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Influence of Weight Reduction and Enhanced Protein Intake on Biomarkers of Inflammation in Older Adults with Obesity

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Abstract

Both aging and obesity are associated with increased levels of pro-inflammatory metabolites, while weight reduction is associated with improvements in inflammatory status. However, few studies have explored the response of key inflammatory markers to the combined settings of weight reduction in an aging population. There are also few studies that have investigated the potential impact of diet composition on inflammatory marker responses. In the MEASUR-UP trial, we evaluated changes in baseline levels of inflammatory markers with post-study levels for a traditional weight loss control group versus a group with generous, balanced protein intake. In this 6-month randomized controlled trial (RCT), older (≥ 60 yrs) adults with obesity (BMI ≥ 30 kg/m²) and Short Physical Performance Battery (SPPB) score of 4–10 were randomly assigned to either a traditional weight loss regimen, (Control, *n*=14) or one with higher protein intake (≥ 30 g) at each meal (Protein, *n*=25). All participants were prescribed a hypo-caloric diet and attended weekly support and education groups and weigh-ins. Protein participants consumed ≥ 30 g of high quality protein/meal, including lean and extra lean beef provided to them for two of the three meals per day. Protein intakes were 0.8 and 1.2 g/kg/day for Control and Protein, respectively. Adiponectin, leptin, C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, serum amyloid A (SAA), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and glycated serum protein (GSP) levels were measured at 0 and 6-month time points. At the 6-month endpoint, there was significant weight loss and decrease in BMI in both the Control (−4.8 ± 8.2 kg; −2.3 ± 2.4 kg/m²; *P*=0.05) and Protein (−8.7 ± 7.4 kg; −2.9 ± 2.3 kg/m²; *P*<0.0001) groups. SPPB scores improved in both arms, with a superior functional response in Protein (*P*<0.05). Body fat (%) at baseline was positively correlated with leptin, hs-CRP, VCAM-1, ICAM-1, and GSP. Several markers of inflammation responded to the Protein group: leptin (*P*<0.001), hs-CRP (*P*<0.01), and ICAM-1 (*P*<0.01) were decreased and adiponectin increased (*P*<0.01). There were no significant changes in any inflammatory markers in the Control arm. In the between group comparison, only adiponectin trended towards a group

difference (more improvement in Protein; $P<0.07$). Our findings in the MEASUR-UP trial show that a weight loss diet with enhanced protein intake is comparable to an adequate protein diet in terms of weight loss success and that it can lead to improvements in inflammatory status, specifically for adiponectin, leptin, hs-CRP, and ICAM-1. These findings are important given current recommendations for higher protein intakes in older adults and justify additional study of the inflammatory impact of an enhanced protein diet. ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01715753) identifier: NCT01715753)

Keywords

Inflammation; protein intake; weight reduction; obesity; older adults

INTRODUCTION

Aging and Inflammation

Older adults account for about 15% of the US population, and by 2030 this number will rise to roughly 20% (1). The aging process is characterized by low-grade systematic inflammation that occurs even in the absence of any infection. More recently, the term “inflammaging” (2) has been used to describe this hallmark of aging. That chronic inflammation is associated with aging is widely accepted; however, the etiology of this inflammation is still unknown. It has been suggested that age-related inflammation is potentially due to a number of causes including, increases in adiposity and changes in body composition with age (3), cellular senescence (4), immunosenescence (4), changes in gut microbiota (4), coagulation system activation (4), and accumulation of damaged macromolecules and cells (4). In older adults, inflammation shows a strong correlation with insulin resistance, heart disease, type II diabetes, cancer, osteoporosis, and macular degeneration, as well as neurological conditions such as Alzheimer’s and Parkinson’s Disease (4). The extensive impact that inflammation has on aging has led to an increased interest (5) in not only gaining a better understanding of this relationship, but also understanding ways to reduce the negative impact of increased inflammation in older adult populations.

Obesity and Inflammation

In the United States, the prevalence of obesity has reached unprecedented levels, including a rate of 38.5% in adults 60 and older (6). Not only are there major health risks posed by obesity, but adults with obesity also have physical deficits. Their physical function is impaired, not only because of decreased mobility, but also because of chronic inflammation (7, 8). Individuals with obesity have a larger adipose stores and thus heightened production and release of inflammatory cytokines, thus giving obesity the categorization of being an inflammatory disease (9, 10). Adiposity level is well known to be correlated with levels of a number of metabolic inflammatory markers, specifically: leptin, C-reactive protein (hs-CRP), serum amyloid A (SAA), tumor necrosis factor (TNF- α), interleukin-1 (IL-1), IL-6, and IL-8 in morbidly obese individuals (11–13). An additional defining characteristic of obesity is low serum levels of adiponectin, a hormone involved in glucose regulation.

Conversely, leptin is found in much larger concentrations in individuals with obesity, and is directly correlated with fat mass (14, 15). Leptin has also been shown to induce the expression of adhesion molecules, such as vascular cell adhesion molecules and intercellular adhesion molecules. The cellular adhesion molecules primarily involved in regulating the inflammatory response are VCAM-1 and ICAM-1. VCAM-1 is identified at higher levels when there is an immune response against disease, thus promoting tissue inflammation (16). It has also been directly correlated with obesity (17). IL-6 is one of the more studied interleukins associated with obesity, has been found at higher levels in adipose tissue of individuals with obesity. IL-1 and IL-8 are two more interleukins closely associated with the inflammatory response. A newer marker, acute-phase protein SAA, is an apolipoprotein associated with lipoproteins, prompting researchers to look at a possible link between SAA and obesity. Several studies have found positive associations between SAA levels and BMI, as well as decreases in SAA levels when obesity reduction occurs (18). Obesity, particularly in the older adult population, continues to be a considerable public health concern because the associated chronic, underlying inflammation also increases the risk of age-related disease (19), and leads to progressive decline in muscle quality (20).

Obesity, Aging, and Function

Because both obesity (pathological) and aging (physiological) are associated with increased inflammation and insulin resistance and impaired metabolic control, their co-occurrence is a double threat to health, resulting in metabolic and inflammatory dysfunction (21). Furthermore, both obesity and the aging process lead to compromised muscle function through multiple mechanisms. Sarcopenia, also known as muscle wasting, is a progressive loss of muscle mass/strength that occurs almost universally with aging (22). There is a substantial loss of lean muscle mass, from about 50% of total body weight in young adults to about 25% at 75–80 yrs (23). Older adults also experience a decline in muscle strength and power, slower movement, and an increased risk for falls (24). Although they utilize different mechanisms, age-related loss of physical function and deterioration of muscle quality also occurs in older adults with obesity. This combination of sarcopenia and obesity markedly accelerates functional decline. Muscle quality is even further reduced due to the accumulation of lipids between muscles associated with greater adiposity. Chronic activation of the innate immune system causes persistent low-grade inflammation in older adults with obesity, which leads to muscle depletion by enhancing protein breakdown and impairing myogenesis in parallel (7, 25). This impairment is further exacerbated by obesity reducing the ability to be physically active, and thus leading to progressive muscle atrophy due to disuse.

Intentional Weight Loss in Older Adults with Obesity

Obesity reduction has been associated with improved inflammatory status. However, weight reduction is difficult to achieve in adults of all ages (26, 27) and it is especially challenging to treat in older adults, who have lower basal metabolic rates, calorie requirements, and rates of physical activity (28–30). Moreover, efforts to reduce body weight in older adults with obesity raise concerns about the concomitant loss of lean body mass (31). Previous studies have found that sarcopenia is exacerbated by weight loss and as such, researchers have been investigating the best approach to combat obesity while limiting the amount of muscle (lean)

mass lost (32). A higher protein intake during obesity reduction could help circumvent loss of muscle mass and strength. Protein intakes exceeding the RDA level (>0.8 g/kg) are linked with better preservation of lean mass (33, 34) and new findings from the Framingham study have confirmed the benefits of high quality (animal) protein for protection of appendicular lean mass (35), preservation of grip strength (36) and decreased risk of function decline in the long-term (37). While both higher protein intake and decreased inflammation are known to benefit muscle, the direct impact of higher protein intake on whole body inflammatory state is a matter of active study and some disagreement in the literature (38–40), due in part to the limited number of published trials. Only a few studies have explored the response of inflammatory markers to enhanced protein intake during obesity reduction (40–42) and we could not find any such studies focusing on older adult participants. The purpose of this analysis of secondary outcomes from a randomized controlled trial of protein enhanced weight loss in physically frail older adults with obesity was to evaluate the responses of a wide array of traditional and novel biomarkers of inflammation, namely adiponectin, leptin, IL-1, IL-6, IL-8, hs-CRP, TNF, ICAM, VCAM, SAA and GSP, to this intervention.

METHODS

Study design and participants

This is an analysis of secondary outcomes from the “The Measuring Eating, Activity and Strength: Understanding the Response-Using Protein” (MEASUR-UP) study, a randomized controlled trial that compared a protein-enhanced diet to a traditional weight loss regimen in frail older adults with obesity during a 6-month intervention. The study design and methods (32), and primary results from the intent-to-treat parent study have been previously reported and published (25). The study was approved by Duke University School of Medicine Institutional Review Board and written consent was obtained from all study participants. Briefly, physically frail men and women aged ≥ 60 years with obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$) living in or near Durham, North Carolina, were enrolled. Physical frailty at baseline was qualified by having a score of 4–10 on the Short Physical Performance Battery (43). Sixty-seven participants were randomly assigned in a 1:2 ratio to a traditional (Control) weight loss regimen or one with higher protein intake ($\geq 30 \text{ g}$) at each meal (Protein). All participants were prescribed a hypo-caloric diet designed to achieve a target of 10% body weight loss. Participants were weighed weekly and attended weekly group support sessions led by Registered Dietitians.

The present analyses were performed on 39 frail, older (≥ 60 years) men and women with obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$), who participated in the MEASUR-UP trial and who had blood samples available at baseline and endpoint to analyze for inflammatory biomarkers (Control $n=14$; Protein $n=25$). This includes 6 participants whose intervention lasted 3 months instead of 6.

Intervention

A supervised 6-month weight loss treatment program was prescribed to both the Control and Protein groups and delivered by Registered Dietitians (Interventionists). Interventionists prescribed a 500 kcal deficit diet and provided individualized kcal prescriptions and meal

plans to achieve a weight 10% weight loss goal. The macronutrient distribution for the Control group was 15% protein, 30% fat, 55% carbohydrate and their protein intake met the Recommended Dietary Allowance of 0.8 g/kg body weight. Protein group macronutrient distribution was 30% protein, 30% fat, 40% carbohydrate and protein intake was 1.2 g/kg body weight. Additionally, the Protein meal plans included 30 grams of lean, high-quality protein three times a day. Protein participants were provided two 30 gram portions of prepared, frozen or chilled, lean and extra lean beef (ground sirloin, deli roast beef, and flank steak) daily and were counseled on ways to achieve 30 grams of lean high quality protein at the third daily meal. Both groups participated in weekly group meetings led by Interventionists that provided education, counseling, peer support, and supervised weekly weigh-ins.

Assessments

Inflammatory markers—Fasting blood samples were collected using standard procedures at baseline ($n=59$) and completion of intervention ($n=39$) by a Registered Nurse. Blood serum was analyzed for biomarkers of inflammation by Dr. Virginia Kraus' laboratory in the Duke Molecular Physiology Institute. Adiponectin assessment was by MSD Human Adiponectin Kit (#K151BXC-1) and leptin by MSD Human Leptin Kit (#K151BYC-1). The MSD Human Proinflammatory 4-plex (#K15052D) was used to quantify IL-1 β , IL-6, IL-8, and, TNF- α . For hs-CRP, SAA, ICAM-1, and VCAM-1 we used the MSD Human Vascular Injury Panel 2 (#K15198D) and for glycosylated serum proteins (GSP) we used the Glycated Serum Protein Assay (Diazyme#DZ112B). For these assays, intra-assay CVs were <7.7% and inter-assay CVs were <11.3%.

Body Composition and Function—Body weight, lean mass, and fat mass were measured in the fasting state using the BOD POD™ air displacement plethysmography method (Life Measurement Inc, Concord, CA). Height was recorded to the nearest 0.5 cm using a stadiometer. Physical function was measured using the Short Physical Performance Battery, a widely used functional assessment developed for use in older adults; it includes three component domains (balance, strength, and gait speed) that yield a total score of 0 (poor) to 12 (high) (43).

Dietary intake—Three-day food intake records (2 week days and 1 weekend day) were collected at baseline and at the endpoint of the intervention. Food records were translated to food codes by Registered Dietitians and analyzed using Food Processor Nutrition Analysis Software (ESHA Research, Salem, OR) for total calories and distribution of macronutrients (protein, carbohydrates, and fat).

Statistical analysis

All statistical analyses were performed in SAS 9.4 (SAS Institute, Cary NC). Descriptive statistics were calculated for baseline characteristics. Spearman correlation coefficients were computed for baseline inflammatory markers and baseline body weight, body fat (%), function (SPPB), and age. For a small subset of participants (6: $n=2$ Protein; $n=4$ Control) who had assessments at three-months but not six-months, the three-month data was carried forward and used as the six-month measure. For each outcome of interest, change scores

were created using baseline and six-month data. Inflammatory marker levels were log transformed to better approximate normality to satisfy linearity assumptions. Two-Sample t-test and paired t-test were used to determine changes between and within groups. Data were then back-transformed according to intervention group for ease of interpretation.

RESULTS

Baseline and Descriptive characteristics

As outlined in the consort diagram (Figure 1), 67 participants were randomized into either Control or Protein group. The data presented in this analysis is from a subset of 39 participants who completed the intervention and had blood serum available for analysis of inflammatory biomarkers (Control $n=14$ and Protein $n=25$). Baseline characteristics by group and for the total subset are shown in Table 1. The average age of study participants was 68.3 ± 5.6 and the population was mostly female (79.5%) and predominantly white (69.2%). Additionally, 21% of participants reported CVD, 16% reported Type 2 Diabetes and 71% reported hypertension. Participants had a mean BMI of $37.0 \pm 6.8 \text{ kg/m}^2$ (Class II obesity). At baseline, there were significant correlations between percent body fat and these inflammatory markers: leptin ($r=0.739$, $P<0.001$), hs-CRP ($r=0.351$, $P=0.031$), ICAM ($r=0.552$, $P<0.001$), and VCAM-1 ($r=0.475$, $P=0.003$) (data not shown).

Intervention and Inflammatory Markers

Intervention effects on weight loss, body composition, and physical function in the MEASUR-UP trial have been previously reported (25). Results in this subsample followed the same pattern. Both groups lost weight ($P<0.05$) and reduced their BMI ($P<0.01$) (Table 2). Physical function, measured by SPPB, improved in both groups, with significantly greater improvement in Protein arm ($P=0.028$) (Table 2).

Table 2 shows the baseline means and change scores for all inflammatory biomarkers in each intervention group between baseline and end of intervention. There were no significant between-group differences; however, there was a trend towards greater improvements in adiponectin in Protein group compared to Control ($P=0.07$). Within the Protein group, improvements in inflammation included, increased adiponectin ($P=0.004$) and decreased leptin ($P<0.001$), hs-CRP ($P=0.005$), ICAM-1 ($P=0.001$), and VCAM-1 ($P=0.07$). No significant with-in group changes were detected in the Control group.

As illustrated in Table 3, both Control and Protein significantly reduced their calorie intakes during the intervention ($P<0.01$). The Protein group significantly increased protein intake from 0.89 to 1.22 g/kg bw per day ($P<0.01$), while there was no significant change from baseline for the Control group ($P=0.28$).

Changes in Inflammatory Status in the Combined Sample

Given that there were no significant differences in intervention responses between Protein and Control (other than a trend for adiponectin to be more improved in Protein), we repeated the analysis with the groups combined. Results for this analysis showed that, between baseline and endpoint, adiponectin increased ($P=0.006$), while Leptin ($P<0.001$), hs-CRP

($P=0.014$), and ICAM-1 ($P<0.001$) decreased. Spearman correlations were then performed between inflammatory marker change scores and fat mass change scores. Change in fat mass was directly correlated with change in leptin levels ($r=0.618$, $P<0.001$) and SAA levels ($r=0.384$, $P=0.017$) and inversely correlated with levels of adiponectin ($r=-0.331$, $P=0.042$).

DISCUSSION

This study is the first to explore the impact of a balanced, higher protein weight loss diet (two of three meals as lean or extra lean beef) compared to a traditional weight loss diet on markers of inflammation in physically frail, older adults with obesity. Generous protein intakes, particularly from high quality protein sources, stimulate muscle protein synthesis and reduce muscle protein breakdown in older adults. Diets higher in protein have been shown to improve weight loss and weight maintenance by increasing satiety hormones, peptide YY and glucagon-like peptide (44), increasing postprandial thermogenesis (45, 46) and maintain resting energy expenditure (45–47). Furthermore, short-term studies have shown preservation of lean mass with higher protein hypocaloric diets as compared to those with protein at the recommended dietary allowance level (48). For older adults with obesity, who are at greater risk of losing lean muscle mass while undergoing intentional weight loss, these findings are particularly relevant and in agreement with our findings for improved function in our Protein treatment group.

In response to equivocal findings in the literature (38, 40, 49) on the inflammatory impact of meat protein and recognizing there is very limited study of higher protein weight loss diets in older adults, our analysis focused specifically on the effect of a higher protein (predominantly lean beef) diet during obesity reduction on markers of inflammatory status in physically frail older adults. We found that this diet did not have a negative impact on inflammatory markers; there were essentially no group differences between Control and Protein group outcomes for inflammatory biomarkers. Additionally, there were several beneficial within-group changes in the Protein group, for adiponectin, leptin, hs-CRP, ICAM-1, and VCAM-1. The absence of a detrimental effect of protein on inflammation agrees with several other studies. A systematic review of five pooled studies showed no difference in inflammation (hs-CRP) between high and low protein diets of middle age (median age of 45) men and women (39). Additionally, Hodgson et al. (49), found no difference in markers of inflammation or oxidative damage when lean red meat replaced carbohydrates in an isoenergetic diet consumed by adults (mean age 58.6 ± 8.6 years). The within-group improvements we found with higher protein intakes are supported by a recently published study investigating higher protein intake plus resistance training (38). In this trial by Daly et al. (38), women 60 to 90 years old of age were provided two servings of lean red meat daily for 6 days per week during a 4-month RCT. Compared to an isocaloric control diet (plus resistance training), the higher protein group had greater reductions in IL-6, and significant within-group reductions in TNF- α (38).

With regards to the influence of higher protein intake during weight loss, our findings are consistent with other studies in middle-aged populations. In the POUNDS LOST study of overweight and obese adults aged 30 to 70, Nicklas et al. (41) reported significant improvements in hs-CRP in diets with average and high protein with no difference between

groups. Similarly, in a 3-month weight loss intervention in overweight and obese women (mean age 42 years), Aadbakht et al. (42) found no difference between control (protein, carbohydrate, and fat: 15%, 55%, and 30% respectively) and high protein (protein, carbohydrate, and fat: 25%, 45% and 30%, respectively) diet groups with regards to inflammatory impact. In contrast to these reports and our own findings, Lopez-Legarrea et al. (40) conducted an 8-week weight loss intervention (the RESMENA project) and found that a control diet (15% of energy from protein) had significantly greater improvements in inflammatory scores than a higher protein diet (30% of energy from protein) diet. A sub-analysis showed that diets higher in animal protein and meat protein were positively associated with inflammation. The reason for the discrepancy between the RESMENA findings and ours is not clear, but it could relate to differences in diet composition. Much of the protein consumed in the MEASUR-UP study was provided directly to participants and it was always lean or extra lean. In fact, despite a considerable increase in protein content, fat intakes were decreased from 37 to 31% in the MEASUR-UP Protein group ($P<0.01$) and their saturated fat intake was also reduced ($P<0.05$), whereas fat intake in the RESMENA study protein group was 37%. It is also possible that the difference in participant age between the MEASUR-UP (mean age = 68 yr) and RESMENA (mean age = 50 yr) trials played a role in the response to protein. Additional studies with larger numbers of older adults with obesity are needed to conclusively describe the impact of higher protein intakes from animal protein sources on indicators of inflammation in this high risk population.

Our findings for the combined results of Control plus Protein participants in the MEASUR-UP trial are important additions to the literature. Between baseline and end of intervention, we documented important improvements in adiponectin, leptin, hs-CRP, and ICAM-1 and a correlation between changes in fat mass and improvements in adiponectin and SAA levels. While not unexpected based on findings in younger populations, these results confirm the benefits of obesity reduction to inflammatory status in older adults, whether they are consuming traditional or higher levels of protein (50–52).

Our study has a number of strengths, including the randomized controlled trial design, the inclusion of a population often excluded from research studies (frail older adults with obesity), careful documentation of protein intake, a substantial and significant difference in protein intake between groups, and the measurement of a very wide array of traditional and novel markers of inflammation. The trial also has its limitations, the most important being the small sample size, which could account for the lack of within-group effects for the Control group and limited between-group differences. The generalizability of the findings may also be limited by the consumption of red meat twice daily. Although no detrimental changes were noted in the Protein group, the consumption of the same meat twice daily for 6 months would not be practical or necessary in community implementation of the enhanced protein diet.

In conclusion, we found that a weight loss diet with enhanced protein intake is comparable to an adequate protein diet in achieving weight loss and superior in terms of benefits to function. This diet caused no detrimental changes in inflammatory status; in fact, the Protein treatment was associated with beneficial changes in levels of adiponectin, leptin, HS-CRP, and ICAM-1. Given current recommendations for higher protein intakes in older adults,

these findings strongly justify further study of the inflammatory impact of an enhanced protein weight loss diet in physical frail older adults with obesity. Given the magnitude of concern about elevated inflammatory status in older adults with obesity, the potential that lean high quality protein could reduce inflammation during weight reduction and the mechanism(s) responsible for this effect merit further exploration.

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*Six participants were enrolled for 3 rather than 6 months due to time constraints on study completion (Control n=4; Protein n=2).

Figure 1.
Consort Diagram

Table 1:

Descriptive Characteristics of Participants

	Control (n=14) Mean ± SD, n (%)	Protein (n=25) Mean ± SD, n (%)	Total (N=39) Mean ± SD, n (%)
Age (y)	68.8 ± 6.1	68.0 ± 5.5	68.3 ± 5.6
Body weight (kg)	101.7 ± 16.1	106.6 ± 24.5	105.0 ± 21.9
BMI (kg/m ²)	36.0 ± 5.3	37.5 ± 7.5	37.0 ± 6.8
Female (%)	11 (78.6)	20 (80.0)	31 (79.5)
Race			
Black (%)	3 (21.4)	8 (32.0)	11 (28.2)
White (%)	11 (78.6)	16 (64.0)	27 (69.2)
GFR (mL/min/1.73 m ²) ^{*†}	74.8 ± 19.4	80.3 ± 14.6	78.4 ± 16.4
Cardiovascular Disease	1 (7.1)	7 (29.2)	8 (20.5)
Type 2 Diabetes	1 (7.1)	5 (20.8)	6 (15.8)
Hypertension	11 (78.6)	16 (66.7)	27 (71.0)

Notes: BMI = Body mass index; GFR = glomerular filtration rate

^{*} GFR was calculated as (mL/min/1.73 m²) = 175×(Scr)-1.154×(Age)-0.203×(0.742 female)×(1.212 Black).

[†] Normal GFR values were defined as ≥ 60 mL/min/1.73 m².

Table 2.

Baseline and Change scores at 6 Months for Body Composition, Function, and Inflammatory Markers by Treatment Group

	Control (N=14) Mean $\pm$ SD	P value	Protein (N=25) Mean $\pm$ SD	P value	P value Control vs. Protein
Body Composition and Physical Function					
Body Weight, kg					
Baseline, mean $\pm$ SD	101.7 $\pm$ 16.1		106.6 $\pm$ 24.5		
Change at 6 months, mean $\pm$ SD	-4.8 $\pm$ 8.2	0.05	-8.4 $\pm$ 7.1	<0.001	0.17
Fat Mass, kg					
Baseline, mean $\pm$ SD	45.4 $\pm$ 18.9		51.7 $\pm$ 18.7		
Change at 6 months, mean $\pm$ SD	-3.7 $\pm$ 6.8	0.06	-7.5 $\pm$ 6.4	<0.001	0.09
Fat Mass, %					
Baseline, mean $\pm$ SD	47.1 $\pm$ 8.6		47.7 $\pm$ 8.3		
Change at 6 months, mean $\pm$ SD	-1.5 $\pm$ 3.7	0.17	-3.6 $\pm$ 3.0	<0.001	0.07
Lean Mass, kg					
Baseline, mean $\pm$ SD	49.4 $\pm$ 16.5		55.0 $\pm$ 11.4		
Change at 6 months, mean $\pm$ SD	-1.0 $\pm$ 2.8	0.21	-0.9 $\pm$ 1.6	0.009	0.91
BMI, kg/m²					
Baseline, mean $\pm$ SD	36.0 $\pm$ 5.3		37.5 $\pm$ 7.5		
Change at 6 months, mean $\pm$ SD	-2.3 $\pm$ 2.4	0.008	-2.9 $\pm$ 2.3	<0.001	0.45
SPPB, Total Score					
Baseline, mean $\pm$ SD	8.9 $\pm$ 1.1		8.2 $\pm$ 0.5		
Change at 6 months, mean $\pm$ SD	0.9 $\pm$ 1.6	0.07	2.4 $\pm$ 1.9	<0.001	0.028
Inflammatory Markers*					
Adiponectin, ng/ml					
Baseline, mean $\pm$ SD	21236.4 $\pm$ 8729.87		20007.3 $\pm$ 16990.33		
Change at 6 months, mean $\pm$ SD	1619.1 $\pm$ 5121.5	0.71	0.12 $\pm$ 0.19	0.004	0.074
Leptin, ng/ml					
Baseline, mean $\pm$ SD	96.2 $\pm$ 108.0		60.6 $\pm$ 45.1		
Change at 6 months, mean $\pm$ SD	-41.0 $\pm$ 85.5	0.16	-15.4 $\pm$ 24.9	0.001	0.70
IL-1, pg/ml					
Baseline, mean $\pm$ SD	0.02 $\pm$ 0.02		0.05 $\pm$ 0.10		
Change at 6 months, mean $\pm$ SD	-0.005 $\pm$ 0.02	0.26	-0.002 $\pm$ 0.16	0.89	0.97
IL-6, pg/ml					
Baseline, mean $\pm$ SD	4.5 $\pm$ 12.3		1.3 $\pm$ 0.9		
Change at 6 months, mean $\pm$ SD	-2.9 $\pm$ 10.2	0.34	0.1 $\pm$ 0.6	0.32	0.14
IL-8, pg/ml					
Baseline, mean $\pm$ SD	14.1 $\pm$ 5.0		22.5 $\pm$ 29.3		
Change at 6 months, mean $\pm$ SD	1.8 $\pm$ 6.2	0.31	-2.3 $\pm$ 8.4	0.62	0.23
TNF-α, pg/ml					

	Control (N=14) Mean ± SD	P value	Protein (N=25) Mean ± SD	P value	P value Control vs. Protein
Baseline, mean ± SD	2.4 ± 0.6		2.6 ± 0.6		
Change at 6 months, mean ± SD	0.2 ± 0.5	0.10	0.1 ± 0.7	0.92	0.21
CRP, ng/ml					
Baseline, mean ± SD	6078.8 ± 6029.2		7556.2 ± 11157.4		
Change at 6 months, mean ± SD	-941.2 ± 7022.5	0.54	-2437.8 ± 4709.3	0.005	0.34
SAA, ng/ml					
Baseline, mean ± SD	12117.4 ± 23020.4		15647.4 ± 33769.0		
Change at 6 months, mean ± SD	-6715.5 ± 23442.9	0.35	-8150.3 ± 33956.4	0.22	0.94
ICAM-1, ng/ml					
Baseline, mean ± SD	504.4 ± 112.9		513.9 ± 114.8		
Change at 6 months, mean ± SD	-21.4 ± 0.1	0.27	-53.0 ± 77.0	0.001	0.11
VCAM-1, ng/ml					
Baseline, mean ± SD	654.5 ± 178.3		649.3 ± 179.5		
Change at 6 months, mean ± SD	6.7 ± 55.0	0.69	-22.3 ± 66.4	0.07	0.14
GSP, ng/ml					
Baseline, mean ± SD	425.2 ± 113.7		429.6 ± 162.9		
Change at 6 months, mean ± SD	38.5 ± 83.9	0.11	-2.5 ± 93.5	0.46	0.11

*
P-values are based on log-transformed change scores

Table 3.

Baseline means and change scores at 6 months for calorie and macronutrient intakes by treatment group

	Control (n=14) Mean ± SD	P value	Protein (n=25) Mean ± SD	P value	P value Control vs. Protein
Total Energy, kcal					
Baseline	1942.6 ± 427.5		2064.8 ± 854.6		
Change at 6 months	-544.2 ± 430.2	0.001	-518.6 ± 759.7	0.003	0.91
Protein Intake, g/d					
Baseline	83.8 ± 16.4		91.6 ± 27.8		
Change at 6 months	-11.7 ± 19.4	0.042	24.4 ± 37.6	0.004	0.002
Average Protein Intake, g/kg of body weight/day					
Baseline	0.83 ± 0.21		0.89 ± 0.32		
Change at 6 months	-0.07 ± 0.22	0.28	0.33 ± 0.38	0.001	0.002
Protein Intake, % of total kcal					
Baseline	18 ± 4		19 ± 7		
Change at 6 months	3 ± 2	0.001	11 ± 7	<0.001	0.001
Carbohydrate Intake, g/d					
Baseline	217.4 ± 61.9		220.1 ± 102.5		
Change at 6 months	-46.7 ± 68.7	0.025	-71.4 ± 91.6	0.001	0.39
Carbohydrate Intake, % of total kcal					
Baseline	44 ± 7		43 ± 8		
Change at 6 months	4 ± 9	0.09	-4 ± 12	0.11	0.029
Fat Intake, g/d					
Baseline	83.3 ± 25.9		88.4 ± 46.6		
Change at 6 months	-34.1 ± 22.0	<0.001	-34.5 ± 44.5	0.001	0.97
Fat Intake, % of total kcal					
Baseline	38 ± 5		37 ± 8		
Change at 6 months	-6 ± 9	0.016	-6 ± 10	0.006	0.92
Saturated Fat Intake, g/d					
Baseline	27.4 ± 10.5		26.7 ± 14.5		
Change at 6 months	-11.8 ± 10.2	0.001	-7.4 ± 15.5	0.029	0.35