in body fat. This study examined the hypothesis proposed by Stock¹⁰ that overeating a low or high protein diet would produce less weight gain than overeating a normal protein diet. The extra energy provided was high relative to the usual life excesses of caloric intake, but matched other

studies on overfeeding in the scientific literature.¹⁰⁻¹⁹ The low protein diet group gained less weight than the normal or high protein groups when extra calories were eaten. When the data are expressed as the energy cost of weight gain, our data (collected

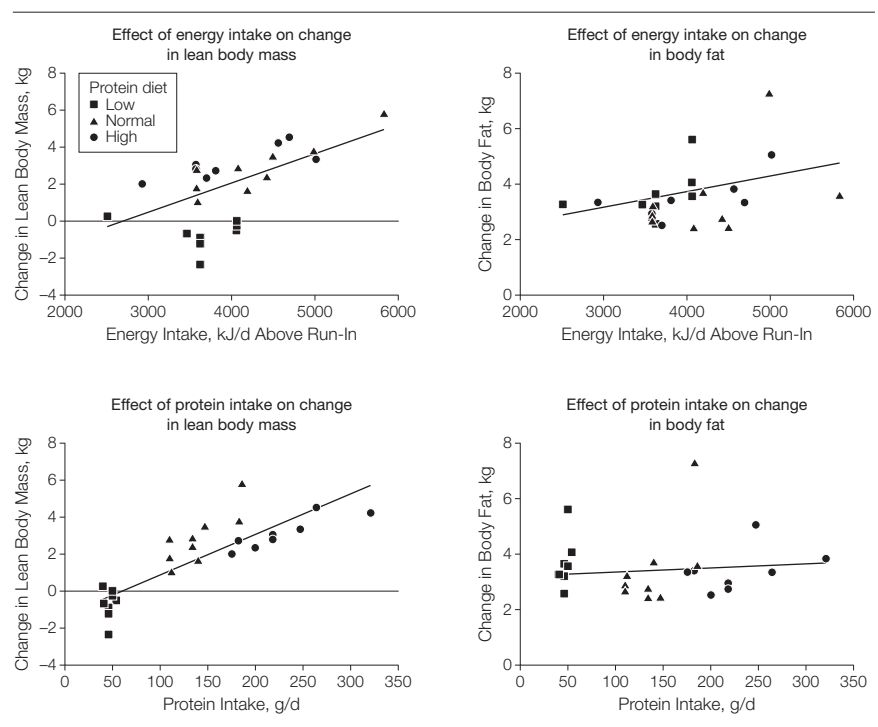
under rigorous experimental conditions in a metabolic ward) are consistent with those of Stock¹⁰ and imply that a diet providing only 5% of energy from protein was metabolically different with a higher energy cost of weight gain compared with diets that

Figure 5. Changes in Resting and Total Energy Expenditure



The error bars indicate 95% confidence intervals.

Figure 6. Relationship of Energy Intake and Protein Intake With Change in Body Fat and Change in Lean Body Mass



contained 15% and 25% of energy from protein. Using the energy cost of weight gain, Joosen and Westerterp³⁰ concluded that 5 of 14 studies showed metabolic inefficiency (ie, a higher value for energy cost of storage than predicted).

The limits of this approach are evident in our study when changes in body composition and energy expenditure are included in the analysis. There were no significant differences between energy intake and energy expenditure between the 3 diets. We can account for all excess energy consumed through energy stored in fat and in protein or expended in higher total energy. With the low protein diet, more than 90% of the extra energy was stored as fat. Because there was no change in lean body mass, the 6.6% increase in total energy expenditure reflects the energy cost of storing fat and is close to the estimate of 4% to 8% for fat storage derived by Flatt.³¹ With the normal and high protein diets, only about 50% of the excess energy was stored as fat with most of the rest consumed (thermogenesis). The high total energy expenditure probably reflects the higher cost of protein turnover and storage.

Resting energy expenditure responded differently to low vs high protein intake. Neither resting energy expenditure, nor lean body mass increased in the low protein group. In contrast, the accretion of lean body mass in the normal and high protein groups was the principal contributor to the increase in resting energy expenditure. Much of the increase in resting energy expenditure occurred within the first 2 weeks in the groups with 15% (normal) and 25% (high) of energy from protein. Over the remaining 6 weeks, there was a further small increase in the normal protein diet group, but little change in the high protein diet group. Harris et al³² also noted that resting energy expenditure increased early during overfeeding, suggesting that it is responding to the increased

thermic effect of feeding more than the increase in body mass. The extra calories in our study were fed as fat, as in several other studies,^{33,34} and were stored as fat with a lower percentage of the excess calories appearing as fat in the high (25%) protein diet group. The higher fat intake in the low protein group probably reduced nutrient absorption (metabolizable energy) relative to the other groups and this would have brought the intake and expenditure closer together in this group.

This study has several strengths. First, only a few studies of overfeeding had 25 or more participants. Second, only 4 other studies fed volunteers for 8 weeks or longer. Third, we measured both resting energy expenditure and total energy expenditure with the doubly labeled water method. To our knowledge, our study is the only one to examine a range of protein intake levels. In our study, the caloric requirement determined from at least 2 weeks of weight stabilization overestimated the energy expenditure that was estimated by doubly labeled water. This suggests that studies using stable body weight may underestimate actual energy requirements in human beings. One limitation of this study is that a majority of the participants were male and black. However, neither sex nor race significantly affected the weight gain produced by overfeeding, suggesting that these data may be generalizable.

In summary, weight gain when eating a low protein diet (5% of energy from protein) was blunted compared with weight gain when eating a normal protein diet (15% of energy from protein) with the same number of extra calories. Calories alone, however, contributed to the increase in body fat. In contrast, protein contributed to the changes in energy expenditure and lean body mass, but not to the increase in body fat.

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National Institutes of Health, Bethesda, Maryland (Dr de Jonge); and St James Place, Baton Rouge, Louisiana (Dr Most).

Author Contributions: Drs Bray and Smith had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Bray and Smith contributed equally to this work.

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Acquisition of data: Bray, Smith, de Jonge, Rood, Martin, Most, Brock, Mancuso.

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Drafting of the manuscript: Bray, Smith, de Jonge, Xie, Brock, Mancuso, Redman.

Critical revision of the manuscript for important intellectual content: Bray, Rood, Martin, Most, Redman.

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