EFFECTS OF A HIGHER PROTEIN WEIGHT LOSS DIET
ON BODY COMPOSITION, METABOLIC DISEASE RISK AND PHYSICAL FUNCTION
IN POSTMENOPAUSAL WOMEN

BY

MINA MOJTAHEDI

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Nutritional Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Doctoral Committee:

       Professor Emeritus Donald K. Layman, Chair
       Associate Professor Ellen M. Evans, Director of Research
       Professor Jeffrey A. Woods
       Professor John G. Georgiadis

ABSTRACT

Adverse increases in adiposity and reductions in lean soft tissue (LST) are age related changes in body composition and are linked to an increased risk for metabolic disease and declines in physical function in older women. Higher protein intake under caloric reduced conditions eliciting weight loss may augment fat mass (FM) loss, which can reduce metabolic disease risk; and attenuate LST loss, which may benefit physical function. This study assessed effects of 6 mo weight loss supplemented with powdered protein or placebo on body composition, metabolic disease risk factors and physical function in older obese women. Twenty-six women (64.7 ± 5.8 yrs, BMI 32.5 ± 4.1 kg/m^2) were prescribed an energy-restricted diet (1400 kcal/d) and randomly assigned to PRO (n=13; 0.8 g/kg/d of dietary protein plus 45g/d of whey protein isolate supplement, resulting in ~30% of energy intake from protein, 40% of energy from carbohydrate and 30% of energy from fat) or CARB (n=13; 0.8 g/kg/d protein plus 50 g/d of an isocaloric maltodextrin supplement, resulting in ~18% of energy from protein, 52% of energy from carbohydrate and 30% of energy from fat) supplementation. Healthy eating based on the USDA My Pyramid diet education classes were taught by a registered dietitian every 2 weeks. Supervised exercise classes, consisting of flexibility and low to moderate intensity walking on an indoor track, were provided 5 d/wk, of which participants were required to attend 2-3 exercise classes/wk. The main outcome measures included the following: whole body FM and LST with DXA; thigh muscle volume, subcutaneous adipose tissue (SAT), intermuscular adipose tissue (IMAT) with MRI; glucose, insulin, C-reactive protein (CRP) and interleukin-6 (IL-6) from fasting blood samples; knee extension and flexion strength with isokinetic and isometric dynamometry; balance using the Star Excursion Balance Test (SEBT); and physical performance assessed from up and go, chair stand, stair climb and transfer tests. Three-day weighed food diaries were collected at baseline, month 3 and post-intervention, and 7-day pedometer step counts were measured monthly. PRO trended towards greater weight loss ($-8.0\pm6.2\%$, $-4.1\pm3.6\%$; p=0.059), had greater reductions in abdominal FM ($-14.8\pm15.0\%$, $-4.1\pm8.1\%$; p=0.037) but no significant differences in losses of FM ($-15.4\pm17.8\%$, $-6.1\pm5.4\%$; p=0.091), greater reductions in SAT (-18.8 ± 13.5, -7.9 ± 6.9; p=0.024) and IMAT (-9.2 ± 9.4, -1.0 ± 7.7; p=0.028) but no difference in IMAT relative to muscle volume (4.7 ± 5.8, 4.3 ± 8.6; p=0.883) compared to CARB, respectively. No differences were seen in LST changes ($-4.0\pm4.9\%$, $-2.0\pm4.1\%$; p=0.414), but PRO had a greater improvement in weight to leg LST ratio (-4.6 ± 3.6, $-$

1.8±2.6; p=0.033), greater reductions in absolute thigh muscle volume (-5.0±3.4, -0.8±3.9; p=0.010) and greater gains in relative thigh muscle volume (10.3±8.8%, 4.5±3.4; p=0.049) compared to CARB. Groups did not differ in changes in metabolic disease risk factors, knee strength, balance or physical performance measures. Change in SAT volume was an independent predictor of % change in CRP explaining 30% of the variance (p=0.021), and change in abdominal fat mass showed a trend towards independently predicting % change in CRP (p=0.057). Changes in weight to leg LST ratio predicted changes in up and go (adjusted r^2=0.189, p=0.02); whereas whole body %LST predicted changes in balance (adjusted r^2=0.179, p=0.04). Higher protein weight loss diet in older women contributes to more weight loss and optimal changes in body composition compared to a greater carbohydrate regimen. Protein intake does not appear to directly impact changes in metabolic risk factors or physical function; however, changes in abdominal fat and SAT reduce systemic inflammation, and preservation of LST with weight reduction improves balance and physical performance.

ACKNOWLEDGMENTS

I shall be telling this with a sigh

Somewhere ages and ages hence:

Two roads diverged in a wood, and I -

I took the one less traveled by,

And that has made all the difference.

Excerpt from "The Road Not Taken" by Robert Frost

Thanks to my mentor, Ellen, for her constant support and guidance through the highs and the lows of my development as a scientist, colleague and lifelong learner.

Thanks to my coach, Frog, for his unwavering support and trustworthy counsel, and for being my rock.

Thanks to my nearest and dearest friends and confidants, whom I carry in my heart no matter how many states, countries, oceans or continents there may be between us.

Lastly but most importantly, thanks to my parents and my sister, whom I love with all my heart, and who patiently bear the brunt of me always choosing the road less traveled.

TABLE OF CONTENTS

CHAPTER 1
Introduction

1.1 Significance

Obesity has reached epidemic proportions in the United States. NHANES II and III data show that the prevalence of overweight and obesity increase with age (1). With approximately 37% and 32% of women 60 years and older being classified as overweight and obese, respectively, combined with the known associations of excess adiposity to adverse health risk obesity in this cohort is a major public health concern (2). According to 2000 U.S. census data, approximately 47 million Americans, or 40% of the adult population, meet the diagnosis criteria for metabolic syndrome (3), with a greater prevalence in women than in men, and higher cardiovascular disease mortality rates in women (4).

Several factors place women at a greater risk for metabolic disease. Longitudinal data from the Health ABC Study shows that associations between abdominal obesity, dyslipidemia and high blood pressure with metabolic syndrome are stronger in older women than men (5). Postmenopausal shifts in hormone levels are associated with women increasing abdominal obesity (3), a strong independent predictor of metabolic disease (6). Abdominal fat is associated with low-grade inflammation, measured by increased circulating levels of C-reactive protein (CRP), a key characteristic of metabolic disease and an independent predictor of future cardiovascular events (7). Novel adipose tissue depots, such as subcutaneous adipose tissue (SAT), measured using magnetic resonance imaging (MRI), have been associated with glucose intolerance (8) and insulin resistance (9); and intermuscular adipose tissue (IMAT) with type 2 diabetes (10) and metabolic syndrome (11). Higher levels of systemic inflammatory markers are found in women than in age-matched men (12-16), further exacerbating gender differences in health risks.

Aging is not only associated with an increase in fat mass, but also a decrease in skeletal muscle mass (17). Moreover, obese older adults tend to have lower skeletal muscle mass relative to body weight (18-20), and poor muscle quality (21-23) relative to non-obese counterparts. These age-related changes in body composition can result in decreased muscle strength (24), mobility limitations (25), an inability to perform daily activities (19), and greater risk for physical disability (18, 26). Gender inequity exists with regard to body composition and physical

function as older women have greater adiposity (27-28), less lean mass (27-28), lower muscle quality (27, 29), worse balance and gait function than men of similar age (30-32), as well as a greater prevalence of falling (33-34) and a higher risk of physical disability (35-36).

Because weight loss in the elderly may result in loss of proportionally more fat free mass (FFM) than fat mass (28), the type of weight loss therapy (i.e. diet vs. exercise, or macronutrient content of diet) and magnitude and rate of weight loss are important factors impacting changes in body composition. Emerging data from the Health ABC Study indicate that older adults consuming greater amounts of protein are less likely to lose lean mass over time (37). Increasing protein intake as part of a low-calorie diet has been shown to promote more total weight and fat mass loss (38-41) while also attenuating loss of muscle relative to changes in adiposity (41-44) compared to an isocaloric diet with a conventional macronutrient composition (i.e. higher carbohydrate). The American Society for Nutrition and NAASO, The Obesity Society, specifically recommend weight loss therapies that maximize fat mass loss and minimize skeletal muscle loss in order to improve metabolic abnormalities and maintain physical function and independence in the elderly (17, 21). In this context, the overarching goal of this study was to assess the relative effects of weight loss diets varying in macronutrient content on a) whole body, regional and ectopic fat or adipose tissue and b) lean mass and muscle volume in overweight older women.

1.2 Specific Aims

Primary Aim 1: To assess the effect of a higher protein, lower carbohydrate (PRO) intake during energy restriction on 1) body fat depots, particularly thigh SAT and IMAT, and 2) indicators of metabolic disease risk, compared with an isocaloric conventional higher carbohydrate, lower protein (CARB) intake in older women.

Secondary Aim 1: To examine the relative association of changes in the various fat or adipose tissue depots with changes in markers of metabolic disease risk.

We hypothesized that a PRO weight loss diet would result in greater reductions in fat mass and adipose tissue compared with an isocaloric CARB diet, and that this would be related to beneficial effects on metabolic disease risk.

Primary Aim 2: To assess the effect of a PRO weight loss diet on lean soft tissue, muscle volume and physical function, compared with an isocaloric conventional higher carbohydrate, lower protein weight loss diet CARB.

Secondary Aim 2: To identify measures of muscle quality which best predict changes in physical function, characterized by strength, balance and physical performance.

We hypothesized that a PRO weight loss diet would result in attenuation of lean mass loss compared with an isocaloric CARB diet, and that these changes may convey beneficial effects on physical function.

1.3 References

1.   Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and Trends in Obesity Among US Adults, 1999-2000. JAMA. 2002 October 9, 2002;288:1723-7.

2.   Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of Overweight and Obesity in the United States, 1999-2004. JAMA. 2006 April 5, 2006;295:1549-55.

3.   Regitz-Zagrosek V, Lehmkuhl E, Weickert M. Gender differences in the metabolic syndrome and their role for cardiovascular disease. Clinical Research in Cardiology. 2006;95:136-47.

4.   Evangelista O, McLaughlin MA. Review of cardiovascular risk factors in women. Gender Medicine. 2009;6:17-36.

5.   Penninx BWJH, Nicklas BJ, Newman AB, Harris TB, Goodpaster BH, Satterfield S, de Rekeneire N, Yaffe K, Pahor M, Kritchevsky SB. Metabolic Syndrome and Physical Decline in Older Persons: Results from the Health, Aging and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2009 January 2009;64A:96-102.

6.   Huffman DM, Barzilai N. Role of visceral adipose tissue in aging. Biochimica et Biophysica Acta (BBA) - General Subjects. 2009;1790:1117-23.

7.   Devaraj S, Singh U, Jialal I. Human C-reactive protein and the metabolic syndrome. Curr Opin Lipidol. 2009 Jun;20:182-9.

8.   Kohrt WM, Holloszy JO. Loss of skeletal muscle mass with aging: effect on glucose tolerance. J Gerontol A Biol Sci Med Sci. 1995 Nov;50 Spec No:68-72.

9.   Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO. Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50-95 year olds. Diabetes Care. 2006 Mar;29:673-8.

10.   Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, Schwartz AV, Kritchevsky S, Newman AB. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care. 2003 Feb;26:372-9.

11.   Goodpaster BH, Krishnaswami S, Harris TB, Katsiaras A, Kritchevsky SB, Simonsick EM, Nevitt M, Holvoet P, Newman AB. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med. 2005 Apr 11;165:777-83.

12.   Abdullah SM, Khera A, Leonard D, Das SR, Canham RM, Kamath SA, Vega GL, Grundy SM, McGuire DK, de Lemos JA. Sex differences in the association between leptin and CRP: results from the Dallas Heart Study. Atherosclerosis. 2007 Dec;195:404-10.

13.   Arena R, Arrowood JA, Fei DY, Helm S, Kraft KA. The relationship between C-reactive protein and other cardiovascular risk factors in men and women. J Cardiopulm Rehabil. 2006 Sep-Oct;26:323-7; quiz 8-9.

14.   Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr., Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol. 2005 Aug 2;46:464-9.

15.   Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Jr., Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J. 2006 Sep;152:593-8.

16.   Pieroni L, Bastard JP, Piton A, Khalil L, Hainque B, Jardel C. Interpretation of circulating C-reactive protein levels in adults: body mass index and gender are a must. Diabetes Metab. 2003 Apr;29:133-8.

17.   Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Am J Clin Nutr. 2005 Nov;82:923-34.

18.   Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc. 2002 May;50:889-96.

19.   Zoico E, Di Francesco V, Guralnik JM, Mazzali G, Bortolani A, Guariento S, Sergi G, Bosello O, Zamboni M. Physical disability and muscular strength in relation to obesity and

different body composition indexes in a sample of healthy elderly women. Int J Obes Relat Metab Disord. 2004 Feb;28:234-41.

20.   Estrada M, Kleppinger A, Judge JO, Walsh SJ, Kuchel GA. Functional impact of relative versus absolute sarcopenia in healthy older women. J Am Geriatr Soc. 2007 Nov;55:1712-9.

21.   Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Obes Res. 2005 Nov;13:1849-63.

22.   Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E, Newman AB. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. J Appl Physiol. 2001 Jun;90:2157-65.

23.   Villareal DT, Banks M, Siener C, Sinacore DR, Klein S. Physical frailty and body composition in obese elderly men and women. Obes Res. 2004 Jun;12:913-20.

24.   Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. J Gerontol A Biol Sci Med Sci. 2006 October 1, 2006;61:1059-64.

25.   Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB, for the Health ABCS. Muscle Mass, Muscle Strength, and Muscle Fat Infiltration as Predictors of Incident Mobility Limitations in Well-Functioning Older Persons. J Gerontol A Biol Sci Med Sci. 2005 March 1, 2005;60:324-33.

26.   Wray LA, Blaum CS. Explaining the role of sex on disability: a population-based study. Gerontologist. 2001 Aug;41:499-510.

27.   Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M, the Health AaBCRG. Strength and Muscle Quality in a Well-Functioning Cohort of Older Adults: The Health, Aging and Body Composition Study. Journal of the American Geriatrics Society. 2003;51:323-30.

28.   Newman AB, Lee JS, Visser M, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Nevitt M, Harris TB. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. Am J Clin Nutr. 2005 October 1, 2005;82:872-8.

29.   Katsiaras A, Newman AB, Kriska A, Brach J, Krishnaswami S, Feingold E, Kritchevsky SB, Li R, Harris TB, et al. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. J Appl Physiol. 2005 Jul;99:210-6.

30.   Musselman K, Brouwer B. Gender-related differences in physical performance among seniors. J Aging Phys Act. 2005 Jul;13:239-53.

31.   Hageman PA, Leibowitz JM, Blanke D. Age and gender effects on postural control measures. Arch Phys Med Rehabil. 1995 Oct;76:961-5.

32.   Wolfson L, Whipple R, Derby CA, Amerman P, Nashner L. Gender differences in the balance of healthy elderly as demonstrated by dynamic posturography. J Gerontol. 1994 Jul;49:M160-7.

33.   Colledge NR, Cantley P, Peaston I, Brash H, Lewis S, Wilson JA. Ageing and balance: the measurement of spontaneous sway by posturography. Gerontology. 1994;40:273-8.

34.   Prudham D, Evans JG. Factors associated with falls in the elderly: a community study. Age Ageing. 1981 Aug;10:141-6.

35.   Penninx BW, Ferrucci L, Leveille SG, Rantanen T, Pahor M, Guralnik JM. Lower extremity performance in nondisabled older persons as a predictor of subsequent hospitalization. J Gerontol A Biol Sci Med Sci. 2000 Nov;55:M691-7.

36.   Murtagh KN, Hubert HB. Gender differences in physical disability among an elderly cohort. Am J Public Health. 2004 Aug;94:1406-11.

37.   Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008 Jan;87:150-5.

38.   Brinkworth GD, Noakes M, Keogh JB, Luscombe ND, Wittert GA, Clifton PM. Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. Int J Obes Relat Metab Disord. 2004 May;28:661-70.

39.   Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. Appl Physiol Nutr Metab. 2007 Aug;32:743-52.

40.   Kerksick C, Thomas A, Campbell B, Taylor L, Wilborn C, Marcello B, Roberts M, Pfau E, Grimstvedt M, et al. Effects of a popular exercise and weight loss program on weight loss, body composition, energy expenditure and health in obese women. Nutrition & Metabolism. 2009;6:23.

41.   Layman DK, Evans EM, Erickson D, Seyler J, Weber J, Bagshaw D, Griel A, Psota T, Kris-Etherton P. A Moderate-Protein Diet Produces Sustained Weight Loss and Long-Term Changes in Body Composition and Blood Lipids in Obese Adults. J Nutr. 2009 March 1, 2009;139:514-21.

42.   Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. J Nutr. 2005 Aug;135:1903-10.

43.   Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006 February 1, 2006;83:260-74.

44.   Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. Dietary Protein, Weight Loss, and Weight Maintenance. Annual Review of Nutrition. 2009;29:21-41.

Figure 1. Hypothesized interrelations among protein intake and primary outcomes in this study. Bold arrows represent primary and secondary aims of this study and dotted arrows represent other possible interactions.
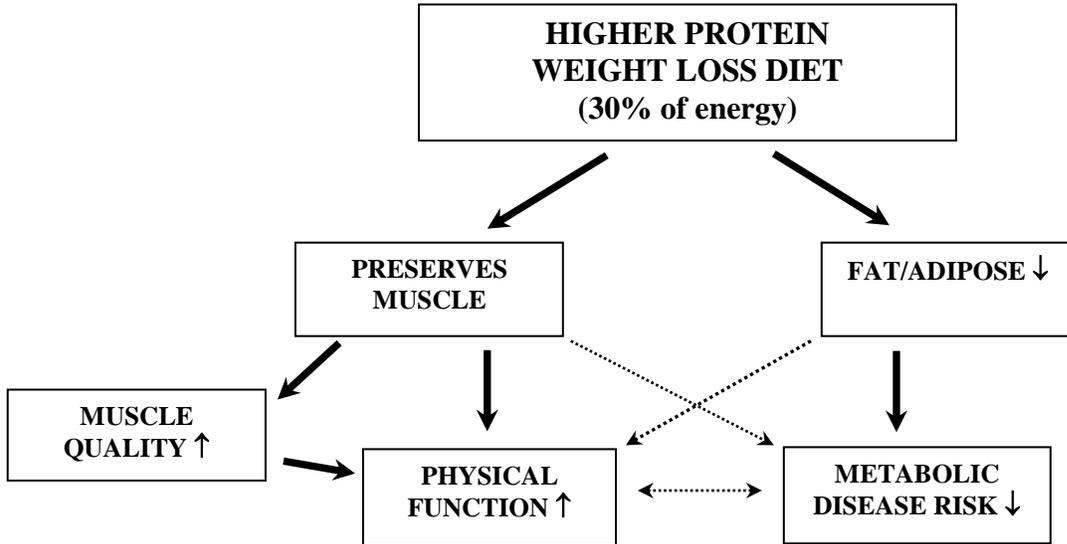
CHAPTER 2
Literature Review

Obesity has reached epidemic proportions in the United States. NHANES II and III data show that the prevalence of overweight [defined as body mass index (BMI) ≥ 25 kg/m^2] and obesity (defined as BMI ≥ 30 kg/m^2) increase with age, and furthermore, the prevalence of obesity has increased in older adults over the last two decades (1). More recent NHANES III data indicate, however, that although the prevalence of overweight and obesity appears to be leveling off in men over the age of 60, prevalence in women of the same age may be slightly decreasing (2). Nevertheless, with approximately 37% and 32% of elderly women classified as overweight and obese, respectively, this is a major health concern (2).

Based on NHANES III data, the prevalence of sarcopenia, defined by skeletal muscle mass relative to body weight or height, increases with age in both men and women, but more so in women (3). Sarcopenia is not simply an issue of thin frailty; rather, severe sarcopenia, defined by a sarcopenia index below two standard deviations of young adult values, is also found in obese elderly men and women (3). Even if muscle mass is maintained in obese elderly, muscle quality becomes increasingly compromised with aging, indicated by lower muscle attenuation (decreased muscle density) and or adipose infiltration of the muscle, measured using computed tomography (4).

This chapter summarizes the current research on health implications of age-related changes in body composition, including 1) the association between adiposity and risk for metabolic disease, and 2) the association between decreased muscle mass and risk for physical limitations. Lastly, weight loss regimens aimed at improving these health risks are addressed, with an emphasis on dietary interventions that incorporate higher protein, lower carbohydrate (PRO) intake compared to a conventional higher carbohydrate, lower protein (CARB) diet.

2.1 Adiposity Associated with Aging Increases Risk for Metabolic Disease

Metabolic syndrome comprised of a cluster of health abnormalities, including central obesity, dyslipidemia, hypertension, and impaired fasting glucose, all of which are also risk factors for other metabolic diseases such as type 2 diabetes and cardiovascular disease. The prevalence for obesity and metabolic syndrome, and thus the risk for other disease states,

increases with age (5). In an aged population, comparing men and women, health inequities exist with older women having greater adiposity (6), and greater subcutaneous thigh adipose tissue than men of the same age (4, 7). Despite equal levels of thigh IMAT, muscle quality, measured by muscle attenuation, is lower (i.e. greater intramyocellular lipid accumulation) in older women than in older men (4, 7). According to 2000 U.S. census data, approximately 47 million Americans, or 40% of the adult population, meet the diagnosis criteria for metabolic syndrome (5), with a greater prevalence in women than in men (8). Furthermore, cardiovascular disease mortality rates are higher in women than in men (9).

Several factors place women at a greater risk for metabolic disease. Firstly, women tend to present with greater insulin resistance and glucose intolerance measured by oral glucose tolerance test (5). Secondly, longitudinal data from the Health ABC Study shows that associations between abdominal obesity, dyslipidemia and high blood pressure with metabolic syndrome are stronger in older women than men (8). Postmenopausal shifts in hormone levels lead to women often developing abdominal obesity (5), a stronger independent predictor of metabolic disease risk than BMI (10). In fact, data from the Baltimore Longitudinal Study of Aging suggests that every 5 cm increase in waist circumference is associated with a 70% higher risk for developing metabolic syndrome over time (11).

Adipocyte dysfunction in visceral adiposity plays a major role in the development of metabolic disease. Although inflammatory markers are not standard in diagnosing metabolic syndrome, low-grade inflammation, measured by increased circulating levels of C-reactive protein (CRP), is a key characteristic and is known to be an independent predictor of future cardiovascular events (12). The pathophysiology of metabolic syndrome seems to be attributable to visceral adipose, a metabolically active tissue which secretes various endocrine and pro-inflammatory products, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), leptin and adiponectin, starting a cascade of events leading to insulin resistance (13). This in turn impairs the function of organs, such as the muscle and liver, in maintaining glucose and lipid homeostasis, and leads to lipid deposition in ectopic tissues (13).

Aging is associated with increased levels of several inflammatory cytokines including IL-6 and CRP (14). Other factors that contribute to the low-grade inflammation typically found in older adults, in addition to the aging immune system and possible sub-clinical infections, are increased adipose tissue and decreased muscle mass (14), both of which are age-related changes

in body composition. In addition, physical activity, which is generally low in the elderly population (15), is inversely associated with CRP levels in the elderly (16), which may be partly related to the link between habitual physical activity and adiposity and decreased muscle mass. Higher levels of systemic inflammatory markers are found in women than in age-matched men (17-21), further exacerbating gender differences in health risks. Moreover, the association between fat mass and inflammation is stronger in older women than men of similar age (22), suggesting that the greater adiposity generally found in women places them at even higher risk for inflammation.

Interestingly, recent findings from the Health ABC Study link obesity and metabolic syndrome with incident mobility limitations (8, 23). Both obesity and metabolic syndrome independently predict development of mobility limitations in older women (23). Furthermore, even after adjusting for whole body obesity, abdominal obesity remained independently associated with incident mobility limitations (8) and elevated inflammatory markers IL-6, TNF-α and CRP, partly explained the association between obesity, metabolic syndrome and mobility limitations (23).

2.2 Physical Function Changes with Aging, Adiposity and Sarcopenia

According to the Behavioral Risk Factor Surveillance System (BRFSS), almost 30% of the population above 65 years of age in the United States are limited by a physical disability (24). Functional limitations can reduce vitality, increase physical pain and have a significant impact on quality of life in the elderly (25). Lower physical function, measured subjectively by reported difficulties performing Activities of Daily Living (ADL) and objectively by physical performance tests, is often associated with age-related changes in body composition, such as increased body fatness and a lower sarcopenic index (3, 26). In addition, low muscle strength and low muscle attenuation are associated with a higher risk for mobility limitations in walking and climbing steps (27).

Due to gender differences in age-related changes in body composition, women are at a greater risk for developing mobility limitations compared to men. The prevalence of sarcopenia according to NHANES III data is greater in women than men above 60 years of age; approximately 70% in older women compared with 50% in older men (3). Although the magnitude of associations between muscle quality and muscle strength appear to be similar in

11

men and women (4) and men lose proportionally more strength due to aging (28), men are nevertheless more fatigue resistant in strength tests (7), have greater leg strength and force production than women of the same age (4, 7, 29). Since both body composition and functional outcomes are less favorable in older women than men, it is not surprising that the prevalence of physical disability and functional impairment in older women is greater than the prevalence in men of similar age, with the differences being even greater between sarcopenic older women and men (3).

Components of physical function include muscle strength, balance and physical performance (i.e. measures of walking, gait, timed tasks), and there is a wide variety of studies in the literature, describing associations between different measures of body composition and components of physical function. Briefly, in cross-sectional studies assessing associations between body composition and measures of physical function, women with obese sarcopenia have a 3.8 times greater risk for functional limitation than women with greater levels of skeletal muscle mass relative to weight (26). Furthermore, lower relative whole body skeletal muscle mass is reported to be associated with lower leg strength (26) and poor physical performance (3). In healthy older women skeletal muscle mass and fat mass relative to body weight are strong predictors of physical performance measured by walking, gait and balance (30). In addition, thigh muscle attenuation (level of fat infiltration of the muscle, i.e. a parameter for muscle quality) has been associated with self-reported mobility limitations (27), therefore it may also be important to measure changes within the muscle.

2.3 Weight Loss Effects on Metabolic Disease Risk and Physical Function

The effects of weight loss on health risks in older women are somewhat controversial. Obesity in older adults is associated with several health risks, including type 2 diabetes mellitus, cardiovascular disease, cancer and arthritis, and weight loss of 10% is recommended for improving mortality risk (31). However, weight loss of this magnitude can have negative consequences in older women. Firstly, some population studies show that weight loss in the elderly is associated with increased mortality, however, this is often involuntary and due to disease (32). Importantly, weight loss in older women decreases bone density and increases risk for osteoporosis (33). Furthermore, weight loss results in not only loss of fat mass, but also FFM (6, 34-35), which in older women may impact physical function. Although intuitive, the

interrelations between changes in fat and lean mass, muscle strength and physical performance are understudied in this population.

Moderate weight loss of 5-10% of initial body weight over 3-6 months via an energy restricted diet generally has a significant impact on body composition in older women: whole body fat mass, subcutaneous fat, FFM, and appendicular lean tissue decrease (34-37). Muscle quality also improves with weight loss. Muscle biopsy samples from obese patients who had undergone weight loss therapy show that muscle fiber lipid content is increased in obesity and decreases as a result of weight loss (38), although this has not been shown in all studies (39). Furthermore, IMAT and low-density muscle area decreases without a change in total muscle area, in moderate weight loss in obese postmenopausal women (35).

Findings on changes in body composition vary when weight loss is induced with exercise in addition to dietary energy restriction. A decrease in fat depots (whole body fat mass, thigh subcutaneous and IMAT areas) is consistent throughout studies (36, 40). As for muscle mass, some studies show a decrease in FFM or lean mass (37), whereas it appears that exercise can help preserve FFM (40) and skeletal muscle (36-37) in other studies. Also, it is not clear whether exercise combined with dietary energy restriction decreases muscle fiber lipid content (39).

Major prospective lifestyle intervention trials have shown that weight loss of 7% of initial weight through diet, exercise or a combination of both can decrease the risk for type 2 diabetes by 58%, or even up to 80% if exercise levels are maintained at 4 h/wk (41). Changes in abdominal obesity, in particular, can have a major impact on metabolic disease risk factors. Decreasing visceral adipose by 25% within 12 weeks with a very low calorie diet can improve insulin sensitivity by 33% (42), although it appears moderate weight loss is preferential for sustained benefits from reductions in abdominal fat (43). Weight loss is a key component of reducing inflammation, as it is well established that changes in weight and fat mass resulting from an energy-restricted diet are associated with proportional changes in CRP levels (44-45), particularly in obese postmenopausal women (46).

Population studies show that overweight and obesity in older women are associated with poor physical function (47-48). Although a limited number of studies have been conducted in older adults assessing the effect of weight loss on physical function, weight loss is shown to help improve physical function and quality of life in the elderly (25). A moderate weight loss regimen that incorporated both energy restriction and exercise resulted in a decrease in fat mass and no

13

change in FFM, and improved measures of physical performance such as balance, gait, walking speed, climbing stairs and knee strength in healthy obese elderly (49) and in obese elderly with knee osteoarthritis (50-52). Limited data in obese elderly with knee osteoarthritis undergoing weight loss therapy indicate that changes in fat and lean mass, assessed with dual energy X-ray absorptiometry, are inversely associated with knee strength (52).

Controversy in recommendations for weight loss in older populations is in regards to the trade-off between improved health outcomes from decreased mortality and metabolic disease risk on the one hand, and poor physical independence from decreased muscle mass leading to reduced strength on the other. Additional research is clearly needed on weight loss regimens that may promote loss of fat while preserving FFM, so that improvements may be seen in both a reduced risk for metabolic disease and improvements in physical function.

2.4 Macronutrient Composition of Weight Loss Diets

Determining the optimal macronutrient composition of a weight loss diet is important because this may have favorable effects on body composition, as weight loss is often accompanied by loss of not only fat mass but also lean mass, which could in turn impact risk of reduced physical function. The most beneficial macronutrient composition of an energy restricted weight loss diet is still debated.

PRO weight loss diets appear to result in greater weight loss compared with a CARB diet (53-56). Also, it appears that more participants are successful in reaching a 10% weight loss goal when on a PRO weight loss diet (57). Furthermore, longer-term PRO weight loss diets are shown to predict weight loss in women (58). Mechanisms for improved weight loss from a PRO diet are likely due to a greater satiety associated with protein intake, and an increased thermogenic effect of food (59-60).

Findings from a meta-analysis on the macronutrient composition of energy-restricted diets indicate that protein intake of >1.05g/kg body weight is associated with greater retention of FFM than diets with protein intake below this level, but protein intake at this level is not associated with greater loss of fat mass (61). Alternatively, lower carbohydrate intake (≤41% of energy intake) is associated with greater FFM and fat mass loss than higher carbohydrate diets (61). In addition, more recent findings not included in this meta-analysis show no effects of the dietary macronutrient composition on weight loss, or changes in fat and lean mass (62-65).

Recent findings from the Health ABC prospective cohort study report an association between protein intake and changes in lean mass over 3 years: elderly men and women with a protein intake 1.2g/kg body weight lost less lean and appendicular lean mass that those with 0.8g/kg (66). This is supported by data from our laboratory showing that a PRO weight loss diet (1.6g protein/kg body weight) is more effective in decreasing fat mass while preserving lean mass in middle-aged women than a CARB (56). Furthermore, the PRO weight loss diet combined with exercise had an additive effect on body composition, decreasing relatively more body fat while preserving more lean mass than a CARB diet combined with exercise or the PRO diet alone (56).

Studies examining effects of a PRO weight loss diet have generally found improved metabolic profile, including fasting glucose, fasting insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and free fatty acids, following weight loss but with no significant effect of the PRO dietary treatment (53, 55, 67-69). Most studies have found that protein content of a weight loss diet does not appear to be a significant factor in decreasing inflammation, as both PRO and CARB weight loss diets appear to decrease CRP levels by a similar magnitude (53, 55, 63). However, CRP was found to be associated with changes in FM following a high protein weight loss diet (70).

Factors that may influence the outcomes and explain differences in findings between studies include different protein sources used to increase intake (food items vs. protein supplementation with powder) and how protein intake was divided over the course of the day (one bolus/day vs. several) which may impact bioavailability as well as satiety, in addition to duration of the study and adherence to the weight loss diet. Also, findings may vary by gender, as lean mass may be preserved more effectively during weight loss in women with PRO regimens (71). Additional studies are needed to assess the effects of a PRO weight loss diet on body composition. In addition, the effect of a PRO diet on adipose tissue infiltration of the muscle measured by MRI is unknown. Furthermore, to our knowledge, no studies have assessed associations between changes in body composition following a PRO weight loss diet and physical function in the older women.

2.5 References

1.   Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and Trends in Obesity Among US Adults, 1999-2000. JAMA. 2002 October 9, 2002;288:1723-7.

2.   Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of Overweight and Obesity in the United States, 1999-2004. JAMA. 2006 April 5, 2006;295:1549-55.

3.   Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc. 2002 May;50:889-96.

4.   Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E, Newman AB. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. J Appl Physiol. 2001 Jun;90:2157-65.

5.   Regitz-Zagrosek V, Lehmkuhl E, Weickert M. Gender differences in the metabolic syndrome and their role for cardiovascular disease. Clinical Research in Cardiology. 2006;95:136-47.

6.   Newman AB, Lee JS, Visser M, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Nevitt M, Harris TB. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. Am J Clin Nutr. 2005 October 1, 2005;82:872-8.

7.   Katsiaras A, Newman AB, Kriska A, Brach J, Krishnaswami S, Feingold E, Kritchevsky SB, Li R, Harris TB, et al. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. J Appl Physiol. 2005 Jul;99:210-6.

8.   Penninx BWJH, Nicklas BJ, Newman AB, Harris TB, Goodpaster BH, Satterfield S, de Rekeneire N, Yaffe K, Pahor M, Kritchevsky SB. Metabolic Syndrome and Physical Decline in Older Persons: Results from the Health, Aging and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2009 January 2009;64A:96-102.

9.   Evangelista O, McLaughlin MA. Review of cardiovascular risk factors in women. Gender Medicine. 2009;6:17-36.

10.   Huffman DM, Barzilai N. Role of visceral adipose tissue in aging. Biochimica et Biophysica Acta (BBA) - General Subjects. 2009;1790:1117-23.

11.   Scuteri A, Morrell CH, Najjar SS, Muller D, Andres R, Ferrucci L, Lakatta EG. Longitudinal Paths to the Metabolic Syndrome: Can the Incidence of the Metabolic Syndrome Be Predicted? The Baltimore Longitudinal Study of Aging. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2009 May 2009;64A:590-8.

12.   Devaraj S, Singh U, Jialal I. Human C-reactive protein and the metabolic syndrome. Curr Opin Lipidol. 2009 Jun;20:182-9.

13.   Lechleitner M. Obesity and the Metabolic Syndrome in the Elderly – A Mini-Review. Gerontology. 2008;54:253-9.

14.   Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. Exp Gerontol. 2004 May;39:687-99.

15.   Center, for, Disease, Control, and, Prevention. Behavioral Risk Factor Surveillance System: Prevalence Data Physical Activity. 2006 April 17 2007 [cited 2008 Feb 5]; Available from: http://apps.nccd.cdc.gov/brfss/sex.asp?cat=EX&yr=2006&qkey=4347&state=UB

16.   Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between Physical Activity and Markers of Inflammation in a Healthy Elderly Population. Am J Epidemiol. 2001 February 1, 2001;153:242-50.

17.   Abdullah SM, Khera A, Leonard D, Das SR, Canham RM, Kamath SA, Vega GL, Grundy SM, McGuire DK, de Lemos JA. Sex differences in the association between leptin and CRP: results from the Dallas Heart Study. Atherosclerosis. 2007 Dec;195:404-10.

18.   Arena R, Arrowood JA, Fei DY, Helm S, Kraft KA. The relationship between C-reactive protein and other cardiovascular risk factors in men and women. J Cardiopulm Rehabil. 2006 Sep-Oct;26:323-7; quiz 8-9.

19.   Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr., Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol. 2005 Aug 2;46:464-9.

20.   Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Jr., Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J. 2006 Sep;152:593-8.

21.   Pieroni L, Bastard JP, Piton A, Khalil L, Hainque B, Jardel C. Interpretation of circulating C-reactive protein levels in adults: body mass index and gender are a must. Diabetes Metab. 2003 Apr;29:133-8.

22.   Thorand B, Baumert J, Doring A, Herder C, Kolb H, Rathmann W, Giani G, Koenig W. Sex differences in the relation of body composition to markers of inflammation. Atherosclerosis. 2006 Jan;184:216-24.

23.   Stenholm S, Koster A, Alley DE, Houston DK, Kanaya A, Lee JS, Newman AB, Satterfield S, Simonsick EM, et al. Joint Association of Obesity and Metabolic Syndrome With Incident Mobility Limitation in Older Men and Women—Results From the Health, Aging, and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2010 January 2010;65A:84-92.

24.	Center, for, Disease, Control, and, Prevention. Behavioral Risk Factor Surveillance System: Prevalence Data Disability. 2005 April 17 2007 [cited 2008 Jan 31]; Available from: http://apps.nccd.cdc.gov/brfss/age.asp?cat=DL&yr=2005&qkey=4000&state=UB

25.	Fine JT, Colditz GA, Coakley EH, Moseley G, Manson JE, Willett WC, Kawachi I. A prospective study of weight change and health-related quality of life in women. Jama. 1999 Dec 8;282:2136-42.

26.	Zoico E, Di Francesco V, Guralnik JM, Mazzali G, Bortolani A, Guariento S, Sergi G, Bosello O, Zamboni M. Physical disability and muscular strength in relation to obesity and different body composition indexes in a sample of healthy elderly women. Int J Obes Relat Metab Disord. 2004 Feb;28:234-41.

27.	Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB, for the Health ABCS. Muscle Mass, Muscle Strength, and Muscle Fat Infiltration as Predictors of Incident Mobility Limitations in Well-Functioning Older Persons. J Gerontol A Biol Sci Med Sci. 2005 March 1, 2005;60:324-33.

28.	Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. J Gerontol A Biol Sci Med Sci. 2006 October 1, 2006;61:1059-64.

29.	Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M, the Health AaBCRG. Strength and Muscle Quality in a Well-Functioning Cohort of Older Adults: The Health, Aging and Body Composition Study. Journal of the American Geriatrics Society. 2003;51:323-30.

30.	Estrada M, Kleppinger A, Judge JO, Walsh SJ, Kuchel GA. Functional impact of relative versus absolute sarcopenia in healthy older women. J Am Geriatr Soc. 2007 Nov;55:1712-9.

31.	Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Am J Clin Nutr. 2005 Nov;82:923-34.

32.	Thomas DR. Weight loss in older adults. Rev Endocr Metab Disord. 2005 May;6:129-36.

33.	Shapses SA, Riedt CS. Bone, body weight, and weight reduction: what are the concerns? J Nutr. 2006 Jun;136:1453-6.

34.	Gallagher D, Kovera AJ, Clay-Williams G, Agin D, Leone P, Albu J, Matthews DE, Heymsfield SB. Weight loss in postmenopausal obesity: no adverse alterations in body composition and protein metabolism. Am J Physiol Endocrinol Metab. 2000 Jul;279:E124-31.

35.   Mazzali G, Di Francesco V, Zoico E, Fantin F, Zamboni G, Benati C, Bambara V, Negri M, Bosello O, Zamboni M. Interrelations between fat distribution, muscle lipid content, adipocytokines, and insulin resistance: effect of moderate weight loss in older women. Am J Clin Nutr. 2006 Nov;84:1193-9.

36.   Janssen I, Fortier A, Hudson R, Ross R. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. Diabetes Care. 2002 Mar;25:431-8.

37.   Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Ehsani AA, Holloszy JO. Lower extremity muscle size and strength and aerobic capacity decrease with caloric restriction but not with exercise-induced weight loss. J Appl Physiol. 2007 Feb;102:634-40.

38.   Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism. 2000 Apr;49:467-72.

39.   Malenfant P, Tremblay A, Doucet E, Imbeault P, Simoneau JA, Joanisse DR. Elevated intramyocellular lipid concentration in obese subjects is not reduced after diet and exercise training. Am J Physiol Endocrinol Metab. 2001 Apr;280:E632-9.

40.   Ryan AS, Nicklas BJ, Berman DM, Dennis KE. Dietary restriction and walking reduce fat deposition in the midthigh in obese older women. Am J Clin Nutr. 2000 Sep;72:708-13.

41.   Horton ES. Effects of Lifestyle Changes to Reduce Risks of Diabetes and Associated Cardiovascular Risks: Results from Large Scale Efficacy Trials. Obesity. 2009;17:S43-S8.

42.   Viljanen APM, Lautamäki R, Järvisalo M, Parkkola R, Huupponen R, Lehtimäki T, Rönnemaa T, Raitakari OT, Iozzo P, Nuutila P. Effects of weight loss on visceral and abdominal subcutaneous adipose tissue blood-flow and insulin-mediated glucose uptake in healthy obese subjects. Annals of Medicine. 2009;41:152-60.

43.   Chaston TB, Dixon JB. Factors associated with percent change in visceral versus subcutaneous abdominal fat during weight loss: findings from a systematic review. Int J Obes. 2008;32:619-28.

44.   Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-reactive protein: a systematic review. Arch Intern Med. 2007 Jan 8;167:31-9.

45.   Dietrich M, Jialal I. The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. Nutr Rev. 2005 Jan;63:22-8.

46.   Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation. 2002 Feb 5;105:564-9.

47.   Blaum CS, Xue QL, Michelon E, Semba RD, Fried LP. The association between obesity and the frailty syndrome in older women: the Women's Health and Aging Studies. J Am Geriatr Soc. 2005 Jun;53:927-34.

48.   Woods NF, LaCroix AZ, Gray SL, Aragaki A, Cochrane BB, Brunner RL, Masaki K, Murray A, Newman AB. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. J Am Geriatr Soc. 2005 Aug;53:1321-30.

49.   Villareal DT, Banks M, Sinacore DR, Siener C, Klein S. Effect of weight loss and exercise on frailty in obese older adults. Arch Intern Med. 2006 Apr 24;166:860-6.

50.   Messier SP, Loeser RF, Miller GD, Morgan TM, Rejeski WJ, Sevick MA, Ettinger WH, Jr., Pahor M, Williamson JD. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum. 2004 May;50:1501-10.

51.   Miller GD, Nicklas BJ, Davis C, Loeser RF, Lenchik L, Messier SP. Intensive weight loss program improves physical function in older obese adults with knee osteoarthritis. Obesity (Silver Spring). 2006 Jul;14:1219-30.

52.   Wang X, Miller GD, Messier SP, Nicklas BJ. Knee strength maintained despite loss of lean body mass during weight loss in older obese adults with knee osteoarthritis. J Gerontol A Biol Sci Med Sci. 2007 Aug;62:866-71.

53.   Brinkworth GD, Noakes M, Keogh JB, Luscombe ND, Wittert GA, Clifton PM. Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. Int J Obes Relat Metab Disord. 2004 May;28:661-70.

54.   Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with Type 2 diabetes: one-year follow-up of a randomised trial. Diabetologia. 2004;47:1677-86.

55.   Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005 Jun;81:1298-306.

56.   Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. J Nutr. 2005 Aug;135:1903-10.

57.   Layman DK, Evans EM, Erickson D, Seyler J, Weber J, Bagshaw D, Griel A, Psota T, Kris-Etherton P. A Moderate-Protein Diet Produces Sustained Weight Loss and Long-Term

Changes in Body Composition and Blood Lipids in Obese Adults. J Nutr. 2009 March 1, 2009;139:514-21.

58.   Clifton PM, Keogh JB, Noakes M. Long-term effects of a high-protein weight-loss diet. Am J Clin Nutr. 2008 Jan;87:23-9.

59.   Layman DK. Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss. J Am Coll Nutr. 2004 December 1, 2004;23:631S-6.

60.   Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. Dietary Protein, Weight Loss, and Weight Maintenance. Annual Review of Nutrition. 2009;29:21-41.

61.   Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006 February 1, 2006;83:260-74.

62.   Bowen J, Noakes M, Clifton PM. Effect of calcium and dairy foods in high protein, energy-restricted diets on weight loss and metabolic parameters in overweight adults. Int J Obes (Lond). 2005 Aug;29:957-65.

63.   Mahon AK, Flynn MG, Stewart LK, McFarlin BK, Iglay HB, Mattes RD, Lyle RM, Considine RV, Campbell WW. Protein intake during energy restriction: effects on body composition and markers of metabolic and cardiovascular health in postmenopausal women. J Am Coll Nutr. 2007 Apr;26:182-9.

64.   Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Foster P, Clifton PM. Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. Am J Clin Nutr. 2005 Apr;81:762-72.

65.   Sargrad KR, Homko C, Mozzoli M, Boden G. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin A1c, and blood pressure in patients with type 2 diabetes mellitus. J Am Diet Assoc. 2005 Apr;105:573-80.

66.   Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008 Jan;87:150-5.

67.   Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. Int J Obes Relat Metab Disord. 2004;28:1283-90.

68.   Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. Appl Physiol Nutr Metab. 2007 Aug;32:743-52.

69.	Kerksick C, Thomas A, Campbell B, Taylor L, Wilborn C, Marcello B, Roberts M, Pfau E, Grimstvedt M, et al. Effects of a popular exercise and weight loss program on weight loss, body composition, energy expenditure and health in obese women. Nutrition & Metabolism. 2009;6:23.

70.	Due A, Toubro S, Stender S, Skov AR, Astrup A. The effect of diets high in protein or carbohydrate on inflammatory markers in overweight subjects. Diabetes, Obesity and Metabolism. 2005;7:223-9.

71.	Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr. 2003 Jul;78:31-9.

CHAPTER 3

The Effects of a Higher Protein Intake During Energy Restriction on Changes in Fat Mass, Adipose Tissue and Metabolic Disease Risk in Postmenopausal Women

3.1 Introduction

Obesity has reached epidemic proportions in the United States, with 69% of women aged 60 and older being classified as overweight, and 32% as obese (1). Adiposity is known to increase risk for metabolic disease, such as cardiovascular disease, metabolic syndrome and type 2 diabetes (2). Weight loss of 10% is recommended for improving mortality (3); however, it remains somewhat controversial in the older population, as weight loss results in loss of not only fat mass but also bone (4) and muscle mass (5-7). Compared to their male counterparts, older women are at an increased risk for obesity (1), osteoporosis and fractures (8), and physical disability (9). Therefore, it is important to determine the most effective weight loss regimens to elicit optimal changes in body composition to decrease obesity related metabolic disease risk in older adults, especially females.

In addition to increased absolute and relative whole body fat associated with aging, obese and older adults appear to have an increased accumulation of adipose infiltration in the muscle (10-11). Novel adipose tissue depots, such as subcutaneous adipose tissue (SAT), measured using magnetic resonance imaging (MRI), has been associated with glucose intolerance (12) and insulin resistance (13); and intermuscular adipose tissue (IMAT) with type 2 diabetes (14) and metabolic syndrome (15).

Regarding macronutrient content of the weight loss diet, increasing protein intake as part of a low-calorie diet has been shown to promote more total weight and fat mass loss (16-19) compared to an isocaloric diet with a conventional macronutrient composition (i.e. higher carbohydrate). However, evidence is equivocal whether there is a beneficial effect of higher protein intake during weight loss on measures of risk for metabolic disease (16-18, 20), especially independent of changes in adiposity. Moreover, to date, no studies have assessed changes in thigh SAT and IMAT resulting from energy restricted diets varying in macronutrient content and the potential impact on risk for metabolic disease in older females.

In this context, the aim of this study was to assess the effects of a higher protein, lower carbohydrate (PRO) intake during energy restriction on 1) fat/adipose depots, particularly thigh SAT and IMAT, and 2) indicators of metabolic disease risk, compared with a conventional higher carbohydrate, lower protein weight loss diet (CARB) in older females. The secondary aim was to examine whether changes in markers of metabolic disease were mediated by changes in fat mass or adipose tissue. We hypothesized that a PRO weight loss diet will result in greater loss of fat mass and adipose tissue compared with an isocaloric CARB diet, and that this would be related to beneficial effects on metabolic disease risk.

3.2 Methods

Study design and participants. Thirty-one healthy, overweight or obese postmenopausal women (mean age±SD: 65.2±4.6 yrs; 29 Caucasian, 1 African American, 1 Latina) were recruited from the community for a 6 month double-blind, randomized weight loss trial. Exclusion criteria were as follows: BMI <28 kg/m^2; >136 kg weight; major weight change defined as >2.3 kg change in the prior 6 mo; postmenopausal <5 yrs; regular physical activity >1 h/wk or >2 sessions/wk in the prior 6 mo; regular smoking within the prior 10 yrs; history of conditions that impact the primary outcomes such as cancer, cardiovascular disease, type 2 diabetes, renal disease, musculoskeletal or neuromuscular impairments; and use of medications that may impact bone metabolism, such as hormone replacement therapy or osteoporosis drugs, within the prior 2 years. Health history was obtained with a questionnaire, which included items on medications, use of non-steroidal anti-inflammatory drugs, supplements and history of conditions other than ones listed as exclusion criteria (e.g. arthritis). Participants were screened with standard blood tests (comprehensive metabolic panel and complete blood count). All participants were required to provide personal physician clearance prior to enrollment. All participants signed a consent form approved by the University of Illinois Institutional Review Board upon enrollment in the study. Participants were blocked on age and BMI with subsequent random assignment to either PRO or CARB treatment. Researchers and participants were blinded to which treatment, PRO or CARB, the participants received.

Dietary treatment. Participants were provided with a supplement: the PRO group received 50 g/d of 90% whey protein isolate, and the CARB group received 50g/d of maltodextrin. Participants were asked to ingest half (25g) of the supplement in the morning with

breakfast and the other half in the afternoon or evening. The PRO diet prescribed was designed to provide 0.8 g/kg/d of dietary protein with an additional 45g/d of protein from the supplement, resulting in approximately 30% of energy intake from protein, 40% of energy from carbohydrate and 30% of energy from fat. The CARB diet was aimed at providing 0.8 g/kg/d protein or approximately 18% of energy from protein, 52% of energy from carbohydrate (~182 g/d) and 30% of energy from fat, with an additional 50 g/d of carbohydrate from the supplement.

Participants were asked to attend diet education classes taught by a registered dietitian every 2 weeks during the 6 mo weight loss program. Diet education was based on healthy eating guidelines according to the USDA My Pyramid (21). The registered dietitian also provided personal feedback monthly to each participant, adjusting energy intake and portions when needed to ensure weight loss. Participants were provided with meal plans and food scales (A&D Weighing, Model SK2000, San Jose, CA, USA) to weigh their food and control portions. Diet education and meal plans were the same for both PRO and CARB groups, and were designed to provide 1400 kcal/d, with a goal of 10% weight loss over 6 mo. The whey protein and maltodextrin supplements provided an additional 160 kcal/d. Participants were also provided with a multivitamin and a calcium and vitamin D supplement to be taken every day.

Post-intervention testing was conducted 6-8 mo after the start of the intervention and diet and exercise treatments were continued until completion of measurements. Adherence to the diet was monitored by measuring body weight monthly and by self-reported weight weekly. Online food records were obtained monthly to track food intake and provide feedback to participants. Three-day weighed food diaries were collected for research purposes at baseline, month 3 and post-intervention, and were reviewed by the dietitian for completeness. All 3-day food diaries were analyzed by the same student using Nutritionist Pro commercial software version 4.1 (Axxya Systems, Stafford TX, USA). Used supplement containers were collected and unused supplement was weighed to assess compliance to the supplement component of the treatment.

Exercise treatment. Supervised exercise classes were provided 5 d/wk, of which participants were required to attend 2-3 exercise classes/wk. Exercise consisted of 20 min of flexibility exercises and 20-30 min of low to moderate intensity walking on an indoor track. Prescribed exercise was the same for both PRO and CARB groups. Participants recorded weekly exercise and rate of perceived exertion (RPE) at classes. Participants were also provided a

pedometer (Accusplit AE170XLGM, Pleasanton, CA, USA), and were asked to record total daily steps over 7 consecutive days monthly.

Body composition. Height was measured at baseline and post-intervention to the nearest 0.1 cm with a digital stadiometer (Seca model 242, Hanover, MD, USA). Weight was measured monthly to the nearest 0.1 kg with a digital scale (Tanita, Model BWB-627 A, Arlington Heights, IL, USA). Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Waist and hip circumferences were measured monthly to the nearest 0.1 cm with a Gulick II retractable measuring tape (Country Technology Inc., Gay Mills, WI, USA).

Whole body fat mass (FM) and whole body relative fat (%Fat) were measured using dual energy X-ray absorptiometry (DXA; Hologic Discovery, software version 12.7.3, Waltham, MA, USA). Regions of interest were drawn in the trunk from T11 to L5 vertebrae for analysis of abdominal fat and %Fat. Participants wore light-weight clothing and removed all jewelry. All DXA scans were analyzed by the same technician. The DXA machine was calibrated daily using manufacturer phantoms. Precision for DXA measurements of interest is between 1 and 1.5% in our laboratory.

Magnetic resonance imaging (MRI) was used to measure subcutaneous (SAT) and intermuscular adipose tissue (IMAT) of the right thigh. For this study, IMAT is defined as adipose tissue located beneath the fascia lata and between muscle groups (Shen et al. 2003; Gallagher et al. 2005). All imaging was performed on a 1.5T General Electric Signa Excite whole-body scanner (Milwaukee, WI, USA) using a phased array torso coil strapped around the thigh of interest. A spoiled gradient-recalled echo sequence was used to acquire in- and out-of-phase images for a two-point Dixon fat separation technique (22). Images were acquired in 10 mm slices over the middle third of the participant's femur (14-16 slices), as measured from the distal and proximal endpoints of the femur in the scouting scan. A field of view of 48 cm and matrix size of 256x256 resulted in an in-plane resolution of 1.875 mm. Determination of SAT and IMAT quantities was performed by segmenting the depots in the fat-only image, and region growing was used to determine the threshold. All images were analyzed by the same operator using Amira 5.2 (Visage Imaging Inc., San Diego, CA, USA).

Indicators of metabolic disease risk. Blood samples were obtained at baseline, month 1 and post-intervention, following an overnight 12 h fast. To ensure that the participant was healthy (i.e. free of acute inflammation) at the time of sampling, the blood draw was repeated if

the participant reported symptoms of illness or sickness at the draw or within 7 days post-draw. Only data from baseline and post-intervention are presented. Plasma samples were sent to an external medical laboratory (Provena Covenant Medical Center, Champaign, IL, USA) for measurement of fasting glucose concentrations. Serum insulin concentrations were measured using a commercial radioimmunoassay kit (ImmuChem, MP Biomedicals LLC, Solon, OH, USA). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as (fasting insulin x fasting glucose) / 405 (23). C-reactive protein (CRP) and interleukin 6 (IL-6) were measured using commercial high-sensitivity ELISA kits (CRP: Diagnostic Automation Inc., Calabasas, CA, USA; IL-6: Quantikine HS, R&D Systems, Minneapolis, MN, USA). Inter-assay CVs for CRP and IL-6 were 9.2% and 14.5%, respectively; intra-assay CVs for CRP, IL-6 and insulin were 1.0%, 5.0% and 1.4%, respectively. Blood pressure was measured in duplicate after sitting quietly for 10 min using a semi-automated blood pressure monitor (Dynamap PRO 100).

 Statistical analysis. All data analyses were conducted using SPSS version 17.0 (Chicago, IL, USA). Only data for participants who completed post-intervention tests were included in the analysis. Data were assessed for normality using the Shapiro-Wilk statistic. Nonparametric tests were used for data that did not meet the assumption of normality. CRP and IL-6 data were normally distributed only after exclusion of two outliers (>400% increase from pre- to post-intervention), therefore these were excluded from analyses on inflammation levels. Change variables were calculated for the difference in values between PRO and CARB groups relative to baseline values. Baseline and change variables were compared between PRO and CARB groups using Student's independent t-test. T-tests were also conducted using two combinations of compliance criteria: 1) weight loss within the 75th percentile of most weight lost; and either 2a) supplement consumption >50% of prescribed amount (PRO n=12, CARB n=7), or 2b) protein intake >1.1 g/kg/d for PRO and carbohydrate intake >52%E (PRO n=8, CARB n=5). Linear regression analyses were used to assess independent associations between fat depots and specific indicators of metabolic disease risk. An α-level of 0.05 was considered significant. Results are expressed as mean±SD.

3.3 Results

Of the 31 women that enrolled in the study, 26 (84%; 13 PRO, 13 CARB; 25 Caucasian, 1 African American) completed post-intervention measurement. Three participants withdrew due to health issues, and two withdrew for other personal reasons (Figure 2). Twelve of the 26 completers (46%; PRO n=5, CARB n=7) reported regular use of non-steroidal anti-inflammatory medications, and 16 (62%; PRO n=7, CARB n=9) used statins. Dose per week of anti-inflammatory medications and statins was calculated as a covariate in data analyses. Sixteen women (62%; PRO n=9, CARB n=7) reported having mild or moderate arthritis in hands, back, or legs. Arthritis was included as a dummy variable in data analyses.

Baseline variables for body composition and indicators of metabolic disease risk did not differ between PRO and CARB (Tables 1, 3 and 4). Of baseline dietary macronutrients, carbohydrate and fat intake as a percentage of energy intake (%E) differed between groups with a +7.7% difference in %E carbohydrate intake and -7.2% lower %E fat intake in the PRO group.

Diet education class adherence was 81.4±19.2% and 71.3±22.2% (p=0.23) and exercise class attendance was 87.9±12.2% and 78.6±14.9% (p=0.09) for PRO and CARB, respectively. On a scale where 6 is extremely easy and 20 is extremely hard, mean RPE over 6 mo of exercise classes was 12.9±1.7 and 12.3±1.2 (p=0.38) for PRO and CARB, respectively. Baseline pedometer step count was 5901±2875 steps and 4108±1484 steps (p=0.07), post-intervention 6463±3217 steps and 6652±2708 steps (p=0.88) and change in pedometer steps relative to baseline +28.8±79.2% and +55.7±40.6% (p=0.35), for PRO and CARB, respectively.

Mean supplement intake over 6 mo was 41.5±6.5 g/d (77.5% of prescribed supplement) for PRO and 38.2±10.8 g/d (74.8% of prescribed supplement) for CARB, and did not differ between groups (p=0.36). Total post-intervention energy and macronutrient intake, displayed in Table 2, includes intake from both diet and supplement combined. Energy intake differed between groups at post-intervention, however, when taking post-intervention weight into account, intake was not different (20.6±4.7 and 21.1±4.8 kcal/kg body weight, for PRO and CARB, respectively; p=0.50). As intended by study design, total protein intake was greater for PRO and total carbohydrate intake was greater for CARB when on treatment. At post-intervention, PRO ingested 1.20±0.14 g/kg/d protein compared to 0.86±0.20 g/kg/d for CARB (p<0.001). Fat intake and %E fat intake were less for PRO compared to CARB, (both p<0.05);

28

however, the % change from baseline did not differ likely somewhat influenced by the higher %E fat intake in CARB at baseline (p<0.05).

Twenty-three % of participants (6 of 26) reduced their weight 10% or more from baseline; 54% (14 of 26) reduced initial weight by 3-9%; and the remaining 23% reduced their weight by 1% or less. Mean weight decreased after 6 months in both groups, with a strong trend for a greater decrease in the PRO group compared to CARB (3.9% difference, p=0.059; Table 1). PRO had greater reductions in all fat or adipose depots, except IMAT relative to muscle volume; however, only reductions in abdominal fat mass, thigh SAT and IMAT volume were significantly greater in PRO compared to CARB (Table 3). No significant changes between groups were found in any of the indicators of metabolic disease risk (Table 4). Although changes in weight, fat mass or adipose tissue and metabolic indicators were greater in the PRO group, they did not differ significantly between PRO and CARB groups when using any of the compliance criteria, possibly due to reduced sample size (data not shown).

Linear regression analyses indicated that % change in SAT volume was an independent predictor of % change in CRP explaining 30% of the variance, when adjusting for PRO vs. CARB and use of anti-inflammatory medication (Table 5b). Covarying for anti-inflammatory dose per week resulted in a better model fit than covarying for statin dose per week (data not shown). Relative change in abdominal fat mass showed a trend towards independently predicting % change in CRP (p=0.057). Changes in any depot of fat or adipose did not predict % change in HOMA-IR, nor did supplement group alone predict % change of either HOMA-IR or CRP.

3.4 Discussion

The primary novel finding in this study is that higher protein intake leads to greater reductions in IMAT. However, when accounting for changes in muscle volume, IMAT actually increases albeit not significantly and changes are not different between PRO and CARB. Changes in abdominal fat and SAT are also impacted by level of protein intake, with greater protein intake resulting in greater fat loss. Furthermore, although indicators of metabolic disease risk did not differ between groups, it appears that changes in abdominal fat and SAT can mediate positive effects on inflammation metabolic disease risk. Effect sizes, which were within a moderate range, suggest that statistically significant differences between groups in the primary outcomes may have been reached with a larger sample size.

Greater protein intake contributed to more successful weight loss compared to greater carbohydrate intake in this study, in terms of percentage of women who lost weight as well as average weight lost, which is similar to other studies with middle-aged and older adults (16-19, 24-25) but conflicts with other findings (19-20, 26). Studies assessing the effect of higher protein intake on weight loss vary in duration, from 2-3 mo (16-17, 26-27) to 6 mo (24-25); and in level of protein, ranging from 24 %E (84 g/d) (17) to ~30 %E (115 g/d) (16, 18-20, 27). Long-term follow-ups indicate that although some weight may be regained, weight loss is better maintained following a moderate to high protein intake (24, 27). Findings from our study confirm that longer-term trials are necessary to show significant weight changes, but that a moderate protein intake, 27% (92 g/d), may be sufficient for maintaining this weight loss over a long period.

Notably, our study design was unique compared with previous research, in that higher protein intake was attained via a powder supplement that could be mixed in with foods or beverages, rather than via diet education on eating more protein-rich foods. Thus we were able to reduce bias from assessing protein intake via diet records, and better control actual ingested protein intake. In addition, in our study, timing of protein intake was controlled and about 22g of whey protein was consumed with breakfast foods, while the rest of the supplement was consumed often as an afternoon snack, and dietary protein consumed at meals. Timing of protein ingestion may be important for maximizing the beneficial effects on weight management (28-29). A proposed mechanism for improved weight management with higher protein intake is increased satiety resulting in decreased energy intake (30). Distributing protein intake throughout the day may have beneficially influenced satiety in our study potentially promoting greater weight loss, as seen in post-intervention energy intake levels which were lower for PRO compared with CARB, which is similar to previous findings (24-25). Interestingly, total protein intake (dietary + supplement) increased in the PRO group by only ~16 g/d, despite supplementary protein intake averaging at 42 g/d. A closer examination reveals that the whey protein supplement displaced dietary protein intake in the PRO group such that a ~18 g/d decrease is seen in dietary protein intake from baseline to post-intervention.

Similarly to weight loss, many studies show greater loss of fat mass (16-19, 25) with higher protein intake, but not all findings are consistent (20). In our study, the PRO group clearly lost more fat mass (effect size = 0.6), but due to a small sample size, only differences in abdominal fat, SAT and IMAT were significant between PRO and CARB. Interestingly, IMAT

decreased with weight loss, and decreased more in the PRO group, but naturally, changes in IMAT are dependent on changes in the surrounding muscle. When IMAT was expressed as relative to muscle volume, not only were there no longer differences between groups, but relative IMAT increased. To our knowledge, IMAT has not been previously measured under weight loss conditions, and an important question remains whether absolute or relative IMAT has more clinical significance.

Similarly to previous higher protein weight loss studies (17-18, 20, 24, 27), although metabolic profile improved following weight loss, no significant effect of dietary treatment was detected, possibly due to large variability. Carbohydrate intake was well above the 10-20 %E (~100 g/d) in the PRO group, and therefore is not considered a restricted carbohydrate diet, which is thought to also have beneficial effects on cardiovascular disease risk (31). CRP has been found to be associated with changes in FM following a high protein weight loss diet (32). We found changes specifically in abdominal fat and SAT to independently predict changes in inflammation. Therefore, greater reductions in these fat depots from a higher protein diet may mediate decreases in inflammation, which in turn is known to impact risk for metabolic syndrome (33), cardiovascular disease (34-36) and type 2 diabetes (2). This question is of high public interest and requires further study.

This small pilot study, although innovative, does have limitations. The main limitation in this study was the small sample size. However, effect sizes calculated for body composition and metabolic markers in this study, ranging from 0.09 (SAT) to 0.77 (IMAT) for body composition outcomes and from 0.21 (insulin) to 0.54 (CRP) for metabolic markers, indicate that with a larger sample size significant differences between groups would have been more apparent (37). Thus, this study serves as excellent preliminary data for further research assessing the effects of protein intake on health status under weight loss conditions in older women.

As with all clinical trials investigating behavioral interventions, adherence and compliance is an issue. Although withdrawal rate was relatively low (16%) compared with most other clinical trials, when evaluated in conjunction with supplement compliance (75%), the effect was present although not well characterized. Indeed, although this trial was designed as an efficacy trial and not an effectiveness trial, it should be noted that both withdrawal and supplement compliance was similar between the groups. Exclusion of data based on compliance criteria, such as weight loss, supplement intake and adherence to dietary guidelines, can also be

problematic in a small trial as it not only decreases the sample size but also reduces generalizability and can create bias. For example, including only data that met compliance criteria in this study resulted in more samples excluded from the CARB group, as the PRO group was overall more successful with weight loss, similar to previous reports (19). Therefore, in this case, excluding subjects based on weight loss criteria may lead to potential bias and type 2 error.

In conclusion, our findings support increasing protein levels in reduced calorie diets to promote weight loss, reduce fat mass and adipose tissue and potentially improve metabolic profile risk. Effects of a higher protein weight loss diet on improvements in insulin sensitivity, glucose tolerance and inflammation may become more apparent in a higher risk population, such as in people with metabolic syndrome, cardiovascular disease or type 2 diabetes. Lastly, we show that using a powdered supplement to increase protein intake as part of a weight loss diet is acceptable to an older population, instead of increasing consumption of protein-rich foods, which may be more difficult for older individuals to ingest and may have a higher fat content. Nevertheless, longer-term studies with larger sample sizes are needed to confirm the feasibility of maintaining these optimal changes beyond six months.

3.5 References

1.   Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of Overweight and Obesity in the United States, 1999-2004. JAMA. 2006 April 5, 2006;295:1549-55.

2.   Shah A, Mehta N, Reilly MP. Adipose Inflammation, Insulin Resistance, and Cardiovascular Disease. JPEN J Parenter Enteral Nutr. 2008 November 1, 2008;32:638-44.

3.   Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Obes Res. 2005 Nov;13:1849-63.

4.   Shapses SA, Riedt CS. Bone, body weight, and weight reduction: what are the concerns? J Nutr. 2006 Jun;136:1453-6.

5.   Gallagher D, Kovera AJ, Clay-Williams G, Agin D, Leone P, Albu J, Matthews DE, Heymsfield SB. Weight loss in postmenopausal obesity: no adverse alterations in body composition and protein metabolism. Am J Physiol Endocrinol Metab. 2000 Jul;279:E124-31.

6.   Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M, the Health AaBCRG. Strength and Muscle Quality in a Well-Functioning Cohort of

Older Adults: The Health, Aging and Body Composition Study. Journal of the American Geriatrics Society. 2003;51:323-30.

7.   Mazzali G, Di Francesco V, Zoico E, Fantin F, Zamboni G, Benati C, Bambara V, Negri M, Bosello O, Zamboni M. Interrelations between fat distribution, muscle lipid content, adipocytokines, and insulin resistance: effect of moderate weight loss in older women. Am J Clin Nutr. 2006 Nov;84:1193-9.

8.   van Helden S, van Geel ACM, Geusens PP, Kessels A, Nieuwenhuijzen Kruseman AC, Brink PRG. Bone and Fall-Related Fracture Risks in Women and Men with a Recent Clinical Fracture. J Bone Joint Surg Am. 2008 February 1, 2008;90:241-8.

9.   Murtagh KN, Hubert HB. Gender differences in physical disability among an elderly cohort. Am J Public Health. 2004 Aug;94:1406-11.

10.   Gallagher D, Kuznia P, Heshka S, Albu J, Heymsfield SB, Goodpaster B, Visser M, Harris TB. Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. Am J Clin Nutr. 2005 Apr;81:903-10.

11.   Song MY, Ruts E, Kim J, Janumala I, Heymsfield S, Gallagher D. Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. Am J Clin Nutr. 2004 May;79:874-80.

12.   Kohrt WM, Holloszy JO. Loss of skeletal muscle mass with aging: effect on glucose tolerance. J Gerontol A Biol Sci Med Sci. 1995 Nov;50 Spec No:68-72.

13.   Racette SB, Evans EM, Weiss EP, Hagberg JM, Holloszy JO. Abdominal adiposity is a stronger predictor of insulin resistance than fitness among 50-95 year olds. Diabetes Care. 2006 Mar;29:673-8.

14.   Goodpaster BH, Krishnaswami S, Resnick H, Kelley DE, Haggerty C, Harris TB, Schwartz AV, Kritchevsky S, Newman AB. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care. 2003 Feb;26:372-9.

15.   Goodpaster BH, Krishnaswami S, Harris TB, Katsiaras A, Kritchevsky SB, Simonsick EM, Nevitt M, Holvoet P, Newman AB. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med. 2005 Apr 11;165:777-83.

16.   Brinkworth GD, Noakes M, Keogh JB, Luscombe ND, Wittert GA, Clifton PM. Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. Int J Obes Relat Metab Disord. 2004 May;28:661-70.

17.   Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. Appl Physiol Nutr Metab. 2007 Aug;32:743-52.

18.   Kerksick C, Thomas A, Campbell B, Taylor L, Wilborn C, Marcello B, Roberts M, Pfau E, Grimstvedt M, et al. Effects of a popular exercise and weight loss program on weight loss, body composition, energy expenditure and health in obese women. Nutrition & Metabolism. 2009;6:23.

19.   Layman DK, Evans EM, Erickson D, Seyler J, Weber J, Bagshaw D, Griel A, Psota T, Kris-Etherton P. A Moderate-Protein Diet Produces Sustained Weight Loss and Long-Term Changes in Body Composition and Blood Lipids in Obese Adults. J Nutr. 2009 March 1, 2009;139:514-21.

20.   Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005 Jun;81:1298-306.

21.   USDA. MyPyramid.gov. 2009 [cited 2010 March 25]; Available from: http://www.mypyramid.gov/

22.   Ma J. Dixon techniques for water and fat imaging. Journal of Magnetic Resonance Imaging. 2008;28:543-58.

23.   Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9.

24.   Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. Int J Obes Relat Metab Disord. 2004;28:1283-90.

25.   Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord. 1999;23:528-36.

26.   Clifton PM, Keogh JB, Noakes M. Long-term effects of a high-protein weight-loss diet. Am J Clin Nutr. 2008 Jan;87:23-9.

27.   Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with Type 2 diabetes: one-year follow-up of a randomised trial. Diabetologia. 2004;47:1677-86.

28.   Layman DK. Dietary Guidelines should reflect new understandings about adult protein needs. Nutr Metab (Lond). 2009;6:12.

29.   Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. Current opinion in clinical nutrition and metabolic care. 2009 Jan;12:86-90.

30.   Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, Purnell JQ. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. Am J Clin Nutr. 2005 July 1, 2005;82:41-8.

31.   Samaha F, Foster G, Makris A. Low-carbohydrate diets, obesity, and metabolic risk factors for cardiovascular disease. Current Atherosclerosis Reports. 2007;9:441-7.

32.   Due A, Toubro S, Stender S, Skov AR, Astrup A. The effect of diets high in protein or carbohydrate on inflammatory markers in overweight subjects. Diabetes, Obesity and Metabolism. 2005;7:223-9.

33.   Devaraj S, Singh U, Jialal I. Human C-reactive protein and the metabolic syndrome. Curr Opin Lipidol. 2009 Jun;20:182-9.

34.   Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation. 2002 Feb 5;105:564-9.
35.   Dietrich M, Jialal I. The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. Nutr Rev. 2005 Jan;63:22-8.

36.   Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-reactive protein: a systematic review. Arch Intern Med. 2007 Jan 8;167:31-9.

37.   Lipsey MW. Design sensitivity: statistical power for experimental research. Newbury Park, CA: Sage Publications; 1990.

Figure 2. Study design flow chart. PRO = higher protein weight loss diet; CARB = higher carbohydrate weight loss diet.
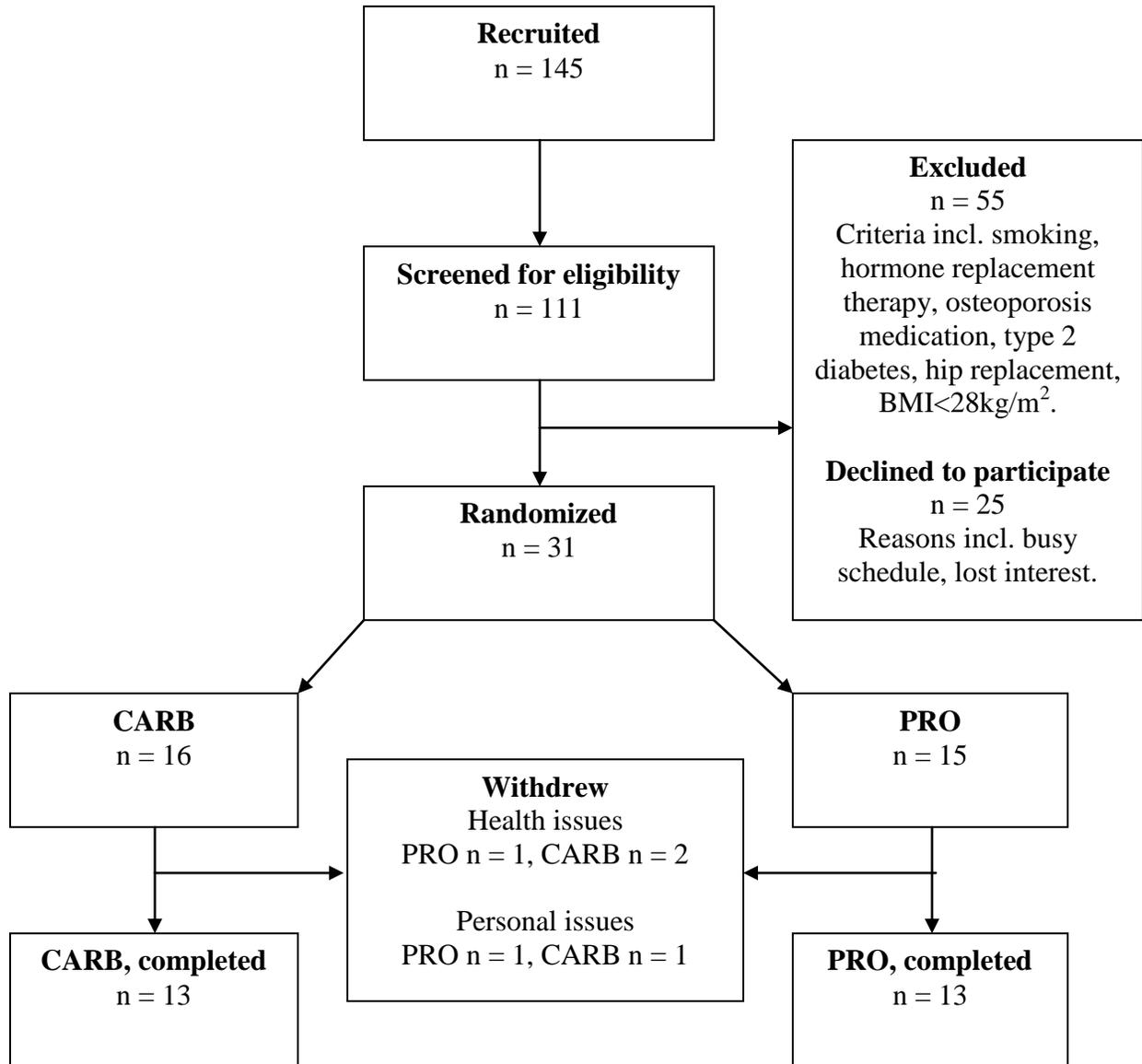
Table 1. Descriptive characteristics of participants (PRO n= 13, CARB n= 13) represented as mean±SD.

	Group	Baseline	P-value*	Post-intervention	% Change†	P-value‡
Age	PRO	64.77±4.4		-	-	
	CARB	64.6±5.2	0.936	-	-	-
Height (cm)	PRO	161.9±4.9		-	-	
	CARB	162.7±5.7	0.678	-	-	-
Weight (kg)	PRO	84.7±10.5		77.7±9.35	-8.0±6.2	
	CARB	86.8±13.4	0.655	83.3±13.7	-4.1±3.6	0.059
BMI (kg/m^2)	PRO	32.3±3.9		29.8±3.7	-7.6±6.2	
	CARB	32.7±4.2	0.804	31.5±4.6	-3.9±3.7	0.075

* P-value for difference in baseline values between PRO and CARB groups.

†% Change = change from baseline relative to baseline value [(post-intervention – baseline) / baseline].

‡ P-value for difference in % Change between PRO and CARB groups.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; BMI = body mass index.

Table 2. Dietary energy and macronutrient intake data, and total energy and macronutrient including supplement (PRO n=11, CARB n=12) represented as mean±SD.

	Group	Energy intake (kcal/d)	Protein intake			Carbohydrate intake		Fat intake	
			(g/d)	(g/kg/d)	(%E)	(g/d)	(%E)	(g/d)	(%E)
Dietary baseline	PRO	1687±264	76.2±24.0	0.91±0.23	18.6±7.4	220.0±47.5	52.1±7.5	56.2±18.5	29.5±6.0
	CARB	1743±321	81.4±18.6	0.98±0.25	18.8±3.6	190.9±49.8	44.4±9.1*	71.4±19.6	36.7±6.9*
Dietary during intervention	PRO	1221±228	58.5±13.3	0.76±0.13	19.4±4.1	169.0±24.5	56.1±7.5	35.3±12.0	25.6±5.7
	CARB	1484±367	71.0±13.7	0.87±0.20	19.8±4.2	178.3±47.3	48.8±10.2	53.8±23.4	31.5±7.6
Total during intervention§	PRO	1369±197	91.8±12.6	1.21±0.14	27.3±5.3	169.0±24.5	49.6±5.6	35.3±12.0	22.8±5.6
	CARB	1627±388*	71.0±13.7*	0.87±0.20*	17.9±3.5*	217.8±53.6*	54.2±9.8	53.8±23.4*	28.8±7.4*
% Change†	PRO	-18.1±18.8	27.5±29.4	40.5±33.8	61.3±44.8	-19.6±26.0	-2.3±18.6	-34.1±31.9	-22.4±24.7
	CARB	-6.0±30.3	-9.8±26.0	-5.8±26.9	0.1±30.6	17.2±41.1	26.8±35.9	-20.3±47.6	-18.8±25.7
p-value‡		0.295	0.004	0.001	0.001	0.019	0.014	0.622	0.737

* Significant difference in values between PRO and CARB groups within the same time point, p<0.05.

† % Change = change from baseline relative to baseline value [(during intervention including supplement − baseline) / baseline].

‡ p-value for difference in % Change between PRO and CARB groups.

§ Includes mean supplement and dietary intake per day assessed at month 6 of intervention.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet.

Table 3. Changes in fat mass and adipose volume in response to the intervention (DXA: PRO n=13, CARB n=13; MRI: PRO n=12, CARB n=12), represented as mean±SD.

	Group	Baseline	Post-intervention	% Change*	P-value†
DXA whole body fat mass (kg)	PRO	36.5±6.6	30.6±8.7	-15.4±17.8	
	CARB	37.4±8.3	36.0±9.1	-6.1±5.4	0.091
DXA whole body %fat	PRO	42.4±3.2	39.7±4.6	-6.3±7.0	
	CARB	42.5±4.1	41.8±4.6	-2.6±3.7	0.101
DXA abdominal fat mass (kg)	PRO	5.8±1.7	4.8±1.7	-14.8±15.0	
	CARB	5.7±1.9	5.5±2.2	-4.1±8.1	0.037
DXA abdominal %fat	PRO	40.4±6.0	36.9±8.6	-8.1±12.2	
	CARB	40.5±6.9	39.6±8.7	-2.0±6.7	0.133
MRI thigh subcutaneous fat (m^3)	PRO	2.20±5.5	1.82±5.0	-18.8±13.5	
	CARB	2.45±8.3	2.37±7.4	-7.9±6.9	0.024
MRI thigh IMAT (m^3)	PRO	0.23±0.04	0.21±0.03	-9.2±9.4	
	CARB	0.23±0.06	0.23±0.06	-1.0±7.7	0.028
MRI Thigh IMAT (% of muscle)	PRO	5.95±0.94	6.22±1.08	4.7±5.8	
	CARB	5.62±0.83	5.63±0.45	4.3±8.6	0.883

* % Change = change from baseline relative to baseline value [(post-intervention – baseline) / baseline].

†P-value for difference in % Change between PRO and CARB groups.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; DXA = dual energy X-ray absorptiometry; whole body %fat = whole body fat mass/whole body mass; abdominal %fat = abdominal fat mass/abdominal mass; MRI = magnetic resonance imaging; IMAT = intermuscular adipose tissue.

Table 4. Indicators of metabolic disease risk (PRO n=13, CARB n=13) represented as mean±SD.

	Group	Baseline	Post-intervention	% Change*	P-value†
Fasting insulin (μIU/ml)	PRO	38.0±25.2	25.2±9.5	-24.0±26.8	
	CARB	36.6±23.3	27.4±11.6	-13.1±30.0	0.338
Fasting glucose (mg/dl)	PRO	96.4±14.0	90.9±9.6	-4.6±11.8	
	CARB	96.9±10.8	94.7±11.2	-2.1±6.6	0.515
HOMA-IR	PRO	9.35±6.69	5.70±2.29	-26.7±30.3	
	CARB	9.08±6.59	6.64±3.38	-14.2±32.4	0.319
CRP‡ (mg/l)	PRO	2.53±1.96	2.12±1.85	-25.7±53.4	
	CARB	2.88±1.51	3.14±1.93	3.9±51.4	0.179
IL-6‡ (pg/ml)	PRO	1.37±0.89	1.38±0.98	-3.4±44.7	
	CARB	1.18±0.56	1.12±0.47	1.2±37.5	0.564
Systolic blood pressure (mmHg)	PRO	134±15	127±9	-3.7±10.2	
	CARB	148±18	138±21	-6.9±8.9	0.441
Diastolic blood pressure (mmHg)	PRO	79±7	73±5	-5.6±6.8	
	CARB	80±8	78±11	-2.7±9.8	0.422
Waist circumference (cm)	PRO	97.6±9.3	91.2±9.7	-6.4±4.5	
	CARB	96.6±9.0	92.8±11.3	-4.1±4.04	0.177
Waist-to-hip ratio	PRO	0.83±0.06	0.80±0.05	-3.4±2.6	
	CARB	0.81±0.07	0.78±0.06	-3.0±4.4	0.756

* % Change = change from baseline relative to baseline value [(post-intervention − baseline) / baseline].

†P-value for difference in % Change between PRO and CARB groups.

‡ Outliers excluded (PRO n=12, CARB n=12).

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; HOMA-IR = homeostasis model assessment of insulin resistance; CRP = C-reactive protein; IL-6 = interleukin 6.

Table 5a. Regression analyses: % change in HOMA as dependent variable (outliers excluded; PRO n=12, CARB n=12).

	Variable	Adjusted R^2	Model p-value	Standardized β-coefficient	p-value
Model 1	Supplement group*	0.037	0.179	-0.277	0.179
Model 2	Supplement group*	0.146	0.177	-0.173	0.424
	WB fat mass[†]			0.282	0.197
Model 3	Supplement group*	0.056	0.203	-0.172	0.434
	Abdominal fat mass[†]			0.263	0.237
Model 4	Supplement group*	0.050	0.230	-0.126	0.612
	SAT volume[†]			0.288	0.253
Model 5	Supplement group*	0.052	0.227	-0.128	0.605
	IMAT volume[†]			0.289	0.248

* PRO = 1, CARB = 0.

† % Change from baseline relative to baseline value [(post-intervention – baseline) / baseline] as independent variables.

PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; HOMA = homeostasis model assessment of insulin resistance; WB = whole body; SAT = subcutaneous adipose tissue; IMAT = intermuscular adipose tissue.

Table 5b. Regression analyses: % change in CRP as dependent variable (outliers excluded; PRO n=12, CARB n=12)

	Variable	Adjusted R^2	Model p-value	Standardized β-coefficient	p-value
Model 1	Supplement group*	0.075	0.100	-0.336	0.100
Model 2a	Supplement group*	0.114	0.101	-0.227	0.284
	WB fat mass[†]			0.295	0.168
Model 2b	Supplement group	0.154	0.091	-0.273	0.196
	WB fat mass[†]			0.297	0.157
	Anti-inflammatory meds[‡]			-0.272	0.169
Model 3a	Supplement group*	0.182	0.042	-0.175	0.394
	Abdominal fat mass[†]			0.404	0.057
Model 3b	Supplement group*	0.208	0.049	-0.225	0.278
	Abdominal fat mass[†]			0.384	0.067
	Anti-inflammatory meds[‡]			-0.242	0.204
Model 4a	Supplement group*	0.282	0.014	-0.037	0.863
	SAT volume[†]			0.568	0.015
Model 4b	Supplement group*	0.299	0.021	-0.089	0.682
	SAT volume[†]			0.543	0.019
	Anti-inflammatory meds[‡]			-0.224	0.234
Model 5a	Supplement group*	0.046	0.241	-0.252	0.313
	IMAT volume[†]			0.163	0.511
Model 5b	Supplement group*	0.085	0.205	-0.288	0.246
	IMAT volume[†]			0.188	0.442
	Anti-inflammatory meds[‡]			-0.282	0.190

* PRO = 1, CARB = 0.

[†] % Change from baseline relative to baseline value [(post-intervention – baseline) / baseline] as independent variables.

[‡] Post-intervention anti-inflammatory medication used as independent variable.

PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; CRP = C-reactive protein; WB = whole body; SAT = subcutaneous adipose tissue; IMAT = intermuscular adipose tissue.

CHAPTER 4

The Effects of a Higher Protein Intake During Energy Restriction on Changes in Lean Soft Tissue, Muscle and Physical Function in Postmenopausal Women

4.1 Introduction

Aging is associated with not only increased adiposity, but also decreased skeletal muscle mass (1). These age-related changes in body composition can result in decreased muscle strength and quality (2-3), mobility limitations (4), an inability to perform daily activities (5) and a greater risk for physical disability (6-7). Gender inequity exists with regard to body composition and physical function as older women have greater adiposity (8-9), less lean mass (8-9), lower muscle quality (8, 10), poorer balance and gait than men of a similar age (11-13), as well as a greater prevalence for falls and risk of physical disability (14-15).

Although limited data is available on effects of intentional weight loss in obese and overweight individuals on physical function and risk of disability in older adults, evidence suggests that intentional weight loss may improve physical performance (16-18). This issue is as yet controversial in the elderly specifically, as weight loss is known to lead to reductions in muscle and decreases in muscle strength (3, 19).

Increasing dietary protein intake in a reduced calorie diet may help maintain muscle mass during weight loss, and subsequently convey a beneficial effect on physical function. Emerging data from the Health ABC Study indicate that older adults consuming greater amounts of protein are less likely to lose lean mass over time (20). Early studies on nitrogen balance in obese individuals undergoing weight loss showed that the current Recommended Dietary Allowance (RDA) for protein is insufficient to support nitrogen equilibrium (21-22). Levels of protein intake of 1.5-1.6 g/kg body weight/d (21, 23), or 25-30% of energy intake (23-24) have been suggested for improved weight loss and maintenance of muscle mass. Moreover, incorporating exercise with a higher protein weight loss diet appears to have additive effects for optimizing changes in body composition (25).

The American Society for Nutrition and NAASO, The Obesity Society, specifically recommend weight loss therapies that maximize fat mass loss and minimize skeletal muscle loss in order to maintain physical function and independence in older adult cohorts (1). However,

consensus on the most appropriate weight loss regimen and the body composition variable most predictive of beneficial changes in physical function for this population has not been adequately characterized.

In this context, the primary aim of this study was to assess the effect of a higher protein, lower carbohydrate (PRO) weight loss diet on lean soft tissue (LST), muscle and physical function, compared with a conventional higher carbohydrate, lower protein weight loss diet (CARB). The secondary aim was to identify measures of muscle quality that best predict changes in physical function, characterized by strength, balance and physical performance. We hypothesized that a PRO weight loss diet would result in attenuation of lean mass loss compared with an isocaloric CARB diet, and that these changes may convey beneficial effects on physical function. To our knowledge, no studies have previously assessed the effects of a higher protein intake on physical function in older individuals undergoing intentional weight loss. Findings from this study will contribute to the understanding of the optimal macronutrient composition of weight loss diets for maintaining lean soft tissue, muscle and physical function in older individuals.

4.2 Methods

Study design and participants. Thirty-one healthy, overweight or obese postmenopausal women (mean age±SD: 65.2±4.6 yrs; 29 Caucasian, 1 African American, 1 Latina) were recruited from the community for a 6 month double-blind, randomized weight loss trial. Exclusion criteria were as follows: BMI <28 kg/m^2; >300 lb weight; major weight change defined as >5 lb change in the prior 6 mo; postmenopausal <5 yrs; regular physical activity >1 h/wk or >2 sessions/wk in the prior 6 mo; regular smoking within the prior 10 yrs; history of conditions that impact the primary outcomes such as cancer, cardiovascular disease, type 2 diabetes, renal disease, musculoskeletal or neuromuscular impairments; and use of medications that may impact bone metabolism, such as hormone replacement therapy or osteoporosis drugs, within the prior 2 years. Health history was obtained with a questionnaire, which included items on medications, supplements and history of conditions other than ones listed as exclusion criteria. Participants were screened with standard blood tests (comprehensive metabolic panel and complete blood count). All participants were required to provide personal physician clearance prior to enrollment. All participants signed a consent form approved by the University

of Illinois Institutional Review Board upon enrollment in the study. Participants were blocked on age and BMI with subsequent random assignment to either PRO or CARB treatment. Researchers and participants were blinded to which treatment, PRO or CARB, the participants received.

Dietary treatment. Participants were provided with a supplement: the PRO group received 50 g/d of 90% whey protein isolate, and the CARB group received 50g/d of maltodextrin. Participants were asked to ingest half (25g) of the supplement in the morning with breakfast and the other half in the afternoon or evening. The PRO diet prescribed was designed to provide 0.8 g/kg/d of dietary protein with an additional 45g/d of protein from the supplement, resulting in approximately 30% of energy intake from protein, 40% of energy from carbohydrate and 30% of energy from fat. The CARB diet was aimed at providing 0.8 g/kg/d protein or approximately 18% of energy from protein, 52% of energy from carbohydrate (~182 g/d) and 30% of energy from fat, with an additional 50 g/d of carbohydrate from the supplement.

Participants were asked to attend diet education classes taught by a registered dietitian every 2 weeks during the 6 mo weight loss program. Diet education was based on healthy eating guidelines according to the USDA My Pyramid (26). The registered dietitian also provided personal feedback monthly to each participant, adjusting energy intake and portions when needed to ensure weight loss. Participants were provided with meal plans and food scales (A&D Weighing, Model SK2000, San Jose, CA, USA) to weigh their food and control portions. Diet education and meal plans were the same for both PRO and CARB groups, and were designed to decrease daily energy intake by approximately 500 kcal/d, with a goal of 10% weight loss over 6 mo. Participants were also provided with a one-a-day multivitamin and a calcium and vitamin D supplement to be taken every day.

Adherence to the diet was monitored by measuring body weight monthly and by self-reported weight weekly. Online food records were obtained monthly to track food intake and provide feedback to participants. Three-day weighed food diaries were collected for research purposes at baseline, month 3 and post-intervention, and were reviewed by the dietitian for completeness. All 3-day food diaries were analyzed by the same student using Nutritionist Pro commercial software version 4.1 (Axxya Systems, Stafford TX, USA). Used supplement containers were collected and unused supplement was weighed to assess compliance to the supplement component of the treatment.

Exercise treatment. Supervised exercise classes were provided 5 d/wk, of which participants were required to attend 2-3 exercise classes/wk. Exercise consisted of 20 min of flexibility exercises and 20-30 min of low to moderate intensity walking on an indoor track. Prescribed exercise was the same for both PRO and CARB groups. Participants recorded daily minutes walked at exercises classes and rate of perceived exertion (RPE) at classes. RPE scale ranged from 6 (extremely easy) to 20 (extremely hard). Participants were also provided a pedometer (Accusplit AE170XLGM, Pleasanton, CA, USA), and were asked to record total daily steps over 7 consecutive days monthly.

Body composition. Height was measured at baseline and post-intervention to the nearest 0.1 cm with a digital stadiometer (Seca, Model 242, Hanover, MD, USA). Weight was measured monthly to the nearest 0.1 kg with a digital scale (Tanita, Model BWB-627 A, Arlington Heights, IL, USA). Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Leg length, waist and hip circumferences were measured monthly to the nearest 0.1 cm with a Gulick II retractable measuring tape (Country Technology Inc., Gay Mills, WI, USA).

Whole body lean soft tissue (LST) and whole body relative LST (%LST) were measured using dual energy X-ray absorptiometry (DXA; Hologic Discovery, software version 12.7.3, Waltham, MA, USA). Right leg LST and %LST (right leg LST/right leg mass) were obtained from the whole body scan. Participants wore light-weight clothing and removed all jewelry. All DXA scans were analyzed by the same technician. The DXA machine was calibrated daily using manufacturer phantoms. Precision for DXA measurements of interest is between 1 and 1.5% in our laboratory.

Magnetic resonance imaging (MRI) was used to measure muscle volume of the right thigh. All imaging was performed on a 1.5T General Electric Signa Excite whole-body scanner (Milwaukee, WI, USA) using a phased array torso coil strapped around the thigh of interest. A spoiled gradient-recalled echo sequence was used to acquire in- and out-of-phase images for a two-point Dixon fat separation technique (27). Images were acquired in 10 mm slices over the middle third of the participant's femur (14-16 slices), as measured from the distal and proximal endpoints of the femur in the scouting scan. A field of view of 48 cm and matrix size of 256x256 resulted in an in-plane resolution of 1.875 mm. Determination of muscle quantity was performed by segmenting in the water-only image, and region-growing was used to determine the threshold.

All images were analyzed by the same operator using Amira 5.2 (Visage Imaging Inc., San Diego, CA, USA).

Muscle strength. Right knee extensor and flexor strength were measured using a Humac Norm Isokinetic Dynamometer (Computer Sports Medicine Inc. Stoughton, MA, USA). Participants performed voluntary maximal contractions, while seated with back supported and the tested thigh strapped down. Isokinetic tests were performed at 60°/s, and the greatest peak torque from four repetitions was used for analysis. Isometric tests were performed with the dynamometer arm fixed at a 45° angle, with the greatest peak torque of three trials used for analysis. The sum of isometric extensor and flexor strength was used for muscle quality indices.

Balance. The Star Excursion Balance Test (SEBT) is a standardized protocol that challenges unilateral dynamic balance (28). It was performed with the participants standing with their right foot in the middle of a "star" formed by eight lines extending out at 45° from each other. The participants were asked to reach as far as possible with their left leg along the lines in the anterior, posteromedial and posterolateral directions, lightly touch the ground with the distal part of their foot and return to a double-leg stance without losing balance. The three best trials were averaged and normalized for right leg length.

Physical performance. The modified nine-item Physical Performance Test (PPT) was used to assess each participant's level of physical function at baseline (29-30). The PPT correlates well with degree of disability, loss of independence and mortality, as well as other objective measures of strength balance and aerobic power (31). Items include climbing a flight of 10 stairs, standing up 5 times from a chair, walking 50 ft, putting on and removing a coat, picking up a penny placed 12 inches in front of the dominant foot, lifting a book onto a shelf 12 inches above shoulder height, climbing up and down 4 flights of stairs, turning 360°, and lastly, standing with feet side by side, semi-tandem and full-tandem. Items were completed once and scored between 0 and 4, with 36 being a perfect total score, and a participant with a total score between 18 and 31 is classified as mildly frail.

Other tests of physical performance included the timed up and go and transfer tasks. Up and go involved the participant starting seated in a chair, standing up without using their arms, walking as quickly as possible around a cone place 2.5 m from the chair, and returning to a seated position in the chair. The transfer task involved a timed test where the participant started from a standing position, moved to a seated position on the floor and then resumed standing. In

addition, the 10-stair climb and the chair stand tests were used as independent measures of physical performance. These, as well as the stair climb and chair stand tests, were conducted twice, and times were averaged.

Statistical analysis. All data analyses were conducted using SPSS version 17.0 (Chicago, IL, USA). Only data for participants who completed post-intervention tests were included in the analysis. Data were assessed for normality using the Shapiro-Wilk statistic. Nonparametric tests were used for data that did not meet the assumption of normality. Change variables were calculated for the difference in values between PRO and CARB groups relative to baseline values. Baseline and change variables were compared between PRO and CARB groups using Student's independent t-test. Linear regression using change variables was used to examine body composition determinants of changes in physical function outcomes (strength, balance and physical performance). For regression analyses, one outlier was removed to meet the assumptions of normality and homoscedasticity. Composite variables were created using reliability analysis for 1) balance and 2) physical performance constructs. Both balance (SEBT posteromedial and posterolateral reach) and physical performance (up and go, chair stand and stair climb) had good internal consistency, with Cronbach's alphas of 0.88 and 0.70, respectively. Z-scores of the measures used in the constructs were then averaged to produce balance and physical performance composite variables. An α-level of 0.05 was considered significant. Results are expressed as mean±SD.

4.3 Results

Of the 31 women that enrolled in the study, 26 (84%; 13 PRO, 13 CARB; 25 Caucasian, 1 African American) completed post-intervention measurements. Three participants withdrew due to health issues, and two withdrew for other personal reasons (Figure 3).

Physical activity levels of participants measured by pedometer did not differ between PRO and CARB at baseline, nor did level of physical function measured by the PPT (Table 6). Of baseline dietary macronutrients, carbohydrate and fat intake as a percentage of energy intake (%E) differed between groups with a +7.7% difference in %E carbohydrate intake and -7.2% lower %E fat intake in the PRO group (Table 7). Baseline variables for strength, balance, physical performance and body composition did not differ between PRO and CARB, except the up and go test (p=0.04; Tables 8 and 9).

Diet education class adherence was 81.4±19.2% and 71.3±22.2% (p=0.23) and exercise class attendance was 87.9±12.2% and 78.6±14.9% (p=0.09) for PRO and CARB, respectively. Walking duration at exercise classes increased for both groups, and although PRO walked 5% longer than CARB, this was not statistically different (p=0.13; Table 6). RPE at exercise classes increased for PRO but decreased slightly for CARB, trending towards a significant difference (p=0.08; Table 6). Pedometer steps increased from baseline to post-intervention for PRO and CARB combined (5054±2536 steps/d, 6516±2997 steps/d, p=0.023), and duration of walking at exercise classes increased from month 1 to post-intervention (22.9±2.6 min/class, 24.6±3.7 min/class, p<0.001).

Mean supplement intake over 6 mo was 41.5±6.5 g/d (77.5% of prescribed supplement) for PRO and 38.2±10.8 g/d (74.8% of prescribed supplement) for CARB, and did not differ between groups (p=0.36). Total post-intervention energy and macronutrient intake, displayed in Table 2, includes intake from both diet and supplement combined. Energy intake differed between groups at post-intervention, however, when taking post-intervention weight into account, intake was not different (20.6±4.7 and 21.1±4.8 kcal/kg body weight, for PRO and CARB, respectively; p=0.50). As intended by study design, total protein intake was greater for PRO and total carbohydrate intake was greater for CARB when on treatment. At post-intervention, PRO ingested 1.20±0.14 g/kg/d protein compared to 0.86±0.20 g/kg/d for CARB (p<0.001). Fat intake and %E fat intake were less for PRO compared to CARB, (both p<0.05); however, the % change from baseline did not differ likely somewhat influenced by the higher %E fat intake in CARB at baseline (p<0.05).

Twenty-three % of participants (6 of 26) reduced their weight 10% or more from baseline; 54% (14 of 26) reduced initial weight by 3-9%; and the remaining 23% reduced their weight by 1% or less. Mean weight decreased after 6 months in both groups, with a trend for a greater decrease in the PRO group compared to CARB (3.9% difference, p=0.059; Table 6).

None of the changes in strength, balance or physical performance measures differed between PRO and CARB (Table 8), including change in the sum of isometric extensor and flexor strengths (p=0.46). Changes in either balance or performance composite variables did not differ between PRO and CARB groups (p=0.95, p=0.84, respectively).

In PRO and CARB combined, all measures of strength decreased, and balance and physical performance improved at post-intervention (Table 8). Isokinetic knee extensor

(p=0.001) and flexor (p=0.006), isometric knee flexor strength decreased significantly (p=0.001); and anterior reach (p=0.019), posterolateral reach (p<0.001), chair stand (p<0.001), and stair climb (p=0.001) improved significantly. Changes in isometric knee extensor (p=0.099), posteromedial reach (p=0.058) and transfer test (p=0.064) approached significance.

Changes in DXA body composition and muscle quality indices did not differ between PRO and CARB, but MRI showed significant changes in muscle volume between groups (Table 9). PRO lost 4.2% more muscle volume than CARB, but when expressed as relative to thigh volume (p=0.01), PRO actually gained 5.8% more muscle than CARB (p=0.049). Furthermore, when expressed as a weight to leg LST ratio, there was a beneficial improvement in this ratio in PRO compared with CARB (p=0.03).

As no differences in the main outcomes were found between PRO and CARB groups, the groups were subsequently combined, and linear regression was used to determine whether changes in weight and body composition predicted changes in physical function. With respect to changes in physical function measures, weight loss contributed to improved performance in up and go (p=0.038), transfer test (p=0.028), trended towards improvement in stair climb (p=0.074), and decreased strength in isometric knee extensor strength (p=0.041). Of LST and muscle measures, changes in weight to leg LST ratio showed a trend towards predicting changes in the composite of performance (adjusted r^2=0.161, p=0.052) and predicted changes in up and go (adjusted r^2=0.189, p=0.02); whereas whole body %LST predicted changes in the composite of balance (adjusted r^2=0.179, p=0.04) and posteromedial reach (adjusted r^2=0.191, p=0.02).

4.4 Discussion

The primary finding in this study confirms previous research and meta-analyses (24-25, 32-33) showing that a higher protein intake during weight loss, compared to a traditional higher carbohydrate, lower protein reduced calorie diet, can help maintain muscle relative to changes in adiposity. However, in absolute terms, similarly to reductions in LST and FFM found in other studies (34-37), we show that there was actually a greater absolute loss in the PRO group. Importantly though, when accounting for greater success with weight loss seen in the PRO group by examining relative muscle volume, there is actually a greater net gain of muscle in the PRO compared to the CARB group. In this study, the benefit of using MRI in assessing changes in

50

body composition was apparent, as the directions of LST changes were similar when measured with DXA.

Intentional weight loss induced primarily by caloric restriction has been shown to decrease thigh muscle volume in 50-60 year old men and women (19), as well as decrease knee flexor strength (19). We found a reduction in isometric knee extensor strength, however, in our study it appears that this loss of strength was due to weight loss and not to changes in LST or muscle, neither absolute or relative to weight. Importantly, despite reduced strength, physical performance improved due to weight loss. Weight loss in older adults, even in short-term trials, has indeed been found to improve physical performance in both healthy individuals (17-18) and those with knee osteoarthritis (16). Incorporating flexibility, endurance and resistance exercise in the weight loss program has even greater benefits for both strength (38-39) and physical performance (39-42). The volume, intensity and modality of exercise in the current study was likely not adequate enough to elicit beneficial effects on strength. Pedometer steps and duration of walking at exercise classes increased throughout the course of the study, and although there was not a statistical effect of these variables on physical function, increased activity at classes and potentially at home may have played a role in improving performance.

Developing optimal weight loss programs for decreasing risk of physical disability and obesity related disease requires information on body composition changes that best predict physical function following weight loss. Very limited data is available, but it has been suggested that decreases in fat free mass are associated with improved physical performance, which is counter-intuitive (16, 18). In our PRO and CARB combined sample, increases in whole body %LST predicted 18-19% of improved balance, and weight to leg LST ratio predicted 16-19% of variance of improved composite performance. In cross-sectional studies assessing associations between body composition and measures of physical function, women with obese sarcopenia have a 3.8 times greater risk for functional limitation than women with greater levels of skeletal muscle mass relative to weight (5). Furthermore, lower relative whole body skeletal muscle mass is reported to be associated with lower leg strength (5) and poor physical performance (6). Overall, it appears that although muscle mass may decrease with weight loss, it is important to measure relative muscle mass, and our findings suggest that an increase in %LST with weight loss can confer beneficial effects on balance and performance. In addition, thigh muscle attenuation (intramyocellular fat accumulation, i.e. a parameter for muscle quality) has been

associated with self-reported mobility limitations (4), therefore it may also be important to measure changes within the muscle itself.

As an indicator of the amount of whole body weight supported by the lower-body, the weight to leg LST ratio conceptually has clinical significance for physical function although empirical work is limited. Findings from the current study confirm previous results in our laboratory, in which relative (i.e. to body mass) leg LST was correlated with physical performance in older women (43). We found that the up and go test, in particular, was independently predicted by weight to leg LST ratio, suggesting that the relative amount of lower-body LST to support whole body weight, is important for the ability to perform everyday activities. The older female population is known to be at an increased risk for physical disability (14-15), therefore losing some of the burden caused by overweight on the leg joints may help prevent injuries (44-45), and furthermore, help maintain physical independence with aging (1). Although the PRO diet did not directly impact measures of strength, balance or physical performance, the change in weight to leg LST ratio differed between PRO and CARB groups, indicating, again, that a higher protein intake can help preserve muscle, which in turn has beneficial effects on physical performance.

The main limitation in this study was the small sample size. However, the small effect sizes in this study for physical function measures, ranging from 0.01 (SEBT posteromedial reach) to 0.32 (isokinetic knee flexor strength) (46), with the exception of the up and go and chair stand tests (effect sizes 0.87 and 0.5, respectively), suggest that our nonsignificant findings are not necessarily due to a small sample size. Alternatively, either dietary treatment is not robust enough or that the elicited weight change was not of a magnitude to produce changes in these outcomes or perhaps the performance measures are not sensitive enough to show potential changes in physical function. Promising findings on the importance of protein for maintaining relative muscle during weight loss, and the subsequent preservation of %LST to enhance physical function provide excellent preliminary data for further research.

In conclusion, increasing protein intake during weight loss can offset the deleterious effects on muscle mass by maintaining more muscle relative to weight lost. Protein intake levels during weight loss do not appear to directly impact physical function. However, the effects of weight loss on changes in relative LST appear to play a role in improving balance and physical performance. In this older female population, although weight loss negatively impacted strength,

the reduced weight helped with other aspects of physical function. This is a significant finding for the development of weight loss programs that can improve health while also contributing to maintaining independence in older individuals. Including exercise beyond walking and flexibility, such as resistance training, will likely prevent some of the strength loss, and further improve physical function. Future clinical trials should include both a dietary and an exercise weight loss group to determine the most beneficial regimen for improving health, as well as strength, balance and performance.

4.5 References

1.   Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. Obes Res. 2005 Nov;13:1849-63.

2.   Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. J Gerontol A Biol Sci Med Sci. 2006 October 1, 2006;61:1059-64.

3.   Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, Boudreau R, Manini TM, Nevitt M, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. Am J Clin Nutr. 2009 December 1, 2009;90:1579-85.

4.   Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB, for the Health ABCS. Muscle Mass, Muscle Strength, and Muscle Fat Infiltration as Predictors of Incident Mobility Limitations in Well-Functioning Older Persons. J Gerontol A Biol Sci Med Sci. 2005 March 1, 2005;60:324-33.

5.   Zoico E, Di Francesco V, Guralnik JM, Mazzali G, Bortolani A, Guariento S, Sergi G, Bosello O, Zamboni M. Physical disability and muscular strength in relation to obesity and different body composition indexes in a sample of healthy elderly women. Int J Obes Relat Metab Disord. 2004 Feb;28:234-41.

6.   Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc. 2002 May;50:889-96.

7.   Woods NF, LaCroix AZ, Gray SL, Aragaki A, Cochrane BB, Brunner RL, Masaki K, Murray A, Newman AB. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. J Am Geriatr Soc. 2005 Aug;53:1321-30.

8.   Newman AB, Haggerty CL, Goodpaster B, Harris T, Kritchevsky S, Nevitt M, Miles TP, Visser M, the Health AaBCRG. Strength and Muscle Quality in a Well-Functioning Cohort of

Older Adults: The Health, Aging and Body Composition Study. Journal of the American Geriatrics Society. 2003;51:323-30.

9.   Newman AB, Lee JS, Visser M, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Nevitt M, Harris TB. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. Am J Clin Nutr. 2005 October 1, 2005;82:872-8.

10.   Katsiaras A, Newman AB, Kriska A, Brach J, Krishnaswami S, Feingold E, Kritchevsky SB, Li R, Harris TB, et al. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. J Appl Physiol. 2005 Jul;99:210-6.

11.   Wolfson L, Whipple R, Derby CA, Amerman P, Nashner L. Gender differences in the balance of healthy elderly as demonstrated by dynamic posturography. J Gerontol. 1994 Jul;49:M160-7.

12.   Hageman PA, Leibowitz JM, Blanke D. Age and gender effects on postural control measures. Arch Phys Med Rehabil. 1995 Oct;76:961-5.

13.   Musselman K, Brouwer B. Gender-related differences in physical performance among seniors. J Aging Phys Act. 2005 Jul;13:239-53.

14.   Penninx BW, Ferrucci L, Leveille SG, Rantanen T, Pahor M, Guralnik JM. Lower extremity performance in nondisabled older persons as a predictor of subsequent hospitalization. J Gerontol A Biol Sci Med Sci. 2000 Nov;55:M691-7.

15.   Murtagh KN, Hubert HB. Gender differences in physical disability among an elderly cohort. Am J Public Health. 2004 Aug;94:1406-11.

16.   Miller GD, Nicklas BJ, Davis C, Loeser RF, Lenchik L, Messier SP. Intensive weight loss program improves physical function in older obese adults with knee osteoarthritis. Obesity (Silver Spring). 2006 Jul;14:1219-30.

17.   Jensen GL, Roy M-A, Buchanan AE, Berg MB. Weight Loss Intervention for Obese Older Women: Improvements in Performance and Function[ast][ast]. Obesity. 2004;12:1814-20.

18.   Sartorio A, Lafortuna CL, Agosti F, Proietti M, Maffiuletti NA. Elderly obese women display the greatest improvement in stair climbing performance after a 3-week body mass reduction program. Int J Obes Relat Metab Disord. 2004;28:1097-104.

19.   Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Ehsani AA, Holloszy JO. Lower extremity muscle size and strength and aerobic capacity decrease with caloric restriction but not with exercise-induced weight loss. J Appl Physiol. 2007 Feb;102:634-40.

20.   Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB. Dietary protein intake is associated with lean mass

change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr. 2008 Jan;87:150-5.

21.   Hoffer LJ, Bistrian BR, Young VR, Blackburn GL, Matthews DE. Metabolic effects of very low calorie weight reduction diets. The Journal of Clinical Investigation. 1984;73:750-8.

22.   Oi Y, Okuda T, Koishi H, Koh H, Waki M, Kurata M, Nambu S. Relationship between protein intake and nitrogen balance in obese patients on low energy diet. J Nutr Sci Vitaminol (Tokyo). 1987;33:219-26.

23.   Layman DK. Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss. J Am Coll Nutr. 2004 December 1, 2004;23:631S-6.

24.   Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. Dietary Protein, Weight Loss, and Weight Maintenance. Annual Review of Nutrition. 2009;29:21-41.

25.   Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. J Nutr. 2005 Aug;135:1903-10.

26.   USDA. MyPyramid.gov. 2009 [cited 2010 March 25]; Available from: http://www.mypyramid.gov/

27.   Ma J. Dixon techniques for water and fat imaging. Journal of Magnetic Resonance Imaging. 2008;28:543-58.

28.   Gribble PA, Hertel J. Considerations for normalizing measures of the Star Excursion Balance Test. Meas Phys Educ Exerc Sci. 2003;7:89-100.

29.   Reuben DB, Siu AL. An objective measure of physical function of elderly outpatients. The Physical Performance Test. J Am Geriatr Soc. 1990 Oct;38:1105-12.

30.   Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol. 1994 Mar;49:M85-94.

31.   Brown M, Sinacore DR, Binder EF, Kohrt WM. Physical and Performance Measures for the Identification of Mild to Moderate Frailty. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2000 June 1, 2000;55:M350-M5.

32.   Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. Am J Clin Nutr. 2006 February 1, 2006;83:260-74.

33.   Layman DK, Evans EM, Erickson D, Seyler J, Weber J, Bagshaw D, Griel A, Psota T, Kris-Etherton P. A Moderate-Protein Diet Produces Sustained Weight Loss and Long-Term Changes in Body Composition and Blood Lipids in Obese Adults. J Nutr. 2009 March 1, 2009;139:514-21.

34.   Brinkworth GD, Noakes M, Keogh JB, Luscombe ND, Wittert GA, Clifton PM. Long-term effects of a high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulinemic subjects. Int J Obes Relat Metab Disord. 2004 May;28:661-70.

35.   Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005 Jun;81:1298-306.

36.   Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with Type 2 diabetes: one-year follow-up of a randomised trial. Diabetologia. 2004;47:1677-86.

37.   Kerksick C, Thomas A, Campbell B, Taylor L, Wilborn C, Marcello B, Roberts M, Pfau E, Grimstvedt M, et al. Effects of a popular exercise and weight loss program on weight loss, body composition, energy expenditure and health in obese women. Nutrition & Metabolism. 2009;6:23.

38.   Wang X, Miller GD, Messier SP, Nicklas BJ. Knee strength maintained despite loss of lean body mass during weight loss in older obese adults with knee osteoarthritis. J Gerontol A Biol Sci Med Sci. 2007 Aug;62:866-71.

39.   Maffiuletti NA, Agosti F, Marinone PG, Silvestri G, Lafortuna CL, Sartorio A. Changes in body composition, physical performance and cardiovascular risk factors after a 3-week integrated body weight reduction program and after 1-y follow-up in severely obese men and women. Eur J Clin Nutr. 2005;59:685-94.

40.   Messier SP, Loeser RF, Miller GD, Morgan TM, Rejeski WJ, Sevick MA, Ettinger WH, Jr., Pahor M, Williamson JD. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum. 2004 May;50:1501-10.

41.   Villareal DT, Banks M, Sinacore DR, Siener C, Klein S. Effect of weight loss and exercise on frailty in obese older adults. Arch Intern Med. 2006 Apr 24;166:860-6.

42.   Frimel TN, Sinacore DR, Villareal DT. Exercise Attenuates the Weight-Loss-Induced Reduction in Muscle Mass in Frail Obese Older Adults. [Article].

43.   Valentine RJMS, Misic MMP, Rosengren KSP, Woods JAP, Evans EMP. Sex impacts the relation between body composition and physical function in older adults. [Article].

44.   Murphy L, Schwartz TA, Helmick CG, Renner JB, Tudor G, Koch G, Dragomir A, Kalsbeek WD, Luta G, Jordan JM. Lifetime risk of symptomatic knee osteoarthritis. Arthritis Care & Research. 2008;59:1207-13.

45.   Niu J, Zhang YQ, Torner J, Nevitt M, Lewis CE, Aliabadi P, Sack B, Clancy M, Sharma L, Felson DT. Is obesity a risk factor for progressive radiographic knee osteoarthritis? Arthritis Care & Research. 2009;61:329-35.

46.   Lipsey MW. Design sensitivity: statistical power for experimental research. Newbury Park, CA: Sage Publications; 1990.

Figure 3. Study design flow chart. PRO = higher protein weight loss diet; CARB = higher carbohydrate weight loss diet.
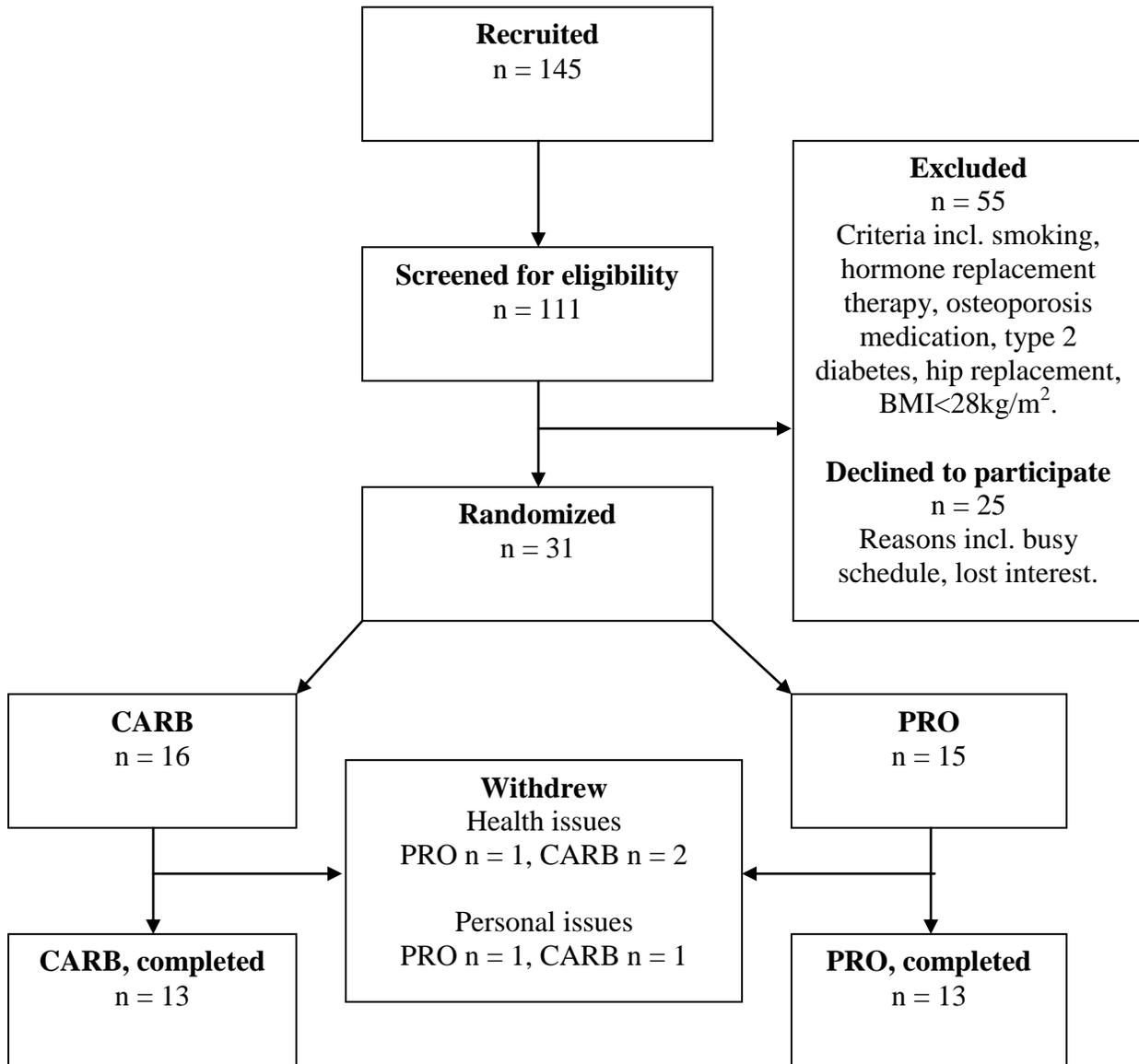
Table 6. Descriptive characteristics of participants (PRO n= 13, CARB n= 13) represented as mean±SD.

	Group	Baseline	P-value*	Post-intervention	% Change†	P-value‡
Age (yrs)	PRO	64.77±4.4		-	-	
	CARB	64.6±5.2	0.936	-	-	-
Height (cm)	PRO	161.9±4.9		-	-	
	CARB	162.7±5.7	0.678	-	-	-
Weight (kg)	PRO	84.7±10.5		77.7±9.35	-8.0±6.2	
	CARB	86.8±13.4	0.655	83.3±13.7	-4.1±3.6	0.059
BMI (kg/m^2)	PRO	32.3±3.9		29.8±3.7	-7.6±6.2	
	CARB	32.7±4.2	0.804	31.5±4.6	-3.9±3.7	0.075
Pedometer (steps/d)	PRO	5902±2875		6463±3217	28.8±79.2	
	CARB	4108±1484	0.068	6652±2708	55.7±40.6	0.347
Walking duration in exercise class (min)	PRO	23.6±2.7§		25.6±3.8	9.8±7.0	
	CARB	22.7±2.5§	0.409	23.7±3.6	4.8±8.1	0.132
RPE at exercise class	PRO	12.3±1.7§		13.0±1.9	9.0±13.6	
	CARB	12.5±1.4§	0.727	12.2±1.5	-2.0±15.0	0.080
PPT score	PRO	31.6±3.1		32.0±2.6	1.5±10.1	
	CARB	32.7±2.5	0.339	33.9±2.0	2.7±5.3	0.726

* P-value for difference in baseline values between PRO and CARB groups, p<0.05.

†% Change = change from baseline relative to baseline value [(post-intervention – baseline) / baseline].

‡ P-value for difference in % Change between PRO and CARB groups.

§ Values at month 1 of the intervention.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; BMI = body mass index; RPE = rate of perceived exhaustion; PPT = physical performance test.

Table 7. Dietary energy and macronutrient intake data, and total energy and macronutrient including supplement (PRO n=11, CARB n=12) represented as mean±SD.

	Group	Energy intake (kcal/d)	Protein intake			Carbohydrate intake		Fat intake	
			(g/d)	(g/kg/d)	(%E)	(g/d)	(%E)	(g/d)	(%E)
Dietary baseline	PRO	1687±264	76.2±24.0	0.91±0.23	18.6±7.4	220.0±47.5	52.1±7.5	56.2±18.5	29.5±6.0
	CARB	1743±321	81.4±18.6	0.98±0.25	18.8±3.6	190.9±49.8	44.4±9.1*	71.4±19.6	36.7±6.9*
Dietary during intervention	PRO	1221±228	58.5±13.3	0.76±0.13	19.4±4.1	169.0±24.5	56.1±7.5	35.3±12.0	25.6±5.7
	CARB	1484±367	71.0±13.7	0.87±0.20	19.8±4.2	178.3±47.3	48.8±10.2	53.8±23.4	31.5±7.6
Total during intervention[§]	PRO	1369±197	91.8±12.6	1.21±0.14	27.3±5.3	169.0±24.5	49.6±5.6	35.3±12.0	22.8±5.6
	CARB	1627±388*	71.0±13.7*	0.87±0.20*	17.9±3.5*	217.8±53.6*	54.2±9.8	53.8±23.4*	28.8±7.4*
% Change[†]	PRO	-18.1±18.8	27.5±29.4	40.5±33.8	61.3±44.8	-19.6±26.0	-2.3±18.6	-34.1±31.9	-22.4±24.7
	CARB	-6.0±30.3	-9.8±26.0	-5.8±26.9	0.1±30.6	17.2±41.1	26.8±35.9	-20.3±47.6	-18.8±25.7
p-value[‡]		0.295	0.004	0.001	0.001	0.019	0.014	0.622	0.737

* Significant difference in values between PRO and CARB groups within the same time point, p<0.05.

[†] % Change = change from baseline relative to baseline value [(during intervention including supplement − baseline) / baseline].

[‡] p-value for difference in % Change between PRO and CARB groups.

[§] Includes mean supplement and dietary intake per day assessed at month 6 of intervention.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet.

Table 8. Strength, balance and physical performance outcomes (knee strength: PRO n=11, CARB n=13; SEBT: PRO n=12, CARB n=11; physical performance: PRO n=11, CARB n=13) represented as mean±SD.

	Group	Baseline	Post-intervention	% Change*	P-value†
STRENGTH					
Isokinetic knee extensor strength (Nm) §	PRO	94.2±25.7	92.8±18.2	-9.0±14.1	
	CARB	94.8±14.6	87.8±13.3	-7.0±8.5	0.680
Isokinetic knee flexor strength (Nm) §	PRO	44.5±20.5	44.9±15.1	-7.3±15.0	
	CARB	52.0±15.1	42.5±11.5	-15.2±23.9	0.369
Isometric knee extensor strength (Nm)	PRO	109.7±26.2	111.8±23.2	-4.5±14.8	
	CARB	113.5±23.0	109.5±17.4	-2.0±14.0	0.674
Isometric knee flexor strength (Nm) §	PRO	57.4±19.0	56.9±16.6	-10.1±11.0	
	CARB	59.2±14.6	49.8±14.4	-15.0±18.9	0.470
BALANCE					
SEBT anterior reach (%) §	PRO	83.0±11.0	88.5±10.4	6.5±9.9	
	CARB	84.5±5.3	87.4±3.5	4.0±8.2	0.519
SEBT posteromedial reach (%)	PRO	65.7±12.3	69.8±12.2	4.9±8.7	
	CARB	65.6±5.4	68.4±7.7	5.0±12.1	0.984
SEBT posterolateral reach (%) §	PRO	51.2±18.3	60.0±13.8	27.8±44.1	
	CARB	48.8±8.9	60.6±8.7	24.7±21.7	0.622
PHYSICAL PERFORMANCE					
Up and go test (sec)	PRO	8.4±1.4	8.0±2.2	-3.2±17.8	
	CARB	7.4±0.9‡	6.8±0.7	-7.4±10.8	0.490
Chair stand test (sec) §	PRO	15.1±3.2	12.9±2.5	-11.9±12.7	
	CARB	13.6±2.4	11.5±1.8	-14.2±11.3	0.654
Transfer test (sec) §	PRO	9.7±5.5	7.9±3.4	-10.6±21.1	
	CARB	8.4±4.5	6.7±1.7	-7.4±10.0	0.658
Stair climb test (sec)	PRO	5.6±1.1	5.4±1.6	-2.7±32.1	
	CARB	5.3±0.9	4.8±0.7	-7.6±16.4	0.703

* % Change = change from baseline relative to baseline value [(post-intervention − baseline) / baseline].

†P-value for difference in % Change between PRO and CARB groups.

‡ Indicates significant difference between PRO and CARB at baseline, p<0.05.

§ Indicates significant difference between baseline and post-intervention, when PRO and CARB groups are combined, p<0.05.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; SEBT = star excursion balance test.

Table 9. Changes in lean soft tissue (LST), thigh muscle volume and indices of muscle quality (DXA: PRO n=13, CARB n=13; MRI: PRO n=12, CARB n=12; muscle quality: PRO n=10, CARB n=13) represented as mean±SD.

	Group	Baseline	Post-intervention	% Change*	P-value†
DXA whole body LST (kg)	PRO	47.2±4.3	45.2±1.1	-4.1±4.2	
	CARB	48.1±6.2	46.9±1.6	-2.4±4.0	0.209
DXA whole body %LST	PRO	55.3±2.8	57.6±4.1	4.2±5.0	
	CARB	55.3±3.9	56.0±4.1	1.3±3.1	0.093
DXA leg LST (kg)	PRO	7.8±0.7	7.5±0.8	-3.6±3.9	
	CARB	8.1±1.1	7.9±1.1	-2.3±3.5	0.356
DXA leg %LST	PRO	52.8±4.2	55.1±3.6	4.5±4.1	
	CARB	51.8±6.4	53.1±6.6	2.4±3.0	0.138
Whole body weight/leg LST ratio	PRO	10.79±0.86	10.28±0.74	-4.6±3.6	
	CARB	10.76±0.76	10.57±0.89	-1.8±2.6	0.033
MRI thigh muscle (m^3)	PRO	1.44±0.17	1.36±0.16	-5.0±3.4	
	CARB	1.50±0.26	1.46±0.23	-0.8±3.9	0.010
MRI thigh muscle (% of thigh volume)	PRO	37.4±6.3	40.7±6.2	10.3±8.8	
	CARB	37.0±9.0	36.8±7.1	4.5±3.4	0.049
IM knee strength/body weight (Nm/kg)	PRO	2.06±0.60	2.18±0.48	0.9±10.4	
	CARB	2.01±0.39	1.95±0.42	-2.7±11.9	0.462
IM knee strength/leg %LST (Nm/%LST)	PRO	3.19±0.76	3.06±0.54	-10.6±11.0	
	CARB	3.35±0.65	3.03±0.58	-8.8±12.2	0.712
IM knee strength/muscle volume (Nm/m^3)	PRO	0.12±0.03	0.13±0.02	-2.7±9.6	
	CARB	0.12±0.02	0.11±0.02	-5.6±13.8	0.590

* % Change = change from baseline relative to baseline value [(post-intervention – baseline) / baseline].

†P-value for difference in % Change between PRO and CARB groups.

SD = standard deviation; PRO = higher protein, lower carbohydrate weight loss diet; CARB = higher carbohydrate, lower protein weight loss diet; DXA = dual energy X-ray absorptiometry; LST = lean soft tissue; whole body %LST = whole body LST/whole body mass; leg %LST = leg LST/leg mass; MRI = magnetic resonance imaging; IM = isometric.

CHAPTER 5

Summary and Discussion

The main findings from this study support increasing protein levels in reduced calorie diets to promote weight loss, and reduce fat mass while attenuating muscle loss relative to the weight reduction in older women. Although higher protein intake did not directly impact the improvements in metabolic disease profile, through enhanced fat mass loss, abdominal fat in particular, some improvements were detected in inflammation, which is known to impact the development of metabolic disease. Similarly, protein intake levels during weight loss do not appear to directly impact physical function. However, improvements were seen in physical performance as a result of weight loss and maintenance of relative lean soft tissue.

Interesting findings that have been recently published from the Health ABC Study link obesity and metabolic syndrome with incident mobility limitations (1-2). Furthermore, even after adjusting for whole body obesity, abdominal obesity remained independently associated with incident mobility limitations and elevated inflammatory markers partly explained the association between obesity, metabolic syndrome and mobility limitations. These Health ABC findings, combined with our results, suggest that there is potential for higher protein weight loss diets to play a key role in both reducing metabolic disease risk and functional limitations, with abdominal fat and inflammation being the targets for improvement. It is the intent that results from this small clinical trial will provide the basis for larger clinical efforts targeting the efficacy and effectiveness of optimal nutritional and physical activity interventions to enhance health in older women.

References

1.   Penninx BWJH, Nicklas BJ, Newman AB, Harris TB, Goodpaster BH, Satterfield S, de Rekeneire N, Yaffe K, Pahor M, Kritchevsky SB. Metabolic Syndrome and Physical Decline in Older Persons: Results from the Health, Aging and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2009 January 2009;64A:96-102.
2.   Stenholm S, Koster A, Alley DE, Houston DK, Kanaya A, Lee JS, Newman AB, Satterfield S, Simonsick EM, et al. Joint Association of Obesity and Metabolic Syndrome With Incident Mobility Limitation in Older Men and Women—Results From the Health, Aging, and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2010 January 2010;65A:84-92.

CURRICULUM VITAE

Mina C. Mojtahedi, PhD
512 W Green St. Apt. 8
Champaign IL 61820
Phone 217-898-3727
mojtahed@illinois.edu
5/24/2010

Education

PhD in Nutritional Sciences   August 2010
University of Illinois at Urbana-Champaign, Urbana IL
Dissertation title: *Effects of a higher protein intake during energy restriction on body composition, metabolic disease risk and physical function in postmenopausal women.*
Dissertation advisor: Ellen M. Evans, PhD

Registered Dietitian Candidate/Intern   May 2010
University of Illinois at Urbana-Champaign, Urbana IL

MS & BS in Nutritional Sciences   2002
University of Helsinki, Helsinki, Finland
Thesis title: *The effect of dietary fat on bone density and bone mineral content in adolescents.*
Thesis advisor: Christel Lamberg-Allardt, PhD

BA in English Philology   2003
University of Helsinki, Helsinki, Finland
Thesis title: *Postmodern play with postcolonial sureties in Janet Frame's* The Carpathians.
Thesis advisor: Mark Shackleton, PhD

Relevant Certifications

Certificate in Business Administration   2009
University of Illinois at Urbana-Champaign, Urbana IL

Minor in Development Studies   2003
Faculty of Social Sciences, Department of Development Studies, University of Helsinki, Finland

Erasmus Student Exchange Program   2000
Department of Human Nutrition and Epidemiology, Wageningen University, The Netherlands

Nutrition in Low-Income Countries Program   1998
Faculty of Medicine, International Maternal and Child Health, University of Uppsala, Sweden

Research Experience
Research Assistant   2004 - 2006, 2007 - present
Bone and Body Composition Laboratory, Department of Kinesiology and Community Health
University of Illinois at Urbana-Champaign, Urbana IL (Lab Director: Ellen M. Evans)
o Designed, developed and implemented cross-sectional and intervention research projects using techniques such as magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DXA), air displacement plethysmography (Bod Pod), skinfold measurements, bioelectrical impedance analysis (BIA), broadband ultrasonic attenuator (BUA), resting energy expenditure (Deltatrac II)
o Analyzed data and on body composition, metabolism, bone health, diet and physical activity using a variety of software, including Nutritionist Pro, Microsoft Access, Amira and SPSS
o Supervised lab personnel and resources, and mentored undergraduate research assistants and Master's level students

Department of Human Nutrition   2006 – 2007
University of Illinois at Chicago, Chicago IL
o Conducted a research study and developed surveys for assessing the impact of the built environment on healthy living in urban low-income neighborhoods
o Managed food frequency and diet recall data using the Minnesota Nutrition Data System, EpiInfo and SAS software

Department of Human Nutrition and Epidemiology   2000
Wageningen University, Wageningen, The Netherlands
o Analyzed data and prepared manuscripts in a study on nitrogen balance during pregnancy

Department of Nutritional Sciences   1997 - 1998
University of Helsinki, Helsinki, Finland
o Planned menus and prepared foods in a study investigating the effect of rape seed oil on serum cholesterol
o Analyzed 7-day diet records for a Calcium Research Unit study investigating calcium intake and bone health in adolescents

Teaching Experience
Teaching Assistant/Grader
Department of Food Science and Human Nutrition   2008
University of Illinois at Urbana-Champaign, Urbana IL
o Teaching assistant in an advanced undergraduate/graduate Nutrition for Sports and Exercise class
o Composed exam questions and graded exams
o Taught body composition laboratory methods and nutrition for travelling athletes

Administrative Experience

Graduate Assistant   2007 – 2008

University of Illinois Women's Wheelchair Basketball Team
- o Assistant coach and manager to the University of Illinois Women's Wheelchair Basketball Team
- o Taught wheelchair basketball skills and strategy
- o Team manager at tournaments
- o Conducted game and shot analysis, and game statistics using various software

Division of Disability Resources and Education Services, College of Applied Life Sciences
University of Illinois at Urbana-Champaign, Champaign IL   2005 - 2006
- o Developed and analyzed data from Leisure Interest Survey for UIUC students with disabilities
- o Coordinated and instructed a cooking class for students with disabilities
- o Organized and coordinated fundraising for a Christmas Holiday Party for children with disabilities
- o Organized UIUC Disability Awareness Month

Project Manager   2002 - 2003

Helsinki Association for People with Mobility Disabilities, Helsinki, Finland
- o Development of 3-year nationwide service project for immigrants with disabilities
- o Conducted cross-sectional research into status of immigrants with disabilities in the greater Helsinki area
- o Planned and coordinated peer counselling program
- o Development of guide book in multiple languages on social services for immigrants with disabilities
- o Counselled immigrants with disabilities in accessing social services and health care
- o Educated social and health care professionals on special issues concerning care of immigrants with disabilities

Team Manager/Coach   2008 - 2009

Champaign Fire Wheelchair Basketball Team/Champaign Wildcats Wheelchair Basketball Team
Champaign Park District, Champaign IL
- o Managed all aspects of a community-based sports program including fundraising, finances, recruitment, personnel and scheduling
- o Coached the community youth wheelchair basketball team

Honors, Awards, Fellowships & Scholarships

Fulbright Scholar	2003 – 2008
William C. Rose Endowment Award	2010
David H. Baker Nutrition Scholar Award	2009
Abbott Nutrition Scholarship	2009
Division of Nutritional Sciences Margin of Excellence Travel Award	
	2005 - 2010
Division of Nutritional Sciences Toshiro Nishida Award	2007

UIUC Graduate College Travel Award   2006 - 2007
MIDWEST Alliance in Science, Technology, Engineering and Mathematics Grant
                                                                2006
ELA Foundation: Lucille Fortier Owen Scholar   2006
UIUC Avery Brundage Scholarship   2005 - 2006, 2006 – 2007
UIUC George Huff Award   2004 – 2006
Student Athlete of the Year, University of Illinois at Urbana-Champaign
                                                                2004 - 2005, 2005 - 2006
Erasmus Exchange Scholarship   2000
Merita Bank Scholarship   1999
Martta Association Scholarship   1999
Nordic Exchange Scholarship   1999

Refereed Publications

Plawecki KL, Evans EM, **Mojtahedi MC**, McAuley E, Chapman-Novakofski, KM. Assessing calcium intake in postmenopausal women. *Prev Chronic Dis 2009;6(4):A124 (Epub ahead of print).*

Mojtahedi MC, Valentine RJ, Evans EM. Body composition assessment methods in athletes with spinal cord injury: comparison of field methods with dual energy X-ray absorptiometry. *Spinal Cord 2009; 47(9):698-704.*

Mojtahedi MC, Snook EM, Motl RW, Evans EM. Bone health in ambulatory individuals with multiple sclerosis: impact of physical activity, glucocorticoid use and body composition. *J Rehabil Res Dev 2008;45(6):851-862.*

Mojtahedi MC, Boblick P, Rimmer JH, Rowland JL, Jones RA, Braunschweig CL. Environmental barriers to and availability of healthy foods for people with mobility disabilities living in urban and suburban neighborhoods. *Arch Phys Med Rehabil 2008;89(11):2174-2179.*

Mojtahedi MC, Valentine RJ, Sigurnbjorn AA, Evans EM. Whole body and regional lean soft tissue and adiposity in physically active individuals with spinal cord injury. *Int J Body Comp Res 2008;6(2):43-50.*

Liang H, **Mojtahedi MC**, Chen D, Braunschweig CL. Elevated C-reactive protein associated with decreased high-density lipoprotein cholesterol in men with spinal cord injury. *Arch Phys Med Rehabil* 2008;89(1):36-41.

Thorpe MP, **Mojtahedi MC**, Chapman-Novakofski KM, McAuley E, Evans EM. A positive association of dietary protein with lumbar spine bone mineral density is suppressed by a negative association of protein sulfur. *J Nutr 2008;138:80-85.*

Mojtahedi MC, Valentine RJ, Arngrimsson SA, Wilund KR, Evans EM. The association between regional body composition and metabolic outcomes in athletes with spinal cord injury. *Spinal Cord 2008;46:192-197.*

Valentine RJ, Misic MM, Kessinger RB, **Mojtahedi MC**, Evans EM. Location of body fat and body size impacts DXA soft tissue measures: a simulation study. *Euro J Clin Nutr 2008; Apr 62(4):553-559.*

Heffernan KS, Jae SY, Lee M, **Mojtahedi MC**, Evans EM, Zhu W, Fernhall B. Gender differences in QTc interval in young trained individuals with lower spinal cord injury. *Spinal Cord 2007;45:518-521.*

Mojtahedi MC, Plawecki KL, Chapman-Novakofski, KM, McAuley E, Evans EM. Older black women differ in calcium intake compared to age and socioeconomic matched white women. J Am Diet Assoc 2006;106(7):1102-1107.

Evans EM, **Mojtahedi, MC**, Kessinger RB, Misic MM. Simulated change in body fatness affects Hologic QDR 4500A whole body and central DXA bone measures. *J Clin Densitom 2006;9(3):315-322.*

Snook EM, **Mojtahedi MC**, Evans EM, Motl RW. Physical activity and body composition among people with multiple sclerosis. *Int J MS Care 2005/2006;7:137-142.*

Mojtahedi M, de Groot LC, Boekholt HA, van Raaij JM. Nitrogen balance of healthy Dutch women before and during pregnancy. *Am J Clin Nutr* 2002 75(6):1078-83.

Invited Publication
Mojtahedi MC, Evans EM. Anthropometry in athletes with spinal cord injury. In: Preedy VR (Ed.) Handbook of Anthropometry. Springer, New York. In press.

Grants
Principal Investigator (Graduate Student Competition)
University of Illinois at Rockford, College of Medicine
Project Export: "Effect of Physical Activity on Risk for Obesity, Type 2 Diabetes Mellitus and Cardiovascular Disease in Individuals with Spinal Cord Injury: A Cross-Sectional Study"; $5,385 (funded summer 2005)

Co-Investigator (Ellen Evans, Principal Investigator)
University of Illinois at Urbana-Champaign, Urbana IL
MJ Neer Research Award: "Effect of Physical Activity on Fat Location, Risk for Type 2 Diabetes Mellitus and Cardiovascular Disease in Individuals with Spinal Cord Injury"; $14,988 (funded 2005 - 2006)

Co-Investigator (Ellen Evans, Principle Investigator)
University of Illinois at Urbana-Champaign, Urbana IL
MJ Neer Research Award: "Habitual Physical Activity and Body Composition in Spinal Cord Injured Individuals"; $15,000 (funded, 2004-2005)

Presentations
Guest Lecturer

Nutrition Lecturer/Consultant for Wheelchair Athletes and Coaches   2004 – present
University of Illinois at Urbana-Champaign, Champaign IL
o Lectured on healthy eating, weight management and sports nutrition
o Organized and instructed cooking classes and shopping excursions with a focus on choices available in grocery stores for preparing healthy and easy-to-cook meals

Department of Kinesiology and Community Health, Urbana IL   2007
o Lectured in Community Health undergraduate class on exercise related nutrition

Parkland College, Champaign IL   2005 - 2006
o Lectured in Health Class on concepts in nutrition, nutritional assessment and weight management

Conference Presentations: Oral
Mojtahedi MC, Thorpe MP, Richey AL, Johnson CL, Karampinos DC, Georgiadis JG, Layman DK, Evans EM. Protein supplementation during 6 mo weight loss enhances body composition changes in older women. *FASEB J 2010;24(4):93.7.*

Mojtahedi MC, Sigurnbjorn AA, Valentine RJ, Evans EM. Gender differences in whole body and regional body composition of athletes with spinal cord injury. *Med Sci Sports Exerc 2008; 40(5):S47.*

Mojtahedi MC, Thorpe MP, Layman DK, Evans EM. Moderate protein intake improves change in lean to fat ratio after 1 year of weight loss in adults. *FASEB J 2008;22(4):442.6 .*

Mojtahedi MC, Valentine RJ, Sigurnbjorn AA, Evans EM. Intermuscular thigh fat mass and insulin sensitivity in athletes with spinal cord injury. *International Spinal Cord Society Annual Meeting 2007:O78.*

Conference Presentations: Poster
Thorpe MP, **Mojtahedi MC**, Richey AL, Layman DK, Evans EM. Higher protein weight loss diet does not affect bone mineral density in a double-blind RCT in postmenopausal women. *FASEB J 2010;24(4):946.6.*

Guest DD, Thorpe MP, **Mojtahedi MC**, Richey AL, Mailey E, Kedem LE, McAuley E, Chapman-Novakofsky K, Evans EM. Peer education initially helps prevent weight changes in freshman women. *FASEB J 2010;24(4):564.10.*

Mojtahedi MC, Chapman-Novakofski KM, Rosengren KS, McAuley E, Evans EM. Protein, carbohydrate and vitamin K related to physical performance in older women. *FASEB J 2009;23(4):550.4.*

Mojtahedi MC, Valentine RJ, Evans EM. Dietary intake and metabolic markers in athletes with spinal cord injury compared with sedentary non-disabled. *Med Sci Sports Exerc 2009;41(5):S445.*

Agiovlasitis S, Heffernan KS, Jae SY, Ranadive SM, Lee M, **Mojtahedi MC**, Fernhall B. Heart rate variability and complexity during static exercise in active individuals with spinal cord injury. *Med Sci Sports Exerc 2009;41(5):S132.*

Mallard DM, **Mojtahedi MC**, Evans EM. Impact of cola consumption on calcaneal quantitative ultrasound in college age females. *J Am Diet Assoc 2008;108(9):A27.*

Mojtahedi MC, Sigurnbjorn AA, Mallard DM, McAuley E, Evans EM. The effect of race and physical activity on bone mineral density is consistent in young adult and older women. *FASEB J 2008;22(4):883.9.*

Mojtahedi MC, Rimmer JH, Boblick P, Rowland JL, Braunschweig CL. Environmental barriers to healthy foods for an urban population with mobility impairments. *American Diabetes Association Scientific Meeting 2007:0948-P.*

Thorpe MP, **Mojtahedi MC**, Chapman-Novakofski KM, McAuley E, Evans EM. Associations of dietary protein, calcium, potential renal acid load and bone mineral density in elderly women. *FASEB J 2007;21(6):548.16.*

Sigurnbjorn AA, **Mojtahedi MC**, Valentine RJ, Evans EM. Intermuscular fat mass and insulin sensitivity in individuals with spinal cord injury. *FASEB J, 2007;21(6):233.2.*

Valentine RJ, **Mojtahedi MC**, Wilund KR, Evans EM. High level physical activity mediates glucose and lipid profiles in individuals with spinal cord injury. *Med Sci Sports Exer 2007;39(5):S288.*

Mojtahedi, MC, Heinrichs KL, Evans EM. Fat free mass affects relative body fat more than fat mass in athletes with SCI. *Med Sci Sports Exer 2006;38(5):S312-313.*

Jae SY, Heffernan KS, Lee M, **Mojtahedi MC**, Evans EM, Zhu W, Fernhall B. Effects of acute isometric exercise on arterial stiffness in persons with paraplegia. *Med Sci Sports Exer 2006;38(5):S182.*

Mojtahedi MC, Evans EM, Plawecki KL, Chapman-Novakofski, KM, McAuley E. Older black women differ in calcium intake compared to age and socioeconomic matched white women. *J Am Diet Assoc 2005;105(8):A19.*

Mojtahedi MC, Evans EM. Prediction equations overestimate RMR of spinal cord injured athletes. *Med Sci Sports Exer 2005;37(5):S437.*