Dietary Protein and Exercise Have Additive Effects on Body Composition during Weight Loss in Adult Women[1,2]

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ABSTRACT This study examined the interaction of 2 diets (high protein, reduced carbohydrates vs. low protein, high carbohydrates) with exercise on body composition and blood lipids in women ($n = 48$, ~46 y old, BMI = 33 kg/m^2) during weight loss. The study was a 4-mo weight loss trial using a 2 × 2 block design (Diet × Exercise). Diets were equal in total energy (7.1 MJ/d) and lipids (~30% energy intake) but differed in protein content and the ratio of carbohydrate:protein at 1.6 g/(kg · d) and <1.5 (PRO group) vs. 0.8 g/(kg · d) and >3.5 (CHO group), respectively. Exercise comparisons were lifestyle activity (control) vs. a supervised exercise program (EX: 5 d/wk walking and 2 d/wk resistance training). Subjects in the PRO and PRO + EX groups lost more total weight and fat mass and tended to lose less lean mass ($P = 0.10$) than the CHO and CHO + EX groups. Exercise increased loss of body fat and preserved lean mass. The combined effects of diet and exercise were additive for improving body composition. Serum lipid profiles improved in all groups, but changes varied among diet treatments. Subjects in the CHO groups had larger reductions in total cholesterol and LDL cholesterol, whereas subjects in the PRO groups had greater reductions in triacylglycerol and maintained higher concentrations of HDL cholesterol. This study demonstrated that a diet with higher protein and reduced carbohydrates combined with exercise additively improved body composition during weight loss, whereas the effects on blood lipids differed between diet treatments. J. Nutr. 135: 1903–1910, 2005.

KEY WORDS: • *obesity* • *low-carbohydrate diets* • *blood lipids* • *insulin* • *adiponectin*

Obesity is an important public health problem associated with multiple chronic health conditions including heart disease, hypertension, hyperlipidemia, diabetes, hyperinsulinemia, and cancer. Recommendations for treatment of adults who are overweight or obese focus on energy balance with lifestyle modifications designed to reduce daily energy intake and increase physical activity (1). Although these recommendations represent public health policy, there are few studies that provide direct evidence about the combined merits and interactions of specific diet and exercise choices. Furthermore, the ideal balance of macronutrients (carbohydrates, lipids, and protein) necessary to optimize the combined effects of exercise and energy restriction is unknown.

There is general consensus that the most critical factor in determining weight loss is total energy intake (1–3). The ideal balance of macronutrients for adult weight loss remains widely disputed (1–5). However, evidence is accumulating that energy-restricted diets with reduced levels of carbohydrates and higher levels of protein are beneficial during weight loss (6–16). These studies report that diets with carbohydrate intake < 150 g/d and protein intake > 1.4 g/(kg · d) result in increased weight loss (6–8,12), increased loss of body fat (7,8,10), attenuated loss of fat-free mass (FFM)[4] (6,8,10,13,14), improved glycemic control (6,10–12), improved blood lipid profiles (10,12,16,17), and enhanced satiety (15).

The importance of exercise in weight loss and prevention of weight regain is well accepted (1–3). In general, exercise during weight loss appears to target loss of fat mass while preserving lean mass. The effects of exercise on total body weight are variable, but generally proportional to the accumulated total energy expenditure (2,3). Variables including duration of activity per day, frequency of activity per week, and intensity ultimately determine energy expenditure and the

[1] Presented in part at the 2004 North American Association for the Study of Obesity meeting, November 15, 2004, Las Vegas, NV [Evans, E. M., Layman, D. K., Baum, J. I., Seyler, J. E., Erickson, D. J. & Heinrichs, K. L. (2004) Additive effects of dietary protein and exercise on body composition and endocrine changes during weight loss in adult women. Obes. Res. 12 (suppl.): A48 (abs.)]; and at Experimental Biology 05, April 2–6, 2005, San Diego, CA [Layman, D., Evans, E., Baum, J., Seyler, J. & Erickson, D. (2005) Interaction of dietary protein and exercise during weight loss in adult women. FASEB J. 19: A419 (abs.)].

[2] Support for this research was provided by the Illinois Council on Food and Agricultural Research, National Cattlemen's Beef Association, The Beef Board and Kraft Foods.

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[4] Abbreviations used: ANCOVA, analysis of covariance; CHD, coronary heart disease; CHO, higher carbohydrate diet; DXA, dual energy X-ray absorptiometry; FFM, fat-free mass; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PRO, higher protein diet; RDA, Recommended Dietary Allowance; TAG, triacylglycerol; TC, total cholesterol.

Manuscript received 16 February 2005. Initial review completed 7 March 2005. Revision accepted 7 May 2005.

potential for weight loss. Studies suggest that low-intensity exercise (i.e., walking) at <150 min/wk has minimal potential to produce weight loss (18,19). Similarly, resistance exercise appears to have beneficial effects on body composition, maintaining FFM while increasing loss of body fat, but has minimal effect on body weight (20,21). In addition to changes in body composition, exercise also has positive effects on blood lipids. Regular endurance exercise is associated with decreases in blood concentrations of triacylglycerol (TAG) and increases in HDL cholesterol (HDL-C) (22). In total, exercise-induced changes in body composition and blood lipids are similar to changes observed with reduced-carbohydrate, high-protein, weight loss diets (7,10,12,17). However, the combined effects of a low-carbohydrate, higher-protein diet with exercise during weight loss have not been examined.

This study was designed to evaluate interactions of diet composition and exercise on body composition changes and blood lipids during weight loss. Based on our review of the scientific literature, we hypothesized that a diet with increased protein and reduced carbohydrates combined with an exercise program of aerobic activity and resistance training would produce an additive effect on loss of body fat and maintenance of FFM. Further, we measured the interactions of diet treatments and exercise on changes in blood lipids and blood concentrations of leptin, adiponectin, ghrelin, and insulin as indicators of metabolic change during weight loss.

SUBJECTS AND METHODS

Design. This study was a randomized 4-mo weight loss trial using a 2 × 2 block design (Diet × Exercise). Diet treatments consisted of either a low carbohydrate:protein ratio (PRO group) or a high carbohydrate:protein ratio (CHO group). Exercise treatments consisted of a control group participating in light walking activity or an exercise group (designated EX) that required walking a minimum of 5 d/wk plus 2 sessions/wk of resistance exercise.

Subjects. Women (n = 48) aged 40–56 y were recruited to participate in a weight loss study. Exclusion criteria were BMI < 26 kg/m^2, body weight > 140 kg (due to DXA scanning bed constraints), smoking, any existing medical conditions requiring medications that would affect primary or secondary outcomes of the study, use of oral steroids or use of antidepression medication. Due to resource and personnel constraints, the first 24 women recruited were randomly assigned to either PRO + EX or CHO + EX groups (n = 12 each). The next 24 women recruited were randomly assigned to either the PRO or CHO groups (n = 12 each). Note that the randomization was blocked on age and BMI for each wave of recruitment. Baseline characteristics did not differ among subjects in each of the treatment groups (**Table 1**). The protocol and aim of the study were fully explained to the subjects, who gave their written informed consent. This study was approved by the Institutional Review Board at the University of Illinois at Urbana-Champaign.

Diet treatments. The CHO diet provided dietary protein at 0.8 g/(kg · d) (~15% of energy intake) with a carbohydrates:protein ratio > 3.5 and dietary lipids at ~30% energy intake. The PRO diet provided dietary protein at 1.6 g/(kg · d) (~30% of energy intake) with a carbohydrate:protein ratio < 1.5 and dietary lipids at ~30% energy intake. These diets were designed to fall within the Acceptable Macronutrient Distribution Range established by the Institute of Medicine (23) with minimum Recommended Dietary Allowance (RDA) intakes for carbohydrates = 130 g/d and protein = 0.8 g/kg and with upper limits for carbohydrates < 65% and protein < 35% of total energy intake. The 2 diets were formulated to be equal in energy (7100 kJ/d; 1700 kcal/d), total fat intake (~57 g/d) and fiber (~17 g/d). With these general criteria, we developed a 2-wk menu plan for each group with meals for each day meeting established nutritional requirements (24) and lipid guidelines (25). The dietary differences between groups were designed to reflect a direct substitution of foods in the protein groups (meats, dairy, eggs, and nuts) for foods in the refined grain/starch groups (breads, rice, cereals, pasta, and potatoes). The education guidelines for the CHO group followed the USDA Food Guide Pyramid (26) and emphasized restricting dietary fat and cholesterol with use of breads, rice, cereals, and pasta. For the PRO group, the education guidelines emphasized use of high-quality proteins including meats, dairy, and eggs. Both diets included 5 servings/d of vegetables and 2–3 servings of fruits.

Exercise treatments. Exercise treatments consisted of a control group that emphasized lifestyle recommendations for physical activity based on the NIH Guidelines for Weight Management (1). These guidelines recommend a minimum of 30 min of walking 5 d/wk. Participation in physical activity for the control groups was voluntary and the education program for this group focused on diet compliance with limited attention to exercise. Exercise was monitored using daily activity logs; on 3 d/mo subjects wore armband accelerometers (Body-Media). Based on these measurements, we estimated that subjects in the control groups averaged <100 min/wk of added exercise. The EX treatment group required a minimum of walking 5 d/wk for 30 min/d plus a resistance training program 2 d/wk consisting of 30 min of stretching and resistance exercise using 7 Nautilus® weight machines. EX subjects were required to complete a minimum of one 12-repetition set on each machine with the resistance weight selected to elicit fatigue by the final repetition using full range of motion with both concentric and eccentric pressure and within the comfort level of the participant. Exercise compliance in the EX group was supervised and averaged >200 min/wk.

Protocol. All subjects participated in a baseline evaluation period that included a 24-h food recall, instructions for weighing and recording of foods, two 3-d weighed food records and measurements of body weight and height, and blood lipids. This evaluation period from first contact with the subjects was 10–20 d and served as an initial control period for each subject. During this baseline period, subjects were instructed to maintain stable body weight and to consume a diet similar to that of the past 6 mo. After the baseline period, subjects reported to the nutrition research laboratory at 0630 h after a 12–14 h overnight fast for weighing and blood sampling.

Body composition and blood measures. Body weight was measured using an electronic scale (Tanita, Model BWB-627A). Whole-body composition and trunk fat (regionally defined with analysis lines drawn through the axilla, proximal femur neck and at the chin line to segregate the arms, legs, and head, respectively) was determined by dual energy X-ray absorptiometry (DXA; Hologic) and scans for a given individual were analyzed by the same technician using standard manufacturer guidelines. The CVs for DXA outcomes of interest were 1.5%. Plasma leptin, adiponectin, and ghrelin (Linco Research; Catalogue # HL-81HK, HADP-61HK and GHRA-88HK, respectively) and insulin (MP Biomedicals; catalogue # 07260105) were determined by commercial RIA kits with all samples for a given participant analyzed in batch. The intra-assay CVs for leptin, adiponectin, ghrelin and insulin were 6.1 1.5, 3.0, and 5.5%, respectively. Serum total cholesterol (TC), HDL-C and TAG were determined by standardized methods (27) by the Washington University School of Medicine Core Laboratory for Clinical Studies. LDL cholesterol (LDL-C) was calculated using the Friedewald equation (28).

Diet education and measures. Subjects were provided electronic food scales and instructed to weigh all food servings at all meals; they were required to report a 3-d weighed food record for each week

TABLE 1

Baseline characteristics of subjects[1]

Group	PRO	PRO + EX	CHO	CHO + EX
Age, *y*	47.0 ± 1.7	46.5 ± 1.2	45.2 ± 1.4	47.9 ± 1.4
Height, *cm*	161.5 ± 2.5	165.8 ± 1.3	162.2 ± 1.4	163.2 ± 1.6
Weight, *kg*	91.1 ± 5.1[a,b]	86.1 ± 4.6[a,b]	93.7 ± 3.5[a]	79.8 ± 2.7[b]
BMI, *kg/m*^{2}	34.8 ± 1.8[a]	31.4 ± 1.7[a,b]	35.4 ± 1.1[a]	30.2 ± 1.3[b]

[1] Values are means ± SEM, n = 12. Means in a row without a common letter differ, $P < 0.05$.

throughout the study. Nutrient intakes were evaluated as mean daily intakes from the 3-d weighed records using Nutritionist Pro software (First DataBank). After baseline data collection, subjects received instruction from a research dietitian about their specific diet and walking program including the menus, food substitutions, portion sizes, and procedures for maintaining weighed diet records. During the 16-wk weight loss program, subjects were required to attend a 1-h meeting each week at the weight management research facility. Meetings were specific for each treatment group and directed by research dietitians who provided diet and exercise information, answered questions, and reviewed diet records for treatment compliance. Each week, subjects were weighed in light clothing without shoes, and turned in the 3-d weighed food records and daily activity logs.

Statistics. All data analyses were conducted using SPSS version 12.0 (SPSS). Differences among groups in baseline measurements of randomization block variables, age and BMI, were evaluated using one-way ANOVA with differences further evaluated using Fisher's Least Significant Test. As a preliminary analysis, changes that occurred in response to treatments (pretest compared to post-test value) within a group were evaluated by a Student's *t* test. The primary analysis, conducted to evaluate the relative effect of the diet and exercise treatments, utilized a two-way ANOVA (Diet × Exercise) and the change over time as the dependent variable. Note that with this analysis, significant main effects in the absence of a significant interaction effect indicate additive effects of the treatment. To evaluate treatment effects on hormones controlling for changes in body composition, an analysis of covariance (ANCOVA) was used where indicated. The percentage change was calculated as follows: $\{[(\text{posttest}) - (\text{pretest})/(\text{pretest})] \times 100\}$. Differences were considered significant at $P < 0.05$. Values are presented as means ± SEM.

RESULTS

Subjects. Baseline characteristics did not differ among subjects in each of the treatment groups **(Table 1)**. Subjects had a mean age of 46.6 y, weight of 87.7 kg, BMI of 32.9 kg/m^2, and relative body fat of 44.1%. Due to the wave recruitment and block randomization strategy, there was a difference in BMI ($P = 0.047$) among groups, largely associated with the difference between the CHO and CHO + EX groups.

Dietary compliance. Daily energy intakes determined from 3-d weighed food records during the prestudy baseline were 8.94 MJ/d (2138 kcal/d) for all subjects **(Table 2)**. Daily menus for the weight loss diets were designed to provide energy at 7.1 MJ/d (1700 kcal); however, subjects were free-living and ultimately determined final choices of daily energy intakes. Reductions in energy intake determined from weekly 3-d weighed food records did not differ among the groups as indicted by nonsignificant Diet and Exercise main effects and nonsignificant interactions.

A summary of the dietary intakes for the 16-wk period illustrates how the subjects applied the 2 diets during the intervention (Table 2). Subjects in the PRO groups maintained protein intakes of 107 g/d and ~30% of energy intake. The ratio of carbohydrate:protein was ~1.24. Total dietary lipid intake decreased to 49 g/d (32% of dietary energy) and SFA decreased to 19.1 g/d (12.4% of energy). Subjects in the CHO groups maintained carbohydrate intake at 198 g/d (61% of energy) and reduced total lipid intake to 37 g/d (25.5% of energy) and SFA to 11.0 g/d (7.5% of energy). Protein intake in the CHO group was 57 g/d (18% of energy intake) and the ratio of carbohydrate:protein was ~3.5.

Body weight. All groups lost significant body weight during the 16-wk treatment period. Body weight changes were larger ($P < 0.05$) in the groups consuming the higher-protein, reduced-carbohydrate diet **(Table 3)**. The PRO and PRO

TABLE 2

Dietary intakes for adult women at baseline and during weight loss (16 wk) while consuming reduced-energy diets with a carbohydrate:protein ratio >3.5 (CHO group) or <1.5 (PRO group)[1]

Group	PRO	PRO + EX	CHO	CHO + EX	*P-value* D[2]	*P-value* E[3,4]
Energy intake, *kJ/d*						
Baseline	8888 ± 384	8362 ± 322	8479 ± 452	7977 ± 339		
Treatment[5]	6062 ± 117*	5540 ± 134*	5377 ± 179*	5644 ± 180*	0.79	0.34
Protein, *g/d*						
Baseline	79.3 ± 4.0	72.6 ± 3.3	82.1 ± 5.5	73.9 ± 4.7		
Treatment[5]	110.0 ± 3.0*	102.1 ± 3.3*	57.6 ± 1.3*	56.5 ± 1.5*	<0.001	0.44
Carbohydrate, *g/d*						
Baseline	262.3 ± 22.3	266.7 ± 9.2	246.0 ± 16.2	266.2 ± 17.8		
Treatment[5]	141.3 ± 4.7*	126.8 ± 4.2*	197.0 ± 6.2*	201.7 ± 5.8*	<0.001	0.34
Lipids, *g/d*						
Baseline	84.7 ± 4.1	73.9 ± 6.7	79.3 ± 6.0	65.0 ± 3.7		
Treatment[5]	52.2 ± 1.3*	46.3 ± 1.4*	34.0 ± 2.3*	40.8 ± 2.1*	0.40	<0.05
Cholesterol, *mg/d*						
Baseline	259.0 ± 44.8	168.4 ± 21.4	236.2 ± 39.1	224.4 ± 28.3		
Treatment[5]	313.5 ± 23.0	283.4 ± 18.9*	103.7 ± 8.5*	95.0 ± 6.0*	<0.001	0.35
SFA, *g/d*						
Baseline	29.5 ± 2.3	23.6 ± 1.9	28.0 ± 3.0	21.8 ± 2.1		
Treatment[5]	20.6 ± 0.6*	17.5 ± 0.7*	10.3 ± 0.6*	11.6 ± 0.6*	<0.01	<0.05
Fiber, *g*						
Baseline	16.7 ± 3.1	21.2 ± 2.0	16.6 ± 2.1	19.2 ± 1.6		
Treatment[5]	18.6 ± 1.2	16.0 ± 1.2*	22.5 ± 1.5*	23.3 ± 1.3*	<0.01	<0.05

[1] Values are means ± SEM, $n = 12$. * Different from baseline, $P < 0.05$.
[2] D = Test for significant main effect of diet (PRO; $n = 24$: CHO; $n = 24$).
[3] E = Test for significant main effect of exercise (EX; $n = 24$: control; $n = 24$).
[4] D × E was not significant for any variable.
[5] Values represent means for the sum of 3-d weighed records for wk 4, 8, 12, and 16.

TABLE 3

Body weight and composition for adult women at baseline and after 16 wk of consuming reduced-energy diets with a carbohydrate:protein ratio >3.5 (CHO) or <1.5 (PRO) with or without a supervised exercise program (EX: 5 d/wk walking and 2 d/wk resistance training)[1]

Group	PRO	PRO + EX	CHO	CHO + EX	*P-value* D[2]	*P-value* E[3,4]
			kg			
Body weight						
Baseline	91.1 ± 5.1	86.1 ± 4.6	93.7 ± 3.5	79.8 ± 2.7		
Post-test	82.4 ± 4.4*	76.3 ± 3.9*	85.9 ± 3.5*	73.1 ± 2.8*	<0.05	0.98
Fat mass						
Baseline	39.0 ± 3.0	40.9 ± 3.6	40.6 ± 2.0	36.3 ± 2.2		
Post-test	33.1 ± 2.4*	32.1 ± 2.9*	35.6 ± 2.1*	30.8 ± 2.3*	<0.05	<0.05
Trunk fat						
Baseline	19.6 ± 2.0	20.1 ± 2.2	20.1 ± 1.3	17.0 ± 1.2		
Post-test	16.0 ± 1.6*	15.1 ± 1.9*	17.1 ± 1.3*	13.8 ± 1.2*	<0.05	0.11
Lean mass						
Baseline	50.6 ± 2.5	42.6 ± 1.4	51.7 ± 1.7	40.6 ± 0.8		
Post-test	48.6 ± 2.4*	42.2 ± 1.4	49.0 ± 1.8*	39.6 ± 0.8*	0.10	<0.001

[1] Values are means ± SEM; n = 12. * Different from baseline, $P < 0.05$.
[2] D = Test for significant main effect of diet (PRO; n = 24: CHO; n = 24).
[3] E = Test for significant main effect of exercise (EX; n = 24: control; n = 24).
[4] D × E was not significant for any variable.

+ EX groups had a weight loss of 9.3 ± 0.8 kg after 16 wk, whereas the CHO and CHO + EX groups reduced body weight by 7.3 ± 0.5 kg ($P < 0.05$). The PRO + EX group had the largest relative weight loss at 11.2% of initial weight and the CHO group had the smallest relative weight loss at 8.4%. The exercise treatment did not affect body weight change. The interaction between Diet and Exercise treatments was not significant for any measurements including body weight, body composition, hormones, or blood lipids.

Body composition. Changes in body composition indicated that weight loss was predominately fat mass, and that there were increased fat losses associated with the higher-protein diet and the exercise program (Table 3). Subjects in the PRO and the PRO + EX groups reduced fat mass by 7.3 ± 0.8 kg and subjects in the CHO and CHO + EX groups reduced fat mass by 5.3 ± 0.3 kg ($P < 0.05$). Subjects in the PRO + EX and CHO + EX groups reduced body fat by 7.2 ± 0.7 kg, whereas the subjects not participating in the supervised exercise program reduced body fat by 5.5 ± 0.5 kg ($P < 0.05$). The significant main effects of Diet and Exercise in the absence of a significant interaction indicate that the PRO and EX effects were independent and additive. Specifically, the combined effects of the PRO diet and the EX program produced a 21.4% decrease in absolute body fat. Subjects in the CHO group without exercise had a 12.8% reduction in absolute body fat. Changes in absolute trunk fat were similar to changes in total fat mass, with reductions in trunk fat greater in the PRO groups compared with the CHO groups. When the change in trunk fat was expressed relative to the change in whole-body fat, all treatment groups experienced a significant reduction with PRO + EX having the greatest ratio reduction (−0.028 ± 0.007; $P = 0.001$) and CHO experiencing the least change (−0.015 ± 0.005; $P = 0.009$); however, there was no significant main effects for diet ($P = 0.32$) or exercise ($P = 0.12$).

Changes in lean mass reflected a significant positive effect of the exercise program ($P < 0.001$) and a trend for a beneficial effect of the PRO diet ($P = 0.10$) during weight loss (Table 3). Notably the PRO + EX group had no significant change in lean mass (−0.9%; $P = 0.39$), whereas the CHO group had the largest decrease in lean mass (−5.4%; $P = < 0.001$).

Net changes in body composition were reflected in relative body fatness (**Fig. 1**). The additive effects of the PRO and EX treatments on body composition were apparent with significant main effects of Diet ($P < 0.01$) and Exercise ($P < 0.001$) in the absence of a significant interaction. Subjects in the PRO and PRO + EX groups reduced relative body fatness by 4.3% (i.e., change in the percentage of body fat), whereas subjects in the CHO and CHO + EX groups reduced relative

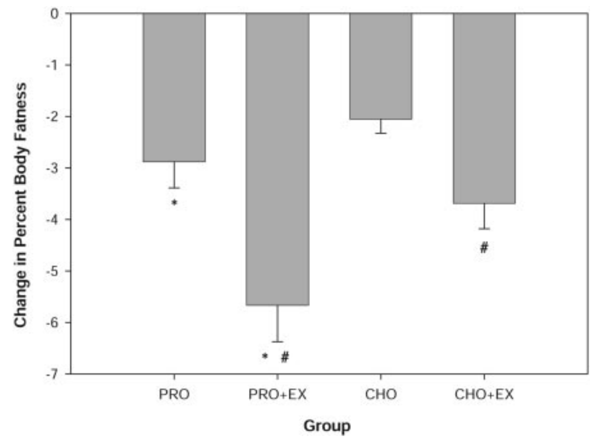

FIGURE 1 Changes in relative body fatness (%Fat) for adult women after 16 wk of consuming reduced-energy diets with a ratio of carbohydrates:protein > 3.5 (CHO) or < 1.5 (PRO) with or without a supervised exercise program (EX: 5 d/wk walking and 2 d/wk resistance training). Values are means ± SEM, n = 12. *Significant main effect of diet, $P < 0.05$; #significant main effect of exercise, $P < 0.05$.

TABLE 4

Endocrine changes for adult women at baseline and after 16 wk of consuming reduced-energy diets with a carbohydrate:protein ratio >3.5 (CHO) or <1.5 (PRO) with or without a supervised exercise program (EX: 5 d/wk walking and 2 d/wk resistance training)[1]

Group	PRO	PRO + EX	CHO	CHO + EX	*P-value* D[2]	*P-value* E[3,4]
Leptin, *μg/L*						
Baseline	38.6 ± 4.9	26.2 ± 3.0	47.6 ± 5.8	37.5 ± 5.6		
Post-test	17.9 ± 4.2*	11.2 ± 2.3*	24.6 ± 3.2*	17.4 ± 4.5*	0.52	0.45
Adiponectin, *mg/L*						
Baseline	2.8 ± 0.4	2.8 ± 0.4	4.4 ± 0.7	3.1 ± 0.4		
Post-test	3.8 ± 0.6	5.5 ± 1.0*	5.0 ± 1.9	7.5 ± 1.7*	0.64	<0.05
Ghrelin, *ng/L*						
Baseline	30.9 ± 2.0	24.2 ± 0.9	32.4 ± 3.0	24.6 ± 1.4		
Post-test	45.2 ± 5.4*	40.5 ± 2.9*	51.2 ± 5.1*	44.6 ± 4.3*	0.34	0.71
Insulin,[5] *pmol/L*						
Baseline	150.6 ± 26.5	189.4 ± 17.2	153.5 ± 27.9	184.4 ± 15.8		
Post-test	125.5 ± 10.8	138.5 ± 7.9*	141.3 ± 9.3	133.4 ± 8.6*	0.78	0.16

[1] Values are means ± SEM; $n = 12$. * Different from baseline, $P < 0.05$.
[2] D = Test for significant main effect of diet (PRO; $n = 24$: CHO; $n = 24$).
[3] E = Test for significant main effect of exercise (EX; $n = 24$: No-EX; $n = 24$).
[4] D × E was not significant for any variable.
[5] Group size: PRO, $n = 9$, PRO + EX, $n = 9$, CARB, $n = 9$ and CHO + EX, $n = 10$.

body fatness by 2.9%. For the main effect of exercise, subjects in the PRO + EX and CHO + EX groups reduced relative body fat by 4.7%, whereas subjects not participating in the supervised exercise program reduced relative body fatness by 2.5%.

Endocrine markers of obesity. Each of the 4 hormones responded to weight loss **(Table 4)**. Plasma concentrations of leptin and insulin decreased and adiponectin and ghrelin increased, but only adiponectin exhibited treatment-specific changes. Blood concentrations of adiponectin more than dou-

TABLE 5

Serum lipid concentrations for adult women at baseline and after 16 wk of consuming reduced-energy diets with a carbohydrate:protein ratio >3.5 (CHO) or <1.5 (PRO) with or without a supervised exercise program (EX: 5 d/wk walking and 2 d/wk resistance training)[1]

Group	PRO	PRO + EX	CHO	CHO + EX	*P-value* D[2]	*P-value* E[3,4]
			mmol/L			
TC						
Baseline	5.59 ± 0.26	5.00 ± 0.23	5.46 ± 0.24	5.09 ± 0.18		
Post-test	5.35 ± 0.28	4.80 ± 0.22	4.91 ± 0.22*	4.63 ± 0.16*	0.06	0.70
LDL-C						
Baseline	3.61 ± 0.19	3.20 ± 0.22	3.52 ± 0.19	3.24 ± 0.15		
Post-test	3.54 ± 0.22	3.11 ± 0.20	3.07 ± 0.15*	2.93 ± 0.12*	<0.05	0.70
HDL-C						
Baseline	1.33 ± 0.09	1.20 ± 0.06	1.30 ± 0.06	1.36 ± 0.07		
Post-test	1.30 ± 0.10	1.25 ± 0.09	1.20 ± 0.04*	1.28 ± 0.07	<0.05	0.19
TAG						
Baseline	1.42 ± 0.15	1.31 ± 0.21	1.40 ± 0.14	1.08 ± 0.13		
Post-test	1.12 ± 0.11*	0.98 ± 0.16*	1.38 ± 0.18	0.91 ± 0.13	<0.05	0.41
LDL-C:HDL-C						
Baseline	2.84 ± 0.23	2.78 ± 0.26	2.79 ± 0.20	2.48 ± 0.19		
Post-test	2.88 ± 0.27	2.64 ± 0.26	2.59 ± 0.13	2.35 ± 0.14	0.35	0.64
TAG:HDL-C						
Baseline	1.11 ± 0.13	1.18 ± 0.25	1.12 ± 0.13	0.84 ± 0.11		
Post-test	0.90 ± 0.10	0.86 ± 0.17	1.15 ± 0.14	0.75 ± 0.12	<0.05	0.25

[1] Values are means ± SEM; $n = 12$. * Different from baseline, $P < 0.05$.
[2] D = Test for significant main effect of diet (PRO; $n = 24$: CHO; $n = 24$).
[3] E = Test for significant main effect of exercise (EX; $n = 24$: No-EX; $n = 24$).
[4] D × E was not significant for any variable.

bled in the EX groups ($P < 0.05$). Furthermore, after controlling for change in fat mass, the main effect of EX remained significant ($P = 0.02$).

Blood lipids. Initial serum lipid concentrations did not differ among all groups (**Table 5**). After 16 wk of weight loss, blood lipid profiles changed in all groups; however, the patterns of changes in TC, LDL-C, HDL-C, and TAG were affected by diets. Changes in TC and LDL-C were greater in the CHO and CHO + EX groups than in the groups that consumed the PRO diets. Serum TC in the CHO and CHO + EX groups decreased by 0.51 ± 0.09 mmol/L (9.2%) and LDL-C decreased by 0.38 ± 0.08 mmol/L (10.4%) from the baseline values. In the PRO and PRO + EX groups, these concentrations decreased by 0.21 ± 0.12 mmol/L (3.7%) and 0.08 ± 0.11 mmol/L (1.7%), respectively. Changes in TAG were largest in the groups consuming the PRO diet. Serum TAG in the PRO and PRO + EX groups decreased by 0.32 ± 0.06 mmol/L (20.2%). In the CHO and CHO + EX groups, TAG concentration decreased by 0.10 ± 0.07 mmol/L (5.2%). HDL-C concentrations changed in opposite directions in the 2 diet treatments producing a significant main effect ($P < 0.05$). The PRO groups had a net increase of 0.01 ± 0.03 mmol/L in HDL-C and the CHO groups decreased by 0.08 ± 0.03 mmol/L. No significant main effects of diet were evident for the ratio of LDL/HDL. Ratios of TAG:HDL were more responsive to the PRO diet than the CHO diet (−0.27 ± 0.07 vs. −0.03 ± 0.07 mmol/L; $P < 0.05$). No significant effects of EX were apparent for any lipid outcomes.

DISCUSSION

This study demonstrates interactions between the macronutrient content of the diet and exercise during weight loss. Subjects consuming diets with more protein and less carbohydrate (PRO and PRO + EX) lost more total weight and fat mass and tended to lose less lean mass ($P = 0.10$) than the groups consuming diets with more carbohydrates and less protein (CHO and CHO + EX). Exercise increased the loss of body fat and preserved lean mass. The combined effects of diet and exercise appeared to be additive for correcting body composition expressed as change in the percentage of body fat (Fig. 1). The PRO + EX group exhibited the greatest loss of body fat (−8.8 kg) with minimal change in lean mass (−0.4 kg), whereas the CHO group had the least change in body fat (−5.0 kg) and the greatest loss of lean mass (−2.7 kg). Changes in body weight and body fat appear to reflect metabolic differences between the diets because total energy intake and daily energy deficits were comparable across all treatment groups.

Although this is the first study to examine the combined effects of exercise with a reduced-carbohydrate, higher-protein diet, the findings are consistent with studies examining the individual effects of diet or exercise. In general, short-term weight loss studies have shown that diets with reduced levels of carbohydrates and increased protein result in increased weight loss (6,7,9,12), increased loss of body fat (7,9), and reduced loss of lean body mass (6,9,13). Similarly, supplemental exercise tends to increase weight loss, but has greater effects on body composition through preserving lean body mass while increasing fat loss (3,18–21,29). In the present study, there was a main effect of diet on total weight loss with no added effect of exercise. The absence of a main effect of exercise on weight loss is consistent with other reports that found that weight loss produced by exercise requires daily activity > 30 min/d and that increases in lean tissue may also reduce changes in total body weight (18–21). For loss of body fat, there were main effects of both diet and exercise, indicating that effects of the higher-protein, reduced-carbohydrate diet and exercise on fat mass loss are independent and additive. Exercise had a main effect of attenuating loss of lean tissue.

Potential explanations for the differential effects of the dietary macronutrient content on body composition and weight loss include: *1*) protein-sparing effects of increased protein on lean body mass (10,14), *2*) increased loss of body fat associated with lower insulin response to the reduced carbohydrate diet (30), and *3*) reduced metabolic efficiency produced by the increased dietary ratio of protein to carbohydrates (31). Although the present study did not directly evaluate fundamental mechanisms, the outcomes are consistent with the combined effects of each of these mechanisms.

Hormone changes in this study largely reflect decreases in body fat mass. Consistent with previous reports, plasma levels of leptin decreased with weight loss (32–34), whereas fasting levels of ghrelin increased (35,36). Only adiponectin exhibited exercise-specific changes.

Adiponectin is an adipocytokine thought to be involved in obesity and insulin sensitivity (37,38). Low plasma concentrations of adiponectin are positively associated with the metabolic Syndrome X characteristics of increased BMI, percentage body fat, and blood TAG and reduced levels of HDL-C (39). Weight reduction achieved by energy-restricted diets increases plasma adiponectin in obese and diabetic patients (38–40). Exercise of short duration that does not alter body weight or body fat does not change adiponectin levels (40,41). The present study found that an exercise regimen that reduced body fat increased adiponectin levels. In addition, when controlling for changes in body fat (ANCOVA), exercise produced positive changes in adiponection concentrations.

As expected, blood lipids were influenced more by diet treatments than exercise. After 4 mo of energy restriction and weight loss, all subjects exhibited improvements in blood lipid profiles, but changes in specific lipoprotein fractions varied among diet treatments. Subjects in the CHO groups had larger reductions in total cholesterol and LDL-C, whereas subjects in the PRO groups had greater reductions in TAG and maintained higher circulating levels of HDL-C. These patterns of changes in blood lipids are consistent with previous studies comparing low- vs. high-carbohydrate diets (9,10,31,42,43).

The ATP III guidelines (22) emphasize that coronary heart disease (CHD) risk is positively related to obesity, TC, LDL-C, and TAG concentrations and inversely related to HDL-C concentrations. However, the relative effect of each of these diet-induced lipid changes on reducing CHD risk is not clear. A meta-analysis of 70 studies evaluating blood lipid changes during weight loss found that weight reduction produced significant correlations with changes in blood lipids (44). They found that for each kilogram decrease in body weight there was a 0.05 mmol/L decrease in TC, a 0.02 mmol/L decrease in LDL-C, a 0.015 mmol/L decrease in TAG, and a 0.007 mmol/L decrease in HDL-C. These relations are consistent with the outcomes of the CHO groups in the present study when values are expressed per kilogram decrease in body weight (0.069, 0.052, 0.014, and 0.011 mmol/L, respectively). Outcomes from the PRO groups differed in magnitude and direction of change. The PRO groups reduced TC, LDL-C, and TAG concentrations by 0.023, 0.009, and 0.034 mmol/L, respectively, when expressed per kilogram of weight loss while increasing the HDL-C concentration by 0.001 mmol/L. These data indicate that blood lipids respond to changes in both body weight and dietary macronutrient content.

The importance of these changes in lipoprotein patterns on long-term health is unknown. In this study, subjects at baseline were obese, but blood lipids and insulin values were within normal ranges. Both dietary approaches were successful for weight loss, improving body composition, and improving blood lipids patterns. However, differential effects of the diet and exercise treatments on blood lipids suggest the possibility that specific treatments may be more beneficial for specific individuals. For example, an individual expressing familial hypercholesterolemia may obtain greater health benefit from use of a low-fat, CHO diet which is likely to produce larger changes in TC and LDL-C (10,30,45), whereas an individual exhibiting hypertriacylglycerolemia and low HDL-C, characteristic of the metabolic syndrome or type 2 diabetes (30,45), may derive greater benefit from use of the PRO diet. Further research is required to determine whether individualization of dietary macronutrient ratios based on phenotype characteristics can improve long-term treatment of obesity and associated health risks.

Dietary outcomes observed in this study raise questions about the relative merits of expressing dietary intakes of macronutrients as a percentage of energy vs. absolute amounts. Diets used in this study were designed to provide protein at 0.8 and 1.6 g/(kg · d) based on specific targets for dietary leucine (10,11). At baseline, subjects in the CHO groups had a mean protein intake of 78.0 g/d or 0.89 g/(kg · d). With an energy intake of 8.23 MJ/d, protein accounted for 15.8% of energy intake. During weight loss, using the teaching model of the Food Guide Pyramid (26), subjects reduced total energy intake emphasizing reduced total lipids and SFA. This energy restriction produced a decrease in protein intake to 57 g/d or 0.71 g/(kg · d). Although this level of protein represents 17% of the reduced energy intake (5.51 MJ/d) it is below the minimum RDA value of 0.8 g/(kg · d) (24). This may explain in part the greater loss of lean mass associated with the CHO groups.

A similar concern about the percentage of energy vs. absolute amounts exists for dietary lipids. Subjects in the PRO groups reduced dietary intake of total lipids by 30.1 g/d and SFA by 7.5 g/d. However, expressed as a percentage of energy, total lipids account for 31.9% of energy intake and SFA account for 12.4% of total energy. Hence, expressing dietary intakes as a percentage of energy intakes would result in the conclusion that subjects in the PRO groups have dietary fat intakes above current guidelines of the AHA (25). Contrary to this conclusion, the PRO groups significantly reduced consumption of total lipids and SFA below baseline values and below national averages (46).

In summary, subjects in both diet treatments were successful in reducing daily energy intakes, achieving the macronutrient goals of the respective diets, and reducing body weight and body fat mass. Subjects consuming the PRO diet with a CHO:PRO ratio < 1.5 lost more total weight and body fat mass and tended to lose less lean mass compared with the groups consuming the CHO diet with a CHO:PRO ratio > 3.5. Exercise increased loss of body fat and preserved lean mass. The combined effects of diet and exercise appear to be independent and additive for enhancing body composition expressed as the absolute or change in the percentage of body fat. This study adds to the increasing body of evidence supporting protein-sparing effects derived from maintaining higher protein intakes during energy restriction (6,8,10,13,14).

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