

Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women¹⁻³

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ABSTRACT

Background: Limited evidence suggests that a higher ratio of protein to carbohydrate during weight loss has metabolic advantages.

Objective: The objective was to evaluate the effects of a diet with a high ratio of protein to carbohydrate during weight loss on body composition, cardiovascular disease risk, nutritional status, and markers of bone turnover and renal function in overweight women.

Design: The subjects were randomly assigned to 1 of 2 isocaloric 5600-kJ dietary interventions for 12 wk according to a parallel design: a high-protein (HP) or a high-carbohydrate (HC) diet.

Results: One hundred women with a mean (\pm SD) body mass index (in kg/m²) of 32 \pm 6 and age of 49 \pm 9 y completed the study. Weight loss was 7.3 \pm 0.3 kg with both diets. Subjects with high serum triacylglycerol (>1.5 mmol/L) lost more fat mass with the HP than with the HC diet (\bar{x} \pm SEM: 6.4 \pm 0.7 and 3.4 \pm 0.7 kg, respectively; P = 0.035) and had a greater decrease in triacylglycerol concentrations with the HP (-0.59 ± 0.19 mmol/L) than with the HC (-0.03 ± 0.04 mmol/L) diet (P = 0.023 for diet \times triacylglycerol interaction). Triacylglycerol concentrations decreased more with the HP (0.30 \pm 0.10 mmol/L) than with the HC (0.10 \pm 0.06 mmol/L) diet (P = 0.007). Fasting LDL-cholesterol, HDL-cholesterol, glucose, insulin, free fatty acid, and C-reactive protein concentrations decreased with weight loss. Serum vitamin B-12 increased 9% with the HP diet and decreased 13% with the HC diet (P < 0.0001 between diets). Folate and vitamin B-6 increased with both diets; homocysteine did not change significantly. Bone turnover markers increased 8–12% and calcium excretion decreased by 0.8 mmol/d (P < 0.01). Creatinine clearance decreased from 82 \pm 3.3 to 75 \pm 3.0 mL/min (P = 0.002).

Conclusion: An energy-restricted, high-protein, low-fat diet provides nutritional and metabolic benefits that are equal to and sometimes greater than those observed with a high-carbohydrate diet. *Am J Clin Nutr* 2005;81:1298–306.

KEY WORDS Weight loss, diet composition, high-protein diet, lipids, dual-energy X-ray absorptiometry, bone turnover, nutritional status

INTRODUCTION

Obesity is a major health concern because it is implicated in the development of many chronic diseases. Strategies recommended for weight control have generally recommended the adoption of

low-fat dietary patterns, which facilitate energy restriction and cardiovascular disease risk reduction. However, studies of the role of a high dietary ratio of protein to carbohydrate in enhancing weight loss and disease risk management have emerged along with an increasing public interest in weight control. From an epidemiologic perspective, a positive health benefit from a high protein intake was observed in the Nurses' Health Study, which found a 26% lower rate of cardiovascular disease in those women in the highest protein intake group than in those in the lowest protein intake group (1). Clinical intervention studies have provided sound evidence that an ad libitum high-protein diet from mixed sources in free-living overweight people increases the amount of weight lost in a 6-mo weight-loss program (by 3.8 kg) compared with a high-carbohydrate diet by enhancing satiety (2). Furthermore, weight-loss studies in overweight women have shown that diets with a high ratio of protein to carbohydrate have positive effects on markers of disease risk, including body composition, blood lipids, and glucose homeostasis, and that these benefits may be mediated partly by the effect of protein on satiety and by a lower glycemic load because of a lower carbohydrate intake (3, 4). A higher protein intake during weight loss may also prevent some of the inevitable loss of lean body mass and, thus, may enhance insulin sensitivity (5, 6), although this has not been observed at very low energy intakes (7). In 2 studies in overweight men and women, with either insulin resistance or type 2 diabetes, we showed that a high-protein weight-loss diet (28–30% of energy from protein) from mixed sources enhances fat loss by 1–2 kg over 12 wk, particularly in women, compared with an isocaloric high-carbohydrate weight-loss diet (8, 9). It is known that different protein sources have different effects on the release of insulin (10, 11), and this may be important to the mechanism of action of both the enhanced satiety (Latner and Schwartz 1999) and the differential fat loss. However, foods and dietary patterns high in protein may vary in saturated fat and

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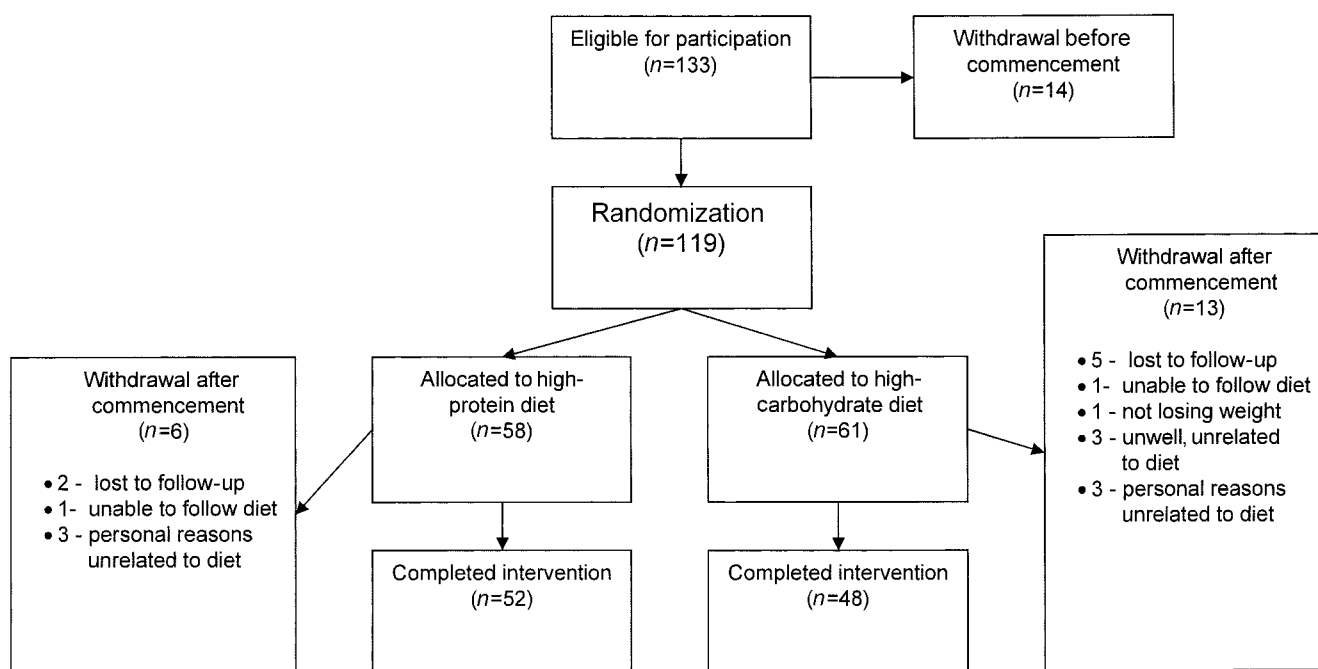


FIGURE 1. Schematic representation of randomization.

nutritional composition, and concerns have been raised regarding the effect of high-protein diets on serum lipids and subsequent cardiovascular disease risk. Evidence also exists that high-protein diets enhance calcium excretion and increase bone loss, which particularly needs clarification (12).

The purpose of the study was to determine the effect of reduced caloric intake, associated with higher dietary protein from low saturated fat sources compared with a high-carbohydrate diet, on weight loss, body composition, cardiovascular disease risk, nutritional status, and markers of bone turnover in overweight and obese women. We hypothesized as our primary outcome that the high-protein diet would enhance fat loss and minimize lean mass loss compared with the high-carbohydrate diet.

SUBJECTS AND METHODS

Subjects

Women were recruited by public advertisement. They were screened by questionnaire and verbally to assess their perceived ability to comply with an energy-restricted dietary regimen. To be eligible for the study, subjects had to be females between 20 and 65 y of age, have a body mass index (BMI; in kg/m^2) between 27 and 40, and have no history of metabolic disease or type 1 or type 2 diabetes. One hundred and nineteen women met the selection criteria and were randomly assigned to treatment (Figure 1).

All subjects gave written informed consent to participate in the study, which was approved by the Human Ethics Committee of the Commonwealth Scientific and Industrial Research Organization, Health Sciences and Nutrition, Adelaide, Australia. Nineteen women withdrew from the study before completion, 6 in the high-protein group and 13 in the high-carbohydrate group. Subject characteristics were not significantly different between diet groups at baseline (Table 1).

Study design

The subjects were randomly assigned to 1 of 2 isocaloric 5600-kJ dietary interventions for 12 wk according to a parallel design: 1) a high-protein, low-saturated-fat dietary pattern [HP group; 34% of energy from protein, 20% from fat (<10% from saturated fat) and 46% from carbohydrate] and 2) a high-carbohydrate, low-saturated-fat dietary pattern [HC group; 17% of energy from protein, 20% from fat (<10% from saturated fat), and 64% from carbohydrate].

The subjects attended individual consultations with 2 dietitians, alternately every 4 wk, throughout the study for instruction on the dietary requirements and methods of recording food intake and for the assessment of compliance. The subjects were issued with digital kitchen scales to weigh food and were advised to consume ≥ 2 cups of low-carbohydrate vegetables per day; they were advised to not eat potato, sweet potato, and avocado. A range of additional low-energy foods was allowed. Two standard servings of alcohol were permitted per week. Eating at restaurants was limited to once every 2 wk. Every 2 wk, the subjects attended the Clinical Research Unit and were supplied with foods consistent with their allocated diet to encourage compliance. Each allocation provided $\approx 60\%$ of the projected total energy intake for 7 d and was isocaloric for both groups.

TABLE 1
Subject characteristics at baseline¹

	HP group (n = 52)	HC group (n = 48)
Weight (kg)	87 \pm 12	86 \pm 12
Age (y)	50 \pm 10	49 \pm 9
BMI (kg/m^2)	32 \pm 6	33 \pm 4

¹ All values are $\bar{x} \pm \text{SD}$. HP, high protein; HC, high carbohydrate. There were no significant differences between groups by two-tailed unpaired *t* test.

TABLE 2Prescriptive food composition of the test diets¹

	HP diet	HC diet
Cereal	25 g bran cereal + 15 g wheat-flake breakfast biscuit	40 g wheat-flake breakfast biscuit
Milk	250 mL/d (<1% fat)	250 mL/d (<1% fat)
Low-fat yogurt	200 g	Nil
Lean meat, poultry, or fish	200 g lean beef or lamb >6 times/wk + extra 100 g other protein food daily (lunch)	80 g chicken, pork, or fish (>6 times/wk) + red meat <1 time/wk
Fresh fruit	300 g	450 g
Pasta, rice	Nil	120 g cooked (6 times/wk)
Salad	100 g	100 g
Vegetables	≤400 g	≤400 g
Canola oil	15 g	15 g
Whole-grain bread	70 g	105 g
Biscuits	Nil	2 shortbread biscuits
Wine or equivalent (optional)	300 mL/wk	300 mL/wk

¹ HP, high protein; HC, high carbohydrate.

The foods prescribed to obtain the planned dietary intakes in both diet groups are outlined in **Table 2**. The total energy content of each diet was initially 5600 kJ, but was adjusted upward for very active subjects so that weight loss would be ≈1 kg/wk for the first 2–3 wk. The fiber content and fatty acid profile were planned to be the same between diets. Key foods were supplied to the subjects, and the energy content of the foods provided was the same for both diet groups. Specifically, lean red meat was provided in 200-g portion packs and lunch meat, chicken, or fish in 100-g portion packs for 6 meals/wk for the HP group; 80-g packs of chicken and pork plus pasta, rice, biscuits, and whole-meal bread were provided to the HC group. To simulate a quasi ad libitum approach, we advised the HP group that it was compulsory to eat 200 g red meat plus 100 g lunch meat, chicken, or fish daily; the other food items could be consumed according to appetite but not to exceed the amounts specified. Similarly for the HC group, we advised that 80 g chicken or pork plus the bread needed to be consumed daily, and this was isocaloric with the meat component in the HP diet. Checklists of all foods consumed were completed daily, and 3-d weighed food records were analyzed in each 2-wk period. The subjects were interviewed by a dietitian individually every 4 wk. Two qualified dietitians provided the dietary counseling and conducted the nutrient analyses. Both dietitians were trained to provide consistent information to the subjects and on the methods for analysis. Advice on physical activity was also consistent with a recommendation to increase physical activity to ≥30 min 3 times/wk and to document these occasions in their daily checklist.

Food-preparation sessions specific to the diet protocol were conducted by a home economist every 4 wk for each diet group, and recipes were provided. The composition of the diets consumed by the subjects and their compliance throughout the study were assessed through 3-d dietary food records that were completed every 4 wk and dietary checklists that were completed daily. Energy and macronutrient intakes were calculated by using Diet 4 nutritional calculation software (Xyris Software, Highgate Hill, Australia), which is based on Australian food-composition tables and data from food manufacturers.

Body weight and composition

Subjects were weighed every 14 d (model AMZ14; Mercury Digital Scales, Japan) while wearing light clothing and no shoes

and after fasting overnight. Height was measured with a stadiometer (Seca, Hamburg, Germany) at week 0. BMI was calculated as weight (kg)/height squared (m). Dual-energy X-ray absorptiometry (Norland Medical Systems Inc, Fort Atkinson, WI) was performed at weeks 0 and 12 (Royal Adelaide Hospital, Adelaide, Australia).

Urinalysis

Collection of total 24-h urine output commenced at 0700 (not including the first morning void) on the day before the subjects attended the research clinic and was completed at 0700 on the day of clinic attendance (including the first morning void) at weeks 0 and 12. Urine samples were measured at the Institute of Medical and Veterinary Science (Adelaide, South Australia) for creatinine, urea, calcium phosphate, and sodium by using proprietary techniques with the Olympus AU5400 chemistry analyzer (Tokyo, Japan). Deoxypyridinoline and pyridinoline were measured by using HPLC (13) and expressed per mmol creatinine.

Biochemistry

Fasting blood samples were collected at weeks 0, 4, 8, and 12 in tubes containing either no additives [for the measurement of lipids, insulin, and C-reactive protein (CRP)] or sodium fluoride EDTA for the measurement of glucose. Plasma or serum was isolated by centrifugation at 2000 × g for 10 min at 5 °C (model GS-6R centrifuge; Beckman, Fullerton, CA) and frozen at –20 °C. Biochemical assays were performed in a single assay at the completion of the study. Plasma glucose and serum total cholesterol and triacylglycerol concentrations were measured with a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) with the use of enzymatic kits (Hoffmann-La Roche Diagnostica, Basel, Switzerland) and control sera. Serum HDL-cholesterol concentrations were measured with a Cobas-Bio analyzer after precipitation of LDL and VLDL cholesterol with polyethylene glycol 6000 solution. A modified Friedewald equation was used to calculate LDL cholesterol (14). Insulin was measured in duplicate with a radioimmunoassay kit (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). CRP was measured with an enzymatic kit (Roche, Indianapolis, IN) on a Hitachi auto analyzer (Roche). Osteocalcin was measured with an immunometric assay (catalogue no. LKOC1) on an Immulite Analyzer (Diagnostics Products Corp, Los Angeles, CA).



TABLE 3
Reported dietary intake data assessed by weighed food records¹

	HP diet (n = 52)	HC diet (n = 47)	<i>P</i> ²
Energy (kJ)	5310 ± 55.5 ³	5219 ± 78.6	NS
Protein (% of energy)	31.3 ± 0.24	17.8 ± 0.21	<0.001
Fat (% of energy)	22.1 ± 0.40	20.1 ± 0.52	0.003
Carbohydrate (% of energy)	44.2 ± 0.42	60.8 ± 0.58	0.000
Alcohol (% of energy)	1.1 ± 0.24	1.1 ± 0.26	NS
Fiber (g)	27.6 ± 0.58	26.1 ± 0.58	NS
Cholesterol (mg)	216 ± 4.8	78 ± 4.6	<0.001
Saturated fat (% of energy)	5.4 ± 0.17	4.6 ± 0.23	0.003
Monounsaturated fat (% of energy)	9.4 ± 0.20	8.3 ± 0.26	0.001
Polyunsaturated fat (% of energy)	4.7 ± 0.11	4.7 ± 0.14	NS
Vitamin A equivalent (μg)	1109 ± 53.9	1149 ± 48.4	NS
Vitamin C (mg)	111 ± 4.6	122 ± 7.0	NS
Thiamine (mg)	1.6 ± 0.02	1.4 ± 0.03	<0.001
Riboflavin (mg)	2.6 ± 0.04	1.5 ± 0.03	<0.001
Niacin equivalent (mg)	48.0 ± 0.43	26.9 ± 0.42	<0.001
Calcium (mg)	777 ± 14.7	594 ± 9.8	<0.001
Iron (mg)	14.8 ± 0.20	9.6 ± 0.22	<0.001

¹ HP, high protein; HC, high carbohydrate.² Unpaired two-tailed *t* test between dietary treatments.³ $\bar{x} \pm$ SEM over 9 d (all such values).

Homocysteine, iron, ferritin, folate, vitamin B-6, and vitamin B-12 were measured at weeks 0 and 12 in a certified commercial laboratory (Institute of Medical and Veterinary Science, Adelaide, South Australia).

Statistical analysis

All subjects who completed the study were included in the data analysis, independent of reported dietary compliance, as indicated by food records, weight loss, and urinary urea excretion relative to creatinine. The statistical analysis was performed with the use of SPSS 11.0 for WINDOWS (SPSS Inc, Chicago, IL). A univariate analysis of variance was used to assess differences between treatment and triacylglycerol status at baseline and to assess changes in weight and body-composition variables. Dietary data were analyzed by using an unpaired *t* test. Univariate analysis using the study endpoints, with baseline variable as a covariate, was used to assess the effects of diet (ie, HP or HC). Analysis of variance with repeated measures was also used to determine the effects of time, diet (within-subject factors), and triacylglycerol status (between-subject factors) and interaction effects. Data were reanalyzed with baseline BMI as a covariate. If an interaction was noted, a post hoc subgroup analysis on the differences was performed by using Tukey's test. Differences were considered significant if *P* < 0.05. All data except baseline characteristics are presented as means ± SEMs.

RESULTS

Dietary intakes

The self-reported composition of the study diets consumed during the 3-mo study period is presented in **Table 3**. There were no significant differences in total energy, alcohol, and dietary

fiber intakes between the diet groups. Total, saturated, and mono-unsaturated fat intakes were significantly lower in the HC group, as was the dietary cholesterol intake. Intakes of the micronutrients thiamine, riboflavin, niacin equivalents, calcium, and iron were significantly higher in the HP group.

Weight and fat loss

The subjects that dropped out were aged 37 ± 8 y, which was significantly younger than those who completed the study (*P* < 0.001), but BMI was not significantly different between groups (*P* = 0.196). When we undertook an intention-to-treat analysis, with baseline weight carried forward for dropouts, there was a significant main effect of diet for weight loss (HP diet: 6.8 ± 3.9 kg; HC diet: 5.4 ± 4.3 kg; *P* = 0.041). When the analysis was carried out by using the last weight carried forward for dropouts, the diet effect was weakened (HP diet: 7.0 ± 3 kg; HC diet: 5.8 ± 4.0 kg; *P* = 0.066). However, we believe that a "completers" analysis was a more conservative and appropriate assessment of our data because this was a controlled clinical trial to examine the metabolic effects of dietary composition. The subjects who completed the 12-wk trial (*n* = 100) had a mean weight loss of 7.6 ± 0.4 kg with the HP diet (*n* = 52) and 6.9 ± 0.5 kg with the HC diet (*n* = 48); these values were not significantly different from each other (*P* = 0.29). There were 84 subjects with weight losses >4 kg. However, there was no statistically significant difference in weight loss or in the number of subjects achieving >4 kg weight loss by diet. When a subgroup analysis was conducted, there was a significant interaction with diet and weight loss according to triacylglycerol status (*P* = 0.032). Triacylglycerol status was categorized about the median of 1.5 mmol/L.

Weight loss was ≈25% greater with the HP diet in subjects with a triacylglycerol concentration >1.5 mmol/L (*P* = 0.005), whereas there was no differential effect of diet in women with a low triacylglycerol concentration (**Table 4**).

Similarly, the DXA data showed no overall effect of diet composition on total fat loss (*P* = 0.16; **Table 5**), but a significant interaction was observed with diet and triacylglycerol status on total (*P* = 0.019) and midriff (*P* = 0.03) fat. In women with high triacylglycerol concentrations, the total fat loss was 6.4 ± 0.7 kg in the HP group and 3.4 ± 0.7 kg in the HC group (*P* = 0.035 for diet difference; **Figure 2**). The amount of weight lost specifically from the midriff area in the HP group was twice that in the HC group, but the difference was not statistically significant by post hoc analysis across the 4 groups (1.0 ± 0.2 kg compared with 0.5 ± 0.1 kg; *P* = 0.12).

Serum and urinary urea, creatinine, and creatinine clearance

The urea-creatinine ratio in urine as well as serum urea were both significantly different by diet (*P* = 0.003 and *P* < 0.001, respectively; **Table 6**). Creatinine clearance decreased with weight loss, from 82 ± 3.3 to 75 ± 3.0 mL/min (8%; *P* = 0.002), with no significant difference between the diets (*P* = 0.346). There was no significant change in serum creatinine (74.0 ± 0.9 μmol/L at baseline compared with 75.4 ± 0.8 μmol/L at week 12); therefore, the difference was due to the amount of creatinine excreted in the urine—from 8.9 ± 0.32 to 8.1 ± 0.21 mmol/d. There was no correlation between weight loss and change in creatinine clearance or creatinine excretion. However, adjustment for the change in weight rendered the change in clearance

TABLE 4Interaction between diet and triacylglycerol (TG) status for weight loss¹

Diet and TG status	TG concentration	Baseline weight	Weight loss
	mmol/L	kg	kg
HP diet			
TG < 1.5 mmol/L (n = 27)	0.89 ± 0.04	89.1 ± 2.8	7.3 ± 0.8
TG > 1.5 mmol/L (n = 25)	1.89 ± 0.16	85.4 ± 1.6	7.9 ± 0.4
HC diet			
TG < 1.5 mmol/L (n = 23)	0.90 ± 0.04	86.5 ± 2.6	8.1 ± 0.7
TG > 1.5 mmol/L (n = 25)	1.99 ± 0.13	86.2 ± 2.5	5.8 ± 0.7 ²

¹ All values are $\bar{x} \pm \text{SEM}$. HP, high protein; HC, high carbohydrate. There was no significant difference in baseline weight by diet or TG-status.

There was a significant interaction between diet and TG status ($P = 0.032$) by repeated-measures ANOVA, with diet and TG status as between-subject factors. The main effect of neither TG status ($P = 0.227$) nor the main effect of diet ($P = 0.286$) was significant.

² Significant difference for change between diets ($P = 0.005$) by univariate analysis, with baseline BMI as a covariate.

insignificant ($P = 0.621$), ie, the change in calculated creatinine clearance was due to the weight change and not to a change in renal function.

Lipids, glucose, insulin, fatty acids, and C-reactive protein

There was no significant effect of diet composition on LDL-cholesterol, HDL-cholesterol, and glucose concentrations (Table 7). LDL cholesterol decreased overall by 6%, HDL cholesterol decreased by 7%, and glucose concentrations decreased by 4% with both diets. Diet composition affected the decrease in triacylglycerols, which decreased by 8% with the HC diet and by 22% with the HP diet ($P = 0.007$). Because subjects with high triacylglycerol concentrations may be more responsive to factors that alter triacylglycerols, we reanalyzed the data according to triacylglycerols status (above or below the median of 1.5 mmol/

L). There was a diet × triacylglycerol status interaction for triacylglycerol ($P = 0.023$). In the women with a high triacylglycerol concentration, the HP diet lowered triacylglycerols

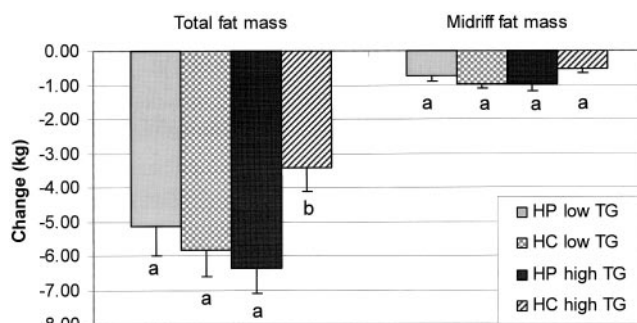


FIGURE 2. Mean (\pm SEM) changes in total and midriff fat assessed by dual-energy X-ray absorptiometry, by serum triacylglycerol (TG) status (high or low concentrations), in subjects who consumed a high-protein (HP) diet or a high-carbohydrate (HC) diet for 12 wk. There was no significant difference at baseline in total or midriff fat mass by diet or TG status. There were no significant main effects of diet or TG status, but there was a significant diet × TG status interaction for total fat ($P = 0.019$) and midriff fat ($P = 0.03$) by repeated-measures ANOVA, with diet and TG status as between-subject factors. Bars with different lowercase letters are significantly different, $P = 0.035$ (Tukey's test for post hoc analysis). HP low TG (n = 27), HC low TG (n = 23), HP high TG (n = 25), HC high TG (n = 25).

TABLE 5Body-composition changes assessed by dual-energy X-ray absorptiometry¹

	HP group (n = 52)	HC group (n = 48)
Total lean mass (kg) ²		
Week 0	41.8 ± 0.8	40.9 ± 0.9
Week 12	40.3 ± 0.9	39.3 ± 1.0
Change	-1.5 ± 0.3	-1.8 ± 0.3
Total fat mass (kg) ²		
Week 0	42.1 ± 1.2	41.9 ± 1.1
Week 12	36.5 ± 1.1	37.1 ± 1.1
Change ³	-5.7 ± 0.6	-4.5 ± 0.5
Midriff lean fat (kg) ²		
Week 0	2.4 ± 0.1	2.5 ± 0.1
Week 12	2.2 ± 0.1	2.4 ± 0.1
Change	-0.2 ± 0.1	-0.2 ± 0.1
Midriff fat (kg) ²		
Week 0	3.6 ± 0.1	3.7 ± 0.2
Week 12	2.7 ± 0.1	3.0 ± 0.1
Change ³	-0.9 ± 0.1	-0.7 ± 0.1

¹ All values are $\bar{x} \pm \text{SEM}$. HP, high protein; HC, high carbohydrate.

There were no significant differences at baseline between diets.

² Main effect of time ($P < 0.01$) by repeated-measures ANOVA with both time points as within-subject variables over both treatments.

³ There were no significant main effects of diet or triacylglycerol status, but there was a significant diet × triacylglycerol status interaction for total fat ($P = 0.019$) and midriff fat ($P = 0.03$) by repeated-measures ANOVA with diet and triacylglycerol status as between-subject factors. See Figure 2 for subgroup analysis.

TABLE 6Markers of renal function¹

	Week 0	Week 12
Urine urea:creatinine		
HP group (n = 50)	33.7 ± 1.29	38.2 ± 0.88 ²
HC group (n = 48)	32.5 ± 1.5	34.2 ± 1.2
Serum urea (mmol/L)		
HP group (n = 50)	5.5 ± 0.2	6.2 ± 0.2 ³
HC group (n = 48)	5.8 ± 0.2	5.1 ± 0.2
Creatinine clearance (mL/min) ⁴		
HP group (n = 50)	82.3 ± 3.3	76.7 ± 2.9
HC group (n = 48)	81.9 ± 3.3	72.9 ± 3.1

¹ All values are $\bar{x} \pm \text{SEM}$. HP, high protein; HC, high carbohydrate. There were no significant differences at baseline between diets.

² Significantly different from HC group, $P = 0.003$ (univariate analysis at week 12 with week 0 as covariate).

³ Significantly different from HC group, $P < 0.001$ (univariate analysis at week 12 with week 0 as covariate).

⁴ No significant difference between diets, $P = 0.346$ (univariate analysis at week 12 with week 0 as covariate).

TABLE 7

Fasting lipid, glucose, insulin, free fatty acid, and C-reactive protein (CRP) concentrations¹

	Week 0	Week 4	Week 8	Week 12	Change	<i>P</i> for diet ²	<i>P</i> for time ³
Triacylglycerol (mmol/L) ⁴							
HP group	1.37 ± 0.11 ⁵	1.08 ± 0.06	1.10 ± 0.06	1.07 ± 0.06	-0.30 ± 0.10	—	—
HC group	1.47 ± 0.11	1.31 ± 0.09	1.3 ± 0.09	1.35 ± 0.10	-0.11 ± 0.06	0.007	<0.001
Total cholesterol (mmol/L)							
HP group	5.75 ± 0.16	4.97 ± 0.14	5.14 ± 0.14	5.26 ± 0.15	-0.48 ± 0.10	—	—
HC group	5.88 ± 0.14	5.12 ± 0.14	5.26 ± 0.15	5.54 ± 0.15	-0.33 ± 0.08	0.164	<0.001
LDL cholesterol (mmol/L)							
HP group	3.79 ± 0.14	3.32 ± 0.13	3.43 ± 0.13	3.53 ± 0.13	-0.26 ± 0.09	—	—
HC group	3.90 ± 0.12	3.39 ± 0.12	3.51 ± 0.13	3.71 ± 0.13	-0.19 ± 0.08	0.399	<0.001
HDL cholesterol (mmol/L)							
HP group	1.33 ± 0.05	1.17 ± 0.04	1.21 ± 0.04	1.25 ± 0.04	-0.09 ± 0.02	—	—
HC group	1.32 ± 0.04	1.15 ± 0.03	1.17 ± 0.04	1.22 ± 0.04	-0.09 ± 0.02	0.657	<0.001
Glucose (mmol/L)							
HP group	6.16 ± 0.65	6.00 ± 0.59	6.13 ± 0.66	5.93 ± 0.61	-0.21 ± 0.05	—	—
HC group	6.08 ± 0.58	5.97 ± 0.53	6.00 ± 0.54	5.83 ± 0.62	-0.25 ± 0.07	0.589	<0.001
Insulin (mU/L)							
HP group	10.0 ± 0.9	7.2 ± 0.5	7.4 ± 0.7	7.3 ± 0.5	-2.7 ± 0.5	—	—
HC group	10.0 ± 0.7	7.5 ± 0.5	7.9 ± 0.8	8.4 ± 1.2	-1.6 ± 0.9	0.278	<0.001
Free fatty acids (mmol/L)							
HP group	0.46 ± 0.03	0.45 ± 0.03	0.39 ± 0.02	0.42 ± 0.03	-0.04 ± 0.03	—	—
HC group	0.41 ± 0.02	0.43 ± 0.02	0.37 ± 0.02	0.39 ± 0.02	-0.02 ± 0.02	0.765	<0.001
CRP (mg/L)							
HP group	6.6 ± 0.7	—	—	4.9 ± 0.6	-1.7 ± 0.4	—	—
HC group	4.8 ± 0.5	—	—	4.0 ± 0.4	-0.8 ± 0.3	0.447	<0.001

¹ HP, high protein (*n* = 52); HC, high carbohydrate (*n* = 48). There were no significant differences in variables at baseline between diets.

² Main effect of diet by univariate analysis at week 12 with diet as the fixed factor and the week 0 data point as the covariate.

³ Main effect of time by repeated-measures ANOVA with all time points as within-subject variables over both treatments.

⁴ Significant diet × triacylglycerol status interaction (*P* = 0.023) by repeated-measures ANOVA with all time points and diet and triacylglycerol status as between-subject factors. See Figure 3 for subgroup analysis.

⁵ $\bar{x} \pm$ SEM (all such values).

significantly, by 28% compared with only 10% with the HC diet. In the low- triacylglycerol group, there was no significant effect of diet composition on triacylglycerol (Figure 3). Fasting glucose, insulin, and free fatty acid concentrations all decreased significantly with weight loss, with no differential effect of diet composition (Table 7). CRP decreased significantly overall, by

19% (*P* < 0.001), with no significant effect of diet (*P* = 0.447). The change in CRP in the low- triacylglycerol group was 0.74 ± 0.27 mg/L, and the change in the high- triacylglycerol group was 1.90 ± 0.41 mg/L (*P* = 0.03 for the difference). This difference was enhanced (*P* = 0.018) after adjustment for weight loss.

Iron status

There was a small but nonsignificant 2% increase in hemoglobin with the HP diet (*P* = 0.116) but no change with the HC diet (Table 8). Transferrin decreased by 9–12% with both diets. There were no significant changes in iron status. Ferritin concentrations were outside the normal range of 150 μg/L in 17 subjects, which suggested that iron stores were likely to be replete and nonresponsive to dietary changes. When these subjects were excluded from the analysis, there was a significant 41% increase in serum ferritin in the HP group but no change in this marker of iron stores in the HC group (*P* = 0.004 for diet effect; Figure 4). It is interesting to note that ferritin, which has been argued to be an additional marker for metabolic syndrome, was positively correlated with serum homocysteine concentrations at baseline (*r* = 0.209, *P* = 0.037).

Vitamins B-12 and B-6, homocysteine, and folate

Vitamin B-12 rose significantly (by 9%) with the HP diet, whereas it decreased (by 13%) with the HC diet (Table 8). The

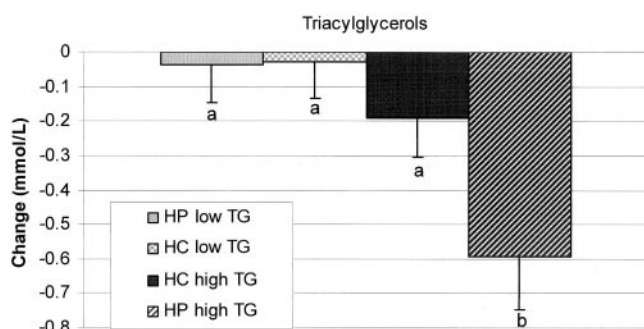


FIGURE 3. Mean (\pm SEM) changes in triacylglycerol (TG) concentrations, by serum TG status (high or low concentrations), in subjects who consumed a high-protein (HP) diet or a high-carbohydrate (HC) diet for 12 wk. There was a significant main effect of time (*P* < 0.0001), a significant main effect of diet (*P* = 0.032), and a significant time × diet × TG status interaction (*P* = 0.023) by repeated-measures ANOVA with diet and TG status as between-subject factors. Bars with different lowercase letters are significantly different, *P* < 0.05 (Tukey's test for post hoc analysis). HP low TG (*n* = 27), HC low TG (*n* = 23), HC high TG (*n* = 25), HP high TG (*n* = 25).

TABLE 8

Iron status, vitamin status, and markers of bone turnover¹

	Week 0	Week 12	Change	<i>P</i> for diet ²	<i>P</i> for time ³
Hemoglobin (g/L)					
HP group	132 ± 1 ^{4,5}	135 ± 1	3 ± 1	—	—
HC group	138 ± 1	138 ± 1	0 ± 1	0.116	0.066
Transferrin (μmol/L)					
HP group	34.3 ± 0.9	30.3 ± 0.8	-4.1 ± 0.4	—	—
HC group	34.0 ± 0.9	30.8 ± 0.9	-3.1 ± 0.5	0.148	<0.001
Transferrin saturation (%)					
HP group	23.9 ± 1.3	24.6 ± 1.3	0.6 ± 1.2	—	—
HC group	27.0 ± 1.3	27.9 ± 1.2	0.4 ± 1.4	0.383	0.554
Ferritin (μg/L)					
HP group	105 ± 23	120 ± 17	15 ± 10	—	—
HC group	83 ± 9	90 ± 12	7 ± 6	0.144	0.064
Iron (μmol/L)					
HP group	16.0 ± 0.7 ⁶	14.6 ± 0.6	-1.1 ± 0.6	—	—
HC group	18.0 ± 0.9	16.2 ± 0.6	-1.8 ± 0.9	0.267	0.010
Serum vitamin B-12 (pmol/L)					
HP group	273 ± 14	311 ± 21	38 ± 13	—	—
HC group	278 ± 14	240 ± 13	-38 ± 7	<0.0001	0.865
Pyridoxyl phosphate activation (%)					
HP group	50.3 ± 1.4	47.0 ± 1.0	-3.1 ± 1.2	—	—
HC group	47.3 ± 1.7	44.9 ± 1.5	-2.4 ± 1.0	0.602	0.001
Serum homocysteine (μmol/L)					
HP group	8.5 ± 0.2	8.5 ± 0.2	0.1 ± 0.2	—	—
HC group	8.8 ± 0.3	8.7 ± 0.2	0.1 ± 0.2	0.733	0.596
Serum folate (nmol/L)					
HP group	26.3 ± 1.0	26.7 ± 0.7	0.4 ± 0.9	—	—
HC group	24.8 ± 1.1	27.1 ± 0.7	2.3 ± 0.9	0.265	0.045
Serum osteocalcin (ng/mL)					
HP group	6.75 ± 0.56	7.95 ± 0.46	1.20 ± 0.3	—	—
HC group	5.49 ± 0.49	7.03 ± 0.48	1.54 ± 0.3	0.997	<0.0001
Deoxypyridinoline:creatinine (nmol/mmol)					
HP group	22.2 ± 1.4	24.7 ± 1.0	2.5 ± 1.1	—	—
HC group	20.5 ± 0.9	24.2 ± 1.3	3.7 ± 1.0	0.786	<0.0001
Pyridinolone:creatinine (nmol/mmol)					
HP group	78.7 ± 5.3	84.3 ± 2.8	5.6 ± 4.0	—	—
HC group	69.3 ± 2.4	77.6 ± 3.1	8.2 ± 2.1	0.493	0.003

¹ HP, high protein (*n* = 52); HC, high carbohydrate (*n* = 48).² Main effect of diet by univariate analysis at week 12 with diet as the fixed factor and the week 0 data point as the covariate.³ Main effect of time by repeated-measures ANOVA with both time points as within-subject variables over both treatments.⁴ $\bar{x} \pm$ SEM (all such values).^{5,6} Significantly different from HC group: ⁵*P* = 0.004, ⁶*P* = 0.045.

difference between diets was significant (*P* < 0.0001). Vitamin B-6 increased with both diets, with no significant difference between them, whereas homocysteine did not change significantly over the intervention. Serum folate increased marginally

with time (*P* = 0.045), with no effect of diet composition (*P* = 0.234 for diet).

Markers of bone turnover

Osteocalcin increased by 23%, with no significant difference between dietary interventions (Table 8). There was no correlation between the amount of weight lost and changes in urinary crosslinks or the calcium-creatinine ratio. The urinary crosslinks and the calcium-creatinine ratio, however, were inversely related (*r* = 0.36 for pyridinoline and *r* = 0.28 for deoxypyridinoline), ie, the greater the decrease in calcium excretion, the smaller the increase in crosslink excretion. Changes in crosslinks or osteocalcin were unrelated to menopausal or triacylglycerol status.

Osteocalcin at week 12 was correlated with the urinary crosslinks at week 12 (*P* < 0.01) after the adjustment for baseline osteocalcin, but was not related to weight changes (*P* = 0.723). However, the urinary crosslinks at week 12 were both correlated with the change in weight (*P* < 0.01) and in osteocalcin (*P* < 0.05) after the adjustment for baseline values. This suggests that weight loss drives increased bone loss and there is partial compensation with increased bone formation.

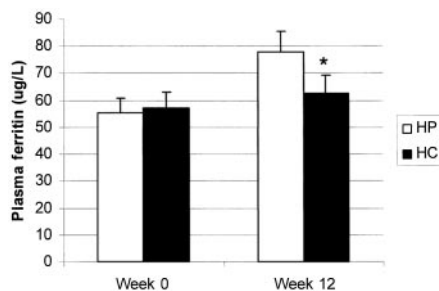


FIGURE 4. Mean (\pm SEM) plasma ferritin concentrations, adjusted to exclude subjects with baseline ferritin concentrations exceeding the normal range (>150 μg/L), in subjects who consumed a high-protein (HP; *n* = 43) diet or a high-carbohydrate (HC; *n* = 40) diet for 12 wk. *Significantly different from the HP diet at week 12, *P* = 0.002 (univariate analysis with diet as the fixed factor and the week 0 data point as the covariate).

DISCUSSION

Although we hypothesized that the HP diet would result in greater fat loss and less lean mass loss than the HC diet, we did not observe this for the group of overweight women overall. This finding contrasts with that of our previous studies in subjects with type 2 diabetes (8) and hyperinsulinemia (9), hence, our subgroup analysis to ascertain whether markers of the insulin resistance syndrome may have predicted responses to the dietary interventions. In our study, overweight women with high triacylglycerol concentrations, one of the key markers of the insulin resistance syndrome, lost 50% more total fat with the HP diet than with the HC diet. Although further confirmation is required, we believe that this is the first study to suggest a phenotype \times diet interaction with respect to the magnitude of weight loss to different diet interventions. Although we speculate that the HP diet provided increased satiety and, hence, subsequent lower energy intake, there was no suggestion of this from reported dietary intakes or differences in physical activity between groups. In a study by Johnston et al (15), subjects who consumed an energy-restricted dietary pattern providing 30% of energy from protein reported less hunger than did those who consumed a high-carbohydrate dietary pattern. However, we cannot rule out the likelihood that the food records may not be suitably accurate to detect a difference of 100 kJ/d between groups, which is the extra energy deficit needed to result in a 1.9-kg weight difference over 12 wk. The mechanism for why this was observed only in the group with elevated triacylglycerol concentrations is of interest. McLaughlin et al (16) showed that the use a cutoff of 1.47 mmol/L is useful in identifying overweight persons who are insulin resistant. However, they showed no differences in weight loss with a hypocaloric diet, on the basis of the degree of insulin resistance (17). A high triacylglycerol concentration may be a marker for the β_2 -adrenoceptor Gln27Glu polymorphism (18). β -Adrenergic receptors play an important role in the regulation of energy expenditure and lipid mobilization. A Gln27Glu polymorphism in the β_2 -adrenergic receptor gene has been shown to be associated with several indexes of obesity in a female, white population, and obesity was shown to be significantly more prevalent in high-carbohydrate consumers with this polymorphism (19). In addition, both lipolysis and fat oxidation appear to be blunted in obese polymorphic Glu27Glu subjects (20), which suggests a rationale for the enhanced fat loss in our subgroup with high triacylglycerol concentrations who consumed the lower-carbohydrate HP diet.

Concerns that diets high in meat protein may have deleterious effects on renal function and bone turnover were not substantiated by this study, which showed similar reductions in creatinine clearance with both dietary patterns as a consequence of body mass change. Skov et al (21) assessed changes in renal function by measuring the glomerular filtration rate during high-protein and high-carbohydrate diets over a 6-mo period and also concluded that the HP diet had no adverse effects on kidney function. Johnston et al (15) observed that creatinine clearance was not altered by dietary protein in the context of weight loss, and nitrogen balance was more positive in subjects who consumed the HP diet than in those who consumed the HC diet. Whether this is also true in subjects with compromised kidney function has not been studied, although we have shown an improvement in microalbuminuria in subjects with type 2 diabetes after weight loss with either a high-protein or a high-carbohydrate diet, which

suggests that weight loss and consequent blood pressure reduction may be more important in ameliorating renal function than is dietary protein. Last, although the amount of dietary protein was proportionally high, it was not high in absolute terms. The HP diet provided 104 g and the HC diet provided 58 g protein, which is within the range of protein intakes in the Australian population (22). In fact, these intakes represent the 95th percentile and the 20th percentile of protein intakes for women of this age group in Australia.

The effect of this level of protein on markers of bone turnover was similarly not deleterious. Although weight loss appears to enhance both bone breakdown and, secondarily, bone formation, these variables were not significantly different between the 2 diet groups. Other studies have shown that diet-induced weight loss in postmenopausal women is associated with general bone loss, probably because of reduced mechanical strain on the skeleton (23), but that premenopausal women do not lose bone even if they have a low calcium intake during weight loss (24). Evidence also indicates that higher protein intakes, particularly higher animal protein intakes, are associated with decreased bone loss in older persons (25). The reduction in urinary calcium in this study was also unusual because dietary protein metabolism is associated with increased urinary calcium (26). The high vegetable consumption with both dietary patterns may have prevented this because high vegetable intakes have been shown to decrease urinary calcium (27). An increase in calcium excretion was observed with the consumption of a high-protein diet in the study by Johnston et al (15), who state that this was due to the high calcium content of the high-protein diet in this study. However, we did not observe this in other studies of high-protein patterns in which dietary calcium was very high, ie, 2400 mg/d (28).


Cardiovascular disease markers improved with weight loss with both diets; triacylglycerol concentrations decreased more with the HP diet in women with elevated triacylglycerol concentrations. This finding reflects a lower carbohydrate load with the HP diet, which results in reduced VLDL TG production (29). CRP, which is known to decrease with weight loss (30), was not influenced by dietary composition, although there was a suggestion that the HP diet lowered CRP more effectively in women with higher triacylglycerol concentrations. This observation warrants further investigation.

Dietary patterns intended for weight loss, which sustain or improve nutritional status, are important for optimum health. The HC diet pattern was designed to provide a contrasting intake of protein and, as such, did not fully meet the recommended dietary allowance (RDA) for some nutrients, notably calcium and iron. In contrast, nutrient intakes with the HP diet were adequate or exceeded the RDA, which reflected the higher proportion of nutrient-dense protein foods from dairy foods and lean meat in the diet. Hemoglobin concentrations were maintained with both diets. This is in contrast with the findings of Kretsch et al (31), who fed dieting obese women dietary iron at twice the US RDA—half of which was from food and half of which was from an oral supplement—yet found a significant reduction in hemoglobin concentrations. This group also found that hemoglobin and transferrin saturation were both positively correlated with mean performance on a measure of sustained attention. The stability of iron status in the HC group was surprising given both the quantitatively lower iron intake and the theoretically lower bioavailability of iron with this diet, but the higher fruit and vegetable intake may have contributed to optimizing iron absorption.



Pyridoxal phosphate activation, a marker of vitamin B-6 status, decreased with weight loss with both diets, which indicated improved vitamin B-6 status. Vitamin B-6 functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids as well as in glycogenolysis as a cofactor for glycogen phosphorylase. Vitamin B-6 is found in a wide variety of foods, including beans, meat, poultry, fish, and some fruit and vegetables. Improved vitamin B-6 status is likely to be a function of the improved nutrient density of both dietary patterns compared with baseline eating patterns.

The greatest difference in nutrient status was observed with serum vitamin B-12, which increased by 9% with the HP diet but decreased by 13% with the HC diet. This finding reflected the difference in animal protein sources between the 2 dietary patterns. Ames (32) postulated that micronutrient deficiencies are a major cause of DNA damage by the same mechanism as radiation and many chemicals. Intervention studies in humans have shown that DNA damage is minimized when, among other micronutrients such as folate, serum concentration of vitamin B-12 are >300 pmol/L, which is precisely the concentration achieved with the HP diet in this study without supplementation.

In conclusion, both the HP and HC, which were intended for weight loss, resulted in significant improvements in markers of cardiovascular disease risk, although the HP diet resulted in a greater reduction in triacylglycerol concentrations and improvements in hemoglobin and vitamin B-12 status. An energy-restricted diet high in protein from lean red meat and low-fat dairy products seems to provide a weight loss advantage in subjects with elevated triacylglycerol concentrations—a marker of the metabolic syndrome. This finding requires confirmation in future studies in hypertriglyceridemic women. There was no evidence of adverse effects on bone or renal metabolism with either diet over the 12-wk study period. 

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MN and PMC designed the study, performed the statistical analysis, and wrote the manuscript. PRF and JBK contributed both to the interpretation of the dietary data and to the preparation of the manuscript and were involved in the dietetic counseling and data analysis. None of the authors had a conflict of interest.

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