

# A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations<sup>1-3</sup>

David S Weigle, Patricia A Breen, Colleen C Matthys, Holly S Callahan, Kaatje E Meeuws, Verna R Burden, and Jonathan Q Purnell

## ABSTRACT

**Background:** Ad libitum, low-carbohydrate diets decrease caloric intake and cause weight loss. It is unclear whether these effects are due to the reduced carbohydrate content of such diets or to their associated increase in protein intake.

**Objective:** We tested the hypothesis that increasing the protein content while maintaining the carbohydrate content of the diet lowers body weight by decreasing appetite and spontaneous caloric intake.

**Design:** Appetite, caloric intake, body weight, and fat mass were measured in 19 subjects placed sequentially on the following diets: a weight-maintaining diet (15% protein, 35% fat, and 50% carbohydrate) for 2 wk, an isocaloric diet (30% protein, 20% fat, and 50% carbohydrate) for 2 wk, and an ad libitum diet (30% protein, 20% fat, and 50% carbohydrate) for 12 wk. Blood was sampled frequently at the end of each diet phase to measure the area under the plasma concentration versus time curve (AUC) for insulin, leptin, and ghrelin.

**Results:** Satiety was markedly increased with the isocaloric high-protein diet despite an unchanged leptin AUC. Mean ( $\pm$ SE) spontaneous energy intake decreased by  $441 \pm 63$  kcal/d, body weight decreased by  $4.9 \pm 0.5$  kg, and fat mass decreased by  $3.7 \pm 0.4$  kg with the ad libitum, high-protein diet, despite a significantly decreased leptin AUC and increased ghrelin AUC.

**Conclusions:** An increase in dietary protein from 15% to 30% of energy at a constant carbohydrate intake produces a sustained decrease in ad libitum caloric intake that may be mediated by increased central nervous system leptin sensitivity and results in significant weight loss. This anorexic effect of protein may contribute to the weight loss produced by low-carbohydrate diets. *Am J Clin Nutr* 2005;82:41-8.

**KEY WORDS** Satiety, energy balance, adipose tissue, obesity, body composition, insulin

## INTRODUCTION

The poor long-term outcome of energy-restricted diets for weight loss (1) has led to great interest in weight-reducing diets in which the macronutrient composition is altered but the caloric intake is not overtly specified. Both low-fat diets (2-4) and low-carbohydrate diets that are high in fat and protein (5-7) have been shown to cause a decrease in ad libitum caloric intake and significant weight loss in humans. Thus, it appears that diets with

fat contents at opposite extremes have the same therapeutic result, despite evidence that excessive dietary fat intake promotes obesity (8, 9). This paradox could be explained if it is the high-protein content rather than the lower carbohydrate content of low-carbohydrate diets that offsets the deleterious effect of high fat intakes and results in weight loss.

Studies of macronutrient effects on energy balance are clouded by the inability to vary dietary protein, carbohydrate, and fat content independently of one another. In recently published studies of ad libitum, low-carbohydrate diets, experimental and control subjects consumed diets in which neither fat content nor protein content were held constant between groups (5-7). In the only published long-term study designed specifically to compare the effects of ad libitum diets of normal- and high-protein content, the fat content of the 2 diets was held constant (10). Thus, it could not be determined whether weight loss observed in the subjects who consumed the high-protein diet was due to the increase in dietary protein or the resulting decrease in dietary carbohydrate.

We undertook the present study to further evaluate the hypothesis that increasing the dietary protein content while maintaining the carbohydrate content lowers body weight by decreasing appetite and spontaneous caloric intake. This study was designed to complement a previous study in which the dietary fat content was lowered but the protein content was held constant (11). Subjects in both investigations served as their own controls and were studied under isocaloric intake and ad libitum feeding conditions. Plasma insulin, leptin, and ghrelin concentrations were measured frequently over 24-h periods to elucidate the mechanism of any observed changes in appetite or body composition. Our goal was to determine whether an increased protein intake

<sup>1</sup> From the University of Washington School of Medicine, Seattle, WA (DSW, PAB, CCM, and HSC), and the Oregon Health and Science University, Portland, OR (KEM, VRB, and JQP).

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<sup>3</sup> Address reprint requests to DS Weigle, Endocrinology, Box 359757, Harborview Medical Center, 325 Ninth Avenue, Seattle, WA 98104. E-mail: weigle@u.washington.edu.

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**TABLE 1**  
Subject characteristics at the time of enrollment<sup>1</sup>

	Value (n = 3 M, 16 F)
Age (y)	41 ± 11 (27–62)
Weight (kg)	72.0 ± 8.9 (56.1–88.3)
BMI (kg/m <sup>2</sup> )	26.2 ± 2.1 (22.5–30.1)

<sup>1</sup> All values are  $\bar{x} \pm$  SD; range in parentheses.

confers some of the therapeutic benefits attributed to the currently popular low-carbohydrate diets.

## SUBJECTS AND METHODS

### Subjects

Nineteen healthy adults were recruited by newspaper and advertisements. Eleven subjects from the Seattle area were studied at the General Clinical Research Center (GCRC) of the University of Washington after approval of the protocol by the University of Washington Human Subjects Review Committee. Eight subjects from the Portland, OR, area were studied at the Oregon Health and Science University (OHSU) GCRC after approval of the protocol by the OHSU Human Subjects Review Committee. Characteristics of the subjects at the time of enrollment are summarized in **Table 1**. All subjects were weight-stable for  $\geq 3$  mo before enrollment and were at their lifetime maximal weight. Exclusion criteria included a body mass index (BMI; in kg/m<sup>2</sup>)  $>30$ , regular aerobic exercise ( $>30$  min 3 times/wk), tobacco use, consumption of  $>2$  alcoholic beverages/d, diabetes, chronic medical illness, or pregnancy. Prospective subjects were informed that this was not a weight-reduction study and were not enrolled if they expressed any expectation of losing weight. The subjects provided informed written consent before enrollment.

### Study protocol

After enrollment, subjects completed a 3-d food record and were interviewed by a GCRC dietitian. Individuals were excluded from the study at this point if they typically consumed a diet containing  $>55\%$  or  $<35\%$  of total calories from carbohydrate. Subjects were then placed for 2 wk on a baseline diet consisting of 35% of total daily energy as fat, 50% as carbohydrate, and 15% as protein. All meals were prepared in the Nutrition Research Kitchens of the GCRC at the University of Washington and OHSU and consisted of typical foods found in a mixed American diet (12). A 3-d cycle of a standardized menu provided the required macronutrient distribution over the course of each day. Subjects visited the GCRC 2–3 times weekly to be weighed, meet with the dietitian, and pick up prepared meals for the next 2 or 3 d. Subjects were instructed to eat all food items provided, and daily caloric intake was adjusted to stabilize body weight to within 1.0 kg of baseline weight. Subjects maintained a daily log in which they recorded all food items consumed and provided answers to 2 appetite-related questions every day using 100-mm visual analog scales (VAS). The questions were “How hungry have you felt between meals today?” with VAS anchor points of “not at all hungry” and “extremely hungry” and “How full have you felt after eating meals today?” with VAS anchor points of “not at all full” and “extremely full.” Recent work has confirmed the reproducibility and validity of VAS data for appetite research (13).

On the last day of the 2-wk baseline period, subjects were admitted to the GCRC for placement of an intravenous catheter (visit CRC1); the study diet for that day of the cycle was administered in 3 meals given at 0800, 1200, and 1730 with a snack at 2000. Blood was drawn into EDTA-coated tubes at 30-min intervals from 0800 to 2100 and then hourly until 0800 the next morning. Plasma was separated and stored at  $-70$  °C. At 0730 on the second morning, supine resting metabolic rate (RMR) was measured by indirect calorimetry over a 30-min period with a ventilated hood connected to a metabolic cart with a model 29n Indirect Calorimeter (SensorMedics, Yorba Linda, CA) at the OHSU and with a TrueOne 2400 (Parvomedics Inc, Sandy, UT) at the University of Washington. Because of a period of equipment unavailability, RMR was measured in only 11 of the 19 study participants. Subjects were discharged from the GCRC after the 0800 blood drawing.

During the 2 wk immediately after visit CRC1, the subjects were placed on an isocaloric high-protein diet consisting of 20% fat, 50% carbohydrate, and 30% protein, with a 3-d cycle menu. Daily caloric intake was fixed at the level that would result in a stable weight with the baseline diet, and subjects were instructed to eat all food provided. The subjects continued to keep a daily food log, record appetite information, and visit the GCRC 2–3 times weekly to meet with a dietitian, be weighed, and pick up meals for the next 2 or 3 d. The subjects were readmitted to the GCRC on the last day of this 2-wk period (visit CRC2). The study diet for that day of the cycle was provided, blood was sampled, and RMR was measured as during visit CRC1. All subjects underwent body-composition assessment by dual-energy X-ray absorptiometry scanning at visit CRC2.

After visit CRC2, the dietary macronutrient distribution remained fixed at 20% fat, 50% carbohydrate, and 30% protein; however, subjects were instructed to eat only as much of the diet as they wished (ad libitum phase). Specifically, they were told to eat when hungry, stop eating when satisfied, and avoid making any conscious effort to modify food intake, physical activity, or body weight. Three additional menu days were added to those of the second dietary period to provide a 6-d menu cycle. Sufficient food was provided on this ad libitum high-protein diet to allow subjects to consume up to 15% more than their weight-maintaining daily caloric intake. To decrease boredom and increase compliance with the diet, the subjects were allowed to eat one nonstudy meal and to consume up to 3 servings of alcoholic beverages in a 7-d period. They were also allowed substitutions for fruit and vegetables, depending on seasonal availability, and were provided with supplemental foods that matched the nutrient composition of the diet. The subjects completed the same daily food logs, recorded the same appetite information, and made the same twice-weekly GCRC visits as described above. At each GCRC visit, the subjects returned their food and appetite logs and all uneaten food items from the previous visit. The GCRC nutrition staff weighed back all returned food items to determine actual daily calorie and macronutrient consumption. The subjects were readmitted to the GCRC (visit CRC3) after 12 wk of ad libitum high-protein meal consumption. The study diet for that day of the cycle was provided, and blood sampling, RMR measurement, and dual-energy X-ray absorptiometry scanning procedures were identical to those used during visit CRC2.

Examples of the 15%-protein and 30%-protein diets are given in **Table 2**. The macronutrient composition of the diets was calculated by using the PRONUTRA database and is given in

TABLE 2

Menu for 1 d of the 15%-protein and 30%-protein diets

	15%-Protein diet		30%-Protein diet	
	Weight	Food	Weight	Food
	<i>g/2000 kcal</i>		<i>g/2000 kcal</i>	
Breakfast	155	Orange	248	Orange juice
	28	Cream cheese (regular)	130	Egg Beaters egg whites <sup>1</sup>
	85	Plain bagel	56	Raisin bread
	244	Reduced fat milk	28	Peanut butter
			14	Jam
			244	Fat-free milk
Lunch	80	Whole-wheat bread	80	Whole-wheat bread
	80	Turkey breast	100	Turkey breast
	20	Mayonnaise (regular)	35	"Light" Jarlsberg cheese
	11	Leaf lettuce	15	Leaf lettuce
	180	Apple	15	Fat-free mayonnaise
	26	Potato chips	244	Fat-free milk
Dinner	85	Chicken fajita strips	556	Beef lasagna
	69	Flour tortilla	85	Green beans
	60	Green pepper strips	50	Lettuce
	30	Onions	60	Tomatoes
	4	Olive oil	4	Olive oil, for dressing
	80	White rice	8	Wine vinegar, for dressing
	8	Margarine		
Snack	85	Vanilla ice cream	85	Mandarin oranges, canned
	15	Chocolate fudge sauce	85	Pineapple, canned

<sup>1</sup> ConAgra Foods, Downers Grove, IL.

**Table 3.** Total dietary fiber averaged 11.8 g/1000 kcal for the 15%-diet and 10.2 g/1000 kcal for the 30%-protein diets. Calcium intake averaged 450 mg/1000 kcal for the 15%-protein diet and 700 mg/1000 kcal for the 30%-protein diets. The average fatty acid composition of the 15%-protein diet as a percentage of total energy was 12.7% saturated, 11.5% monounsaturated, and 9.9% polyunsaturated; that of the 30%-protein diets was 7.6% saturated, 7.4% monounsaturated, and 3.9% polyunsaturated.

### Hormone assays

Plasma insulin was measured with a double-antibody radioimmunoassay (14). The lower and upper detection limits were 2.2 and 300  $\mu$ U/mL, respectively, and the intraassay CV was 7%. Leptin was measured with a commercially available radioimmunoassay (Linco Research, St Charles, MO) with lower and upper detection limits of 0.5 and 100 ng/mL, respectively, and an intraassay CV of 5%. All insulin and leptin samples from a single subject were run in duplicate in a single assay. Plasma immunoreactive ghrelin was measured with a commercially available

radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA) with lower and upper detection limits of 80 and 2500 pg/mL, respectively; an intraassay CV of 8.7%; and an interassay CV of 14.6%. Because of the greater variability and lower capacity of the ghrelin assay, 24-h integrated plasma ghrelin concentrations were measured in pools of plasma created from the timed blood samples by combining 50  $\mu$ L plasma from each blood sample drawn at 30-min intervals and 100  $\mu$ L plasma from each blood sample drawn at 1-h intervals. Ghrelin concentrations in these pools were measured in duplicate in 2 separate assays that were normalized to one another by using internal controls as described previously (15).

### Statistical analysis

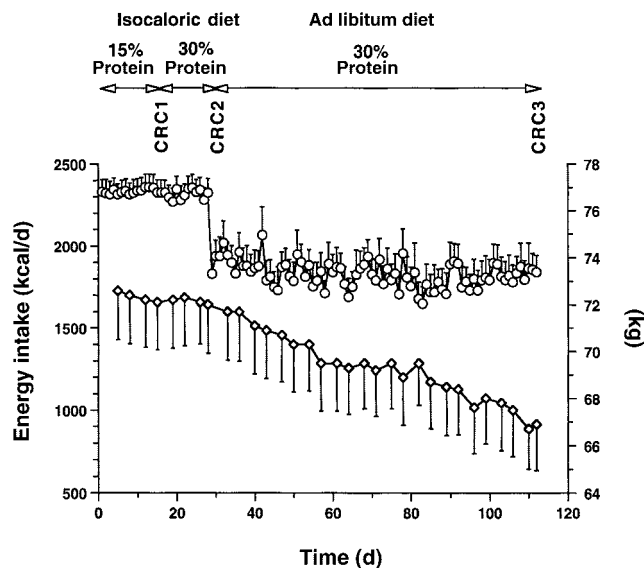
Concentrations of all plasma hormones or fuel molecules, body composition, RMR, and caloric intake data are expressed as means  $\pm$  SEs, unless noted otherwise. Nadirs of leptin time series data were defined as the average of the 2 lowest consecutive values, and peaks were defined as the average of the 2 highest consecutive values occurring over a 24-h period. Values for the 24-h integrated area under the curve (AUC) of plasma leptin, insulin, and glucose concentrations versus time were calculated above zero concentration by using the trapezoidal rule. In addition, AUC was calculated for plasma leptin concentrations minus the morning nadir value (AUC of the change in leptin). Ghrelin concentrations in pooled plasma were multiplied by 24 to calculate AUC ghrelin values. Within-subject comparisons among variables measured at visits CRC1, CRC2, and CRC3 were made by using repeated-measures analysis of variance with the Bonferroni correction applied to pairwise post hoc comparisons. Within-subject comparisons between variables measured only at visits CRC2 and CRC3 were made by using paired-samples *t*

TABLE 3

Composition of the study diets<sup>1</sup>

	Energy	Fat	Carbohydrate	Protein
	<i>kcal</i>	<i>% of energy</i>	<i>% of energy</i>	<i>% of energy</i>
Isocaloric diet				
15%-Protein <sup>2</sup>	1997 $\pm$ 2	35.0 $\pm$ 0.2	50.0 $\pm$ 0.3	15.0 $\pm$ 0.1
30%-Protein <sup>2</sup>	2001 $\pm$ 5	20.2 $\pm$ 0.6	50.3 $\pm$ 0.4	29.5 $\pm$ 0.4
Ad libitum diet				
30%-Protein <sup>3</sup>	2000 $\pm$ 3	20.2 $\pm$ 0.6	50.2 $\pm$ 0.4	29.6 $\pm$ 0.3

<sup>1</sup> All values are  $\bar{x} \pm$  SD.<sup>2</sup> Average of 3 meals in menu cycle.<sup>3</sup> Average of 6 meals in menu cycle.

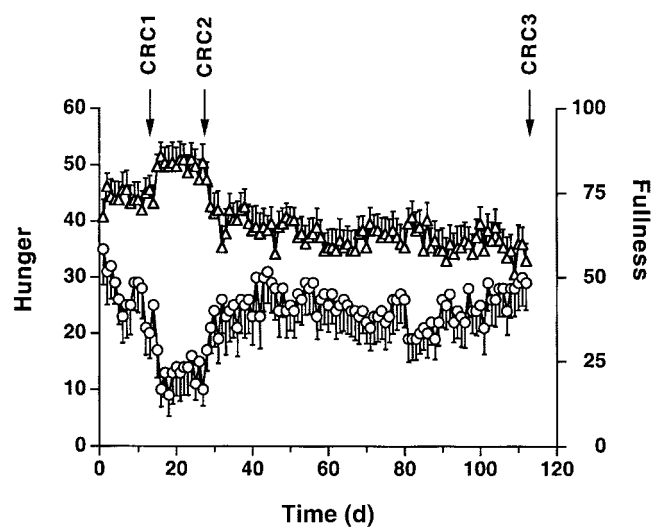


**FIGURE 1.** Mean total daily energy intake ( $\circ$ ) and body weight ( $\diamond$ ) for 19 healthy subjects plotted against day of study. The sequence of study diets and timing of visits 1, 2, and 3 to the General Clinical Research Center (CRC1, -2, and -3) are indicated. The bars represent SEs.

tests. Relations between pairs of variables were assessed by univariate regression analysis with the use of a linear model. All statistical analyses were carried out by using STATVIEW 5.0.1 software (SAS Institute Inc, Cary, NC).

## RESULTS

We studied the consequences of a 15% increase in energy from dietary protein in 19 subjects under weight-stable conditions (isocaloric diets) during the first 4 wk of the protocol and during active weight loss (ad libitum diet) in the final 12 wk of the protocol. The dietary carbohydrate content was constant at 50% of total daily caloric intake throughout the study. The subjects successfully maintained a stable body weight during the isocaloric diet phase, with no significant change in mean weight through day 28 (**Figure 1**). The subjects reported a clear decrease in hunger and increase in fullness during weeks 3 and 4 of the study after the transition to the isocaloric high-protein diet (**Figure 2**). This increase in satiety was confirmed by a decrease in spontaneous caloric intake of  $494 \pm 74$  kcal/d within 24 h of starting the ad libitum diet phase of the study (**Figure 1**). Hunger and fullness scores returned to baseline shortly after the ad libitum high-protein diet began. The subjects maintained a significant decrease in spontaneous caloric intake, relative to baseline (CRC2 compared with CRC3:  $441 \pm 63$  kcal/d;  $P < 0.001$ ), through the end of the study. This sustained decrease in spontaneous caloric intake resulted in a constant rate of weight loss during the 12-wk ad libitum high-protein diet, which amounted to  $4.9 \pm 0.5$  kg lost by the end of the study (**Figure 1**). A decrease in body fat mass accounted for  $3.7 \pm 0.4$  kg (76%) of the weight lost between CRC2 and CRC3, and the overall weight loss was fully explained by the cumulative reduction in caloric intake. There were no significant differences in RMR measured during visits CRC1, CRC2, and CRC3 for the 11 subjects in whom these data could be obtained. Mean energy intake, body composition, and RMR data are summarized in **Table 4**.



**FIGURE 2.** Daily visual analogue scale ratings (scores of 0–100) of hunger ( $\circ$ ) and fullness ( $\Delta$ ) for 19 healthy subjects plotted against day of study. The arrows indicate the time of visits 1, 2, and 3 to the General Clinical Research Center (CRC1, -2, and -3). The bars represent SEs.

The 24-h plasma leptin profiles measured during visits CRC1, CRC2, and CRC3 are shown in **Figure 3**, and the variables characterizing these profiles are summarized in **Table 5**. The isocaloric high-protein diet led to no significant change in nadir plasma concentrations, peak plasma concentrations, or AUC values for the 24-h leptin profiles obtained during CRC2 compared with those obtained during visit CRC1. In contrast, 12 wk of ad libitum high-protein diet consumption led to significant reductions in all of the variables characterizing the leptin profiles at visit CRC3 (**Figure 3**). The percentage change in body fat mass from visit CRC2 to visit CRC3 accounted for 32% of the variability in the percentage change in leptin AUC over this interval (**Figure 4**).

Leptin pulse amplitude was assessed by calculating the difference between peak and nadir plasma leptin concentrations and by calculating the AUC of the leptin profiles after subtracting nadir leptin concentrations (AUC for the change in leptin). As shown in **Table 5**, the peak minus nadir leptin concentration was significantly lower at visit CRC3 than at visit CRC1 and was lower at visit CRC2 than at visit CRC1 ( $P = 0.09$ ). There was a trend toward a reduction in the AUC for the change in leptin during visits CRC2 and CRC3 relative to visit CRC1 ( $P = 0.15$ ; **Figure 3**).

The meal-related changes in plasma insulin and glucose concentrations were as expected, as shown in **Figure 5** and **Figure 6**, respectively. No significant differences were observed in fasting plasma concentrations of free fatty acids, glucose, or insulin measured during visit CRC1, CRC2, or CRC3 (**Table 6**). The isocaloric high-protein diet led to significant increases in AUC values for the 24-h insulin profiles obtained during visit CRC2 compared with those obtained during either visit CRC1 or CRC3. Glucose AUC values were similar during all GCRC admissions.

Aliquots of the 38 plasma samples collected during each 24-h GCRC admission were pooled as described in Subjects and Methods for measurement of average hourly plasma ghrelin concentrations. Ghrelin AUC values were calculated by multiplying the average hourly ghrelin concentrations by 24. This approach minimized errors by allowing all samples to be run in just 2

TABLE 4

Body composition and energy intake data obtained during the final 24-h periods of the weight-maintaining 15%-protein diet (visit CRC1), the isocaloric weight-maintaining 30%-protein diet (visit CRC2), and the ad libitum 30%-protein diet (visit CRC3)<sup>1</sup>

	CRC1	CRC2	CRC3
Weight (kg)	72.1 ± 2.0 <sup>a</sup>	72.0 ± 2.1 <sup>a</sup>	67.1 ± 1.9 <sup>b</sup>
Fat mass (kg)	—	24.1 ± 1.3 <sup>a</sup>	20.4 ± 1.2 <sup>b</sup>
Percentage body fat (%)	—	33.7 ± 1.5 <sup>a</sup>	30.5 ± 1.6 <sup>b</sup>
Energy intake (kcal/d)	2356 ± 80 <sup>a</sup>	2325 ± 85 <sup>a</sup>	1884 ± 101 <sup>b</sup>
Resting metabolic rate (kcal/d)	1542 ± 63 <sup>a</sup>	1568 ± 58 <sup>a</sup>	1560 ± 52 <sup>a</sup>

<sup>1</sup> All values are  $\bar{x} \pm SE$ ;  $n = 19$  for all measurements except resting metabolic rate ( $n = 11$ ). CRC, Clinical Research Center. Means with different superscript letters are significantly different,  $P < 0.05$  [repeated-measures ANOVA with the Bonferroni correction applied to pairwise post hoc comparisons (weight, energy intake, and resting metabolic rate) or paired-samples  $t$  tests (fat mass and percentage body fat)].

ghrelin assays that were tightly linked by means of multiple internal control samples. Ghrelin AUC values measured after 12 wk of ad libitum high-protein diet consumption during CRC3

were significantly greater than were the values measured during visit CRC1 (Table 6). Ghrelin AUC values measured during visit CRC2 did not differ significantly from those measured during visit CRC1.

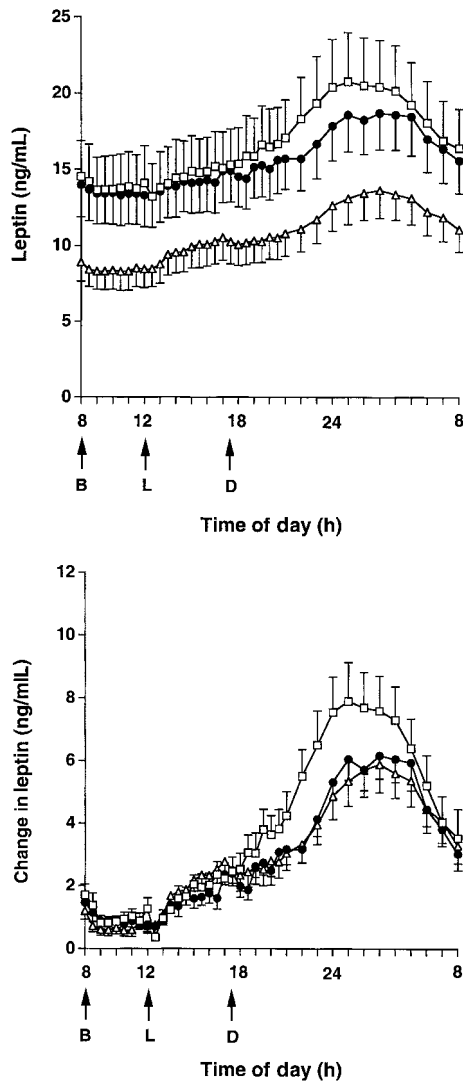


FIGURE 3. Mean 24-h unmodified plasma leptin concentrations and the change in leptin concentrations (eg, minus the morning nadir plasma leptin concentration) in 19 healthy subjects during visits 1 (□), 2 (●), and 3 (△) to the General Clinical Research Center. The bars represent SEs. The arrows indicate the times at which the major meals (B, breakfast; L, lunch; D, dinner) were consumed.

## DISCUSSION

We found that an increase in dietary protein content comparable with that observed in popular low-carbohydrate diets, but no reduction in dietary carbohydrate content, resulted in rapid losses of weight and body fat. This favorable change in body composition was due to a sustained decrease in appetite and ad libitum caloric intake. It is likely that a reduction in dietary fat by 15% of total energy contributed to weight loss in the present study. However, a larger 20% reduction in dietary fat with no change in the percentages of calories from protein produced less weight loss ( $3.7 \pm 0.6$  compared with  $4.9 \pm 0.5$  kg;  $P = 0.13$ ) in our previous study (11). The protocol and 12-wk ad libitum diet periods were identical in both studies. These results suggest that substituting protein for fat in the diet may lead to greater weight loss than can be obtained by substituting carbohydrate for dietary fat. A larger study in which subjects are concurrently randomly assigned to both types of diets will be required to be certain of their relative ability to promote weight loss.

There are 2 mechanisms by which increased dietary protein intakes can promote a negative energy balance and loss of body fat. The first is the ability of dietary protein to increase energy expenditure. This is a small but significant increase that may depend on the relative proportion of animal and vegetable protein in the diet (16) and is partly due to greater diet-induced thermogenesis after protein consumption than after consumption of equal caloric loads of carbohydrate or fat (16–18). Increased dietary protein has also been shown to raise total daily energy expenditure in subjects at energy balance and to attenuate decreases in both sleeping metabolic rate and total daily energy expenditure in subjects following energy-restricted diets (19–21). We found that RMR, the major component of total daily energy expenditure, did not increase with the high-protein diets and that overall weight loss during ad libitum feeding was fully explained by the cumulative reduction in caloric intake. Thus, with the use of real food items and the natural feeding conditions used in the present study, increased thermogenesis did not appear to contribute significantly to the observed weight loss.

A more important mechanism by which dietary protein promotes weight loss appears to be its ability to produce greater satiety than do other macronutrients. This effect was shown in short-term feeding studies that used subjective appetite measures

**TABLE 5**

Plasma leptin data obtained during the final 24-h periods of the weight-maintaining 15%-protein diet (visit CRC1), the isocaloric weight-maintaining 30%-protein diet (visit CRC2), and the ad libitum 30%-protein diet (visit CRC3)<sup>1</sup>

	CRC1	CRC2	CRC3
Nadir (ng/mL)	12.8 ± 2.2 <sup>a</sup>	12.5 ± 1.9 <sup>a</sup>	7.7 ± 1.1 <sup>b</sup>
Peak (ng/mL)	21.1 ± 3.2 <sup>a</sup>	19.3 ± 2.6 <sup>a</sup>	13.8 ± 1.8 <sup>b</sup>
Peak – nadir (ng/mL)	8.3 ± 1.2 <sup>a</sup>	6.8 ± 0.8 <sup>a,b</sup>	6.1 ± 0.9 <sup>b</sup>
AUC leptin (ng · 24 h/mL)	402 ± 62 <sup>a</sup>	375 ± 51 <sup>a</sup>	259 ± 35 <sup>b</sup>
AUC for change in leptin (ng · 24 h/mL) <sup>2</sup>	94 ± 14 <sup>a</sup>	75 ± 8 <sup>a</sup>	74 ± 11 <sup>a</sup>

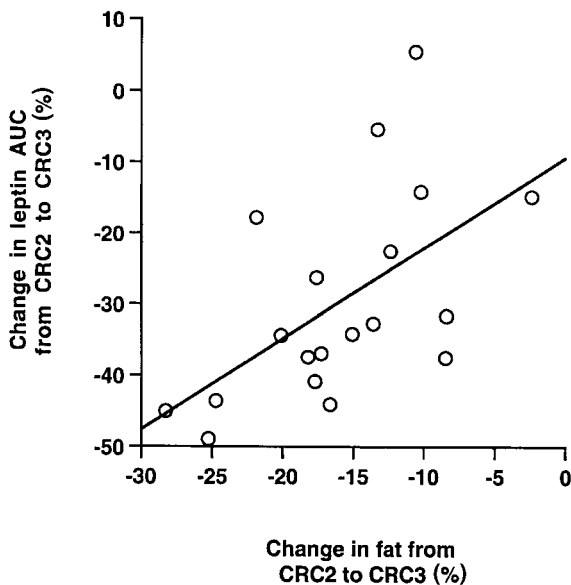
<sup>1</sup> All values are  $\bar{x} \pm SE$ ;  $n = 19$ . CRC, Clinical Research Center. Means with different superscript letters are significantly different,  $P < 0.05$  (repeated-measures ANOVA with the Bonferroni correction applied to pairwise post hoc comparisons).

<sup>2</sup> Calculated as described in Subjects and Methods.

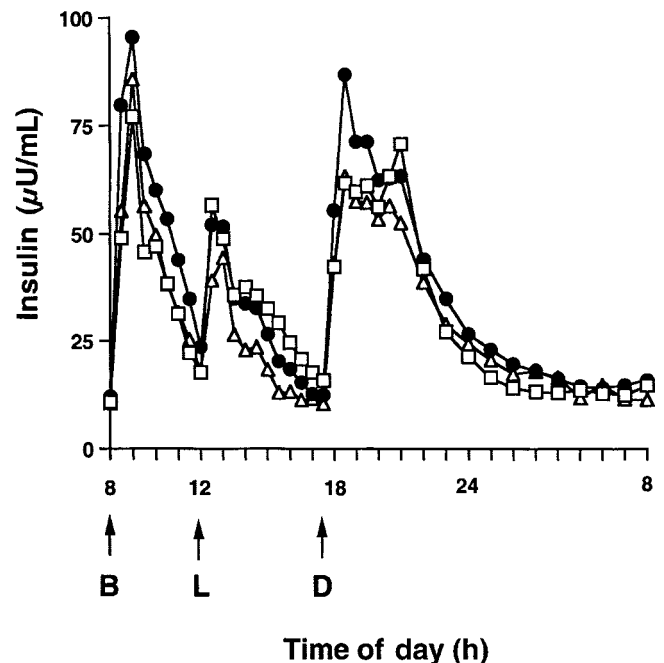
or test meal consumption as endpoints (22–25). In most long-term studies, the effect of increased protein consumption on appetite was obscured by caloric restriction imposed on subjects in addition to the altered macronutrient content of the diet (26–29). Only one long-term, controlled ad libitum feeding study by Astrup et al (10, 30) has been designed specifically to investigate the effect of increased dietary protein content on spontaneous energy intake and body composition. In this study, both the reduction in caloric intake and magnitude of weight loss at 6 mo in the subjects who consumed a 25% protein diet were significantly greater than the values observed in subjects who consumed a 12% protein diet (10). These differences persisted, but were attenuated after consumption of the study diets for 12 mo (30). Although the overall conclusion of this study agrees with ours, no information was provided regarding the effects of the diet on hunger, satiety, or circulating concentrations of hormones known to be involved in body weight regulation.

Our subjects reported a marked increase in satiety with the isocaloric high-protein diet despite an insignificant change in leptin AUC between visit CRC1 and visit CRC2. Their ad libitum

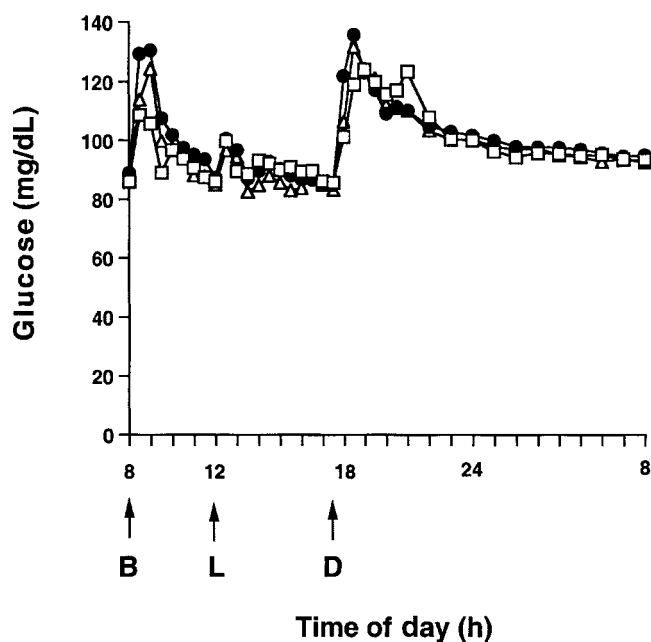
energy intake after 12 wk of the high-protein diet remained  $441 \pm 63$  kcal/d lower than baseline, despite a significant decrease in leptin AUC between visit CRC2 and visit CRC3. This decrease in spontaneous caloric intake was significantly greater ( $P = 0.04$ ) than the  $222 \pm 81$  kcal/d decrease noted at 12 wk in our previous study of carbohydrate substitution for dietary fat at constant protein intake (11). Taken together, these results suggest that increased protein intake enhances the satiating effect of circulating leptin in the central nervous system (CNS). Unlike our previous study, ghrelin AUC values were significantly increased after 12 wk of ad libitum high-protein intake (11). A growing body of evidence suggests that an increase in circulating ghrelin concentrations should increase appetite, thereby attenuating the reductions in caloric intake and body weight that we observed in the present study (31). The anorexic effect of dietary protein, which may be due in part to increased CNS leptin sensitivity, is apparently stronger than any orexigenic effect of increased ghrelin concentrations accompanying weight loss with a high-protein diet.



**FIGURE 4.** Percentage change in the area under the plasma leptin concentration versus time curve (AUC) in 19 healthy subjects between visits 2 and 3 to the General Clinical Research Center (CRC2 and CRC3) plotted against the percentage change in body fat mass over the corresponding time period. The line indicates the best least-squares fit to the data:  $y = 1.28x - 9.35$  ( $r = 0.568$ ,  $P < 0.01$ ).



**FIGURE 5.** Mean 24-h plasma insulin concentrations in 19 healthy subjects during visits 1 (□), 2 (●), and 3 (△) to the General Clinical Research Center. The arrows indicate the times at which the major meals (B, breakfast; L, lunch; D, dinner) were consumed. The error bars were omitted for clarity.




**FIGURE 6.** Mean 24-h plasma glucose concentrations in 19 healthy subjects during visits 1 (□), 2 (●), and 3 (△) to the General Clinical Research Center. The arrows indicate the times at which the major meals (B, breakfast; L, lunch; D, dinner) were consumed. The error bars were omitted for clarity.

Havel et al (32) reported that substitution of carbohydrate for dietary fat increased the diurnal circulating leptin pulse amplitude (peak minus nadir plasma leptin concentration). These authors speculated that, as for other endocrine systems (33, 34), the CNS might interpret an increase in leptin pulse amplitude as a signal calling for a decrease in appetite independently from any change in the integrated circulating leptin concentration (32). Our data directly address this hypothesis because we observed a decrease in leptin pulse amplitude between visit CRC1 and visit CRC2 (decreased peak minus nadir plasma leptin concentration and decreased AUC of the change in leptin) without a significant change in integrated circulating leptin concentration (leptin AUC). The subjects reported a marked increase in satiety despite this isolated reduction in leptin pulse amplitude. These data suggest that if diurnal leptin pulse amplitude is a signal regulating energy balance, it is less important than the putative change in CNS leptin sensitivity observed in the present study.

We found insulin AUC to be significantly higher at visit CRC2 than at visit CRC1, which possibly reflects the better ability of protein than of fat, which it was isocalorically substituted for, to

stimulate insulin secretion (35). Because insulin appears to act synergistically with leptin in the hypothalamus (36), this increase in insulin AUC may have contributed to the increased satiety observed with the isocaloric high-protein diet. The decrease in insulin AUC to baseline values after 12 wk of the ad libitum high-protein diet most likely reflects a decrease in the stimulus for insulin secretion resulting from the overall decrease in energy intake by this point in the study.

In conclusion, a 15% increase in energy from dietary protein at constant carbohydrate intake produces a sustained decrease in ad libitum caloric intake that may be mediated by increased CNS leptin sensitivity and results in clinically significant weight loss. This salutary effect of protein may help to explain the paradoxical weight loss observed in subjects placed on low-carbohydrate diets, because an increase in protein intake accompanies the high fat content of such diets (5–7). Our results suggest that less emphasis should be placed on carbohydrate restriction without regard for concomitant increases in dietary fat. Replacing a portion of dietary fat with protein may result in weight loss comparable with that reported with low-carbohydrate diets while minimizing the adverse long-term effects of increased dietary fat. However, further study of the effects of dietary protein intake on renal function and calcium balance will be required before high-protein diets can be widely recommended for weight loss. 

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**TABLE 6**

Plasma fuel molecule, insulin, and ghrelin data obtained during the final 24-h periods of the weight-maintaining 15%-protein diet (visit CRC1), the isocaloric weight-maintaining 30%-protein diet (visit CRC2), and the ad libitum 30%-protein diet (visit CRC3)<sup>1</sup>

	CRC1	CRC2	CRC3
Fasting free fatty acids (mEq/L)	0.581 ± 0.037	0.571 ± 0.028	0.644 ± 0.034
Fasting glucose (mg/dL)	86.2 ± 2.0	89.1 ± 1.8	85.9 ± 2.3
AUC glucose (mg · 24 h/dL)	2360 ± 31	2414 ± 27	2339 ± 46
Fasting insulin (μU/mL)	10.8 ± 1.0	12.0 ± 1.3	10.5 ± 1.1
AUC insulin (μU · 24 h/mL)	762 ± 47 <sup>a</sup>	875 ± 62 <sup>b</sup>	717 ± 38 <sup>a</sup>
AUC ghrelin (pg · 24 h/mL)	13,979 ± 1072 <sup>a</sup>	14,640 ± 1124 <sup>a,b</sup>	15,456 ± 1173 <sup>b</sup>

<sup>1</sup> All values are  $\bar{x} \pm SE$ ;  $n = 19$ . CRC, Clinical Research Center. Means with different superscript letters are significantly different,  $P < 0.05$  (repeated-measures ANOVA with the Bonferroni correction applied to pairwise post hoc comparisons).

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