

# A Moderate-Protein Diet Produces Sustained Weight Loss and Long-Term Changes in Body Composition and Blood Lipids in Obese Adults<sup>1,2</sup>

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## Abstract

Diets with increased protein and reduced carbohydrates (PRO) are effective for weight loss, but the long-term effect on maintenance is unknown. This study compared changes in body weight and composition and blood lipids after short-term weight loss (4 mo) followed by weight maintenance (8 mo) using moderate PRO or conventional high-carbohydrate (CHO) diets. Participants (age =  $45.4 \pm 1.2$  y; BMI =  $32.6 \pm 0.8$  kg/m<sup>2</sup>;  $n = 130$ ) were randomized to 2 energy-restricted diets ( $-500$  kcal/d or  $-2093$  kJ/d): PRO with  $1.6$  g·kg<sup>-1</sup>·d<sup>-1</sup> protein and  $<170$  g/d carbohydrates or CHO with  $0.8$  g·kg<sup>-1</sup>·d<sup>-1</sup> protein,  $>220$  g/d carbohydrates. At 4 mo, the PRO group had lost 22% more fat mass (FM) ( $-5.6 \pm 0.4$  kg) than the CHO group ( $-4.6 \pm 0.3$  kg) but weight loss did not differ between groups ( $-8.2 \pm 0.5$  kg vs.  $-7.0 \pm 0.5$  kg;  $P = 0.10$ ). At 12 mo, the PRO group had more participants complete the study (64 vs. 45%,  $P < 0.05$ ) with greater improvement in body composition; however, weight loss did not differ between groups ( $-10.4 \pm 1.2$  kg vs.  $-8.4 \pm 0.9$  kg;  $P = 0.18$ ). Using a compliance criterion of participants attaining  $>10\%$  weight loss, the PRO group had more participants (31 vs. 21%) lose more weight ( $-16.5 \pm 1.5$  vs.  $-12.3 \pm 0.9$  kg;  $P < 0.01$ ) and FM ( $-11.7 \pm 1.0$  vs.  $-7.9 \pm 0.7$  kg;  $P < 0.01$ ) than the CHO group. The CHO diet reduced serum cholesterol and LDL cholesterol compared with PRO ( $P < 0.01$ ) at 4 mo, but the effect did not remain at 12 mo. PRO had sustained favorable effects on serum triacylglycerol (TAG), HDL cholesterol (HDL-C), and TAG:HDL-C compared with CHO at 4 and 12 mo ( $P < 0.01$ ). The PRO diet was more effective for FM loss and body composition improvement during initial weight loss and long-term maintenance and produced sustained reductions in TAG and increases in HDL-C compared with the CHO diet. J. Nutr. 139: 514–521, 2009.

## Introduction

Obesity is a major public health crisis in the United States. Diet strategies for successful long-term weight loss and maintenance remain relatively untested. High-carbohydrate, low-protein, low-fat (CHO)<sup>6</sup> diets are often recommended for weight management (1–3). However, recent studies have shown that diets with increased protein and reduced carbohydrates (PRO) are often

more effective, at least for short-term weight loss (4,5). PRO diets with protein  $>1.4$  g·kg<sup>-1</sup>·d<sup>-1</sup> and carbohydrates  $<150$  g/d tend to increase weight and fat loss and reduce lean mass loss compared with CHO diets (6–8). Although PRO diets appear beneficial during periods of short-term weight loss, long-term compliance and effect on body weight and composition are unknown (9).

Most studies evaluating PRO diets have lasted  $<4$  mo and examined fewer than 40 participants (4). Longer studies report that greater initial weight losses associated with PRO diets are not maintained at 12 mo, largely due to poor long-term compliance (9–12). However, few long-term studies exist and these studies usually lack continuing nutrition education and diet monitoring, a fact that provided the impetus for the current study (6,10–18).

We previously completed 2 clinical trials comparing the effects of PRO and CHO diets designed within Dietary Reference Intake (DRI) guidelines for macronutrients (6) to produce short-term weight loss (8,16,17). The first study was a 10-wk

<sup>1</sup> Supported by the National Cattlemen's Beef Association, Beef Checkoff, and Kraft Foods (Principal Investigator: D. K. Layman).

<sup>2</sup> Author disclosures: D. K. Layman serves on Speakers Bureau for National Cattlemen's Beef Association. E. M. Evans, D. Erickson, J. Seyler, J. Weber, D. Bagshaw, A. Griel, T. Psota, and P. Kris-Etherton, no conflicts of interest.

<sup>6</sup> Abbreviations used: AMDR, Acceptable Macronutrient Distribution Range; apoB, apolipoprotein B; CHO, high-carbohydrate, low-protein, low-fat diet; DRI, Dietary Reference Intake; %Fat, relative body fatness; FM, fat mass; ITT, intention-to-treat; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PRO, diet with increased protein and reduced carbohydrate; RDA, Recommended Daily Allowance; TAG, triacylglycerol; TC, total cholesterol.

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highly controlled feeding study designed to minimize behavioral aspects of diet compliance. Participants were assigned diets with defined meal composition with a required 14-d menu rotation and ~60% of meals prepared at our Food Research Center (8,16). Participants also completed weekly 3-d weighed food records. The PRO diet resulted in increased fat loss, greater improvement in body composition (greater reduction in fat mass (FM) and attenuated loss of lean mass), reduced triacylglycerides (TAG), and increased HDL cholesterol (HDL-C) (8,16). The 2nd study was a 16-wk evaluation of the same diets with or without exercise (17). Participants were required to use the same meals tested in study 1 but were allowed free choice for daily meal selection based on personal diet preferences. Participants were required to complete weekly 3-d weighed food records and ~20% of meals were consumed at the Food Research Center. The primary outcomes of increased fat loss, attenuated loss of lean mass, reduced TAG, and increased HDL-C were consistent with study 1 (17). Exercise further increased fat loss and attenuated loss of lean mass with greater effects with the PRO diet (17).

In this study, our goal was to extend our previous research with a large multi-center trial to examine compliance and long-term changes in body composition and blood lipids in response to energy-restricted PRO and CHO diets. This study addresses the important question of how free-living participants will implement these diets and if short-term changes (4 mo) in body composition and blood lipids associated with active weight loss are maintained at 12 mo. This study is unique in its use of a comprehensive nutrition education program accompanied by a high degree of nutrition monitoring throughout the 12 mo. Participant compliance was evaluated for participants who completed the 12-mo protocol (completers) with participation in weekly meetings that included monitoring body weight, weighed food records, and diet instruction and participants who completed the study protocol and also achieved a minimum of 10% weight loss. The first criterion focused on compliance with the prescribed macronutrient regimens and participation in weekly education meetings and the 2nd criterion focused on adherence to the macronutrient regimens and a prescribed energy deficit. We anticipated that regardless of the compliance definition, the PRO diet would invoke more favorable body composition and blood lipid changes both after active weight loss and through the 12-mo weight loss and maintenance periods.

## Participants and Methods

This study was a 12-mo, multi-center weight loss trial (4 mo active weight loss; 8 mo weight maintenance) with participants randomized after stratifying for BMI, gender, age, and total cholesterol (TC). Diet treatments consisted of either a low carbohydrate:protein ratio (PRO group: ~40% of energy from carbohydrates, 30% protein, and 30% fat) or a high carbohydrate:protein ratio (CHO group: 55% carbohydrates, 15% protein, and 30% fat). Percentages provide a general description of the diet; however, dietary protein was defined in proportion to body weight and not energy intake (see "Diet treatments" below).

**Participants.** One-hundred thirty men ( $n = 58$ ) and women ( $n = 72$ ) aged 40–56 y were recruited to participate in the weight loss study. Exclusion criteria were BMI  $< 26 \text{ kg/m}^2$ ; body weight  $> 140 \text{ kg}$  (due to dual X-ray absorptivity scanning bed constraints); smoking; any existing medical conditions requiring medications that affected primary or secondary outcomes of the study, i.e. lipid-lowering medications; use of oral steroids; or use of antidepressant medication. This study was approved by the Institutional Review Boards at the University of Illinois at Urbana-Champaign and The Pennsylvania State University. Parti-

cipants provided written informed consent prior to participation in the study.

Thirty-one individuals screened for participation ( $n = 182$ ) did not meet the exclusion criteria and 21 declined to participate. Eligible individuals ( $n = 130$ ) were randomized to treatment groups (CHO,  $n = 66$ ; PRO,  $n = 64$ ). Prior to the 4-mo data collection, 15 participants withdrew from the CHO group (9 stated personal reasons and 6 stated unable to comply with diet protocol) and 12 participants withdrew from the PRO group (11 stated personal reasons and 1 stated inability to comply with diet protocol). Between the 4- and 12-mo data collection points, 21 participants withdrew from the CHO group (16 stated personal reasons and 5 stated unable to comply with diet protocol) and 11 participants withdrew from the PRO group (8 stated personal reasons and 1 stated unable to comply with diet protocol).

All participants participated in a baseline evaluation period that included 2 3-d weighed food records during separate weeks and measurements of height, weight, and blood lipids. This evaluation period from first contact with the participants was 10–20 d and served as an initial control period for each participant. During the baseline period, participants were instructed to maintain stable body weight and to consume a diet similar to the past 6 mo. After the baseline period, participants reported to the nutrition research laboratory at 0630 after a 12-h overnight fast for measurements of body weight, body composition, and blood sampling (time 0 = baseline).

**Diet treatments.** The PRO diet provided dietary protein at  $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (~30% of energy intake) with a carbohydrate:protein ratio  $< 1.5$  and dietary lipids ~30% energy intake. The CHO diet provided dietary protein equal to  $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (~15% of energy intake) with a carbohydrate:protein ratio  $> 3.2$  and total fat ~30% of energy intake. These diets were designed to fall within the Acceptable Macronutrient Distribution Range (AMDR) of the DRI as established by the Institute of Medicine (15) with minimum Recommended Daily Allowance (RDA) intakes for carbohydrates  $> 130 \text{ g/d}$  and protein  $> 0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  and with upper ranges for carbohydrates  $< 65\%$  and protein  $< 35\%$  of total energy intake. The 2 diets were formulated to be equal in energy [7.10 MJ/d (1700 kcal/d for females; 7.94 MJ/d (1900 kcal) for males], total fat intake (30% of energy), and fiber (17 g/4.18 MJ). Each diet group received a menu plan with meals for each day meeting established nutritional requirements (15) and dietary fat guidelines (18). Diet differences between groups were designed to reflect direct substitution of foods in the protein groups (meats, dairy, eggs, and nuts) for foods with high-carbohydrate content (breads, rice, cereals, pasta, and potatoes). The education guidelines for the CHO group followed the USDA Food Guide Pyramid (19) and emphasized restricting dietary fat and cholesterol with use of whole-grain breads, rice, cereals, and pasta. For the PRO group, the education guidelines emphasized use of high-quality, low-fat proteins including lean meats, reduced-fat dairy, and eggs or egg substitutes. Both diets included 5 vegetable servings/d and 2–3 fruit servings/d.

**Education program, participant monitoring, and compliance.** Participants were provided with electronic food scales and were instructed to weigh all food servings at all meals. Participants were required to report 2 3-d weighed food records during the baseline period prior to assignment to diet groups. Nutrient intakes were evaluated as mean daily intakes from the 3-d weighed records using Nutritionist Pro software (First DataBank). After baseline data collection, participants received specific diet program instructions from a research dietitian, including the menus, food substitutions, and portion sizes. Throughout the 12-mo study, participants 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 information, answered questions, and reviewed diet records for treatment compliance. Each week, participants were weighed in light clothing without shoes and submitted 3-d weighed food records, which were reviewed each week with feedback to participants about diet compliance. Any participant with an unexcused absence at a weekly meeting was called by a staff dietitian to promote attendance and any participant with 3 consecutive absences was asked to define their participation status in the study. All participants completing the 12-mo

data collection (completers) participated in >75% of meetings, including weigh-ins and diet records. Compliance with dietary protocols was also monitored with plasma TAG at 0, 4, and 12 mo as a marker of carbohydrate intake (20) and 24-h urinary urea at 0, 4, and 8 mo as a marker of protein intake (8).

The exercise guidelines emphasized physical activity lifestyle recommendations based on the NIH Guidelines for Weight Management (21). These guidelines recommend a minimum of 30 min of walking 5 d/wk; however, participation was voluntary. Physical activity was monitored using daily activity logs and armband accelerometers (BodyMedia) worn 3 d/mo. Activity logs were collected each week. Based on these measurements, participants averaged just under 100 min/wk of added exercise and daily activity levels did not differ between the diet treatment groups ( $P > 0.05$ ).

**Body composition and blood measurements.** After 4 and 12 mo, participants reported to the nutrition research laboratory at 0630 after a 12-h overnight fast for measurements of body weight, body composition, and blood sampling. Body weight was measured using an electronic scale (Tanita, Model BWB-627A). Height was measured using a stadiometer. Body composition was determined by dual energy X-ray absorptiometry (Illinois: Hologic QDR 4500A, software version 11.1.3; Penn State: Hologic QDR 4500W, software version 12.5) and scans for a given individual were analyzed by the same technician using standard manufacturer guidelines. The CV for dual energy X-ray absorptiometry outcomes of interest were 1.0–2.0%.

Serum samples were stored at  $-80^{\circ}\text{C}$  prior to measurements. Serum TC, HDL-C, TAG, and apolipoprotein B (apoB) were determined using standardized methods (22) by the Washington University School of Medicine Core Laboratory for Clinical Studies (St. Louis, MO). LDL cholesterol (LDL-C) was calculated using the Friedewald equation (23). Apolipoprotein B was determined for Illinois participants only.

**Data analyses and statistics.** All data analyses were conducted using SPSS version 14.0. Differences among groups at baseline were evaluated using a  $t$  test. The primary outcome variable was FM, with secondary weight-related outcomes being defined as absolute and relative weight, FM, and fat-free mass. FM change at 12 mo was used to determine statistical power calculations and required sample size. Previous work in our laboratory (17) determined a calculated effect size for FM changes between PRO and CHO was  $\sim 1.0$  (Cohen's  $d$ ) in response to 16 wk of weight loss. Assuming that this effect size would be sustained in response to an additional 8 mo of maintenance, and using an  $\alpha$  (significance) level of 0.05 and a power of 90%, a sample size of  $\sim 25$  participants per group would be required to find differences in FM change between the PRO and CHO groups should it exist at this magnitude. Given an estimated retention rate of 50% based on previous weight loss studies of this length, we planned to recruit 130 individuals into the study. Secondary outcomes included changes in TC, LDL-C, HDL-C, TAG, and apoB.

The primary analysis utilized repeated-measures ANOVA to evaluate separately the relative effect of the diet treatments on weight loss at 4 mo and weight maintenance at 12 mo. Long-term efficacy of the dietary treatments was analyzed using 3 approaches. First, an intention-to-treat

(ITT) analysis was conducted using the last value carried forward approach with participants who had completed the active weight loss phase (4-mo time point). Second, an analysis was conducted on all individuals completing the protocol regardless of weight loss success (completers). A 3rd analysis utilized data from participants maintaining  $\geq 10\%$  weight loss at 12 mo. The degree of weight loss was defined based on work by Wing and Hill (24) that suggested maintenance of 10% loss of initial weight for a minimum of 12 mo is an indication of success. This criterion supports diet compliance with prescribed energy deficit. A chi-square analysis on protocol completion rates was used to determine differences in treatment efficacy. To assess the independent effects of dietary treatment on serum lipids independent of fat loss, an ANCOVA was used controlling for FM change. Note that due to nonnormal distribution of TAG values, a log transformation was performed for statistical analyses; however to enhance data interpretation, nontransformed values are presented in the text and figure. All other variables were normally distributed. Percentage change was calculated as  $\{[(\text{posttest}) - (\text{pretest})]/(\text{pretest})\} \times 100\}$ .  $P < 0.05$  was considered significant. All values are presented as means  $\pm$  SEM.

Results

**Participants.** Randomization after stratifying participants for gender, age, BMI, and TC was successful with no significant differences between treatment groups in baseline characteristics (Table 1). Age of the participants was  $45.4 \pm 1.2$  y and BMI was  $32.6 \pm 0.8$  kg/m<sup>2</sup>. As expected, men were taller, heavier, had less FM, and more lean mass than women ( $P < 0.05$ ); however, there was no significant gender-by-group interactions for any baseline characteristics.

**Compliance with dietary protocols.** Compliance with macronutrient goals was monitored with weekly 3-d weighed food records, 24-h urinary urea, and plasma TAG concentrations. Compliance with the energy deficit goal was also evaluated for participants with a minimum 10% weight loss at 12 mo. The 3-d weighed food records were reviewed each week with detailed analyses provided at 4 and 12 mo (Table 2).

Mean energy and protein intakes were  $\sim 14\%$  higher in the PRO group at baseline, whereas carbohydrate intakes did not differ between the groups. Both groups had carbohydrate:protein ratios of  $\sim 3$ . Energy intake did not differ between the groups during the study with data presented for 4 and 12 mo (Table 2). Differences in protein and carbohydrate intakes were consistent with treatment goals throughout the study. Participants in the PRO group had protein intakes of 115 g/d or  $\sim 29\%$  of energy intake during the active weight loss period (4 mo). The ratio of carbohydrate:protein was  $\sim 1.4$ . At 4 mo, total dietary fat intake was 58 g/d (32% of dietary energy) and SFA was

TABLE 1 Baseline characteristics of participants<sup>1</sup>

	PRO group			CHO group		
	Male	Female	All	Male	Female	All
<i>n</i>	28	36	64	31	35	66
Age, y	46.0 $\pm$ 1.9	44.5 $\pm$ 1.7	45.2 $\pm$ 1.2	46.5 $\pm$ 1.5	45.6 $\pm$ 1.2	46.0 $\pm$ 1.0
Height, cm	69.7 $\pm$ 0.5	63.9 $\pm$ 0.5	66.5 $\pm$ 0.5	69.1 $\pm$ 0.6	64.6 $\pm$ 0.4	66.7 $\pm$ 0.43
Weight, kg	100.2 $\pm$ 3.1	85.1 $\pm$ 2.0	91.7 $\pm$ 2.0	100.1 $\pm$ 2.0	88.3 $\pm$ 2.0	93.8 $\pm$ 1.6
BMI, kg/m <sup>2</sup>	31.8 $\pm$ 0.8	32.4 $\pm$ 0.8	32.2 $\pm$ 0.5	32.6 $\pm$ 0.7	32.8 $\pm$ 0.7	32.7 $\pm$ 0.5
FM, kg	28.7 $\pm$ 1.5	34.6 $\pm$ 1.2	32.0 $\pm$ 1.0	30.5 $\pm$ 0.9	36.8 $\pm$ 1.3	33.8 $\pm$ 0.9
Lean mass, kg	68.6 $\pm$ 2.0	48.3 $\pm$ 1.0	57.2 $\pm$ 1.6	67.4 $\pm$ 1.5	49.2 $\pm$ 1.1	57.8 $\pm$ 1.4

<sup>1</sup> Values are means  $\pm$  SE. No significant group  $\times$  gender effects exist for baseline characteristics.

**TABLE 2** Dietary intakes at baseline and during the intervention while consuming reduced-energy diets with a carbohydrate:protein ratio <1.5 (PRO group) or 3.2 (CHO group)<sup>1</sup>

	PRO group	CHO group	P-value <sup>2</sup>
Energy, MJ/d			
Baseline	10.06 ± 0.38	8.78 ± 0.38	0.01
4 mo	6.73 ± 0.23	6.20 ± 0.24	NS
12 mo	7.18 ± 0.28	6.80 ± 0.35	NS
Protein, g/d			
Baseline	97.1 ± 4.5	83.3 ± 4.6	0.04
4 mo	115.3 ± 3.2	68.6 ± 3.3	<0.001
12 mo	116.2 ± 4.4	70.2 ± 5.5	<0.001
Carbohydrate, g/d			
Baseline	294.2 ± 12.9	263.3 ± 13.2	NS
4 mo	162.3 ± 7.2	219.3 ± 7.4	<0.001
12 mo	168.3 ± 10.0	231.7 ± 16.3	0.001
Total fat, g/d			
Baseline	93.6 ± 4.4	76.7 ± 4.5	0.004
4 mo	58.2 ± 2.1	42.4 ± 2.2	NS
12 mo	66.8 ± 3.0	50.8 ± 3.7	NS
Saturated fat, g/d			
Baseline	32.1 ± 1.6	25.4 ± 1.7	0.003
4 mo	21.9 ± 0.8	13.3 ± 0.8	NS
12 mo	24.8 ± 1.1	16.2 ± 1.4	NS
Cholesterol, mg/d			
Baseline	314.1 ± 36.0	275.4 ± 36.8	NS
4 mo	332.0 ± 14.9	144.3 ± 15.2	0.007
12 mo	212.4 ± 6.1	206.1 ± 7.5	NS
Fiber, g/d			
Baseline	20.0 ± 1.1	19.2 ± 1.2	NS
4 mo	22.1 ± 1.3	23.9 ± 1.4	NS
12 mo	20.2 ± 1.5	25.1 ± 1.9	0.04

<sup>1</sup> Values are means ± SE for 3-d weighed records obtained at each time point. *n* at each time were (PRO, CHO): 64, 66 at baseline; 52, 51 at 4 mo; and 41, 30 at 12 mo.

<sup>2</sup> P-values represent *t* tests between groups at baseline or treatment effect compared with baseline. NS, *P* > 0.05.

22 g/d (12% of energy). Participants in the CHO group had a carbohydrate intake of 219 g/d (59% of energy), total fat intake of 42 g/d (26% of energy), and SFA intake of 13 g/d (8% of energy). Protein intake in the CHO group was 69 g/d (18% of energy intake) and the carbohydrate:protein ratio was 3.2. Energy, protein, and carbohydrate intakes remained consistent with treatment goals for both groups throughout the weight maintenance period.

Compliance with the diet protocols was supported further with biomarkers of urinary urea (measured at 0, 4, and 8 mo) and serum TAG (measured at 0, 4, and 12 mo). At baseline, 24-h urinary urea did not differ between treatment groups (381 ± 13 and 392 ± 12 mmol/d for PRO and CHO, respectively). At 4 and 8 mo, urea excretion by the PRO group (489 ± 13 and 502 ± 27 mmol/d, respectively) was higher (*P* < 0.05) than for the CHO group (252 ± 22 and 283 ± 15 mmol/d, respectively) and in proportion to dietary records. Likewise, TAG did not differ between groups at baseline (1.68 ± 0.80 and 1.63 ± 0.11 mmol/L for PRO and CHO, respectively) but responded at 4 and 12 mo consistent with the changes in carbohydrates reported in dietary records (4 mo, 1.09 ± 0.05 vs. 1.42 ± 0.08 mmol/L, *P* < 0.05; 12 mo, 1.18 ± 0.09 vs. 1.36 ± 0.09 mmol/L, *P* < 0.05 for PRO vs. CHO, respectively).

Total fat and SFA intakes were lower in the CHO group throughout the study largely due to lower baseline values (Table

2). There were no treatment differences for total fat or SFA between groups (i.e. the decreases in total fat and SFA from baseline to 4 mo did not differ between the groups). Cholesterol intake was higher in the PRO group. Fiber intakes achieved dietary guidelines of 17 g/1000 kcal for both groups (15). During maintenance, the CHO group consumed more dietary fiber than the PRO group.

**Weight and body composition.** Weight loss and compliance did not differ between the groups at 4 mo (Table 3), but the PRO group had a 22% greater loss of body fat (*P* < 0.04) compared with the CHO group (Table 4). Groups did not differ in lean mass loss.

At 12 mo, the primary difference between treatments was that the PRO group had greater loss of FM (Table 4) and improvement in body composition as reflected by relative body fatness (%Fat; Fig. 1). Participants in the PRO group had 35 or 38% greater loss of FM than the CHO group analyzed by ITT (*P* < 0.05) or completers (*P* = 0.06), respectively. Changes in lean mass did not differ between the diet groups, resulting in greater net changes in the proportion of fat to lean mass, i.e. %Fat (Fig. 2). Although both diet treatments reduced FM, the PRO diet produced greater body composition improvement as reflected by %Fat, which indirectly accounts for both fat and lean mass changes.

Assessing long-term weight loss using ITT or completers, weight loss did not differ between treatments at 12 mo. However, the PRO group had a greater number of participants (64%) complete the 12-mo study than the CHO group (45%; *P* < 0.05). For completers, the mean weight loss was 23% greater for the PRO group, but individual weight loss ranged from 0.6 to 30.8 kg within the PRO group and 1.7 to 23.2 kg for the CHO group, suggesting large differences in participant compliance with energy intake targets within each treatment group. Controlling for gender or site did not alter the effect of dietary treatment on either weight or body composition outcomes.

With obvious differences in participant compliance with energy goals of the respective diets, we also utilized a predefined goal of 10% loss of initial body weight as a compliance criterion that assured negative energy balance. Selecting participants who achieved ≥10% loss of initial body weight, 31% of the PRO

**TABLE 3** Absolute and relative body weight changes in response to initial weight loss or long-term weight loss maintenance in response to diets with a carbohydrate:protein ratio < 1.5 (PRO group) or >3.2 (CHO group)<sup>1</sup>

	PRO group	CHO group	PRO group	CHO group
	kg		% of baseline	
Initial loss (4 mo)	-8.2 ± 0.5	-7.0 ± 0.5	-8.7 ± 0.5	-7.6 ± 0.5
<i>n</i>	52	51		
Long term (12 mo)				
ITT <sup>2</sup>	-9.3 ± 1.0	-7.4 ± 0.6	-9.6 ± 0.9	-8.0 ± 0.7
<i>n</i>	52	51		
Completers	-10.4 ± 1.2	-8.4 ± 0.9	-10.7 ± 1.1	-9.1 ± 0.9
<i>n</i>	41	30		
>10% weight loss	-16.5 ± 1.5 <sup>a</sup>	-12.3 ± 0.9	-16.6 ± 0.9 <sup>a</sup>	-13.5 ± 0.9
<i>n</i>	20	14		

<sup>1</sup> Values are means ± SEM. Sample sizes are listed below values. <sup>a</sup>Indicates significant treatment effect compared with baseline, *P* < 0.05.

<sup>2</sup> ITT analyses using last value carried forward for all individuals who completed the 4-mo time point.

**TABLE 4** Fat and lean mass changes in response to initial weight loss or long-term weight loss maintenance in response to diets with a carbohydrate:protein ratio < 1.5 (PRO group) or >3.2 (CHO group)<sup>1</sup>

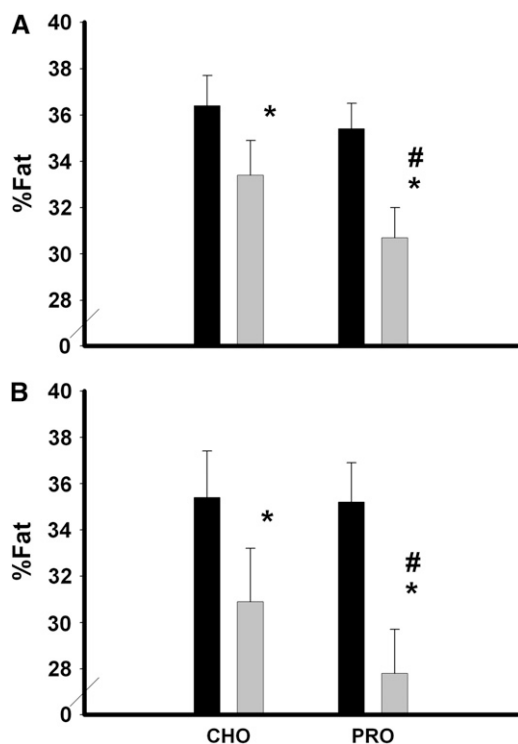
	FM		Lean mass	
	PRO group	CHO group	PRO group	CHO group
<i>kg</i>				
Initial (4 mo)	-5.6 ± 0.4*	-4.6 ± 0.3	-2.6 ± 0.2	-2.4 ± 0.2
<i>n</i>	52	51		
Long term (12 mo)				
ITT <sup>2</sup>	6.6 ± 0.7*	4.9 ± 0.4	-2.4 ± 0.3	-2.2 ± 0.3
<i>n</i>	52	51		
Completers	7.3 ± 0.9	5.3 ± 0.6	-2.6 ± 0.4	-2.7 ± 0.4
<i>n</i>	41	30		
>10% weight loss	11.7 ± 1.0*	7.9 ± 0.7	-4.3 ± 0.6	-4.2 ± 0.5
<i>n</i>	20	14		

<sup>1</sup> Values are means ± SEM. \*Treatment effect compared with baseline,  $P < 0.05$ .

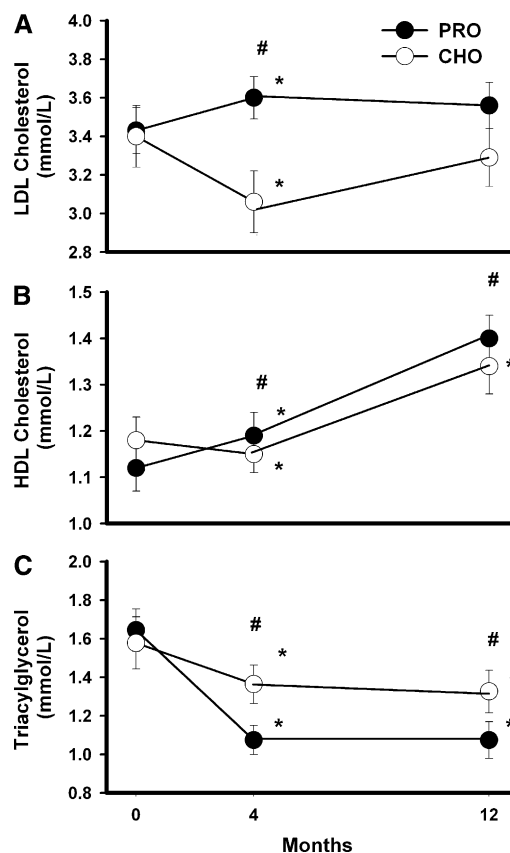
<sup>2</sup> ITT analysis using last value carried forward including data for individuals who completed the 4-mo time point.

group had lower body weight by at least 10% with a mean loss of  $16.5 \pm 1.5$  kg compared with the CHO group, with 21% of the participants achieving the 10% goal with a mean weight loss of  $12.3 \pm 0.9$  kg ( $P < 0.05$ ). Participants in the PRO group also lost 48% more FM than participants in the CHO group ( $P = 0.001$ ; Table 4).

**Serum lipids.** Blood lipids and lipoproteins did not differ at baseline between the treatment groups (Fig. 2). Participants in



**FIGURE 1** %Fat at baseline and 12 mo in individuals who completed the 12-mo PRO ( $n = 41$ ) and CHO ( $n = 30$ ) treatments regardless of compliance (A) and individuals who successfully maintained a 10% weight loss at 12 mo (PRO,  $n = 20$ ; CHO,  $n = 14$ ) (B). \*Change from baseline within group,  $P < 0.05$ ; #treatment effect compared with baseline,  $P < 0.05$ .



**FIGURE 2** Changes in serum LDL-C (A), HDL-C (B), and TAG (C) during initial weight loss and maintenance phase in individuals who completed the 12-mo intervention (PRO,  $n = 41$ ; CHO,  $n = 30$ ) \*Change from baseline within group,  $P < 0.05$ ; #treatment effect compared with baseline,  $P < 0.05$ .

both treatment groups completing the initial weight loss period (4-mo time point) improved lipoprotein profiles, but the changes differed between treatments. Specifically, the CHO group had lower TC and LDL-C concentrations compared with the PRO group ( $P < 0.01$ ), whereas the PRO group had greater HDL-C and lower TAG and TAG:HDL-C ( $-0.30 \pm 0.10$  vs.  $-0.18 \pm 0.09$ ) compared with the CHO group ( $P < 0.01$ ). The treatment effects on serum lipids were unchanged when loss of FM was included as a covariate (ANCOVA: LDL-C,  $P < 0.004$ ; HDL-C,  $P < 0.015$ ; TAG,  $P < 0.007$ ). apoB concentrations did not differ between the treatment groups at any time (data not shown).

The time courses for short-term (i.e. active weight loss) and longer-term (i.e. weight maintenance) changes in LDL-C, HDL-C, and TAG for completers are presented (Fig. 2). LDL-C had an initial decrease in the CHO group at 4 mo during weight loss, with a return to baseline levels during weight maintenance with no sustained treatment difference. Changes in TC mirrored LDL-C values (data not shown). During the maintenance period, the increase in HDL-C concentration in the PRO group ( $0.26 \pm 0.03$  mmol/L) was greater than that for the CHO group ( $0.15 \pm 0.03$  mmol/L;  $P = 0.025$ ). Similarly, TAG concentrations were lower in both treatment groups at 12 mo, with the PRO group having a greater reduction and maintaining the treatment difference ( $P = 0.049$ ). Although both groups improved, the PRO group had a more favorable change in TC:HDL-C ( $-0.89 \pm 0.14$  vs.  $-0.50 \pm 0.12$ ;  $P = 0.044$ ) and TAG:HDL-C ( $-0.94 \pm 0.22$  vs.  $-0.38 \pm 0.13$ ;  $P = 0.016$ ) at 12 mo.

## Discussion

During periods of active weight loss, moderate PRO diets have been reported to produce greater weight loss, increase loss of FM, minimize loss of lean mass, and improve dyslipidemia (lower TAG and increased HDL-C) compared with commonly recommended CHO diets (4). Improvements in body composition and TAG with PRO diets are the most consistent findings (4). The current study extends the findings of previous short-term studies and demonstrates that the PRO diet produces long-term improvements in body composition due to reduced FM and attenuated loss of lean tissue, sustained benefits for serum TAG and HDL-C, and greater participant compliance.

This study evaluated primary outcomes of changes in FM and lean mass using 3 levels of compliance: 1) an ITT analysis; 2) all participants completing measurements at 12 mo (completers); and 3) a predefined compliance criterion of  $\geq 10\%$  weight loss. The ITT analysis utilized all participants completing the initial 4 mo weight loss with values brought forward. This analysis emphasizes outcomes from early weight loss and is the least discriminatory for diet compliance. Participants designated as completers maintained weekly attendance at diet meetings and completed all measurements at 12 mo. They also maintained high compliance with the dietary ratios for carbohydrates and protein, even though some participants did not achieve weight loss goals. Participants achieving the 10% weight loss criterion maintained high compliance with the dietary ratios for carbohydrates and protein plus compliance with energy deficit goals (i.e. weight loss). Across all 3 analyses, the PRO group averaged  $\sim 21\%$  greater weight loss and 27% greater fat loss than the CHO group. Further, irrespective of the amount of weight lost, participants in the PRO group obtained greater improvements in body composition as reflected by greater FM loss and attenuated relative lean mass loss.

In addition to the beneficial effects of the PRO diet for changes in body composition, more participants in the PRO group completed the study (64%) than in the CHO group (45%) and attained  $\geq 10\%$  weight loss (31 vs. 21%, respectively). These findings demonstrate greater compliance with a moderate-PRO diet designed within the DRI guidelines for macronutrients for long-term weight management than the CHO diets often advocated for weight loss (1–3).

Multiple mechanisms have been reported to explain increased loss of body weight and body fat with higher protein diets. Higher protein diets appear to increase satiety (6,25,26), increase energy expenditure (27–29), and/or maintain lean tissue with higher metabolic activity (8,17,30). Findings from this study support reduced food intake and improved body composition. Daily energy deficits, although not different between groups, were similar to theoretical predictions for weight loss (31,32). Schoeller and Buchholz (31) reviewed 9 studies of short-term weight loss that included data for energy balance and concluded that a primary cause of increased weight loss was reduced energy intake. They calculated that the higher weight loss with higher-protein diets was derived from reducing food intake by  $\sim 233$  kcal/d (975 kJ/d). The difference between groups in the current study was 194 kcal/d (812 kJ/d), which is consistent with the weight difference of 1.2 kg at 4 mo (31).

Improvements in body composition, including reducing body fat and maintenance of lean tissue, are critical for prevention of weight regain (30) and long-term health status (33). Evidence is accumulating that the RDA for protein is inadequate to maintain muscle mass in adults during aging (34,35), with a physically inactive lifestyle (36,37), or during energy restriction for weight

loss (17,38). The current RDA for protein represents the minimum protein needs for healthy young adults with adequate energy intakes (33,38,39). During weight loss, energy restriction increases the protein needed to maintain muscle mass (38,40) and protein needs expressed as percentage of the reduced energy intake nearly double (17,38,41). In the present study, participants in the PRO group achieving  $\geq 10\%$  weight loss had a greater reduction in %Fat (Fig. 1B) due to greater loss of FM in proportion to changes in lean mass. These findings reflect greater partitioning of the weight loss to body FM in the PRO group.

The secondary objective of this study was to evaluate changes in serum lipids and lipoproteins during active weight loss and subsequent long-term weight maintenance. After 4 mo of active weight loss, the CHO group had significant reductions in TC, LDL-C, and TAG. These findings are consistent with predicted changes associated with weight loss and reduced dietary intakes of total fat, SFA, and cholesterol (42–45). As expected, the PRO group had a higher daily consumption of cholesterol (272 vs. 175 mg/d;  $P < 0.01$ ) and SFA ( $23.3 \pm 1.0$  vs.  $14.7 \pm 1.2$  g/d;  $P < 0.01$ ). However, diet treatment effects on SFA intake did not differ between the groups, because both groups had lower SFA ( $-8.8$  vs.  $-10.7$  g/d, respectively) than at baseline.

At 12 mo, TC and LDL-C no longer differed from baseline regardless of treatment. Initial changes in TC and LDL-C associated with diet changes and weight loss at 4 mo were not sustained during long-term weight maintenance. These findings for LDL-C are similar to previous reports documenting that diet-induced changes in LDL-C are short-term and transient, with LDL-C concentrations returning to baseline (44,45). During maintenance, HDL-C increased and TAG decreased in the CHO group.

The PRO diet also improved blood lipids but with greater effects on the characteristics of atherogenic dyslipidemia (41–46). After 4 mo, the PRO diet resulted in lower TAG, increased HDL-C, and improved TC:HDL-C and TAG:HDL-C ratios. The significant changes in TAG have been attributed to reductions of dietary carbohydrate (20,42–44). These changes continued at 12 mo, increasing net differences from baseline and maintaining differences between treatment groups. These changes in blood lipids represent important findings for long-term prospective studies with free-living participants that investigate the use of PRO diets for treatment or prevention of metabolic syndrome (20,43,46,47) or type 2 diabetes (7,20,48).

This study is unique in its level of nutrition education and diet monitoring. Previous studies provided instruction about the use of a specific diet protocol during the initial period of weight loss but provided little or no additional instruction or monitoring during the maintenance period (6,12,14–16). In this study, participants in both treatment groups met every week with study dietitians who monitored weight change, reviewed 3-d weighed food records, and provided individual feedback about diet compliance and weight changes. Weekly meetings also included a structured education program addressing nutrition goals, menu development, and food selection. With this level of instruction and monitoring, the prescribed macronutrient ratios were maintained (i.e. lower carbohydrates and greater protein), producing sustained changes in body composition and plasma TAG and HDL-C. Further, the 10% weight loss criterion, which assured compliance with both macronutrient and energy deficit goals, produced sustained changes in body weight and FM as well as body composition and blood lipids.

This study demonstrates the merit of a moderate PRO diet for weight loss and long-term weight maintenance. The DRI guidelines allow for diets with diverse macronutrient ratios but

provide no guidance on how to apply the AMDR to specific health conditions or individual lifestyles. The findings of the current study demonstrate that although energy deficit is the major factor for body weight loss, the macronutrient composition affects body composition, blood lipids, and long-term compliance. Specifically, a PRO diet with protein at the upper end and carbohydrates at the lower end of the AMDR is more effective for reducing %Fat and improving dyslipidemia. Future research is needed to define specific mechanisms that explain the unique benefits of a moderate-PRO diet and to establish dietary guidelines for specific amounts or ratios of protein and carbohydrate required for individuals to achieve these benefits.

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