Published in final edited form as:

Nutr Res. 2011 February 1; 31(2): 104–112. doi:10.1016/j.nutres.2011.01.004.

Varying protein source and quantity does not significantly improve weight loss, fat loss, or satiety in reduced energy diets among midlife adults.¹

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Abstract

We hypothesized that a whey protein diet would result in greater weight loss and improved body composition compared to standard weight loss diets. Weight change, body composition, and renin angiotensin aldosterone system activity in midlife adults was compared between diet groups. Eighteen subjects enrolled in a 5 month study of 8 weeks controlled food intake followed by 12 weeks *ad libitum* intake. Subjects were randomized to one of three treatment groups: control diet (CD) (55% carbohydrate: 15% protein: 30% fat), mixed protein (MP) (40% carbohydrate: 30% protein: 30% fat), or whey protein (WP) (40% carbohydrate: 15% mixed protein: 15% whey protein: 30% fat). Measurements included weight, metabolic measures, body composition by dual energy x-ray absorptiometry (DXA), and resting energy expenditure. No statistically significant differences in total weight loss or total fat loss were observed between treatments, however, a trend toward greater total weight loss ($p = 0.08$) and total fat loss ($p = 0.09$) was observed in the WP group compared to the CD group. Fat loss in the leg and gynoid regions was greater ($p < 0.05$) in the WP group than the CD group. No RAAS mediated response was observed, but a decrease in systolic blood pressure was significantly greater ($p < 0.05$) in the WP group compared to the CD group. In summary, increased whey protein intake did not result in statistically significant differences in weight loss or in total fat loss, but significant differences in regional fat loss and in decreased blood pressure were observed in the WP group.

Keywords

adults; fat loss; weight loss; whey protein; satiety; resting energy expenditure

¹Preliminary data presented as a poster at the North American Research Conference on Complementary and Alternative Medicine, May 2009.

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1. Introduction

In the United States, there is a greater likelihood of obesity among men and women 40 – 59 years of age compared to men and women 20 – 39 years[1]. Obesity in midlife is associated with higher risk of metabolic disease[2,3]. Energy restriction diets of various compositions have been proposed to control weight gain and promote weight loss. Multiple studies have reported successful weight loss and maintenance of lean mass using energy restricted diets of moderate to high protein content ($\geq 25\%$ energy from protein)[4-6]. Generally, these diets have increased protein intake from meat, eggs, dairy, and nuts and decreased the proportion of energy from carbohydrates and fats.

In a single meal study of macronutrient intake, protein decreased postprandial appetite and subsequent energy intake compared to either carbohydrate or fat[7]. *Ad libitum* intake of high protein diets enhanced weight loss compared to diets with high carbohydrate content [8-9]. In a long term study, subjects following a moderate protein diet were more successful in maintaining significant weight loss and improved body composition after 12 months compared to subjects following an average protein diet plan [10].

Benefits associated with high protein reduced energy diets include enhanced satiety [11], increased thermogenesis [8], and improved insulin sensitivity [12,13]. These effects are thought to be associated with the branch chain amino acids (BCAA) found in complete proteins [13,14]. In addition, the BCAA leucine signals intracellular pathways regulating amino acid oxidation and gluconeogenesis [15]. Whey protein (100 grams) contains about twenty-four grams of BCAA [16], which have been associated with improved insulin sensitivity and satiety [17]. Some studies have investigated the use of whey protein in an *ad libitum* high protein weight loss diet [18,19], but to our knowledge whey protein has not been compared against other protein sources in a weight loss protocol.

Ideal weight loss results in reduction of fat mass with minimal loss of lean tissue; therefore, a better understanding of the mechanisms governing adipose activity is required. Recently, adipose metabolism has been found to be influenced by hormones previously identified in blood pressure homeostasis as the renin-angiotensin aldosterone system (RAAS) [20,21]. With weight loss, decreases in renin, angiotensinogen, angiotensin converting enzyme (ACE), and aldosterone have been observed [22]. In a study of the pharmaceutical ACE inhibitors enalapril and captopril, a slight loss of body weight was noted in the treatment groups[23-25], although the metabolic pathway is still unclear. Whether the decrease in RAAS activity is an effect of weight loss, or if the inhibition of the RAAS pathway also results in a loss of weight has not been specifically tested.

Recent studies have proposed that the RAAS system can be inhibited with bioactive peptides found in food sources. ACE inhibition by small peptide groups found in whey protein has been observed *in vitro*[26]. There have been mixed results in human clinical studies with some studies reporting an average decrease in systolic blood pressure of 7.0 mmHg [27-29] and another study showing no significant difference in blood pressure[30]. The specific mechanism of action by whey peptides has not been determined. However, it is believed to involve direct inhibition of ACE by binding with the zinc fingers of the molecule resulting in the inability of ACE to cleave angiotensin I into angiotensin II[31]. It is not clear whether a decrease in blood pressure after consuming whey protein is directly related to ACE inhibition or some other mechanism.

Whey protein may be a preferred protein choice for a weight loss diet because of its high BCAA content, the potential bioactivity with the RAAS of the adipose tissue, the potential to increase satiety, and its ease of incorporation into a diet plan. We hypothesized that a whey protein diet would result in greater weight loss and improved body

composition compared to standard weight loss diets. Using a randomized, parallel design of three diets, our objectives were to determine weight loss, body composition, insulin sensitivity, satiety, blood pressure, and RAAS activity in midlife adults who were following an energy restricted protocol to promote weight loss. Based upon the literature, increased consumption of whey protein may decrease ACE activity and may be related to maintenance of lean mass. Measures of changes in ACE activity, plasma renin activity, and aldosterone concentration were compared to changes in body composition for each diet group.

2. Methods and materials

2.1. Study design

A randomized, parallel design was used to compare the effects of diets containing varying protein quantities from different protein sources. After signing consent forms, subjects were randomized to a control diet (CD), mixed protein diet (MP), or whey protein diet (WP) (Figure 1). Subjects were directed to maintain their daily activity habits and not increase physical activity during the study.

For the first 8 weeks, all the food to be consumed was provided to the subjects. Subjects came daily to the General Clinical Research Center (GCRC) of the University of Minnesota to pick up meals. At daily visits, each subject completed a compliance questionnaire, a satiety questionnaire, and was weighed.

On completion of the 8-week feeding phase, subjects continued on their assigned diet for an additional 12-week *ad libitum* phase. Subjects were instructed by a registered dietitian on how to continue the assigned diet. Those subjects assigned to WP were given additional Designer Whey® protein powder and Pure Protein® bars. Subjects were expected to report daily intake on a secure, personal dietary analysis web program (www.nutrihand.com), which the principal investigator could evaluate for compliance. Every four weeks, each subject returned to the GCRC to be weighed and have blood drawn for fasting glucose and insulin levels. At baseline, 8, and 20 weeks, each subject came to the GCRC for evaluations, including indirect calorimetry to measure resting energy expenditure (REE), fasting glucose and insulin, RAAS activity, and a dual energy x-ray absorptiometry (DXA) scan to measure body composition. Concentrations of fasting glucose and insulin were used to calculate the homeostatic model assessment of insulin resistance (HOMA IR: = [fasting glucose (mmol/l) * fasting insulin (μU/ml)] / 22.5) [32] at these three clinical visits.

Approval of the study was obtained from the University of Minnesota Committee for the Use of Human Subjects and Research. The entire study was performed at the University of Minnesota GCRC.

2.2. Subjects

Healthy, midlife (40 – 60 yrs) adults were recruited from the Minneapolis-St. Paul metropolitan area through local posters and advertisement. Eligibility was determined by responses to a telephone interview and subjects were disqualified if they reported a current medical problem, were pregnant, or were using prescription medication. Subjects were asked about their willingness to comply with dietary treatments. Inclusion criteria included BMI 27 – 32 kg/m², no recent weight changes, a fasting blood glucose measure < 126 mg/dL, and a desire to lose weight. Of 85 individuals screened by telephone interview, 27 were eligible for further screening at the GCRC. A total of 18 participants consented to the protocol and were enrolled in the study.

2.3. Resting Energy Expenditure

At the screening visit, eligible subjects had their REE measured with the ParvoMedics True One Metabolic Monitor (Provo, UT). All REE measures were completed in a fasting state after the subject had rested in a supine position for about thirty minutes. Fifteen minutes of stable, continuous measure were used to calculate the baseline REE for each subject. [33] The baseline REE was evaluated along with the activity level reported by each subject to determine total energy needs. To determine the activity level, each subject was asked if they regularly engaged in specific activities and what duration of time was spent in each given activity. From this evaluation, a custom reduced energy diet was tailored to promote 0.75 kg weight loss weekly. Each subject's usual food consumption prior to starting the study was assessed by the Diet History Questionnaire (NCI, 2003).

2.4. Dietary Treatments

The macronutrient composition for each dietary treatment is shown in Table 1. All three diets were similar in dietary fiber, cholesterol, calcium intake, and fatty acid balance. *Ad libitum* additions of salt were not controlled with these diets. Diets were designed to differ in the percent of energy from protein and the protein source. The two high protein diets contained 30% energy from protein and 40% energy from carbohydrate compared to CD, which contained 15% energy from protein and 55% energy from carbohydrate. The CD and the MP diets contained protein from the same sources, but in different quantities. The WP diet contained the same quantity of protein by mass as the MP, but the entire increase of protein above the level of the CD came from the addition of Designer Whey®, a commercial whey protein isolate. Daily calcium intake was balanced across groups by providing supplemental calcium tablets (Caltrate 600) to subjects in the CD and MP groups.

Experimental diets were formulated with commonly available food items, including whey protein, as shown in Table 2. A five day menu rotation was developed and then modified for each experimental group. The nutrient composition of the test diets was calculated with Nutritionist V nutrient analysis software (Hearst Corporation, 2005). The nutrient data for all food items used in the menus were obtained from the USDA standard reference database as included in Nutritionist V.

2.5. Dietary Compliance and Satiety Assessment

Subjects completed compliance questionnaires on a daily basis during the 8 week feeding phase. The first questionnaire asked the subject to report all quantities of provided food left uneaten, and to specify any additional food that had been consumed during the previous 24 hours. In the second questionnaire, satiety was evaluated by use of a Labeled Affective Magnitude scale (LAM) [34,35]. Similar to a Visual Analogue Scale (VAS), this scale contained phrases describing different degrees of hunger or fullness on a 100 mm vertical line (0mm = 'Greatest Imaginable Hunger', 50mm = 'Neither hungry nor full', 100mm = 'Greatest Imaginable Fullness'). Subjects were instructed to mark the vertical line at the place that best described their current feeling of hunger or fullness.

During the 12 week *ad libitum* phase, compliance was monitored by daily online recording via the Nutrihand.com website. The online records were reviewed weekly by investigators with feedback provided to each subject by email. Records were evaluated for maintenance of restricted energy intake and assigned protein intake. The average weekly intake for each subject was calculated from the selection of consecutive days in each week with three or more meals recorded each day. The mean intake per treatment group was calculated using the average weekly intake of each subject.

2.6. Blood analyses

After fasting overnight, plasma samples were collected from subjects in EDTA vacutainer tubes for analysis of plasma renin concentrations. Serum samples were collected in vacutainer tubes for glucose, insulin, aldosterone, and ACE analysis. Samples were separated by centrifuge at $2000 \times g$ and 5°C for 20 minutes and then individually labeled and stored at -80°C until analysis was completed.

Insulin was measured with a commercially available ELISA kit (LINCO Research, St. Charles, MO) with a range of $2\text{--}200 \mu\text{U/mL}$. The procedure used microtiter plates coated with an antibody for human insulin. Samples were added to the prescribed wells and additional antibodies were added to sandwich the captured insulin. Quantification of the captured insulin was accomplished by measuring the activity of horseradish peroxidase in tetramethylbenzidine with an ELISA reader. Glucose was measured with a spectrophotometric protocol developed by Morin et al [36]. A 2.5 mL glucose oxidase-preoxidase reagent was added in duplicate to $50 \mu\text{L}$ serum samples, vortexed, and then incubated two minutes before measuring absorbance in the spectrophotometer (Varian Cray 50 2.0). ACE activity was measured with a commercially available spectrophotometric assay (ALPCO Immunoassays, Salem, NH) with a reference range of $27\text{--}68 \text{ U/L}$. The production of hippuric acid by ACE was stopped with the addition of HCl and the product then combined with cyanuric chloride to a measurable absorbance at 382 nm . [37] Plasmarenin activity was measured with a commercially available immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Webster, TX) with lower and upper detection limits of 5 and 500 pg/mL , respectively. The assay measured the renin that was attached to two antibodies in a non-competitive enzyme reaction. [38] Aldosterone was measured with a commercially available radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) with lower and upper detection limits of 25 and 1600 pg/mL , respectively. This competitive radioimmunoassay measured the aldosterone bound to the antibody, which is inversely proportional to the aldosterone present in the original sample. [39]

2.7. Body Composition Measures

Body weight was measured on an electronic scale (Scale-Tronix 5005 Stand-On Scale) to the nearest $\pm 0.1 \text{ kg}$ with subjects wearing light clothing and no shoes. Height was measured to the nearest $\pm 1.0 \text{ cm}$ on a stadiometer attached to the scale. BMI was calculated as kg/m^2 . DXA measures of total body, lumbar spine, and dual femur were completed at baseline, 8, and 20 weeks by a trained technician. The DXA software (Prodigy, General Electric Medical, Madison, WI) calculated total tissue, total fat tissue, total lean tissue, percent body fat, and bone mineral density from the total body scan. [40] In addition to total body measures, the DXA software identified five regions for additional analysis: trunk, arm, leg, gynoid, and android regions. The android region is defined as the waist area starting from a longitudinal line directly above the pelvis and progressing upward a height that is 20% of the measured distance from the pelvis to the neck base. The gynoid region is defined from a longitudinal line at the top of the pelvis and progressing downward a total height twice as high as the android region [41].

2.8. Statistical analyses

All data are presented as means ($\pm \text{SEM}$) of each dietary treatment group. The groups were compared using analysis of variance in Procedure General Linear Models (Proc GLM) (SAS version 9.2, SAS Institute, Inc. Cary, NC). Area under the curve (AUC) was measured for sodium and potassium intake during the *ad libitum* phase of the trial. The overall F-test is reported, with the results of pair-wise group comparisons and tests of within-group changes from baseline. Results were considered significant at $p < 0.05$.

3. Results

3.1. Subjects

Randomization of subjects to the three treatment groups was successful. At baseline, no statistically significant differences existed between treatment groups for age, weight, BMI, or percent body fat (Table 3). All subjects completed the 8 week feeding phase of the trial and one female from each group did not complete the 12 week *ad libitum* phase (Figure 1). Follow up with non-completers indicated the reasons for not returning for follow up clinical visits were life challenges and dissatisfaction with rate of weight loss.

3.2. Weight loss and body composition

Significant weight loss within each treatment group was observed ($p < 0.05$ for CD, MP, and WP ranging from 6 to almost 10 kg). Differences in weight loss between all three treatments were not significant. However, there was a trend ($p = 0.08$) toward greater weight loss in the WP group compared to the CD group.

Body composition improved with a reduction in fat mass in all treatment groups. There was an observed trend of greater total fat mass loss ($p = 0.09$) in the WP group compared to the CD group, especially in the leg and gynoid regions (Figure 2). In addition, there were some regional differences in lean mass changes between treatments (Figure 3) with the WP group showing slightly greater loss of lean mass in association with greater total body weight reduction. In comparison by fat : lean ratio, the CD group showed a loss of 5.6 : 1 g compared to a loss of 8.4 : 1 g with the WP group. The difference in total lean mass loss between groups at week 20 was not significantly different.

3.3. RAAS activity, blood pressure and metabolic measures

Plasma renin and ACE activities, and aldosterone concentration are reported in Table 4. Renin activity was not significantly different between groups and did not change over the five month protocol. ACE activity in the CD group was higher at baseline compared to the other treatment groups but the difference was not statistically significant. By week 8, all the groups had similar ACE measures with a significant decrease within the CD group ($p < 0.05$). Over the 20 weeks, ACE activity decreased in the WP group, but was not statistically different from other groups. Aldosterone concentration was similar at baseline between treatments, and a trend toward decreasing aldosterone concentration ($p = 0.08$) was observed in the CD and WP groups at week 20.

Systolic blood pressure decreased in all three treatments during the first 8 weeks. At the conclusion of the study, there was a significant difference ($p < 0.04$) in change of systolic blood pressure between the CD and WP treatment. There were no significant changes in diastolic blood pressure between treatments.

No significant differences were observed between groups for fasting glucose or insulin measures at any time point in the study. HOMA IR values at baseline were similar between groups and the decreasing values observed with all treatments were not significantly different at weeks 8 or 20.

3.4. Dietary Results

Protein intake between diets was significantly different throughout the 20 weeks. As designed, protein intake during the feeding phase for the CD group averaged 0.8 g/kg/day, while protein intake of the MP and WP groups averaged 1.4 g/kg/day. A comparison of the essential amino acid composition average between the MP and WP treatment during the feeding phase showed an increase by mass in tryptophan (48%), threonine (63%),

isoleucine(27%), leucine (45%), cystine (46%), and valine (21%) with daily intake in the WP treatment. This increase is based primarily on the additional whey protein consumed in the diet.

During the *ad libitum* phase,protein intake of the CD group increased to 1.1 g/kg/day and protein intake of the MP group was 1.3 g/kg/day, while the WP group maintained 1.4 g/kg/day. Subjects maintained a protein and carbohydrate intake that was significantly different ($p < 0.05$) between the CD treatment and both high protein treatments. No significant difference in AUC analysis for sodium and potassium intake was observed. However, the self-reported online dietary records did not ask subjects to report added salt. Ninety-three percent of the subjects reported *ad libitum* intake for more than six weeks, and 53% of the subjects reported all weeks of *ad libitum* intake.

3.5. Satiety

Average satiety responsesduring the feeding phase showed that even on a reduced calorie diet most subjects reported “fullness” (>50 mm). Average weekly responses did not show a significant difference between the groups. However, the WPgroup reported the least variability during the entire 8 weeksas shown in Figure 4. By the week 8, a trend ($p=0.08$) toward differences between the WP and the other treatments was observed at the ‘Neither hungry nor full’ level.

4. Discussion

In our study design incorporating custom reduced energy diets, all subjects showed similar weight loss and circulating hormone activity even though dietary composition was significantly different. Therefore, the hypothesis that a whey protein diet would result in greater weight loss and improved body compositioncompared to standard weight loss diets was rejected in this study. Although the measured outcomes were not statistically different, there was a trend in the WP group toward greater weight loss, greater fat loss, and reduced blood pressure compared to the other treatment groups. There was no evidence to support a RAAS mediated effect as a possible mechanism to explain the blood pressure changes in the WP group.

Some previous weight loss studies [42,43] have reported decreases in plasma renin and ACE activities as well as aldosterone concentrations when subjects have achieved greater than 3% weight loss. It is important to note thatall of our subjects achieved greater than 3% weight loss, and showedno significant differencesamong the three diets in RAAS activity as assessed by renin and ACE activity, and aldosterone level. Therefore, our results fail to identify a weight loss mechanism related to a decrease in circulating ACEactivity and the consumption of whey protein at the levels provided (15% of energy). In a previous study, when a high protein diet (2.0g/kg/day) was fed in a weight maintenance diet to normal weight subjects, increased plasma renin activity and aldosteronewere observed[44]. Two of our treatments were high protein diets, which may have contributed to sustaining RAAS measures even during weight loss. However, the CD group did not show any significant decrease in RAAS with the comparatively lower protein levels. It is possible that the sample size of each group in the pilot study was not sufficient to identify a RAAS mediating effect. It is important to note that dietary sodium and potassium differences between groups may have confounded examination of this issue. Additionally, we only examined circulating RAAS biomolecules. Therefore, we cannot rule out the possibility of RAAS related adipose and muscle-specific mechanisms related to weight loss under these conditions.

Consumption of prepared milk proteins in other studies has resulted in decreased blood pressure in moderately hypertensive subjects[28,29,45]. All of the subjects in our study were

normotensive; therefore, no elevated RAAS levels were observed at baseline, which might have negated the ability to demonstrate a RAAS mediating effect by whey protein during the course of the study. At the end of our study, subjects in the WP group showed a significant decrease in systolic blood pressure compared to the CD group with no significant change in RAAS activity.

Changes in circulating ACE activity did not correlate with the observed decrease in blood pressure. In contrast to our results, Harp et al reported a decrease in blood pressure and ACE activity with weight loss[42]. However, Harp et al also observed that decreases in plasma renin activity and aldosterone were more strongly associated with weight loss than was ACE activity. A trend of decreasing aldosterone concentration ($p = 0.08$) was observed in the CD and WP groups compared to the MP group. However, the similar decrease in aldosterone concentration in the CD and WP groups was not associated with similar weight loss between groups.

High protein reduced energy diets have been shown to maintain lean tissue while promoting decreases in fat tissue[5]. In this pilot study, there were no significant differences between groups for change in lean tissue. On average, the MP group gained lean tissue and the WP group lost lean tissue. Because of the high availability of essential amino acids and BCAA, the observed decrease in lean tissue for the WP group was surprising. The measure of lean tissue loss in the WP group was comparable to the measure of lean tissue loss in the CD group, yet the WP group lost more weight than the CD group. Since exercise was not part of the protocol, these changes in lean tissue reflect the diet effect on lean tissue without an exercise program. If an exercise program were added to the weight loss protocol, a greater retention of lean tissue might be anticipated[46].

Trends of decreased fat tissue were observed with the MP and WP diets compared to the CD diet. These results agree with earlier studies by Layman et al that demonstrated improved body composition among those subjects assigned to a high protein diet [5,10]. Differences in body composition between treatments were most clearly observed in measures of the leg and gynoid regions. It is not clear whether or to what extent the difference in amino acid composition between treatments was related to the differences observed in fat loss and lean tissue gain between the two high protein groups.

The strengths of this pilot study include the control feeding and parallel design, which provided opportunity to evaluate the effect of the different diets. The three treatments offered comparison between a standard diet and two variations of a high protein diet. The comparison between high protein diets allowed the assessment of outcome differences when different sources of protein are favored in the diet composition.

One limitation of this pilot study was small sample size. A parallel-arms design with 6 subjects in each arm was 80% power to detect an effect size larger than 1.8 between groups, at the .05 level. This corresponds to a difference between groups of at least 5.6 kg in weight, 5.6 kg in total body fat mass, or 6.5 ng/L in renin. Therefore, the hypothesis of this study was rejected since these differences were not observed.

These preliminary results based on our feasibility study establish the basis for further studies regarding the effectiveness of incorporating whey protein into custom reduced-energy diets for weight loss at midlife. The trend in the WP group toward greater weight loss, greater fat loss, and reduced blood pressure compared to the other treatment groups warrants further investigation in a larger sample.

Acknowledgments

Funding for this work was provided by grants from the National Center for Research Resources, National Institutes of Health, M01-RR00400, and from USDA National Needs Graduate Fellowship Competitive Grant No. 2005-38420-15786 from the National Institute of Food and Agriculture, and from Next Proteins, LLC.

Abbreviations

ACE	Angiotensin Converting Enzyme
AUC	Area Under the Curve
BCAA	Branch Chain Amino Acids
BMI	Body Mass Index
DXA	Dual Energy X-ray Absorptiometry
GCRC	General Clinical Research Center
HOMA IR	Homeostatic Model Assessment Insulin Resistance
LAM	Labeled Affective Magnitude
RAAS	Renin-Angiotensin Aldosterone System
REE	Resting Energy Expenditure
SEM	Standard Error of the Mean
VAS	Visual Analogue Scale

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Fig 1.

Flow diagram for the study design and subjects

CD – control diet; MP- mixed protein diet; WP – whey protein diet

Fig. 2. Fat loss by Region and by Diet at Five Months. Values measured by DXA and reported as mean ($\pm$ SEM). Different lower case letters indicate significance between groups as determined by GLM.

Fig. 3. Lean Tissue Change by Region and by Diet at Five Months. Values measured by DXA and reported as mean ($\pm$ SEM). Different lower case letters indicate significance between groups as determined by GLM.

Fig. 4.

The weekly average satiety score during the eight week feeding phase. The Labeled Affective Magnitude (LAM) Scale consisted of labeled terms on a 100 mm scale with 0 mm being 'Extremely Hungry', 50 mm being 'Neither Hungry nor Full', and 100 mm being 'Extremely Full'. Data shown are means ($\pm$ SEM). Week 8 difference between groups $p = .08$.

Table 1

Macronutrient composition of the dietary groups*

Macronutrient	CD	MP	WP
Kilocalories	1599.9 ± 2.0	1605.8 ± 0.5	1599.7 ± 2.8
Protein (g)	63.4 ± 0.2	124.1 ± 0.5	124.2 ± 0.4
Carbohydrate (g)	225.3 ± 0.2	163.9 ± 0.6	166.9 ± 0.5
Fat, Total (g)	54.9 ± 0.4	54.0 ± 0.2	54.7 ± 0.4
Saturated Fat (g)	16.1 ± 0.1	16.0 ± 0.2	16.2 ± 0.1
Monounsaturated Fat (g)	16.1 ± 0.1	16.1 ± 0.2	16.3 ± 0.1
Polyunsaturated Fat (g)	16.1 ± 0.1	15.9 ± 0.2	16.1 ± 0.1
Dietary Fiber (g)	25.0 ± 0.5	24.0 ± 1.0	23.2 ± 0.7
Cholesterol (mg)	239.8 ± 0.8	240.0 ± 1.6	268.5 ± 3.2
Calcium (mg)	1316.1 ± 41.0 [#]	1668.0 ± 90.9 [#]	1536.0 ± 47.8

Data are means (± SEM)

* Calculated by Nutritionist V software

[#] includes Caltrate 600 supplement

CD – control diet; MP- mixed protein diet; WP – whey protein diet

Table 2**Sample Menu (1600 Kcal)**

Meal	Menu Item Diet	CD (g)	MP (g)	WP (g)
Breakfast	Apple Juice	200	100	70
	Wheat Chex Cereal	37	25	25
	2% Milk	162	180	200
	Whole Wheat English Muffin	62	23	30
	w/ Butter	10	3	0
	w/ Canola Margarine	0	0	6
	Jam – Low Sugar	4	3	3
	Egg Beaters	0	75	0
	w/ Butter	0	3	0
	w/ 2% milk	0	20	0
	Cheddar Cheese, Low fat	0	35	0
	Designer Whey – Chocolate	0	0	28
Lunch	Rye Bread	46	0	0
	Reduced Calorie Wheat Bread	0	46	46
	Corned Beef	44	70	35
	Mayonnaise, Regular	14	6	0
	Mayonnaise, Lite	0	0	20
	w/ Egg Yolk	10	1.5	0
	Romaine Lettuce	25	20	20
	Red Tomato	37	30	30
	Yellow Mustard	4	3	3
	Carrots, raw	25	20	25
	Celery, raw	25	20	25
	Blueberries	187	80	75
	GENISOY Deep Sea Soy Nuts	0	35	0
	Designer Whey – French Vanilla	0	0	28
Dinner	Chicken Breast, cooked	56	135	55
	Egg Noodles, cooked	187	75	0
	Rotini, cooked	0	0	85
	Chicken Gravy	75	50	75
	w/ Olive oil	3	4	4
	Green Beans, cooked	125	90	100
	Whole Wheat Dinner Roll	44	0	0
	Reduced Calorie Wheat Bread	0	25	30
	w/ Butter	0	3	8
	w/ Canola Margarine	5	0	0
	Mandarin Oranges, canned	175	90	70
Snack	Milk Chocolate Chips	30	0	0

Meal	Menu Item Diet	CD (g)	MP (g)	WP (g)
	Walnuts	14	14	15
	Skim Milk	150	240	220
	Designer Whey – Chocolate	0	0	28

CD – control diet; MP- mixed protein diet; WP – whey protein diet

Table 3

Subject characteristics by group at baseline and change in study parameters at 2 and 5 months.

	CD	MP	WP	Overall <i>p</i>
<i>value</i>				
N (F/M)	6 (5/1)	6(5/1)	6(5/1)	
Age (years)	51.3 ± 2.1	49.6 ± 3.5	49.2 ± 1.6	0.824
Baseline				
BMI	29.9 ± 0.6	30.3 ± 0.7	30.6 ± 0.6	0.712
% Body Fat	43.0 ± 2.5	42.7 ± 2.5	45.2 ± 2.9	0.777
HOMA-IR	0.89 ± 0.18	0.96 ± 0.28	0.99 ± 0.26	0.954
Weight (kg)				
Baseline	81.6 ± 1.9	84.4 ± 1.2	85.2 ± 4.2	0.626
2 mo. Δ	− 5.3 ± 0.76 *	− 5.9 ± 0.59 *	− 7.3 ± 0.73 *	0.167
5 mo. Δ	− 6.1 ± 0.82 *	− 7.6 ± 1.72 *	− 9.7 ± 1.27 *	0.198
Systolic				
Baseline	117.8 ± 4.3	117.7 ± 2.7	116.2 ± 3.2	0.933
2 mo. Δ	− 2.2 ± 5.1	− 8.7 ± 3.9	− 3.3 ± 3.8	0.541
5 mo. Δ	3.2 ± 4.5 ^a	− 3.6 ± 2.7 ^a	− 7.2 ± 1.7 ^{b*}	0.106
Diastolic				
Baseline	67.7 ± 2.9	66.8 ± 2.4	66.2 ± 3.4	0.936
2 mo. Δ	3.8 ± 4.2	− 4.3 ± 2.1	− 2.3 ± 3.8	0.255
5 mo. Δ	1.2 ± 3.2	− 2.6 ± 1.3	− 6.0 ± 4.0	0.289
Fasting REE				
Baseline	1627 ± 139	1582 ± 152	1452 ± 81	0.613
2 mo. Δ	− 269 ± 93 *	− 160 ± 125	− 43 ± 69	0.296
5 mo. Δ	− 264 ± 237	− 247 ± 184	− 21 ± 88	0.583
Total Body Fat mass (kg)				
Baseline	34.65 ± 1.9	35.61 ± 2.1	37.78 ± 1.6	0.498
2 mo. Δ	− 4.29 ± 0.7 *	− 5.17 ± 0.5 *	− 5.99 ± 0.7 *	0.211
5 mo. Δ	− 5.45 ± 1.1 *	− 7.54 ± 1.4 *	− 8.77 ± 1.3 *	0.216
Total Body Lean mass (kg)				
Baseline	43.83 ± 2.6	45.67 ± 1.8	44.95 ± 4.9	0.927
2 mo. Δ	− 1.12 ± 0.9	− 0.32 ± 0.8	− 1.14 ± 0.4 *	0.680
5 mo. Δ	− 0.32 ± 0.4	+0.43 ± 1.1	− 1.09 ± 0.1 *	0.300

Data are means ± SEM

* marks significant within-group change from baseline ($p < 0.05$)Row values displaying different lowercase letters are significantly different ($p < .05$, Proc GLM) 624CD – control diet; MP- mixed protein diet; WP – whey protein diet

Table 4

Renin –Angiotensin Aldosterone System concentrations by group

	CD	MP	WP	p value
N (F/M)	6 (5/1)	6(5/1)	6(5/1)	
Renin (ng/L)				
Baseline	7.2 ± 0.5	7.2 ± 1.3	7.7 ± 1.8	0.941
2 mo.	8.4 ± 0.8	8.5 ± 1.6	8.3 ± 0.7	0.984
5 mo.	9.5 ± 1.4	8.4 ± 1.8	9.0 ± 1.2	0.871
ACE (U/L)				
Baseline	61.2 ± 3.2	50.3 ± 4.4	50.0 ± 5.9	0.187
2 mo.	51.7 ± 2.1*	50.0 ± 5.4	48.0 ± 6.0	0.867
5 mo.	52.2 ± 7.0	54.4 ± 5.7	46.8 ± 4.6	0.651
Aldosterone (ng/L)				
Baseline	46.5 ± 6.4	53.8 ± 8.7	54.5 ± 11.1	0.789
2 mo.	48.9 ± 8.6	77.8 ± 21.5	73.1 ± 18.6	0.461
5 mo.	49.0 ± 4.4 ^a	67.8 ± 11.1 ^b	42.8 ± 4.4 ^a	0.077

Data are means ± SEM

* marks significant within-group change from baseline (p < 0.05)

Row values displaying different lowercase letters are significantly different (p < .05, Proc GLM)

CD – control diet; MP- mixed protein diet; WP – whey protein diet