

Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women¹⁻³

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ABSTRACT

Background: It is not clear whether varying the protein-to-carbohydrate ratio of weight-loss diets benefits body composition or metabolism.

Objective: The objective was to compare the effects of 2 weight-loss diets differing in protein-to-carbohydrate ratio on body composition, glucose and lipid metabolism, and markers of bone turnover.

Design: A parallel design included either a high-protein diet of meat, poultry, and dairy foods (HP diet: 27% of energy as protein, 44% as carbohydrate, and 29% as fat) or a standard-protein diet low in those foods (SP diet: 16% of energy as protein, 57% as carbohydrate, and 27% as fat) during 12 wk of energy restriction (6–6.3 MJ/d) and 4 wk of energy balance (≈ 8.2 MJ/d). Fifty-seven overweight volunteers with fasting insulin concentrations > 12 mU/L completed the study.

Results: Weight loss (7.9 ± 0.5 kg) and total fat loss (6.9 ± 0.4 kg) did not differ between diet groups. In women, total lean mass was significantly ($P = 0.02$) better preserved with the HP diet (-0.1 ± 0.3 kg) than with the SP diet (-1.5 ± 0.3 kg). Those fed the HP diet had significantly ($P < 0.03$) less glycemic response at weeks 0 and 16 than did those fed the SP diet. After weight loss, the glycemic response decreased significantly ($P < 0.05$) more in the HP diet group. The reduction in serum triacylglycerol concentrations was significantly ($P < 0.05$) greater in the HP diet group (23%) than in the SP diet group (10%). Markers of bone turnover, calcium excretion, and systolic blood pressure were unchanged.

Conclusion: Replacing carbohydrate with protein from meat, poultry, and dairy foods has beneficial metabolic effects and no adverse effects on markers of bone turnover or calcium excretion. *Am J Clin Nutr* 2003;78:31–9.

KEY WORDS Weight loss, protein, body composition, glycemic response, lipids, humans

INTRODUCTION

Insulin resistance increases the risks of cardiovascular disease (1, 2) and type 2 diabetes (2–4). Moderate weight loss—ie, on the order of 4.5–13 kg (5–7)—particularly that involving a reduction in visceral adipose tissue (8, 9), has been shown to improve insulin sensitivity, glycemic control, and dyslipidemia in overweight subjects with insulin resistance. Since the 1960s, high-protein diets with an emphasis on some degree of carbohydrate restriction have been popular with the dieting public (10).

The replacement of some dietary carbohydrate with protein, combined with low total ($< 30\%$) and saturated ($< 10\%$) fat contents, was shown to enhance weight loss in free-living subjects (11, 12). There is minimal evidence (13) for the amelioration of insulin resistance, independent of weight loss, with the consumption of a high-protein diet. The improvement in insulin resistance observed in that study (13) may have resulted from the preservation of lean mass combined with the loss of fat. Two other studies reported that lean mass was preserved after weight loss with an energy-restrictive high-protein diet (14, 15), but neither study examined whether there was an associated improvement in insulin resistance. One weight-loss (12) and 2 weight-maintenance (16, 17) studies also showed that replacing some carbohydrate with protein improves the fasting lipid profile. In 13 obese hyperinsulinemic men (12), an increase in HDL-cholesterol concentrations was reported after greater weight loss with consumption of an energy-restrictive high-protein diet (45% of energy) than that with consumption of an isoenergetic standard-protein diet (15% of energy). The beneficial effect of high-protein diets on serum lipids during either energy restriction or weight maintenance requires confirmation.

Concern has been expressed over the safety of high-protein diets (10). They may enhance calcium excretion and increase bone resorption (18). Calcium loss may occur only when calcium intake is increased but phosphorous intake is fixed (19). An increase in the consumption of meat protein has no effect on bone turnover (19, 20). Diets high in animal protein may also increase blood pressure (21, 22), although cross-sectional data revealed an inverse association between blood pressure and animal protein intake (23, 24). There are no data on the effects of high-protein diets on changes in bone resorption and blood pressure after moderate weight loss.

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² Supported by National Health and Medical Research grant 158012 and Dairy Research and Development Corporation grant CSHN10003.

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Received June 26, 2002.

Accepted for publication February 19, 2003.

TABLE 1
Subject characteristics at screening¹

	SP diet		HP diet	
	Men (n = 7)	Women (n = 22)	Men (n = 7)	Women (n = 21)
Age (y)	48.6 ± 3.2	50.6 ± 2.1	51.9 ± 3.3	50.6 ± 2.7
Weight (kg)	109.4 ± 5.2	88.3 ± 2.2 ²	107.4 ± 5.8	89.5 ± 2.1 ²
BMI (kg/m ²)	34.5 ± 1.5	33.6 ± 0.8	33.7 ± 1.1	34.5 ± 0.9
Insulin (mU/L)	21.6 ± 2.2	17.1 ± 1.6	17.6 ± 1.4	18.1 ± 1.6
Glucose (mmol/L)	5.9 ± 0.4	5.2 ± 0.1 ²	5.4 ± 0.1	5.3 ± 0.2 ²

¹ $\bar{x} \pm \text{SEM}$. SP, standard-protein; HP, high-protein. There were no significant differences between the SP and HP diet groups.

²Significantly different from men, $P < 0.001$ (two-factor ANOVA, with diet and sex as the factors).

The aim of this study was to compare the effects of 2 isoenergetic, energy-restricted diets with either a high or standard content of dietary protein (27% or 16% of energy, respectively, as protein) on body composition, glucose and insulin homeostasis, lipid concentrations, bone turnover, and blood pressure in obese subjects with hyperinsulinemia. We hypothesized that the high-protein diet would lead to weight loss similar to that of the standard-protein diet but would spare lean mass and would result in greater decreases in fasting and postprandial insulin concentrations.

SUBJECTS AND METHODS

Subjects

Sixty-six obese and overweight subjects were recruited by public advertisement. Subjects were included if they were aged 20–65 y and had a fasting serum insulin concentration > 12 mU/L and a body mass index (BMI; in kg/m²) of 27–43. Exclusion criteria included diabetes mellitus; proteinuria; and a history of liver, unstable cardiovascular, respiratory, or gastrointestinal disease or of malignancy. All subjects attended detailed informational sessions and gave written informed consent to their participation in the study. The study was approved by the human ethics committees of the Commonwealth Scientific Industrial Research Organisation and the Royal Adelaide Hospital.

Fifty-seven subjects (14 men, 43 women) completed the study (Table 1). Six subjects withdrew before study commencement because of family and work commitments; in addition, one subject withdrew because of pregnancy during the course of the study, the clinic lost contact with one subject, and data from one subject were excluded from the analysis because of the subject's non-compliance with the diet. Subjects taking antihypertensive or lipid-lowering medication were asked to maintain all medications and supplements at prestudy doses. Most subjects were sedentary at baseline and were asked to maintain their usual levels of physical activity and to refrain from drinking alcohol throughout the study.

Diets

The prescribed diets were either high in protein [HP diet: 30% of energy as protein (≈ 110 g/d), 40% as carbohydrate, and 30% as fat] or contained a standard amount of protein [SP diet: 15% of energy as protein (≈ 60 g/d), 55% as carbohydrate, and 30% as fat]. The fatty acid profiles for each diet were matched (8% of energy as saturated, 12% as monounsaturated, and 5% as polyunsaturated fatty acids). The diets were prescriptive fixed-menu plans, and subjects were supplied with key foods that made up 60% of their energy intake. Some minor adjustments were made

if the amount of food specified was too little or too much for some subjects. The key foods supplied to both diet groups were preweighed meat and poultry, shortbread biscuits, canola margarine (Canola Lite; Meadow Lea Foods Ltd, Mascot, Australia), and high-oleic acid sunflower oil (Sunola; Meadow Lea Foods Ltd). The HP diet group also received low-fat (3% fat) cheese (Kraft Free; Kraft Foods Ltd, Melbourne, Australia) and skim milk powder, whereas the SP diet group received rice and rice noodles. Other differences between the diets are outlined in Table 2. Forty-five percent of the protein in the HP diet came from dairy foods and 45% came from meat and poultry, whereas in the SP diet, only 18% of the protein came from dairy foods and 42% came from meat and poultry.

Each subject completed weighed daily checklists of all foods consumed and was assessed by the same dietitian at 2-wk intervals according to the method described by Parker et al (25). Group training in the use of scales and in keeping food records was provided. Three consecutive days (1 weekend day and 2 weekdays) of the checklist from each 2-wk period were analyzed with the use of DIET/1 NUTRIENT CALCULATION software, 1998 version (Xyris Software, Highgate Hill, Australia). This program had no missing values for the nutrients of interest (26), and, because the

TABLE 2
Food composition of the test diets¹

Foods	HP diet	SP diet
Milk, reduced-fat (mL)	250	250
Cereal ²	1 Wheat biscuit	1 Wheat biscuit
Skim milk powder (g)	30	—
Fruit (g)	200	300
Wholemeal bread (g)	70	105
Low-fat (3% fat) cheese (g) ³	60	—
Lean meat or poultry (g)	200	100
Rice (g dry wt)	—	65
Low-calorie vegetables		
(cup)	2.5	2.5
(mL)	0.6	0.6
Margarine (g) ⁴	10	10
High-oleic acid sunflower oil (g) ⁵	15	15
Shortbread biscuits (g) ⁶	45	60
Diet yogurt (g)	200	—

¹SP, standard-protein; HP, high-protein.

²Wheatbix Sanitarium, Sydney.

³Kraft Free (3% fat); Kraft Foods Ltd, Melbourne, Australia.

⁴Canola Lite; MeadowLea Foods Ltd, Mascot, Australia.

⁵Sunola; MeadowLea Foods Ltd.

⁶Three biscuits in the HP diet and 4 in the SP diet.

diet was very prescriptive, unusual foods were rarely encountered. Recipes were entered as proportions of the original ingredients. The database had been extensively modified by our group to include new foods and recipes.

Experimental design

The study was conducted on an outpatient basis over 16 wk. Subjects were matched on the basis of fasting serum insulin concentrations at screening, BMI, age, and sex. Subjects from each group were then randomly assigned to either the HP or SP diet group. Screening occurred 4–6 wk before the study commenced. Both dietary groups underwent 12 wk of energy restriction ($\approx 30\%$ restriction of total energy, or 6.4 MJ on average), followed by 4 wk of energy balance with the same macronutrient composition.

On 2 consecutive days at each of weeks 0, 4, 8, 12, and 16, body weight and blood pressure were measured and venous blood samples were taken in the morning after an overnight fast for determination of plasma glucose and serum insulin and lipid concentrations. At weeks 0 and 16, a 3-h meal-tolerance test was performed with meals that were representative of the diet to which subjects were assigned. Baseline venous blood samples were taken before consumption of an HP diet (2715 kJ; 32% of energy as protein, 54% as carbohydrate, and 14% as fat) or SP diet (2747 kJ; 10% of energy as protein, 77% as carbohydrate, and 13% as fat) test meal, and thereafter blood was sampled at 30, 60, 120, and 180 min for assessment of plasma glucose and serum insulin and fatty acid concentrations. At weeks 0 and 16, each subject's body composition was measured with the use of dual-energy X-ray absorptiometry (DXA) (Norland densitometer XR36; Norland Medical Systems, Fort Atkinson, WI; CVs of $2.3 \pm 0.9\%$ for total fat mass and of $2.1 \pm 0.4\%$ for total lean mass), and a 24-h urine sample was collected. The ratio of urinary urea to creatinine was measured to determine dietary compliance, and markers of bone turnover and calcium excretion were also measured.

Biochemical analysis

Fasting blood samples were analyzed for concentrations of plasma glucose, insulin, fatty acids, triacylglycerol, and total, HDL, and LDL cholesterol with the use of methods described in detail elsewhere (25). Urine samples taken for assessment of compliance with the diet were frozen, and urea and creatinine were

measured in one run at the end of the study with the use of the urease enzymatic assay (27) and the Jaffe reaction technique (28), respectively. Urinary pyridinium cross-links (markers of bone turnover) were measured with the use of HPLC.

Statistical analysis

One subject was excluded from the lipid analysis because the subject began taking lipid-lowering medications, and one subject declined to undergo a DXA scan for personal reasons. All data are presented as means (\pm SEMs). Statistical analysis was performed with the use of SPSS FOR WINDOWS software, version 10.0 (SPSS Inc, Chicago). Baseline measurements were assessed by using two-factor analysis of variance with diet and sex as the fixed factors. Dietary intake was assessed with the use of an unpaired *t* test. The effect of the diet intervention was assessed by using repeated-measures analysis of variance (with covariates of baseline weight, total fat mass, and total lean mass in specific analyses) with variables measured at weeks 0, 4, 8, 12, and 16. Diet and sex were the between-subject factors. The incremental area under the glucose and insulin response curves during the 3-h meal-tolerance tests was calculated geometrically by using the trapezoidal rule (29). The homeostasis model assessment (HOMA) for insulin resistance was calculated as (fasting insulin \times fasting glucose/22.5) (30). Significance was set at $P < 0.05$ (without Bonferroni correction). The *t* tests were two-sided.

RESULTS

Screening characteristics

The physical characteristics of subjects at screening are shown in Table 1. There were no significant differences in any of the variables between the diet groups. Body weight and fasting plasma glucose concentrations were significantly greater in the men than in the women at screening. There was no effect of sex on any of the other variables.

Diet composition and subject compliance

Energy intake did not differ between the 2 groups during either the 12-wk energy-restriction phase or the 4-wk energy-balance phase (Table 3). As prescribed, the protein intake was higher and

TABLE 3
Nutrient composition of the study diets derived from subjects' daily weighed food records¹

	SP diet (<i>n</i> = 29)		HP diet (<i>n</i> = 28)	
	ER	EB	ER	EB
Energy (MJ/d)	6.5 \pm 0.1	8.2 \pm 0.2	6.3 \pm 0.1	8.0 \pm 0.2
Protein (% of energy) ²	15.7 \pm 0.2	15.4 \pm 0.3	27.4 \pm 0.2	27.3 \pm 0.3
Carbohydrate (% of energy) ²	57.3 \pm 0.3	56.9 \pm 0.3	44.4 \pm 0.4	44.6 \pm 0.5
Total fat (% of energy)	26.8 \pm 0.3	27.5 \pm 0.4	27.0 \pm 0.3	26.9 \pm 0.5
SFA (% of energy)	7.9 \pm 0.1	8.4 \pm 0.3	8.5 \pm 0.1	8.3 \pm 0.2
MUFA (% of energy)	13.3 \pm 0.2	13.0 \pm 0.3	13.3 \pm 0.2	13.1 \pm 0.3
PUFA (% of energy)	3.0 \pm 0.1	3.5 \pm 0.2	2.8 \pm 0.1	2.9 \pm 0.1
Fiber (g/d) ²	29.4 \pm 0.6	35.8 \pm 0.8	21.8 \pm 0.7	28.4 \pm 1.1
Cholesterol (mg/d) ²	87.4 \pm 2.5	117.2 \pm 6.4	178.0 \pm 6.1	239.4 \pm 13.5

¹ $\bar{x} \pm$ SEM. SP, standard-protein; HP, high-protein; ER, energy-restrictive; EB, energy balance; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. No significant differences (unpaired *t* test) were found between the 4 diet records in the ER phase or between the 2 records for the EB phase, so data for recordings in each phase were averaged.

²Significant main effect of diet (SP compared with HP) in both the ER and EB phases, $P < 0.05$.

TABLE 4
Body weight and body composition of subjects at weeks 0 and 16¹

	SP diet		HP diet	
	Men (n = 7)	Women (n = 21)	Men (n = 7)	Women (n = 21)
Body weight (kg) ^{2,3}				
Week 0	108.8 ± 5.2	88.1 ± 2.3	107.8 ± 5.9	89.1 ± 2.2
Week 4	103.5 ± 4.8	84.7 ± 2.2	102.7 ± 5.2	86.4 ± 2.2
Week 8	100.7 ± 4.2	82.7 ± 2.3	98.5 ± 4.9	84.4 ± 2.2
Week 12	99.0 ± 4.1	81.1 ± 2.3	96.0 ± 4.4	82.5 ± 2.2
Week 16	99.2 ± 4.1	80.7 ± 2.3	96.3 ± 4.3	82.5 ± 2.3
Change	-9.6 ± 1.7	-7.4 ± 0.5	-11.4 ± 2.1	-6.6 ± 0.5
Total fat mass (kg) ^{2,3}				
Week 0	38.2 ± 3.3	42.7 ± 2.0	37.8 ± 3.3	43.2 ± 1.3
Week 16	30.6 ± 2.9	35.6 ± 1.9	28.8 ± 2.0	36.6 ± 1.4
Change	-7.6 ± 3.1	-7.1 ± 2.0	-9.0 ± 2.7	-6.6 ± 1.4
Abdominal fat (kg) ^{2,3}				
Week 0	15.7 ± 1.6	15.6 ± 0.6	15.6 ± 1.4	15.4 ± 0.5
Week 16	12.2 ± 1.3	12.7 ± 0.7	11.1 ± 0.8	12.5 ± 0.5
Change	-3.5 ± 0.7	-2.6 ± 0.2	-4.5 ± 1.0	-2.8 ± 0.3
Total lean mass (kg) ^{2,4}				
Week 0	67.0 ± 2.1	42.4 ± 0.9	66.1 ± 3.1	42.5 ± 1.1
Week 16	65.1 ± 2.1	40.9 ± 0.9	63.6 ± 2.4	42.4 ± 1.2
Change	-1.9 ± 2.1	-1.5 ± 0.3	-2.5 ± 2.8	-0.1 ± 0.3

¹ $\bar{x} \pm$ SEM. SP, standard-protein; HP, high-protein. Data from weeks 0, 4, 8, 12, and 16 were compared by using three-factor repeated-measures ANOVA with time, diet, and sex as the factors.

²Significant effect of time from week 0 to week 16, $P < 0.0001$.

³Significant time-by-sex interaction, $P < 0.03$.

⁴Significant time-by-diet-by-sex interaction, $P = 0.002$; significant time-by-diet interaction in women, $P = 0.02$; time-by-diet interaction in men, $P > 0.05$.

the carbohydrate intake was lower with the HP diet than with the SP diet, and there was no difference between the energy-restrictive and energy-balance phases (effect of diet, $P < 0.0001$). Total and saturated fat content did not differ between the diets or phases, but dietary fiber was lower and dietary cholesterol was higher with the HP diet than with the SP diet during both phases (Table 3). The ratio of urinary urea to creatinine rose from 30.3 ± 1.5 at week 0 to 36.5 ± 1.6 at week 16 of the HP diet, and it remained constant from week 0 to week 16 of the SP diet (32.2 ± 1 compared with 31.8 ± 1) ($P < 0.001$ for time-by-diet effect; $P < 0.01$ for time). Measured compliance was excellent.

Body weight and body composition

After 12 wk of energy restriction and 4 wk of energy balance, the overall mean weight loss was 7.9 ± 0.5 kg ($P < 0.0001$), but the decrease in weight was not affected by the diet composition (7.8 ± 0.7 compared with 7.9 ± 0.6 kg in the HP and SP diet groups, respectively). The men lost more weight overall than did the women (Table 4), but the percentage of weight change did not differ significantly between the sexes (men: 9.7%; women: 7.9%). Weight was maintained during the energy-balance phase, with no difference between diets or sexes.

Total fat mass was 6.9 ± 0.4 kg less after 16 wk ($P < 0.0001$), but the decrease was not affected by diet composition (Table 4). There was a difference in the reduction in fat mass between the sexes, with the men losing a greater amount of fat (Table 4). At week 16, abdominal fat mass was 3.1 ± 0.2 kg less ($P < 0.0001$), with no effect of diet composition. A time-by-sex interaction ($P = 0.005$) was present, and the reduction in abdominal fat was greater for the men than for the women (Table 4). After 16 wk, total lean mass had decreased 1.2 ± 0.3 kg ($P < 0.0001$). There

was no overall effect of diet on the reduction in lean mass. A time-by-diet-by-sex interaction ($P = 0.002$) was present, even after control for baseline lean mass, such that the women lost significantly ($P < 0.02$) less lean mass with the HP diet than with the SP diet (Table 4).

Glycemic control, insulin sensitivity, and fatty acids

Fasting plasma glucose did not differ between weeks 0 and 16, and there was neither an effect of diet nor a time-by-diet interaction (Table 5). There was no effect of sex on fasting glucose at baseline; however, at screening, fasting glucose was higher in the men than in the women. Fasting serum insulin decreased by $33 \pm 3.3\%$ at week 12 and by $29 \pm 3.4\%$ at week 16 ($P < 0.001$) (Table 5), with no effect of either diet composition or sex. The HOMA index for insulin resistance decreased 32 ± 4%, from 4.3 at week 0 to 2.5 at week 12 ($P < 0.001$), and by 27 ± 4% to 2.8 at week 16 ($P < 0.001$). Neither diet nor sex affected the HOMA index.

Overall, the area under the plasma glucose curve was smaller after the HP meal than after the SP meal, at both weeks 0 and 16 ($P = 0.027$) (Table 5). The area under the plasma glucose curve was smaller at week 16 than at week 0 ($P < 0.001$) (Table 5), but the reduction tended to be greater in the HP diet group than in the SP diet group ($8.7 \pm 2.2\%$ compared with $1.9 \pm 2.1\%$; $P = 0.08$). If the glucose values are analyzed with a repeated-measures ANOVA, the response of plasma glucose to the test meals decreased after 16 wk ($P < 0.001$) (Figure 1, top), and there was a time-by-diet interaction ($P < 0.05$) such that the plasma glucose response decreased more after the HP diet meal than after the SP diet meal (Figure 1, top). At week 16, plasma glucose concentrations at all time points were lower

TABLE 5
Glucose, insulin, and fatty acid concentrations in the 2 diet groups¹

	SP diet (n = 29)	HP diet (n = 28)
Fasting glucose (mmol/L)		
Week 0	5.3 ± 0.1	5.4 ± 0.1
Week 4	5.3 ± 0.1	5.3 ± 0.1
Week 8	5.2 ± 0.1	5.6 ± 0.09
Week 12	5.2 ± 0.1	5.4 ± 0.06
Week 16	5.2 ± 0.1	5.5 ± 0.08
Change	-0.1 ± 0.08	0.16 ± 0.1
Glucose AUC (mmol·180 min/L) ^{2,3}		
Week 0	1300 ± 49	1202 ± 32
Week 16	1265 ± 48	1099 ± 40
Change	-35 ± 45	-103 ± 0.21
Fasting insulin (mU/L) ²		
Week 0	17.7 ± 2.4	17.5 ± 1.2
Week 4	12.5 ± 1.3	12.6 ± 1.1
Week 8	10.7 ± 0.9	13.5 ± 1.4
Week 12	10.4 ± 0.6	10.5 ± 0.7
Week 16	11.5 ± 0.9	11.9 ± 1.1
Change	-6.2 ± 2.0	-5.6 ± 0.9
Insulin (mU·180 min/L) ^{2,3}		
Week 0	9108 ± 1094	7935 ± 762
Week 16	8719 ± 718	5922 ± 533
Change	389 ± 300	2013 ± 337
Fatty acids ²		
Week 0	0.4 ± 0.03	0.4 ± 0.03
Week 4	0.4 ± 0.02	0.4 ± 0.02
Week 8	0.3 ± 0.03	0.3 ± 0.02
Week 12	0.3 ± 0.02	0.3 ± 0.03
Week 16	0.3 ± 0.02	0.3 ± 0.03
Change	-0.10 ± 0.03	-0.11 ± 0.02

¹ $\bar{x} \pm$ SEM. SP, standard-protein; HP, high-protein; AUC, area under the curve. Data were compared by using three-factor repeated-measures ANOVA with time, diet, and sex as the factors. There were no significant three-factor or two-factor interactions and no significant main effect of sex.

²Significant effect of time, $P < 0.001$.

³Significant effect of diet, $P < 0.05$.

after the HP diet meal than after the SP diet meal ($P < 0.001$ for diet effect). At week 16, the response of serum insulin to the test meals was less than it had been at week 0 ($P < 0.001$) (Figure 1, bottom). There was no effect of diet composition on the reduction in postprandial serum insulin at week 16 (Figure 1, bottom).

After 16 wk, fasting serum fatty acids decreased 26% ($P < 0.001$), with no effect of either diet or sex (Table 5). At week 0 during the meal-tolerance test, serum fatty acid concentrations decreased from 0.43 ± 0.02 mmol/L at baseline to 0.006 ± 0.003 mmol/L at 120 min, but no further decrease occurred after 16 wk. There was no effect of either diet or sex on the fatty acid response curves after the meal-tolerance tests.

Concentrations of total, LDL, and HDL cholesterol and triacylglycerol

Overall, fasting serum total cholesterol at week 12 decreased by 10.0% compared with that at week 0 and decreased by 5.3% at week 16 ($P < 0.0001$), with no effect of diet (Table 6). There was an effect of sex on the decrease in fasting serum total cholesterol from week 0 to week 12 ($P < 0.005$) such that total cholesterol decreased more in the men (1.0 ± 0.2 mmol/L) than in the women (0.42 ± 0.1 mmol/L). Fasting serum LDL cholesterol was 12% lower at week 12 and 6% lower at week 16 ($P < 0.0001$), with

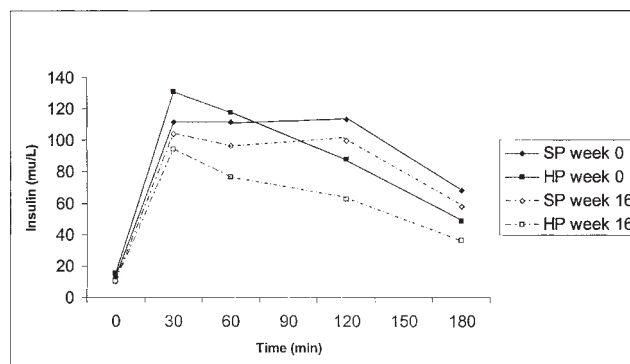
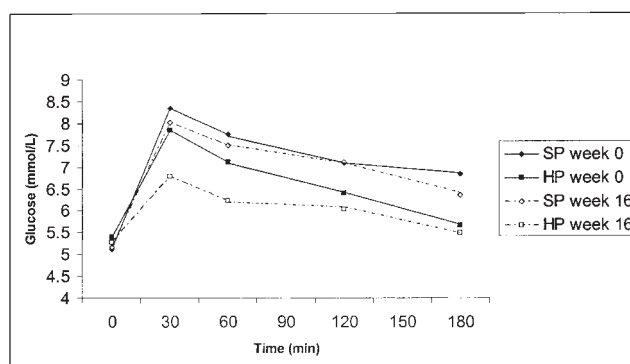


FIGURE 1. Mean (\pm SEM) plasma glucose and insulin concentrations at baseline and 30, 60, 120, and 180 min after the ingestion of a standard-protein (SP; $n = 29$) or high-protein (HP; $n = 28$) test diet, which was representative of the subjects' study diet at weeks 0 and 16. Week 0 and week 16 data were compared with the use of 4-way repeated-measures ANOVA with week and blood-sampling time as the within-subject factors and diet and sex as the between-subject factors. Significant time-by-diet interaction (ie, glucose concentrations decreased more by week 16 with the HP diet than with the SP diet), $P < 0.05$. Significant effect of time on glucose and insulin concentrations from week 0 to week 16, $P < 0.001$.

no effect of diet (Table 6). At both weeks 12 and 16, the decrease in LDL cholesterol was greater for the men than for the women ($P < 0.02$). Fasting serum HDL cholesterol increased 2% by week 12 and 5% by week 16 ($P = 0.001$), with no effect of either diet or sex (Table 6). Fasting serum triacylglycerol concentrations decreased 15.8% by week 12 and 14.1% by week 16 ($P < 0.0001$). A time-by-diet effect was observed ($P < 0.05$) such that the decrease in serum triacylglycerol concentrations was 29% by week 12 and 23% by week 16 with the HP diet, but the decrease was only 12% by week 12 and 10% by week 16 with the SP diet (Table 6).

Urinary calcium, markers of bone turnover, and blood pressure

Urinary calcium excretion was unchanged at week 16 compared with week 0 (4.7 ± 0.4 compared with 4.4 ± 0.3 mmol/24 h), and there was no effect of either diet or sex. The concentrations of bone-turnover markers (pyridinoline: 62.5 ± 2.2 compared with 62.1 ± 2.6 nmol/mmol creatinine; deoxypyridinoline: 18.6 ± 0.7 compared with 18.5 ± 0.9 nmol/mmol creatinine) also did not differ between baseline and week 16 in both groups. Systolic blood

TABLE 6
Fasting lipid concentrations in the 2 diet groups¹

	SP diet		HP diet	
	Men (n = 7)	Women (n = 21)	Men (n = 7)	Women (n = 21)
Total cholesterol ^{2,3}				
Week 0	5.25 ± 0.35	5.70 ± 0.18	6.11 ± 0.41	5.36 ± 0.24
Week 4	4.39 ± 0.29	5.34 ± 0.21	4.66 ± 0.20	4.84 ± 0.23
Week 8	4.59 ± 0.35	5.26 ± 0.20	4.70 ± 0.24	4.91 ± 0.20
Week 12	4.55 ± 0.38	5.31 ± 0.25	4.69 ± 0.26	4.92 ± 0.22
Week 16	4.83 ± 0.37	5.51 ± 0.21	5.22 ± 0.31	5.12 ± 0.23
Change	-0.42 ± 0.10	-0.19 ± 0.16	-0.89 ± 0.33	-0.24 ± 0.12
LDL cholesterol ^{2,3}				
Week 0	3.51 ± 0.32	3.84 ± 0.18	4.11 ± 0.33	3.65 ± 0.21
Week 4	2.89 ± 0.28	3.59 ± 0.21	3.10 ± 0.15	3.32 ± 0.21
Week 8	2.93 ± 0.34	3.47 ± 0.20	3.09 ± 0.16	3.26 ± 0.17
Week 12	2.90 ± 0.35	3.53 ± 0.24	3.05 ± 0.18	3.76 ± 0.16
Week 16	3.14 ± 0.33	3.66 ± 0.21	3.51 ± 0.20	3.32 ± 0.20
Change	0.38 ± 0.15	-0.17 ± 0.15	-0.71 ± 0.31	0.14 ± 0.11
HDL cholesterol ²				
Week 0	0.89 ± 0.06	1.00 ± 0.06	0.90 ± 0.06	1.00 ± 0.05
Week 4	0.89 ± 0.07	0.97 ± 0.05	0.93 ± 0.04	0.93 ± 0.03
Week 8	0.90 ± 0.07	0.96 ± 0.04	0.83 ± 0.11	0.97 ± 0.04
Week 12	0.92 ± 0.06	1.01 ± 0.04	1.03 ± 0.08	1.00 ± 0.04
Week 16	0.97 ± 0.06	1.05 ± 0.04	1.03 ± 0.05	1.00 ± 0.04
Change	0.08 ± 0.02	0.04 ± 0.04	0.13 ± 0.04	0.03 ± 0.3
Triacylglycerol ^{2,4}				
Week 0	1.84 ± 0.30	1.89 ± 0.12	2.39 ± 0.33	1.71 ± 0.13
Week 4	1.33 ± 0.14	1.69 ± 0.10	1.36 ± 0.15	1.37 ± 0.11
Week 8	1.52 ± 0.25	1.66 ± 0.12	1.56 ± 0.28	1.43 ± 0.12
Week 12	1.62 ± 0.25	1.67 ± 0.13	1.31 ± 0.18	1.34 ± 0.11
Week 16	1.58 ± 0.28	1.74 ± 0.12	1.71 ± 0.23	1.34 ± 0.12
Change	-0.27 ± 0.16	-0.15 ± 0.11	-0.68 ± 0.21	-0.37 ± 0.08

¹ $\bar{x} \pm$ SEM. SP, standard-protein; HP, high-protein. Fasting lipid concentrations were measured on 2 consecutive days at weeks 0, 4, 8, 12, and 16. Data from weeks 0, 4, 8, 12, and 16 were compared by using three-factor repeated-measures ANOVA with time, diet, and sex as the factors. There was no significant three-factor interaction and no significant main effects of diet or sex.

²Significant effect of time, $P < 0.01$.

³Significant time-by-sex interaction, $P < 0.01$.

⁴Significant time-by-diet interaction, $P < 0.05$.

pressure decreased from 130 ± 1.9 mm Hg at week 0 to 126 ± 1.8 mm Hg at week 12 ($P = 0.022$), but, by week 16, systolic blood pressure (126 ± 2.3 mm Hg) not differ from that at week 0. At both weeks 12 and 16, diastolic blood pressure (72 ± 1.3 and 72 ± 1.4 mm Hg, respectively) was significantly ($P < 0.04$) lower than that at week 0 (74 ± 1.4 mm Hg). There was no effect of either diet or sex on systolic or diastolic blood pressure.

DISCUSSION

These data show that total energy intake, rather than the protein content of the diet, is the most important determinant of weight loss, at least over a span of 16 wk. This observation is consistent with previous findings reported by our group in subjects with type 2 diabetes (25) and reported by others (31) in subjects without diabetes (13–15). In contrast, a small study of 13 hyperinsulinemic men showed 28% more weight loss (8.3 compared with 6.0 kg) over 4 wk with consumption of a diet containing 45% protein than with consumption of an isoenergetic (≈ 7.4 MJ/d) diet containing 12% protein (12). A significant difference in loss of total body water (-1.0 compared with 0.3) between the HP and SP diets, rather than the nonsignificant difference in loss of fat (-7.1 compared with -6.3 kg), may explain some of the differential

weight loss in that study (12). Another study (11) showed that, in a span of 6 mo, a 3.3-kg greater fat loss occurred with an ad libitum diet that contained 25% of total energy from dietary protein than occurred with a diet that contained 12% protein. Subject compliance with the diets was aided by convenient access to a shop that supplied a large selection of high-protein and standard-protein low-fat foods. Two studies showed that protein is more satiating than is either carbohydrate or fat (32, 33). Increased satiation may facilitate weight loss by reducing energy intake, and together these factors may aid compliance over the longer term. Because our HP and SP diets were fixed-menu isoenergetic diets, the effect of the enhanced satiety with protein would be minimal.

There is little information on whether the source of dietary protein may affect metabolic responses to protein. Protein type as a factor in weight loss has been examined in only one study by Yamashita et al (34), who contrasted a weight-loss diet based on meat (150 g/d) with a weight-loss diet based on soy protein (130 g/d) with protein providing 25% of energy. No differences were seen between these diets in weight loss over 16 wk or in changes in lipids, blood pressure, insulin, or arterial compliance. Laboratory, clinical, and population data suggest a possible anti-obesity effect of dietary calcium (35). To date, no clinical trials have yet been conducted to definitively show this effect in humans. There is a

suggestion that dietary calcium may be involved in the regulation of adiposity in animal models (36). Our dietary interventions differed in calcium as well as in protein, with the SP diet providing 600 mg dietary calcium and the HP diet providing 1600 mg; we did not set out to answer this question. Therefore, the results we obtained could be related to the increased dietary protein or calcium or possibly to the reduction in carbohydrate intake.

The preservation of total lean mass in the women who consumed the HP diet is consistent with the findings of Piatti et al (13). That the effect was observed only in our female subjects may reflect the small number of men studied or the possibility that the protein content of the HP diet was not sufficient to preserve lean mass in the men. Piatti et al (13) and Hoffer et al (14) showed that the proteolysis of lean tissue was suppressed in subjects consuming 1.5 g protein/kg ideal body weight. Proteolysis was not measured in the current study, but the reported protein intake in the HP diet group was 1.1 g/kg ideal body weight for the men and 1.4 g/kg ideal body weight for the women. Presumably, there was sufficient dietary protein to suppress proteolysis and preserve lean mass in the women, but the lower protein intake was not adequate for the men. We previously showed that lean mass was not spared in either women or men with type 2 diabetes, who consumed on average 1.3 g protein/kg during energy restriction (25).

Fasting plasma glucose concentrations were not significantly reduced during energy restriction or after weight loss in the current study. Consistent with previous research in subjects with and without type 2 diabetes (37, 38), fasting insulin concentrations decreased during energy restriction and subsequent weight loss. As a surrogate measure of insulin resistance, we used the HOMA insulin resistance index, which has been shown to correlate highly with clamp techniques in several large populations without diabetes (39). The HP diet had no benefit in ameliorating insulin resistance, over and above energy restriction and weight loss. This finding is in contrast with the improvement in insulin resistance reported by Piatti et al (13), who used a euglycemic hyperinsulinemic clamp to evaluate changes in insulin sensitivity. The HOMA index provides only a qualitative estimate of insulin resistance, and therefore small changes in insulin sensitivity may not have been detected.


When protein is added to a carbohydrate meal, a significant attenuation in the glucose response has been reported by some investigators (40–42) but not by others (43, 44). In the current study, there was no difference in postload insulin concentrations between the HP and SP diet groups, and therefore the lower glucose response after the HP diet meals presumably reflects the smaller carbohydrate load of the HP diet compared with the SP diet. After weight loss, the glucose response area decreased 6.8% more in the HP diet group than in the SP diet group. Prospective data from the Nurses' Health Study showed that dietary glycemic load, which one would expect to relate to postmeal glucose concentrations, was related to cardiovascular disease and diabetes (45, 46). Decreasing the postmeal glucose concentration by reducing carbohydrate and increasing protein, even in "glucose-tolerant" subjects, may reduce the risk of future cardiovascular morbidity and mortality.

Our finding of a greater reduction in fasting triacylglycerol concentrations in both men and women consuming the HP diet is similar to the findings of other investigators who examined the effect of such a diet during weight maintenance in subjects with mildly high cholesterol concentrations (16) and in normolipidemic subjects (17). The beneficial effect of the diet on triacylglycerol

concentrations presumably reflected the lower carbohydrate content of the diet. Low-fat, high-carbohydrate diets have been reported to elevate triacylglycerol concentrations (47, 48), particularly in insulin-resistant subjects (49). The greater carbohydrate content of the SP diet may have decreased the clearance of triacylglycerol-rich VLDL because of a reduction in the efficiency of lipoprotein lipase (47). We previously reported in subjects with type 2 diabetes (25) that an HP diet had no effect on serum triacylglycerol concentrations but did reduce fasting serum total and LDL-cholesterol concentrations. Disparate findings between our current and previous (25) studies may be due to the greater weight loss achieved in the current study. Wolfe and Giovannetti (16) also reported a greater reduction in total and LDL cholesterol and a greater increase in HDL cholesterol in response to increased dietary protein. In their study, only subjects with high baseline serum cholesterol concentrations were included, whereas in the current study, subjects were not excluded on the basis of their baseline serum cholesterol concentrations. Raeini-Sarjaz et al (50) made similar observations in relation to triacylglycerol and diets rich in carbohydrate. They also observed a decrease in LDL cholesterol with energy restriction alone, as we did during the energy-restriction phase.

We observed no deleterious effect of the HP diet on bone turnover. Our findings are consistent with a study that showed no change in urinary hydroxyproline, pyridinoline, and deoxypyridinoline when protein was increased from 0.44 to 2.71 g/kg in 15 young subjects (20). In the Framingham Osteoporosis Study (51), persons in the highest quartile of protein intake (1.24–2.78 g · kg⁻¹ · d⁻¹, or 17–27% of energy) had the smallest loss of bone mineral density over 4 y.

One study reported an increase in systolic blood pressure after an increase in animal protein intake over 4 wk in 21 subjects (22). We observed that the protein content had no effect on systolic or diastolic blood pressure. No studies have examined the effect of a high-protein, energy-restrictive diet on blood pressure, and therefore our finding requires confirmation.

Replacing some dietary carbohydrate with protein during energy restriction does not enhance weight or fat mass loss or have any deleterious effects on bone turnover or blood pressure in subjects with insulin resistance, at least over the short term. Protein intakes from meat, poultry, and dairy foods that are moderately higher than those typically consumed by Western populations do, however, reduce postload glucose and fasting triacylglycerol concentrations. Long-term studies are required to determine whether high-protein diets will reduce the risks of cardiovascular disease and possibly delay the progression to type 2 diabetes in obese adults with insulin resistance. Further studies are also warranted to determine whether these findings are enhanced by ad libitum consumption of high- and low-protein low-fat foods and whether the mechanism for these changes relates to higher protein or calcium intakes or is a function of lower carbohydrate intake. This study supports the concept that weight loss can be achieved with widely divergent dietary strategies. 

We acknowledge Rosemary McArthur, Anne McGuffin, and Jodie Avery for assistance in performing these studies.

PMC, MN, and GW were involved in the design of the study, analysis of the data, and writing of the manuscript; EF and NDL were involved in the collection and analysis of the data and writing of the manuscript; and EA was involved in the collection of the data. None of the authors had financial or personal conflicts of interest.

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