AN ABSTRACT OF THE DISSERTATION OF

Kari D. Pilolla for the degree of Doctor of Philosophy in Nutrition presented on March 21, 2013.

Title: Changes In Body Composition And Metabolic Syndrome Risk Factors: Response to Energy-Restriction, Protein Intake, and High Intensity Interval Training

Abstract approved:

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Metabolic syndrome (MetS) and abdominal obesity (AbOb) increase the risk of developing cardiovascular disease and diabetes. Energy restriction (ER), high-protein (PRO) intake and high-intensity interval training (HIT) can independently improve MetS and AbOb. However, ER reduces metabolically active lean body mass (LBM) in addition to body fat (BF). **Purpose:** To determine the effects of a 16-wk ER diet with 2 levels of PRO (15% or 25% of energy), plus HIT, on MetS risk factors, AbOb, and body composition in women. **Methods:** Sedentary, premenopausal women (age=35±10y) with AbOb (waist circumference [WC] ≥80cm) were randomized to a 16-wk ER diet (-300kcals/d) with 15% (15PRO; n=17) or 25% (25PRO; n=18) of energy from PRO, plus 45min/d, 3d/wk HIT and 45min/d, 2d/wk continuous moderate-intensity exercise (CME) (-200kcals/d). Diet and physical activity (PA) were assessed using 4-d weighed food and PA

records, respectively; diet and exercise compliance were assessed monthly with multiple-pass 24-h recalls and weekly tracking logs. Body weight (BW), WC, DXA-assessed body composition (BF [%], BF [kg], trunk fat [kg], and LBM [kg]), blood lipids (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], triglycerides [TG]), glycemic markers (fasting plasma glucose [FPG], insulin, and homeostatic model assessment for insulin resistance [HOMA-IR], beta cell function [HOMA-%β] and insulin sensitivity [HOMA-%S]) and resting blood pressure (BP) (systolic BP [SBP]; diastolic BP [DBP]) were assessed pre/post-intervention. Repeated measures analysis of variance and two sample t-tests were used at analyze the date. Results are reported as means±standard deviations. **Results:** There were significant time, but not group, differences in BW (-5.1±2.6kg, p=0.0141), WC (-7.3±3.6cm, p<0.0001), TC (-18.1±17.4mg/dL, p<0.0001), LDL-C (12.2± 16.2mg/dL, p<0.0001), TG (-25.3±56.2mg/dL, p=0.0064), insulin (-2.1±4.2mg/dL, p=0.0048), HOMA-IR (-0.2±0.5, p=0.0062), HOMA-%β (-12.1±35.2%, p=0.0497), HOMA-%S (28.5±78.4%, p=0.0357), and SBP (-3±9mmHg, p=0.214). There were significant group x time differences in DBP (15PRO=-5±8mmHg, 25PRO=-2±8mmHg; p=0.0024). There were no time or group differences in FPG or HDL-C. There were significant time, but not group, effects on changes in BW (-5.1kg± 2.6, p<0.0001), BF (-3.3±1.6%, p<0.0001), and LBM (-0.6kg±1.5, p=0.0283). The 15PRO group lost more absolute whole BF (-5.2kg vs. -3.9kg, p=0.0355) and trunk fat (-3.1kg vs. -2.2kg) vs. the 25PRO group. **Conclusion:** Both diets significantly improved BW, AbOb, MetS risk factors, glycemic control, and BF (%); LBM (kg) loss was similar in both groups. Compared to the 15PRO diet had significantly greater absolute BF-kg and trunk fat-kg losses. Increased PRO intake did not improve AbOb or MetS risk beyond ER and HIT/CME. The impact of HIT/CME and the greater (-1.3kg) changes in BW in the 15PRO group may have contributed significantly to the changes in absolute BF and trunk fat. More

research is needed to separate the impact of HIT/CME and weight loss from the impact of PRO during ER.

Changes In Body Composition And Metabolic Syndrome Risk Factors: Response to Energy-Restriction, Protein Intake, and High Intensity Interval Training

by
Kari D. Pilolla

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Kari D. Pilolla, Author

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CONTRIBUTION OF AUTHORS

Melinda M. Manore, PhD, RD, CSSD, assisted with the design and implementation of the research project, data analysis, and drafting of the manuscripts presented in Chapter 2 and 3. Whitney M. Sweat, MS, RD, assisted with the implementation of the research project, and data collection and analysis for the manuscripts presented in Chapter 2 and 3. Dr. Gianni F. Maddalozzo, PhD, assisted with the data collection for the manuscript presented in Chapter 3.

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Dedicated to…

The loves of my life: my husband and beautiful daughters ~

Michael ~ your support, encouragement, and love has endured many challenges during this journey, yet they have been unwavering. Your belief in me has made me a stronger person, and I am forever thankful to you for allowing me to pursue my dreams!

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CHAPTER 1 – GENERAL INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States (US), and stroke and diabetes are close behind in as the fourth and seventh causes of death, respectively.[2] Metabolic syndrome (MetS), a collection of cardiometabolic risk factors, is linked to the development of CVD and diabetes.[3] It is hypothesized that abdominal obesity (AbOb), especially increased visceral fat (VF), is a primary factor contributing to MetS.[4]

For women, advancing age and increased waist circumference (WC) contribute to the prevalence of AbOb and MetS, which now exceeds 62% and 37%, respectively.[5,6] Unfortunately, age and menopause are two non-modifiable risk factors that increase the risk for developing MetS, CVD, and diabetes. As women age and transition into menopause, WC, VF, and AbOb, all increase.[7] If women gain weight during the premenopausal years, subcutaneous abdominal fat (SAF) increases; however, after menopause, there is a shift in fat deposition and VF increases.[8,9] Lovejoy et al[9] demonstrated this point in a 4-year observational longitudinal study with 156 premenopausal women (age>43y). They found that during the 4 years prior to menopause, total abdominal fat increased as follicle stimulating hormone (FSH) increased. FSH levels increase as women approach menopause and stay elevated for 1 year after the final menstrual period.[10] Conversely, the decreases in estradiol levels that accompany menopause are associated with increases in total abdominal fat.[11] For many women, the approach and transition through menopause, and the decrease in physical activity that typically comes with aging, contribute to the increase in AbOb and MetS.[6,9] Taken together, as overweight premenopausal women age and transition through menopause, they are at high risk for developing obesity, particularly AbOb, which increases the risk for MetS, CVD, and diabetes.

PREVENTING METABOLIC SYNDROME

To prevent an individual from transitioning from overweight (body mass index [BMI] 25kg/m^2 – 29.9kg/m^2) to obesity (BMI≥30 kg/m^2), or developing MetS, targeting and reducing AbOb is critical. Improving diet and increased physical activity is a cost effective, low-risk, approach to reducing body weight and AbOb to prevent the onset of MetS. Energy-restricted diets that are higher in protein (25-35% energy from protein), compared to typical protein intake (12-15% energy from protein),[12] have shown promise for promoting weight loss due to increased satiety and preservation of lean tissue.[13] Satiety is especially high after consuming a higher protein morning meal (0.867g/kg BW).[14] In addition to dietary modifications, exercise modifications can also be effective for weight loss and reducing AbOb. Although the energy expenditure related to exercise can promote weight loss, the incorporation of higher intensity exercise into an exercise program has shown benefit for reducing WC.[15] However, higher intensity exercise is also associated with higher levels of amino acid oxidation,[16] which would increase the need for high quality protein, especially post-exercise when muscle tissue repair is high.[16] Thus a higher protein diet combined with higher intensity exercise may be more successful than either diet or exercise alone to decrease body weight and abdominal fat, while preserving lean body mass (LBM).

Over the years, MetS has been defined and redefined by a number of health organizations. In 2009, the leading organizations finally agreed on a universal MetS definition.[3] Table 1.1 outlines the 2009 Joint Scientific Statement components and cut-points for clinical diagnosis of MetS. The metabolic connections between the MetS risk factors were first identified by Reaven in 1988,[17] but health organization established a specific definition until 1998. The World Health Organization (WHO) was the first to release a definition for MetS.[18] Since then, a number of other health organizations have released differing

definitions of MetS, including the American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI) definitions in 2001[19] and 2005,[20]and the International Diabetes Federation (IDF) definition in 2005.[21] Although the 2005 AHA/NHLBI and IDF definitions agreed on the components of MetS, the IDF required the presence of AbOb for diagnosis, but the AHA/NHLBI did not. The two organizations also disagreed on the cutpoints for AbOb (measured by WC). The IDF cutpoints were lower than those of the AHA/NHLBI (WC ≥80 cm and WC >88 cm for females, respectively), and were sex and ethnic specific.[20,21] The remaining point of controversy is still the criteria for AbOb. The current recommendation uses the AHA/NHLBI cutpoints for Europids and the IDF cutpoints for non-Europids. The ethnic diversity of the US requires careful consideration of which cutpoint to apply for assessment purposes; the AHA/NHLBI cutpoints may not always be appropriate. The cutpoints identified by WHO bridges the gap between the IDF and AHA/NHLBI recommendations. WHO has defined two levels of AbOb: 1. Increased risk (Men: WC≥94cm; Women: WC≥80cm), and 2. Higher risk (Men: WC≥102cm; Women: WC≥88cm)[22]. The criterion for increased risk status aligns with the IDF AbOb cutpoints for Europids and some other ethnic groups; the criterion for higher risk status aligns with the AHA/NHLBI cutpoints for AbOb.

Using the IDF definition of AbOb for Europids, it has been estimated from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) that 43% of men and nearly 63% of women in the US are abdominally obese.[5] However, if IDF ethnic-specific cutpoints are applied to the appropriate populations, these numbers may be even greater. For example, using the IDF Hispanic cutpoints for AbOb, 78% of the Hispanic population has AbOb, vs. 50% when using the Europid cutpoints. Thus, the inclusion of AbOb and the use of sex and ethic cutpoints make a significant difference in the prevalence of MetS. However, regardless of the cut points used, the high prevalence of AbOb in the

US emphasizes the need for identifying and implementing effective intervention approaches to reduce the prevalence of MetS.

THE ROLE OF ABDOMINAL OBESITY IN METABOLIC SYNDROME

According to the IDF, the choice to include AbOb as a required component in their 2005 MetS diagnosis criterion was due to the strength of the evidence associating WC to CVD and MetS risk factors. In particular, the IDF pointed to the evidence linking AbOb to the early steps in the MetS cascade.[21] AbOb involves storing excess fat in one of two fat compartments: SAF and VF. Whereas SAF is located below the surface of the skin and above the abdominal musculature, VF is located around the internal organs, including the heart and liver. Increased abdominal fat could be in either of these compartments, with VF being associated with cardiometabolic risk.[5] Unfortunately, for a given WC, women have a larger amount of total adipose tissue than men; as women age, they experience a greater increase in VF compared to SAF.[7] This increase in VF increases the prevalence of MetS in older women.

Although the specific mechanisms linking AbOb to MetS have not been completely identified, contributing factors include the release and uptake of excess free fatty acids (FFA), increased secretion of adipokines, and reduced adiponectin.[23] The proximity and specific characteristics of VF adipocytes have been hypothesized to play significant roles in the development of MetS.[4] The proximity of VF to the portal vein exposes the liver to high levels of FFAs, which can lead to undesirable changes in blood pressure, blood lipids, and glucose regulation[4]. Because of the link between VF and portal circulation, this proposed cascade has been referred to as the '*portal hypothesis'*.[4] Excess VF contributes to increased lipolysis and release of FFAs,[24,25] due to increased levels of lipolytic β-adrenoreceptors[26] and decreased anti-lipolytic activity of α-adrenoreceptors.[25] For a given exposure to catecholamines, such as norepinephrine, obese individuals have a higher release of FFAs from VF.[25] Using a canine model, Kabir

et al[27] found changes in VF gene expression resulting from induced obesity. In obese canines, the lipid storing enzyme lipoprotein lipase and lipid mobilizing enzyme hormone sensitive lipase were found to be two fold greater in VF than subcutaneous abdominal fat, whereas the levels were near equal in the non-obese canines. Additionally, peroxisome proliferator-activated receptor-γ, a lipid accumulating transcription factor, was found to be four-fold higher in VF compared to subcutaneous abdominal fat in obese canines. The increased mobilization of FFAs results in higher concentrations of FFAs being released into the portal vein and taken up by the liver. Taken together, these results show molecular changes in VF allowing increased storage and mobilization of lipids.[24]

Increased mobilization of FFAs alone cannot explain the metabolic dysfunctions that result in MetS; however, the uptake of excess FFAs by the liver may. A review by Bjorntorp[28] addresses many of the proposed and documented mechanisms. First, the availability of excess FFAs allows increased endogenous synthesis of triglycerides (TG). TGs synthesized in the liver can be stored in the liver or packaged into very low-density lipoproteins (VLDL) for delivery to peripheral tissues. As the liver synthesizes TGs and sends them out in VLDLs, there is an uncompensated peripheral uptake response, resulting in an increased level of circulating low-density lipoproteins (LDL). Excess TGs can also provide the liver with glycerol for gluconeogenesis. Kabir et al found significantly increased expression of gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of the obesity-induced canines,[27] showing molecular support for increase hepatic gluconeogenesis. Combine increased hepatic gluconeogenesis with reduced hepatic insulin clearance, resulting from specific FFA binding to inhibitor sites of insulin receptors, and glucose dysregulation results.

In addition to the mechanisms posed by the '*portal hypothesis,'* there appear to be other mechanisms contributing to the relationship between excess

VF and MetS. Excess VF can induce non-infectious inflammation through the release of higher levels of adipokines.[29] In particular, interleukin-6 (IL-6) has been found to be nearly 50% higher in the portal vein of obese individuals compared to non-obese individuals. Elevated IL-6 levels contribute to impaired insulin-mediated glycogenesis and stimulate hepatic gluconeogenesis, as well as systemic inflammation via hepatic acute-phase reactant production.[30] This elevation of IL-6 is positively correlated with higher inflammatory C-reactive protein levels.[24] Additionally, adiponectin, an anti-inflammatory protein release by adipocytes, is lower in obese individuals than normal weight individuals.[31] Further, adiponectin levels appear to be more closely related to the level of VF than total body fat.[32] Adiponectin is linked to both blood pressure regulation and glycemic control.[33] A review by Lihn, Pedersen, and Richelsen[33] found reduced levels of adiponectin to be associated with reduced insulin sensitivity and increases in atherogensis. Thus, VF can be linked to multiple MetS risk factors through an increased release of acute phase proteins and reduced secretion of adiponectin.

Take together, a number of physiological mechanisms link increased VF to MetS. The evidence is strong and warrants further research to determine effective approaches for reducing AbOb, especially VF, and minimizing the risk for developing MetS.

MEASURING ABDOMINAL OBESITY

Similar to overall body composition, AbOb can be measure through both clinical and field methods. The gold standard for measuring AbOb is computed tomography (CT);[34] magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DXA) are two other clinical methods that can assess the quantity of trunk fat. Whereas CT scans and MRIs can provide estimates of VF and SAF, access to CT scans and MRIs are both limited and costly, and CT scans expose individuals to a significant amount of ionizing radiation. DXA scans involve much

less radiation than CT scans, cost less than both MRI and CT scans, and may useful in clinical settings, but they cannot distinguish VF from SAF. Aside from the clinical approaches for measuring AbOb, WC is a reliable, safe, simple, and inexpensive field tool that provides a strong prediction of cardiometabolic risk and is the best surrogate marker for VF.[35-37] The rational for measuring AbOb is to objectively measure cardiovascular risk;[35] although neither DXA nor WC can distinguish VF from SAF, both provide good correlations to VF.[34,36]

In 2007, the American Society for Nutrition released a position statement supporting the use of WC over waist-to-hip ratio and body mass index (BMI) for measuring cardiometabolic risk.[35] According to this position stand, WC predicts cardiometabolic risk as well as BMI and also provides incremental prediction of diabetes, coronary heart disease, and mortality above and beyond that provided by BMI. Additionally, WC is a better predictor of visceral body fat than BMI.[35]

Although WC can be reliability measured, a number of measurement sites are currently used. It is also not known if one WC site is a better predictor of cardiometabolic risk; however, most literature reports on the use of four measurement sites.[35,38]

1. Immediately above the iliac crest (National Institutes of Health [NIH] site)[39]
2. Midway between the lowest rib and the iliac crest (World Health Organization [WHO] site)[18]
3. Narrowest point between the lowest rib and the iliac crest (Anthropometric Standardization Reference Manual [ASRM] site)[40]
4. Level with the umbilicus

The NCEP Adult Treatment Panel III measures WC immediately above the iliac crest, while IDF bases their definition using NCEP's cutpoints.[20,21]

THE ROLE OF DIET AND EXERCISE FOR REDUCING ABDOMINAL OBESITY AND BODY WEIGHT WHILE PRESERVING LEAN BODY MASS

To prevent an individual from developing MetS, it is imperative to target AbOb while reducing body fat and preserving LBM. The combination of an energy-restricted diet higher in protein and an exercise program incorporating higher intensity exercise may be an effective means of achieving these results.

Influence of Diet on Abdominal Obesity

It is well established that energy-restricted diets are effective in reducing body weight, which result in WC reductions.[41,42] In addition to energy restriction, diet composition can play an important role in reducing body weight and AbOb. Sacks et al[41] compared the effects of 4 different diets of varying macronutrient distribution on weight loss, body composition and anthropometric measurements. Though they did not see a statistically significant difference in WC, there was ~1-2cm greater reduction in WC with the energy-restricted high protein diet (25% energy from protein) compared to the lower protein diet (15% energy from protein). This study did not incorporate physical activity into the protocol. Arciero et al[43] found significant reductions in total abdominal fat after 12wks on an ad libitum high protein diet (40% energy from protein, 40% energy from carbohydrates, 20% energy from fat) compared to a more typical ad libitum diet (15% energy from protein, 55% energy from carbohydrate, 30% energy from fat). However, the 40% protein diet intervention included high intensity cardiovascular training and resistance training while the 15% protein diet included moderate intensity cardiovascular training; thus, the contribution of the two different exercise programs to the reductions in abdominal fat is difficult to factor out. In a later study, Arciero et al[44] controlled the exercise component, offering high intensity interval training and resistance training to both groups, but only compared the effects of an ad libitum 40% protein diet with an ad libitum 25% protein diet. This study did not include a 15% protein diet intervention including

high intensity cardiovascular training, but did include a 40% protein diet group without exercise. At the end of the 12-wk intervention, there were significant differences in WC and abdominal fat percent, measured by DXA, over time in each group; however, there were no significant differences between groups.[44] Taken together, these data show that ad libitum, higher protein (25% and 40% of energy) diets appear to improve AbOb. Unfortunately, the impact of high intensity cardiovascular training cannot be accounted for by these studies. To date, there are no reports evaluating the effectiveness of a 25% of energy from protein diet compared to a 15% of energy from protein diet when both are combined with energy-restriction and high intensity cardiovascular training. These levels of protein intake (25% and 15% of energy from protein) are both within the recommended range of intake[45] and are easier to achieve in the typical diet.

Increasing dietary protein intake while on an energy-restricted diet may be a more effective way to reduce body weight, WC, and AbOb. In a recent review by Westerterp-Plantenga, Lemmens, and Westerterp,[46] research has shown that higher protein diets (20-30% energy from protein) can benefit energy-restricted diets and weight loss through two main mechanisms: 1) Decreased energy intake from increased satiety and 2) Increased energy expenditure from increased diet-induced thermogenesis (DIT) of protein and preservation of LBM. Leidy et al[47] used two energy-restricted diets (-750 kcal/d), one containing 30% of energy from protein vs. 18% of energy from protein for 12-wks and found significantly less pronounced reductions (10% from the higher protein diet vs. 27% from the lower protein diet, $p<0.005$) in post-prandial feelings of fullness levels in the higher protein group.[47] In addition to the greater feelings of fullness, energy intake reduces with higher protein intake. Vander Wal et al[48] reported significantly reduced energy intake for 36h following a higher protein breakfast (meal containing 20% of energy from protein) compared to a similar typical/moderate protein breakfast (meal containing 15% of energy from protein). Taken together,

higher levels of protein promote weight loss through satiety and reduced energy intake.

A second possible mechanism for weight loss and reductions in WC from energy-restricted higher protein diet stems from increased energy expenditure. Higher protein intakes increase DIT from the energy cost of digesting, absorbing, and storing protein. Protein ranks the highest for energy cost compared to the other macronutrients, contributing 20%-30% compared to 0%-3% and 5%-10 for fat and carbohydrates, respectively.[46] Further, higher protein intake prevents negative protein turnover and preserves protein synthesis[46] and LBM.[47,49] Leidy et al[47] reported less loss of LBM with 30% vs. 18% of energy from protein, whereas Krieger et al[49] reported less loss with protein intake ≥1.05g/kg body weight. By preserving the more metabolically active LBM, resting energy-expenditure may also be preserved. Taken together, the decreases in energy intake and increases in energy expenditure are two substantial benefits of including higher protein intake during energy restriction.

Amount of protein needed on an energy-restricted diet plus exercise. When altering the macronutrient composition of a diet it is important to consider the Recommended Dietary Allowances (RDA) for these nutrients. The current adult RDA form protein is 0.8g/kg/d and the Acceptable Macronutrient Distribution Range (AMDR) is 10-35% of total energy intake.[45] Typical weight loss diets incorporate ~15% of energy from protein.[50] However, when exercise is added to an energy restricted diet, higher intakes of protein are recommended to prevent negative nitrogen balance and to preserve lean tissue.[51] Research has also established a positive relationship between protein turnover and exercise intensity.[16] Thus, feeding a higher protein diet with an exercise program involving high intensity exercise would be beneficial, especially when energy intake is reduced for weight loss. Finally the American Dietetic Association (ADA), Dietitians of Canada, and the American College of Sports Medicine (ACSM) have

recommended that individuals participating in endurance and/or strength-training consume 1.2-1.7g of protein/kg/d.[52] For example, using a 70kg female, the minimum protein recommendation would be 56g protein/d (224kcal/d; 12.4% of 1800kcal/d diet) and the upper recommendation for an endurance or strength-trained female would be 119g protein/d (476kcal/d; 26.4% of 1800kcal/d diet). Using either recommendation, the female's recommended protein intake remains within the AMDR.

Timing of protein intake while on an energy-restricted diet plus exercise. Timing of food intake, especially protein consumption during the day, may also be important while on an energy-restricted diet. In particular, consuming higher protein meals for breakfast and after exercise leads to greater satiety[14, 48] and preservation of LBM through increased protein synthesis,[53] respectively.

Breakfast. Higher protein intake at breakfast has been shown to elicit higher satiety and weight loss[14,48]. Leidy et al[14] found that when overweight and obese individuals were on an energy-restricted (-750kcal/d), high protein (25% of energy from protein) diet, greater meal satiety was experienced following a breakfast meal containing ~62% (0.867g/kg BW) of the daily total protein (25% of energy from protein) compared to a dinner meal containing the same amount of protein ($p=0.003$). Furthermore, greater overall fullness (15h composite of measures) was achieved with a higher protein (~62% of total daily [25% of energy from protein] protein) breakfast compared to higher protein lunch and dinner meals ($p=0.009$; $p=0.05$)[14]. Vander Wal et al[48] also reported enhanced body weight loss ($p<0.05$) and a trend toward greater WC reductions ($p<0.06$) after 8-wks in obese individuals on an energy-restricted high protein breakfast (~20% of energy from protein) diet compared to a high carbohydrate (~15% of energy from protein) breakfast diet. Taken together, higher protein intake at breakfast can influence daily energy intake and aid in weight loss efforts.

Post-exercise. In addition to eliciting satiety when consumed at breakfast, higher protein consumed post-exercise is beneficial for lean tissue repair and maintenance.[52,54-56] Exercise can elicit a catabolic state, which is typically followed by an anabolic state during recovery.[57] Thus, availability of protein substrates is critical during exercise recovery. Whey protein, in particular, has been found to be more beneficial for muscle protein synthesis than consumption of a bolus of essential amino acids[58] and it is quickly absorbed.[53] Boirie et al[53] tested the effects of casein and whey proteins on total body protein synthesis and found a 68% average increase in protein synthesis between 40 and 140 minutes with whey protein ingestion compared to 31% average increase with casein protein ingestion. Thus, the fast action of whey protein makes it ideal for consumption post-exercise to promote protein accretion and prevent significant losses of LBM.

Benefits of whey protein while on an energy-restricted diet plus exercise. In addition to the quantity and timing of protein in the diet, the quality of the protein should be considered when restricting energy intake for weight loss. Whey protein, in particular, is a high quality source of protein containing the complete array of essential and nonessential amino acids combined together forming multiple bioactive peptides. When used in supplement form during energy-restriction, whey protein provides a non-fat, low calorie source of amino acids that can be incorporated into foods and beverages. Further, whey protein is quickly absorbed[53] and increases satiety due to its rapid digestion and alteration of gut hormones, including the satiety-related hormones cholecystokinin and glucagon-like peptide 1.[59,60] Independent of its satiating effects, whey protein also appears to enhance weight loss efforts. Frestedt et al[61] examined the effects of a whey protein supplement on weight loss and body composition in midlife men and women by comparing a energy-restricted diet supplemented with whey protein (-500kcal/d, 15% of energy from protein plus 20g whey protein, 30% of

energy from fat) with an energy-restricted control diet (-500kcal/d, 15% of energy from protein, 55% of energy from carbohydrates, 30% of energy from fat). Those subjects who lost ≥2.25kg were considered 'responders' and their data were analyzed separately from the total group. Although all groups lost weight and reduced WC, the subjects consuming whey protein lost significantly more body fat regardless of group designations (completers: 2.8kg [whey] vs. 1.62kg [no whey], p=0.03; responders: 3.63kg [whey] vs. 2.11kg [no whey], p=0.02). The subjects consuming whey protein in the responders group also lost less LBM (-1.07kg [whey] vs. -2.41kg [no whey], p=0.02).

The results of Frestedt et al[61] support the use of whey protein as an aid to increase protein intake for improving body fat loss, while preventing LBM loss, during dietary energy restriction. Unfortunately, this study did not incorporate exercise into their intervention protocol, so effects whey protein combined with exercise are not known.

Health benefits of whey protein related to MetS. The health benefits of whey protein are primarily attributed to specific bioactive peptides and/or the amino acid content. In a review by Ha and Zemel,[62] whey protein was found to have antioxidant, lipid lowering, antihypertensive, and glucose control properties, which are all issues relevant to persons with MetS. Additionally, whey protein promotes skeletal muscle synthesis through its amino acid composition and relative proportions, combined with its relatively rapid absorption rate.[62] Skeletal muscle synthesis is important during weight loss and recovery from exercise in order to maintain LBM.

Antioxidant benefits. Physical activity stresses metabolic pathways that lead to increased production of reactive oxidative species. Animal studies show whey protein to be an effective antioxidant;[63] however, results from human studies are mixed.[64-66] According to Ha and Zemel,[62] the possible sources of whey protein's antioxidant functions include the abundant content of cysteine

amino acid, and the content of lactoferrin and lactoferricin. Cysteine is a known component of the antioxidant glutathione. In a study by Micke et al,[66] glutathione levels improved in HIV patients after 2wks and 6mos of whey protein supplementation. Lands et al[65] also saw improvements in lymphocyte glutathione, peak power, and 30sec work capacity in healthy young adults after supplementing with whey protein for 3mos. However, Middleton et al[64] reported lack of improvements in antioxidant status, assessed by whole blood glutathione concentrations, after 6wks of whey protein isolate supplementation in trained cyclists.

Lipid lowering benefits. Although the specific mechanisms are not yet identified, whey protein appears to improve post-prandial lipid responses. The ability of β-lactoglobulin to sequester hydrophobic molecules has been suggested as a mechanism for plasma lipid reductions.[67] Data on lipid responses to whey protein are limited; however, Krissansen[68] recently cited evidence of β-lactoglobulin binding TG and long chain fatty acids in nursing calves. Mortensen et al[69] recently reported lower plasma TG responses in individuals with type 2 diabetes mellitus after consuming a high fat (80g fat) meal combined with 45g of whey protein compared to high fat meals combined with similar amounts of casein, cod, or gluten protein. No differences were seen in plasma total cholesterol or HDL-chol concentrations. Similarly, Pal, Ellis, and Ho[70] found significant lower TG in arterial blood of post-menopausal overweight and obese women after consuming a meal combined with 45g of whey protein compared to similar meals with additional 45g of casein or glucose. Given the limited research, whey protein may play a role in reducing blood lipids; however more research is needed to establish dose and duration recommendations.[71]

Antihypertensive benefits. Whey protein may also play a role in reducing blood pressure. In a review by Krissansen,[68] the β–lactoglobulin peptide in whey protein inhibits angiotensin-I-converting enzyme (ACE). ACE is partly responsible

for peripheral blood pressure control by converting angiotensin I to angiotensin II, which is a potent vasoconstrictor;[68] thus, consumption of whey protein may assist in blood pressure control.

Glucose control benefits. Whey protein has been linked to improved glycemic control. In addition to providing amino acids for hepatic gluconeogenic substrates and branched chain amino acids for glucose recycling via the pyruvate-alanine cycle, whey protein also has an insulinotrophic effect.[72,73] Nilsson et al[72] examined the effect of whey protein or amino acid mixtures, incorporated into glucose-equivalent drinks, on blood levels of insulin and glucose. They found whey protein to have the greatest insulinotrophic effect, resulting in a 56% lower postprandial blood glucose level compared to the control drink. A review by Layman and Walker[73] identifies leucine as the key amino acid in whey protein that is responsible for modulating the insulin-signaling pathway. However, Nilsson et al[72] reported that the composition of whey protein as a whole is more critical than its individual amino acids.

Skeletal muscle benefits. As mentioned previously, higher protein diets helps preserve LBM. Katsanos et al[58] reported increased muscle protein accrual in elderly individuals in response to whey protein consumption. Tipton et al[74] also reported increased net muscle protein synthesis following ingestion of 20g of whey protein before and after exercise. Two recent reviews discuss the ability of leucine, a branched chain amino acid found in whey protein, to inhibit protein degradation[75] and stimulate protein synthesis during energy restriction and after exercise.[73] Though it is arguable that some peptides and/or amino acids are more beneficial than others, the overall composition of whey protein appears to be the crucial factor, especially in regard to amino acid content. Katsanos et al[58] isolated the essential amino acids and the non-essential amino acids from whey protein and determined that it is the combination of these two groups of amino acids that makes ingested whey protein effective in skeletal muscle protein

accrual. Thus, keeping the protein intake appears to be functionally critical. Table 1.2 below shows a comparison between the amino acid composition of a serving of a whey protein supplement and an 8oz serving of non-fat milk.

Influence of Exercise on Abdominal Obesity

When diet and exercise provide a similar negative energy balance, they both elicit similar weight loss. However, when compared to an energy restricted diet alone, exercise plus energy restriction elicits an additive effect on weight loss.[76] Further, exercise during energy balance has been shown to reduce AbOb independent of energy-restricted diets.[77] In a 14-wk study looking at changes in total and regional body fat in premenopausal women, Ross et al reported greater reductions in total body and total abdominal fat in the exercise-induced weight loss group compared to the diet-induced weight loss group, exercise without weight loss group, and control group.[77] Volpe et al found similar results in overweight midlife women when comparing diet, exercise, and diet plus exercise.[78] At the conclusion of a 6-mo supervised intervention, WC significantly decreased in the exercise group, despite remaining weight stable. Together, these studies support the use of exercise for targeting AbOb.

Whereas mode of exercise does not appear to make a significant difference in amount of abdominal fat lost,[79] the intensity of exercise may. Current exercise recommendations for the overweight, obese, and diabetic populations typically include low-to-moderate intensity exercise training (150-250min/wk).[76,80] However, a joint recommendation by the ACSM and the AHA indicates that individuals may use shorter bouts of high intensity exercise training in place of low-to-moderate intensity exercise for promoting health.[81] Recent research suggests that adding high intensity exercise to an exercise program can reduce abdominal fat, especially VF, with or without energy restriction.[15,43,82] In a 1990 observational study, Tremblay et al[82] examined the intensity of physical activity, body fatness, and anthropometric measures from the 1980 Canadian

Fitness Survey. The results showed an inverse relationship between high intensity exercise and both WC and body fatness, even after controlling for the energy cost of exercise.[82] More recently, Irving et al compared isocaloric high intensity (ratings of perceived exertion [RPE]=15-17 on a 6-20 point scale) and low intensity (RPE 10-12) exercise programs, without dietary changes, and found significant reductions in visceral and total abdominal fat in obese women in the high intensity group after 16wks.[15]

To date, there is no universal definition of high intensity exercise, but it can be related to vigorous intensity or >6.0 metabolic equivalents (METs) as described by ACSM and AHA[81]. Others have used a range of RPE of 15-17,[15] ranges of 70-85% VO_{2peak},[83] and a target of 90% maximal HR.[84] Examples of moderate and vigorous activities listed by Ainsworth et al[85] in the Compendium of Physical Activities, and based on the ACSM description of vigorous activities,[81] are listed in Table 1.3.

In recent years, research has shown the health benefits of alternating high intensity exercise with low-moderate intensity exercise, commonly referred to as high intensity interval training, or HIT.[86] Although there is no consensus with regard to the type or dose of HIT, there is a growing body of evidence supporting the use of HIT in place of traditional endurance exercise programs.[86] The physiological mechanisms behind the health benefits of HIT are still unclear; however, the use of HIT compared to continuous moderate intensity endurance training offers some insight. In a review of HIT interventions, Gibala and McGee[87] summarized the rapid metabolic adaptations of HIT and found improvements in glucose and lipid metabolism.[87] These improvements are due, in part, to increases in skeletal muscle glucose transporter 4, oxidative enzymes such as citrate synthase and cytochrome oxidase, and fatty acid binding and transport proteins.[87] It is also possible that the exercise-induced release of catecholamines binds the large number of adrenergic receptors on VF adipocytes and increases

lipolysis, mobilization and oxidation of FFA. Taken together, these changes can translate into better glucose control and increased fat oxidation, cascading to an overall improvement in body composition, including reductions in abdominal fat.

Whereas exercise has been shown to preserve LBM when on a weight loss diet[88] increases in intensity may elicit significant LBM breakdown.[16] As exercise intensity increases, amino acid oxidation also increases and higher intakes of high quality protein may be needed to compensate, especially post-exercise when amino acids are needed as repair and maintenance substrates.[16]

CONCLUSION

Taken together, a modest energy-restricted diet higher in protein (25% of energy from protein or at least ≥1.05g/kg body weight), combined with high intensity exercise, particularly HIT, may work together to reduce abdominal fat and reduce body weight while preventing significant losses of LBM. Whereas an energy-restricted diet and increased exercise will elicit a negative energy balance, HIT may preferentially target VF via increased release of catecholamines. Higher protein intake will elicit reduced energy intake through increased satiety and increased energy expenditure through DIT, as well as preservation of LBM while on a weight loss diet combined with the demands of high intensity exercise.

Research combining high dietary protein, energy-restriction, and HIT are limited. Studies that have been completed were not designed to specifically test the effects of higher protein intake on AbOb, MetS risk factors, and changes in body composition during energy restriction combined with aerobic HIT. Thus, the purpose of this study is to compare the effects of a high protein (25% of energy from protein), energy-restricted diet with a typical protein (15% of energy from protein), energy-restricted diet intake on reductions in MetS risk factors, including AbOb, and body composition in sedentary, overweight premenopausal women participating in a weight loss program incorporating aerobic HIT.

Table 1.1. Criteria for clinical diagnosis of metabolic syndrome[a]

Risk Factors	Categorical Cut-Points for Diagnosis[b]	
Abdominal Obesity (Waist Circumference)	Increased risk[c]: Men: ≥ 37in (94cm) Women: ≥31.5in (80cm)	Higher risk[c]: Men: ≥40in (102cm) Women: ≥ 34.6in (88cm)
Elevated Triglycerides	≥150mg/dL (1.7mmol/L)	
Reduced High Density Lipoprotein Cholesterol	Men: ≤40mg/dL (1.0mmol/L) Women: ≤50mg/dL (1.3mmol/L)	
High Fasting Glucose	≥100mg/dL (5.6mmol/L)	
High Blood Pressure	Systolic: ≥130mmHg, and/or Diastolic: ≥85mmHg	

[a]Criteria from the 2009 Joint Scientific Statement: Harmonizing the Metabolic Syndrome[3]

[b]At least 3 categorical cut-points must be met for diagnosis of metabolic syndrome.

[c]Waist Circumference (WC) cut-points for increased risk and higher risk are based on the World Health Organization's recommended cut-points for Caucasians;[22] the higher risk cut-points are recognized as the WC criteria for United States by the American Heart Association and the National Heart, Lung, and Blood Institute.[39]

Table 1.2. Nutrient composition comparison of Whey Pro Complete[a] and non-fat milk[89]

Nutrients per serving	Whey Pro Complete 100g	Non-fat Milk 100g	Whey Pro Complete 1oz, dry (1serv)	Non-fat Mik 8oz, fluid (1serv)
Calories	267	34	80	83
Total Fat (g)	3	0	1	0.2
Carbohydrates (g)	6.67	4.96	2	12.15
Protein (g)	50	3.37	15	8.26
Alanine (g)	4.49	0.14	1.35	0.35
Arginine (g)	2.27	0.10	0.68	0.24
Aspartic Acid (g)	9.46	0.29	2.84	0.71
Cysteine (g)	2.02	0.02	0.61	0.05
Glutamic Acid (g)	14.81	0.76	4.44	1.85
Glycine (g)	1.57	0.07	0.47	0.16
Histidine (g)	1.58	0.10	0.47	0.25
Isoleucine (g)	5.29	0.17	1.59	0.43
Leucine (g)	10.15	0.32	3.05	0.78
Lysine (g)	8.45	0.28	2.54	0.69
Methionine (g)	1.86	0.09	0.56	0.22
Phenylalanine (g)	2.84	0.18	0.85	0.43
Proline (g)	5.18	0.33	1.55	0.81
Serine (g)	3.83	0.20	1.15	0.50
Threonine (g)	5.30	0.14	1.59	0.35
Tryptophan (g)	1.79	0.04	0.54	0.11
Tyrosine (g)	2.69	0.17	0.81	0.42
Valine (g)	4.95	0.22	1.49	0.54

[a]Nutrient composition provided by Standard Process Inc, Palmyra, WI

Table 1.3. Examples of moderate and vigorous physical activities[90]

Moderate Intensity Activities (3.0-6.0 METs)	**Vigorous/High Intensity Activities (>6.0 METs)**
Walking 3.0 mph (3.3 METs)	Aerobic Dancing (6.5 METs)
Mopping (3.5 METs)	Tennis, general (7.0 METs)
Gardening, general (4.0 METs)	Rope jumping, slow (8.0 METs)
Softball, general (5.0 METs)	Jogging 5.0 mph (8.0 METs)

MET=metabolic equivalent

CHAPTER 2 – MANUSCRIPT #1: MODERATE AND HIGH-PROTEIN, ENERGY-RESTRICTED DIETS WITH HIGH-INTENSITY INTERVAL TRAINING YIELD SIMILAR IMPROVEMENTS IN BODY WEIGHT, WAIST CIRCUMFERENCE, AND METABOLIC SYNDROME RISK FACTORS IN OVERWEIGHT PREMENOPAUSAL WOMEN.

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ABSTRACT

Metabolic syndrome (MetS) and abdominal obesity (AbOb) increase the risk of developing cardiovascular disease and diabetes. Energy restriction (ER), high protein (PRO) intake and high-intensity interval training (HIT), independently improve MetS and AbOb. **Purpose:** To evaluate a 16-wk ER diet with 2 levels of PRO, plus HIT, for reducing MetS and AbOb in women. **Methods:** Sedentary, premenopausal women (age=35±10y) with AbOb (waist circumference [WC] ≥80cm) were randomized to a 16-wk ER (-300kcals/d) diet with 15% (15PRO; n=17) or 25% (25PRO; n=18) of energy from PRO, plus 45min/d, 3d/wk HIT and 45min/d, 2d/wk continuous moderate-intensity exercise (CME) (-200kcals/d). Diet and physical activity (PA) were assessed using 4-d weighed food and PA records; diet and exercise compliance were assessed with monthly multiple-pass 24-h recalls and weekly tracking logs. Body weight (BW), WC, blood lipids (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], triglycerides [TG]), glycemic markers (fasting plasma glucose [FPG], insulin [Ins], and homeostatic model assessment for insulin resistance [HOMA-IR], beta cell function [HOMA%β] and Ins-sensitivity [HOMA%S]), and resting blood pressure (BP) (systolic BP [SBP] and diastolic BP [DBP]) were assessed pre/post-intervention. Repeated measures analysis of variance and two sample t-tests were used at analyze the date. Results are reported as means±standard deviations. **Results:** There were significant time, but not group, differences in BW (-5.1±2.6kg, p=0.0141), WC (-7.3±3.6cm, p<0.0001), TC (-18.1 ±17.4mg/dL, p<0.0001), LDL-C (-12.2±16.2mg/dL, p<0.0001) TG (-25.3 ±56.2mg/dL, p=0.0064), insulin (-2.1±4.2mg/dL, p=0.0048), HOMA-IR (-0.2 ±0.5, p=0.0062), HOMA%β (-12.1±35.2%, p=0.0497), HOMA%S (28.5±78.4%, p=0.0357), and SBP (-3±9mmHg, p=0.214). There were significant group x time differences in DBP (15PRO=-5±8mmHg, 25PRO=-2±8mmHg; p=0.0024). There were no time or group differences in FPG or HDL-C.

Conclusion: Both diets effectively reduced AbOb and MetS risk factors; thus, increasing PRO above typical intake did not improve AbOb or MetS risk beyond ER and HIT/CME.

INTRODUCTION

Obesity is a public health priority in the United States (US). Currently, nearly 69% of adults are either overweight or obese[91] and 62% of women and 42% of men are abdominally obese (AbOb).[5] Excess abdominal fat, particularly visceral fat (VF), is associated with abnormal plasma lipid concentrations,[92,93] impaired glycemic control,[94] and hypertension.[95] Collectively these abnormalities characterize metabolic syndrome (MetS), which confers a 5-fold greater risk for developing type 2 diabetes and 2-fold greater risk of developing cardiovascular disease (CVD) within 5-10y of diagnosis. Using the most recent categorical MetS risk factor cut-points from the 2009 Joint Scientific Statement (Table 1.1),[3] it is now estimated that two in five men and one in three women in the US has MetS.[6]

There is strong evidence that AbOb, especially increased VF, is a primary factor contributing to MetS.[4] Abdominal VF is more sensitive to catecholamine-stimulated lipolysis,[24,29] which increases the release of free fatty acids (FFAs) into the portal vein and increases uptake by the liver. Hepatic exposure to excess FFAs eventually contributes to insulin dysregulation and dyslipidemia.[4] Excess abdominal VF also increases inflammatory adipokine secretion contributing to hypertension.[96] Measurement of AbOb and VF can be done directly with magnetic resonance imaging (MRI) and computed tomography (CT), and indirectly with dual energy x-ray absorptiometry (DXA) and waist circumference (WC). All of these methods provide good estimates of cardiometabolic risk;[34,36,97] however, WC is the most inexpensive, easy, safe, and portable method to use. In the field and clinical setting, WC is recommended by leading health organizations to assess AbOb and cardiometabolic risk.[35]

The high prevalence of obesity and MetS, combined with the pathogenic role of AbOb in their metabolic complications, has driven research for effective methods for reducing AbOb. Sex and age are two non-modifiable AbOb risk factors; for most women, WC, VF, and AbOb increase with age.[7-9] The approach

and transition through menopause is also associated with age-related declines in physical activity (PA), contributing further to AbOb.[9] Thus, for women, aging and menopause increase the risk for weight gain, AbOb and MetS. Fortunately, changing diet (e.g. modest energy restriction (ER); changes in macronutrient composition)[50,98] and PA[99] are two modifiable, low-risk, and cost-effective approaches for reducing AbOb and improving MetS risk factors.

Research suggests that diets higher in protein (PRO) promote reductions in body weight (BW), WC, and MetS risk factors.[98,100] Two primary mechanisms associated with increased PRO intake and weight loss have been proposed: 1) PRO-induced satiety leading to reduced energy intake,[47,101] and 2) PRO-associated increases in thermogenesis leading to increased energy expenditure.[101] Combined, these two factors would alter energy balance favoring weight loss. Further, during periods of ER, a higher PRO diet may preserve lean tissue. Thus, higher PRO intake may indirectly assist in maintaining resting metabolic rate and preventing further decreases in energy expenditure.[101] Finally, increased PA, especially high-intensity exercise, has also been shown to improve body composition and reduce AbOb.[15] Further, high-intensity interval training (HIT), alternating short bouts of high-intensity with low/moderate-intensity exercise, has been shown to elevate fatty acid oxidation[84] and reduce WC.[102]

Although weight loss alone can improve MetS in obese women,[103] research has not examined the combined effects of ER, higher PRO intake, and HIT in premenopausal, overweight women with AbOb. Thus, the purpose of this study was to compare two ER diets with different levels of PRO (15% and 25% of energy from PRO) combined with HIT on BW, WC, and MetS risk factors in premenopausal women. We hypothesized that a higher PRO diet would result in greater improvements in BW, WC, and MetS risk factors.

METHODS

Study Design

This was a non-blinded randomized control trial (16wks) with two treatment groups (See Figure 2.1). After completing eligibility screening and baseline assessments, participants were randomized to a diet containing either 15% (15PRO) or 25% (25PRO) of energy from PRO. All participants consumed an ER diet (~2100kcal/wk) and participated in a PA program (~1400kcal/wk) that included HIT. All assessments were completed pre/post-intervention, while diet and exercise compliance, BW, WC, resting blood pressure (RBP) were monitored weekly and/or monthly. The 15PRO diet served as the control diet with a PRO level representing typical PRO intake in the United States;[12] inclusion of the same exercise program in both groups served as a control for exercise. Budget limitations restricted our ability to include diet- and exercise-only groups. The Oregon State University Institutional Review Board approved the study and all participants provided written informed consent and received modest monetary compensation for participation.

Participants

Sedentary (<1h/wk moderate-intensity exercise for ≥6mo), overweight/obese (body mass index [BMI] 25 to 34kg/m^2), premenopausal women with AbOb (WC≥80cm) were recruited and screened (n=261) for eligibility based on measured BMI, WC and a health and exercise questionnaire. All participants were recruited from the university and surrounding communities. Participants were excluded if they were smokers, pregnant or lactating, or unable to consume lactose-containing products. Medical clearance to participate in HIT was obtained from the participants' primary care physician prior to initiation of exercise. In all, 52 participants were eligible and were randomly assigned to a treatment group; seven participants withdrew prior to the intervention due to discontent with group assignment and time conflicts, seven withdrew during the intervention due to time

conflicts (n=6) and unrelated illness (n=1), three were excluded due to noncompliance. Overall, data from 35 participants were used for final analysis.

Intervention

After randomization, each participant met with researchers, including a registered dietitian (RD) and exercise specialist, to review group assignment, intervention protocol, and their individual diet and exercise plans. Participants completed a 16-wk intervention that was divided into two phases: Phase I=wk 1-4, Phase II=wk 5-16. During Phase I, participants attended weekly nutrition education classes and engaged in progressive exercise training, including Zumba® (explained below). Nutrition classes included education on portion size estimation, a low energy-density eating plan, and application of the exchange system to their own diet.

Energy-restricted diet. Energy needs were assessed using the 2005 Institute of Medicine Dietary Reference Intake equation for estimating energy requirements (EER) based on total energy expenditure (EE) equations for women ages ≥19y. At baseline, a PA coefficient of 1.0 was used to represent a sedentary PA level, and was increased as necessary as the level of PA increased during the study. After energy needs were determined, an ER diet plan (EER - 300kcals/d) was created for each participant using the Exchange List from the Academy of Nutrition and Dietetics and the American Diabetes Association.[104] In general, all participants were encouraged to follow the Dietary Guidelines for Americans, including increasing low energy-dense foods, such as whole fruits and vegetables, whole grains, and low-fat dairy and lean meats, and reducing/eliminating intake of high-energy drinks. The 15PRO diet had an energy distribution of 15% PRO, 60% carbohydrate (CHO), and 25% fat; the 25PRO diet had an energy distribution of 25% PRO, including 18g of supplemented whey protein (WP; Whey Pro Complete®, Standard Process Inc., Palmyra, WI), 50% CHO, and 25% fat. Participants in the 25PRO group were instructed to consume

the supplemental WP daily at breakfast (9g) and immediately following exercise (9g); on non-exercise days, the WP was to be consumed at breakfast and during any snack or meal of their choosing. The WP was a non-fat, high quality PRO source that was easy to incorporate into the diet. Table 2.1 gives an example of each diet for a 70kg female. To increase compliance and meet individual food preferences, participants were allowed the flexibility of 1-2 exchange servings accounting for ~50-200kcal/d; participants were cautioned to use this flexibility sparingly.

Exercise Program. During Phase I, participants engaged in a moderate-intensity (65-80% Heart Rate $[HR]_{max}$; RPE=13-14) Zumba® dance-style fitness class, led by a certified instructor, 2d/wk for 30-45min/d. During Phase II, attendance and duration increased to 3d/wk, 45-60min/d and the intensity of the Zumba® class increased to meet our requirements of HIT (alternating bouts of high [85-90% HR_{max}] and low/moderate-intensity [50-80% HR_{max}]). Additionally, participants were instructed to increase their participation in moderate-intensity exercise to ≥2d/wk (total PA min/wk ≥150). The overall goal was for ~200 kcal/d to be expended in PA. Exercise intensity was monitored using Polar Xtrainer Plus HR monitors (Polar Electro Inc., Lake Success, NY) during Zumba® classes.

Estimates of EE were based on BW (kg) and estimated metabolic equivalents (MET) for the exercise sessions. Using the 2011 Compendium of Physical Activities,[90] the HIT sessions were assigned 6.5 MET, which is listed as the MET level for general aerobic dancing; the moderate-intensity exercise sessions were assigned 3.5 MET, which is equivalent to walking 3.0mph on a level, firm surface.[90] Table 2.2 shows the exercise routine for both groups and the estimated EE for a 70kg female. Together, the diet and exercise during Phase II was designed to provide an energy deficit of ~-3300 to -3600kcal/wk.

Assessments

Anthropometrics. Anthropometrics were assessed pre/post-intervention. BW and body composition (body fat and LBM [excludes bone mineral content]) were measured using a Hologic QDR Discovery Dual Energy X-Ray Absorptiometry (DXA) (Hologic, Inc., Bedford, MA) with participants wearing minimal clothing and no shoes. Height was measured to the nearest 0.5cm using a wall-mounted stadiometer. All WC were measured directly on the skin to the nearest 0.1cm using an inelastic tape measure (Country Technology, Inc., Gays Mills, WI). Measurements were taken at two different sites: 1) Immediately above the iliac crest following the National Institutes of Health (NIH) protocol (WC_{NIH})[39] and 2) At the narrowest point between the lowest rib and the iliac crest following the Anthropometric Standardization Reference Manual (ASRM) protocol (WC_{ASRM}).[40] Data presented are the averages of two WC measurements within 0.7cm.

Resting Blood Pressure and Blood Chemistry. Resting blood pressure (systolic [SBP] and diastolic [DBP]) were measured using a Welch Allyn® automated blood pressure machine (Welch Allyn®, Skaneateles Falls, NY) with the participant seated following 10min of rest. The presented values are the means of two separate measurements taken within 1wk.

Fasting blood samples were collected and immediately centrifuged, aliquoted, refrigerated, and sent to a local laboratory (Good Samaritan Regional Medical Center, Corvallis OR) for analysis of blood lipids (total cholesterol [TC], high density lipoprotein cholesterol [HDL-C], low density lipoprotein cholesterol [LDL-C], triglycerides [TG]), glucose, and insulin. The updated homeostasis model assessment (HOMA) computerized calculator[105] was used to evaluate insulin resistance (HOMA-IR), percent insulin sensitivity (HOMA-%S), and percent pancreatic beta cell functioning (HOMA-%β).[105]

Fitness. Fitness level was assessed by estimating maximal oxygen consumption (VO_{2max}, ml/kg/min) from the modified Balke treadmill protocol.[106] Prior to testing,

participants were instructed to avoid stimulants, including caffeine, heavy meals for 3h, and strenuous activity for 24h. Oxygen consumption (ml O_2/kg BW/min) was estimated using the American College of Sports Medicine's equation for estimating oxygen consumption.[106]

Diet and Physical Activity. To assess pre/during-intervention energy and nutrient intakes and pre/post-intervention PA, participants were given a calibrated food scale (Cuisinart® Weight Mate™, model KS-55, East Windsor, NJ) to complete 4-d weighed food intake and PA records. Verbal and written instructions were provided to each participant. To monitor diet and PA compliance during the intervention, participants completed weekly tracking logs and monthly multiple-pass 24-h recalls and Zumba® participation was monitored with HR monitors and attendance records. Tracking logs were analyzed for energy and nutrient intake and total daily activity, including exercise, using The Food Processor SQL (ESHA, version 10.3.0, Salem, OR). Food labels and recipes were entered into the software program, as necessary, to obtain the most accurate results. If an activity was not listed in the database, staff selected the closest activity matching the movement, the muscle group(s) used, and the MET level.

Statistical Analysis

Data were analyzed from participants who completed all phases of the study. SAS statistical software (version 9.3; SAS Institute Inc., Cary, NC, USA) was used to conduct repeated measures two-way analysis of variance (ANOVA) on two groups (15PRO/25PRO) with two time periods for body composition and MetS variables (pre/post-intervention) and for diet variables (pre/during intervention). Two-sample t-tests were used to assess differences between groups at baseline. Power calculations to detect pre/post-intervention changes in WC of ≥3.0cm (1.2in) between the groups determined that 16 subjects/group

were needed to provide 80% power. Significance was set at $p \leq 0.05$. All values are reported as means ± standard deviations (SD), unless otherwise noted.

RESULTS

Baseline Characteristics

Overall, 35 participants (15PRO: n=17; 25PRO: n=18) completed all phases of the study. Baseline characteristics did not differ between groups for age (35.3±10.2y), weight (80.5±8.4kg), BMI (28.8±2.1kg/m^2), total body fat (40.8±3.1%), WC_{NIH} (101.4±6.5cm) or estimated VO_2max (29.3±4.9ml/kg/min) (Table 2.3). All participants self-reported less than 1h/wk of moderate-intensity exercise prior to beginning the intervention, classifying them as 'sedentary' based on study criteria.

Anthropometrics

Mean pre/post-intervention, and changes in BW, BMI, WC_{NIH}, and WC_{ASRM} values are presented in Table 2.4. Overall, both groups significantly reduced WC_{NIH} (-5.8±3.0cm; $p<0.0001$) and WC_{ASRM} (-7.3±3.6cm; $p<0.0001$) with no differences between PRO levels. These changes represent an ~7% decrease in WC. Overall, BMI remained unchanged; however, four participants moved from the obese (BMI≥30.0kg/m^2) category to overweight (BMI=25.0-29.9kg/m^2), and six moved from the overweight to normal weight category (BMI=18.5-24.9kg/m^2).

MetS and Chronic Disease Risk Factors

Both groups significantly decreased their number of MetS risk factors ($p<0.01$) with no difference between groups. See Table 2.5 for group and combined data for MetS and other chronic disease risk factors. Overall, 18 participants (51%) improved their MetS risk factors; six (17%) improved two MetS risk factors, and 12 (34%) improved one risk factor. All participants began the study with at least one MetS risk factor (WC≥80cm) and eight of them finished the study with no risk factors. Further, eight participants began the study with three MetS risk factors and five of them improved by at least one MetS risk factor.

There were no significant time or group effects for blood glucose; however, there was a significant time effect for serum insulin and HOMA markers, with no differences between groups. Reductions occurred in fasting insulin ($p=0.0048$), HOMA-IR ($p=0.0062$), and HOMA-%β ($p=0.0497$) and HOMA-%S increased significantly ($p=0.0357$); all indicating improved insulin resistance and glycemic control.

Blood lipids also improved due to the intervention. There were significant reductions for TG ($p=0.0064$), TC ($p<0.0001$), and LDL-C ($p<0.0001$), with no differences between groups; HDL-C remained unchanged over time in both groups. Overall, with groups combined, TG decreased ~26mg/dL (20.5% from baseline), TC decreased ~18m/dL (8.9% from baseline), and LDL-C decreased ~12mg/dL (11.8% from baseline).

Baseline SBP and DBP were within normal limits (SBP<120mmHg; DBP<80mmHg), but were significantly higher in the 15PRO group (SBP: $p=0.0436$; DBP: $p=0.0028$). There was a significant time effect for SBP ($p=0.0214$) and a significant diet x time effect for DBP ($p<0.0024$) (Table 2.5). The intervention reduced SBP in both groups and DBP in the 15PRO group.

Fitness

As expected, all participants improved their fitness level due to the intervention (+5.6±2.7ml/kg/min, $p<0.0001$; 19% increase), with no group differences (15PRO: +5.4±2.5ml/kg/min; 25PRO: +5.9±3.0ml/kg/min; $p>0.05$). Using age and sex-based normative data for cardiorespiratory fitness (poor, fair, good, excellent, superior),[107] all participants fell at or below the 'good' fitness category at baseline; 26 (74%) participants had 'poor', six had 'fair,' and three had 'good' cardiorespiratory fitness. After the intervention, only 14 (40%) participants remained in the 'poor' fitness category and seven participants moved up into the 'excellent' fitness category. In all, 10 participants increased their fitness by one

category, eight increased by two categories, and one increased by three categories.

Diet and Physical Activity

Dietary intake data are given in Table 2.6. Overall, participants adhered to the diet with energy intake reduced by an average of -550±370kcals/d, with no differences between groups. When groups are combined, mean energy intake during the intervention was 1527kcals/d. By design, the groups differed in their PRO intake during the intervention (15PRO=16.5±2.1% of energy, 25PRO=25.6±5.0%; $p<0.0001$). Fat intake did not differ between the groups (15PRO=24.4±4.3% of energy, 25PRO= 22.5±4.7%; $p<0.001$). Saturated fat and cholesterol (mg/d) intakes were also similar between the groups ($p<0.001$ and $p=0.0002$, respectively). CHO intake was significantly different between the groups ($p=0.0493$); 15PRO consumed 58.2±4.3% energy from CHO while the 25PRO consumed 51.5±5.4%.

At baseline, participants spent similar amounts of time in moderate (4-6 METs) (15PRO=7.4±12.7min/wk, 25PRO=10.7±25.4min/wk; $p=0.6246$) and high-intensity PA (>6METs) (15PRO=4.4±13.9min/wk, 25PRO=1.3±2.7; $p=0.3781$). Both groups participated in the same PA program during the intervention; thus, we expected the level of PA to be similar. Time spent in Zumba® (15PRO=88.6±25.2min/wk; 25PRO=80.7±21.7; $p=0.3347$) and self-selected moderate-intensity PA (15PRO=94.3±62.3min/wk; 25PRO=98.3±54.3; $p=0.8436$) was similar. Overall, participants spent ~85±24min/wk in Zumba® at intensities >65% HR_{max} and ~96±58min/wk in self-selected moderate/high-intensity PA.

DISCUSSION

To our knowledge, this is the first study to compare typical/moderate (0.85g/kg; 15% energy; CHO:PRO=3.5:1) and high-PRO (1.25g/kg; 25% energy; CHO:PRO=2:1), ER diets combined with aerobic HIT on changes in BW, WC, and MetS risk factors in sedentary, overweight, premenopausal women. Prior

research has focused on moderate-intensity aerobic exercise, resistance training, or a combination of these; we incorporated both moderate-intensity PA (2 d/wk) and HIT (3 day/wk). We hypothesized that the ER, 25PRO diet would result in greater improvements in BW, WC, and other MetS risk factors. However, we found similar improvements in BW, WC, MetS risk factors and HOMA markers of glycemic control, regardless of PRO intake. Additionally, 18 (51%) of our participants (15PRO=9; 25PRO=7) reduced their overall risk for MetS. Thus, because both groups followed the same ER and participate in the same exercise program, our results indicate that a modest ER diet (~-550kcals/d) combined with moderate/HIT exercise appear to have had a greater impact on outcomes than level of PRO intake. The higher PRO intake did not confer any additional improvements in BW, WC or MetS risk factors beyond ER and moderate/HIT exercise. However, it is important to know mean PRO intake, regardless of group, exceeded the Recommended Dietary Allowance (RDA) for PRO (0.8g pro/kg BW).

Body Weight and Waist Circumference

The intervention, which included an ER diet (~-550kcal/d) and exercise-induced energy expenditure (~-200kcal/d), resulted in significant lost weight (-5.1kg ± 2.6) with no difference between groups. Numerous studies have investigated the effects higher PRO intakes combined with ER on weight loss and cardiometabolic factors.[13,108] Two recent meta-analyses concluded that high-PRO ER diets, where PRO intake was at least 10% of energy higher than the control diet, had a small but significant effect on weight loss (-0.79 to -1.2 kg).[13,108] However, these meta-analyses excluded studies with structured exercise as part of the weight loss protocol[13] or did not distinguish between the study protocols that did/did not include exercise.[108] Santesso et al[108] included studies (n=54) with and without exercise, provided that the studies included the same exercise treatment across diet groups. They then assumed the effects of

PRO intake would not be masked by exercise. However, this assumption has not been tested, especially with different amounts and intensities of exercise.

Our results suggest that HIT may mask the impact of a higher PRO intake (~10% increase as percentage of energy) on weight loss. Others[41,109-112] have found equivocal results when examining the impact of exercise combined with ER high-PRO diets on weight loss. Layman et al[109] reported greater weight loss with an ER high-PRO diet (1.6g/kg/d vs. 0.8g/kg/d) when combined with a structured aerobic exercise (5d/wk walking) and resistance training (2d/wk) program. However, others[41,110-112] have failed to see an effect of PRO intake on weight loss with ER and exercise. Two studies by Kerksick et al,[111,113] using a supervised circuit-style resistance exercise (3d/wk; 30min/wk) and ER (1200kcal/d) program, found comparable weight loss across groups, regardless of PRO intake (15%, 50%, and 63% of energy from PRO). In addition, Walker Lasker, Evans, and Layman[112] and Sacks et al[41] combined 90min/wk of moderate-intensity exercise with ER and did not see a significant effect of PRO intake (15%-30% of energy) on weight loss.[41,112] In these studies, the exercise interventions were unsupervised; whereas, exercise was supervised in the current study and in the studies by Kerksick et al.[111,113] Finally, Josse et al[110] conducted a study that was similar to ours in population, duration, energy deficit from intake (-500kcals/d), and exercise (-250kcals/d from 5-7d/wk aerobic exercise plus 2d/wk resistance training). They found no effect of PRO (15% and 30% energy from PRO) on weight loss.

AbOb, determined by elevated WC, is considered a primary contributor to MetS.[3,21] At baseline, all of our participants had a WC greater than 80cm, which increases their risk for MetS and mortality.[22,114] After 16wks, the mean reduction of 7.3cm (WC_{NIH}) resulted in 7 of 35 (20%) participants reducing their WC below the World Health Organization (WHO)[22] category of 'increased risk' (<80cm) and 11 of 35 (~31%) reducing their WC below the WHO 'higher risk' category

(<88cm). Combined, over 50% of our participants reduced their chronic disease risk based on WC alone. However, similar to others[110,113] we did not see an impact of PRO intake on changes in WC.

MetS and Chronic Disease Risk Factors

MetS risk factors improved over time regardless of PRO intake. At baseline, all participants had at least one MetS risk factor (WC≥80cm) and 22 of 35 (~63%) had a least two MetS risk factors. After 16wks, only 15 of 35 (~43%) remained with two or more MetS risk factors. Overall, MetS reduction was similar between groups.

Glycemic Control. Fasting glucose has been found to improve with weight loss and/or exercise.[115] Similarly, higher PRO intakes have been suggested to improve post-prandial glycemic control.[112] At baseline all participants had normal fasting blood glucose levels, which did not change over time regardless of group. However, the intervention did significantly improved fasting serum insulin, HOMA-IR, HOMA-S, and HOMA-β. While the improvements seen in serum insulin, HOMA-IR, and HOMA-S indicate an improved ability to manage blood glucose through insulin receptor sensitivity, the improvement in HOMA-β indicates possible improved pancreatic β-cell activity. Similar to our findings, Walker Lasker, Evans, and Layman[112] found no effect of higher PRO intakes (1.6g/kg/d; ~30% energy from PRO) and moderate-intensity exercise (90min/wk) on fasting glucose, but did find improvements in HOMA-IR from higher PRO intake. HIT has been shown to improve markers of insulin sensitivity beyond moderate-intensity exercise;[99] thus, it is possible that the improvements observed were a results of the HIT program.

Blood Lipids. Weight loss and improved glycemic control can translate into an improved blood lipid profile. In the current study, we saw no effect to PRO intake on the reductions in TG (26mg/dL), TC (18mg/dL), and LDL-C (12mg/dL). The significant weight loss and improved glycemic control we observed, may have

masked any effect of PRO intake on changes in blood lipids. Our data are supported by others,[110,111] who found no effect of PRO intake on weight loss or blood lipids. The weight lost in Josse et al[110] (~5% combined mean) and Kerksick et al[111] (3.9-5.3%) was not significantly different between groups and similarly, they did not see an effect of PRO on their significantly[110], or favorably[113] improved blood lipids. Sacks et al [41] suggest that the impact of weight loss on improvements in blood lipids is stronger than the impact of PRO. Thus, weight loss may be an underlying factor for our lipid changes, as well. However, not all studies agree. Two studies[109,112] reporting a PRO effect on weight loss also found a PRO effect on changes in blood lipids. Layman et al[109] reported a significantly greater weight loss their high-PRO (1.6g/kd/d; ~30% of energy) group compared to their low-PRO (0.8g/kg/d; ~15% of energy) group (11.4% vs. 8.4% BW loss respectively) and also reported significant PRO effects on changes in blood lipids. Walker Lasker, Evans, and Layman[112] saw a non-significant trend (p=0.07) for greater weight loss from their higher PRO group compared to their lower PRO group (9.4% vs. 7.3%, respectively) and saw significant changes in blood lipids, as well. Thus, it is possible that weight loss was an underlying factor for blood lipid improvements.

Blood Pressure. In the current study, hypertension was an exclusion criteria due to safety concerns associated with participation in HIT. Thus, baseline RBP values were normal; however, within the range of normal, our 15PRO group had a significantly higher DBP (78 vs. 70mmHg). The intervention was successful in reducing SBP in both the 15PRO and 25PRO groups, while DBP only reduced in the 15PRO group. Others[41,116] have reported improved RBP (SBP and DBP) with ER and exercise, regardless of PRO intake.

Strengths & Limitations

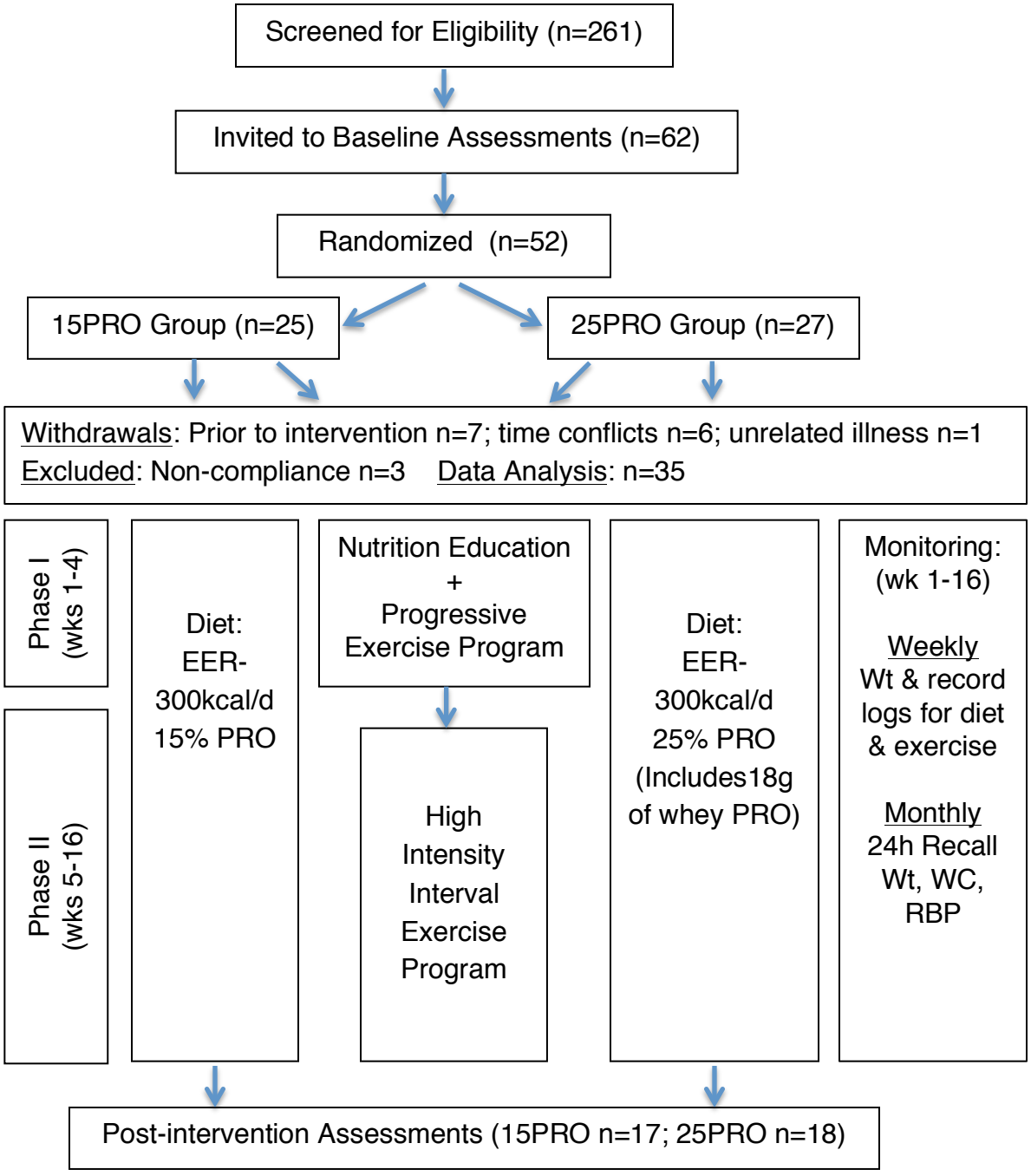
Although both arms of the intervention were successful in reducing AbOb (WC) and MetS risk factors, the design of the study was not without

limitations. First, one must consider the inherent limitations of weight loss research in free-living participants with regard to compliance (diet and PA), data reporting (including diet and PA), collection and analysis. Second, the data reported here are applicable to apparently healthy, premenopausal overweight/obese women who were capable of participating in HIT. Third, the participants involved in this study were motivated to lose weight and improve health. Fourth, the participants in this program were provided with personalized guidance and education to assist with intervention compliance, which is often not available in traditional weight loss programs. However, the diet and exercise program was specifically designed to be easily transferable without the cost of continued guidance, membership fees, or special foods to be purchased. Finally, this study did not include a HIT-only group or a moderate-intensity exercise only group; thus, limiting our ability to evaluate the effect of the PRO level when participating in HIT compared to more traditional moderate-intensity exercise.

CONCLUSIONS

Our results suggest that prior to menopause, overweight women with AbOb can effectively reduce their risk of chronic disease and MetS by incorporating HIT and consuming an ER diet with 15-25% of energy coming from lean PRO. Higher PRO intakes did not add additional benefits. However, both groups had mean PRO intakes at or above the RDA for PRO (0.8g/kg/d). Adding HIT to an ER diet results in the same weight loss and metabolic benefits a shorter period of time, which will appeal to those with limited time to exercise.

Figure 2.1. Study design



PRO=protein (15% or 25% energy from PRO)
EER=Estimated Energy Requirement [1]
Wt=body weight
WC=waist circumference
RBP=resting blood pressure

Table 2.1. Sample macronutrient distribution for a diet based on a 70-kg female

Diet	Energy (kcals/d)	Fat (g)	CHO (g)	PRO (g)	PRO (g/kg)	CHO:PRO (g/g)
25PRO	1880-300 =1580	53	178	99	1.4	1.8
15PRO	kcal	53	217	59	0.84	3.7

PRO=protein; CHO=carbohydrate
25PRO=25% energy from PRO, including 18g/d supplemental whey PRO
15PRO=15% energy from PRO, no supplemental whey PRO

Table 2.2. Exercise intervention and estimated energy (kcals) expenditure from exercise[a] for a 70kg female

Wk	Type	Frequency (d/wk)	Intensity (MET)	Time (min/d)	kcals/ d	kcals/ wk	Total kcals/wk
1	Class	2	6.5	30	240	480	610
	Home	1	3.5	30	130	130	
2	Class	2	6.5	30	240	480	740
	Home	2	3.5	30	130	260	
3	Class	2	6.5	30	240	480	870
	Home	2	3.5	45	195	390	
4	Class	2	6.5	45	360	720	1110
	Home	2	3.5	45	195	390	
5 - 16	Class	3	6.5	45	360	1080	1470
	Home	2	3.5	45	195	390	

[a]Energy expenditure from exercise[90] = 0.0175 kcal/min/MET x METs x Body Weight (kg) x Time (min)

Class = Supervised, programmed Zumba® exercise (high-intensity interval exercise); excludes warm-up & cool-down

Home = Unsupervised, self-directed exercise (e.g. moderate-intensity walking); excludes warm-up & cool-down

Table 2.3. Baseline characteristics by group and with groups combined[a]

Variable	Combined (n=35)	15PRO (n=17)	25PRO (n=18)	p-value[b]
Age (y)	35.3 ± 10.2	34.7 ± 10.9	35.8 ± 9.8	0.7622
Body Weight kg)	80.5 ± 8.4	80.4 ± 8.6	80.6 ± 8.5	0.9348
BMI (kg/m^2)	28.8 ± 2.1	29 ± 2.3	28.7 ± 1.9	0.5832
WC_{ASRM} (cm)	89.4 ± 5.4	89.3 ± 4.9	89.5 ± 6.0	0.9274
WC_{NIH} (cm)	101.4 ± 6.5	101.1 ± 6.9	101.6 ± 6.2	0.8379
Body Fat (%)	40.8 ± 3.1	41.2 ± 3.4	40.4 ± 3.0	0.4697
LBM (kg)	45.2 ± 4.5	44.8 ± 4.5	45.7 ± 4.6	0.5517
Energy Intake (kcals/d)	2077 ± 376	2160 ± 391	1998 ± 354	0.2080
PRO Intake (% kcals)	14.4 ± 2.4	14.0 ± 2.5	14.7 ± 2.3	0.3817
PRO Intake (g/kg/d)	0.94 ± 0.2	0.94 ± 0.3	0.94 ± 0.2	0.9929
CHO Intake (% kcals)	50.4 ± 6.2	51.1 ± 6.4	49.8 ± 6.1	0.5260
Fat Intake (% kcals)	33.4 ± 5.4	33.3 ± 4.9	33.5 ± 5.6	0.9147
Submaximal VO_2 (ml/kg/min)	29.3 ± 4.9	29.9 ± 5.5	28.7 ± 4.2	0.4558

[a]Values are reported as means ± SD; Significant difference = $p<0.05$

[b]p-values for baseline group differences using Welch's Modified Two-Sample t-Test

BMI = body mass index

WC = waist circumference

WC_{ASRM} = Anthropometric Standardization Reference Manual method for measuring WC[117]

WC_{NIH} = National Institutes of Health method for measuring WC[39]

Table 2.4. Anthropometric measures by group and with groups combined[a, b]

Variable	15PRO (n=17)	25PRO (n=18)	Combined (n=35)	Effect	p-value
Body Weight (kg)					
Pre	80.4 ± 8.6	80.6 ± 8.5	80.5 ± 8.4	Diet	0.7492
Post	74.5 ± 8.4	76.1 ± 8.6	75.4 ± 8.4	**Time**	**<0.0001**
Change	-5.8 ± 2.7	-4.5 ± 2.4	-5.1 ± 2.6	DxT	0.1279
Body Mass Index (kg/m2)					
Pre	29.0 ± 2.3	28.7 ± 1.9	28.8 ± 2.1	Diet	0.8000
Post	27.1 ± 2.1	27.1 ± 22.2	27.1 ± 2.1	**Time**	**<0.0001**
Change	-1.9 ± 1.8	-1.5 ± 0.9	-1.7 ± 1.4	DxT	0.3661
Waist Circumference$_{NIH}$[c]					
Pre	101.1 ± 6.9	101.6 ± 6.2	101.4 ± 6.5	Diet	0.5827
Post	93.1 ± 5.5	94.9 ± 6.4	94 ± 6.0	**Time**	**<0.0001**
Change	-8.0 ± 3.6	-6.7 ± 3.5	-7.3 ± 3.6	DxT	0.2721
Waist Circumference$_{ASRM}$[d]					
Pre	89.3 ± 4.9	89.5 ± 6.0	89.4 ± 5.4	Diet	0.6683
Post	82.9 ± 4.3	84.3 ± 6.7	83.6 ± 5.6	**Time**	**<0.0001**
Change	-6.4 ± 3.3	-5.2 ± 2.5	-5.8 ± 3.0	DxT	0.2239
Total Body Fat (%)					
Pre	41.2 ± 3.4	40.4 ± 3.0	40.8 ± 3.1	Diet	0.7707
Post	37.4 ± 3.8	37.5 ± 3.9	37.4 ± 3.8	**Time**	**<0.0001**
Change	-3.8 ± 1.3	-2.9 ± 1.8	-3.3 ± 1.6	DxT	0.1023

15PRO=15% protein from energy group; 25PRO=25% protein from energy group

[a]Values are means ± SD; $p<0.05$; Significant change=$p<0.05$; Non-significant change (NS)=$p\geq0.05$

[b]Repeated measures ANOVA analysis

[c]Waist Circumference measured at the iliac crest using NIH guidelines[39]

[d]Waist circumference measured at the narrowest waist using the Anthropometric Standardization Reference Manual guidelines[117]

Table 2.5. Metabolic syndrome and other chronic disease risk factors for groups and with groups combined[a,b]

Variable	15PRO (n=17)	25PRO (n=18)	Combined (n=35)	Effect	p-value
Resting Blood Pressure – Systolic (mmHg)					
Pre	119 ± 12	111 ± 11	115.0 ± 12	Diet	0.0944
Post	114 ± 15	109 ± 9	111.0 ± 12	**Time**	**0.0214**
Change	-5 ± 8	-2 ± 8	-3 ± 9	DxT	0.1995
Resting Blood Pressure – Diastolic (mmHg)					
Pre	78.0 ± 8	70 ± 8	74 ± 9	Diet	0.0646
Post	71 ± 8	71 ± 7	71 ± 7	Time	0.0162
Change	-7 ± 6	1 ± 8	-3 ± 8	**DxT**	**0.0024**
Total Cholesterol (mg/dL)					
Pre	210.1 ± 40.3	197.8 ± 40.1	203.8 ± 40.1	Diet	0.4819
Post	188.8 ± 40.9	182.7 ± 34.9	185.6 ± 37.5	**Time**	**<0.0001**
Change	-21.3 ± 16.5	-15.2 ± 18.2	-18.1 ± 17.4	DxT	0.3051
Low Density Lipoprotein Cholesterol (mg/dL)					
Pre	129.5 ± 35.1	117.3 ± 35.0	123.2 ± 35.1	Diet	0.4235
Post	114.2 ± 36.8	107.9 ± 32.1	111.0 ± 34.1	**Time**	**0.0001**
Change	-15.2 ± 19.4	-9.4 ± 12.4	-12.2 ± 16.2	DxT	0.2927
High Density Lipoprotein Cholesterol (mg/dL)					
Pre	53.5 ± 13.2	58.3 ± 15.4	55.9 ± 14.4	Diet	0.5340
Post	54.7 ± 12.3	55.3 ± 11.5	55.0 ± 11.7	Time	0.5015
Change	1.2 ± 8.3	-3.0 ± 7.0	-0.9 ± 7.9	DxT	0.1124
Triglycerides (mg/dL)					
Pre	136.0 ± 59.1	111.5 ± 56.9	123.4 ± 58.4	Diet	0.3711
Post	99.0 ± 40.5	97.2 ± 41.6	98.1 ± 40.5	**Time**	**0.0064**
Change	-37.0 ± 61.0	-14.3 ± 42.1	-25.3 ± 52.6	DxT	0.2074
Insulin (mg/dL)					
Pre	9.8 ± 4.0	8.0 ± 3.9	8.9 ± 4.0	Diet	0.3118
Post	6.8 ± 2.5	6.7 ± 2.9	6.8 ± 2.6	**Time**	**0.0048**
Change	-3.0 ± 3.5	-1.3 ± 4.7	-2.1 ± 4.2	DxT	0.2228
Glucose (mg/dL)					
Pre	89.7 ± 5.6	90.5 ± 9.9	90.1 ± 8.0	Diet	0.4452
Post	86.2 ± 6.1	88.9 ± 8.8	87.6 ± 7.6	Time	0.0617
Change	-3.5 ± 4.7	-1.6 ± 9.8	-2.5 ± 7.7	DxT	0.4582
HOMA-IR					
Pre	1.3 ± 0.5	1.1 ± 0.5	1.2 ± 0.5	Diet	0.3372
Post	0.9 ± 0.3	0.9 ± 0.4	0.9 ± 0.4	**Time**	**0.0062**
Change	-0.4 ± 0.5	-0.2 ± 0.6	-0.2 ± 0.5	DxT	0.2667

HOMA-S (%)								
Pre	87.8	± 32.3	111.7	± 46.8	100.1	± 41.6	Diet	0.5375
Post	131.4	± 63.6	126.0	± 80.5	128.6	± 71.8	**Time**	**0.0357**
Change	43.6	± 60.8	14.3	± 91.5	28.5	± 78.4	DxT	0.2762
HOMA-β (%)								
Pre	115.1	± 36.6	98.1	± 32.8	106.4	± 35.3	Diet	0.1780
Post	98.7	± 34.0	90.1	± 7.0	94.3	± 30.5	**Time**	**0.0497**
Change	-16.5	± 32.8	-8.0	± 37.9	-12.1	± 35.2	DxT	0.4831

15PRO=15% protein diet group; 25PRO=25% protein diet group
DxT=Diet x Time interactive effect
HOMA-IR=HOMA Insulin Resistance factor[105]
HOMA-S=HOMA percent insulin sensitivity[105]
HOMA-β=HOMA percent pancreatic β-cell function[105]
[a]Values are means ± SD; $p<0.05$; Significant=$p<0.05$; Non-significant (NS)=$p\geq0.05$
[b]Data analyzed with repeated measures ANOVA

Table 2.6. Dietary intake at baseline and during the intervention[a,b]

Variable	15PRO (n=17)	25PRO (n=18)	Effect	p-value[b]
Energy (kcals/d)				
Baseline	2160 ± 391	1998 ± 354	Diet	0.4633
Interventin	1515 ± 371	1537 ± 181	**Time**	**<0.0001**
Change	-645 ± 373	-461 ± 354	DxT	0.1436
Protein (%kcals/d)				
Baseline	14.0 ± 2.5	14.7 ± 2.3	Diet	<0.0001
Intervention	16.5 ± 2.1	25.6 ± 5.0	Time	<0.0001
Change	2.5 ± 3.3	10.8 ± 4.4	**DxT**	**<0.0001**
Protein (g/kg/d)				
Baseline	0.94 ± 0.26	0.94 ± 0.16	Diet	0.0003
Intervention	0.85 ± 0.17	1.26 ± 0.21	Time	0.0279
Change	-0.09 ± 0.30	0.31 ± 0.27	**DxT**	**0.0002**
Carbohydrate (%kcals/d)				
Baseline	51.1 ± 6.4	49.8 ± 6.1	Diet	0.0077
Intervention	58.2 ± 4.3	51.5 ± 5.4	Time	0.0012
Change	7.1 ± 7.1	1.7 ± 7.7	**DxT**	**0.0396**
Fat (%kcals/d)				
Baseline	33.3 ± 4.9	33.5 ± 6.0	Diet	0.5269
Intervention	24.4 ± 4.4	22.5 ± 4.7	**Time**	**<0.0001**
Change	-8.9 ± 4.9	-11.0 ± 7.0	DxT	0.3006
Saturated fat (%kcals/d)				
Pre	11.0 ± 3.3	11.2 ± 2.7	Diet	0.8355
During	7.8 ± 1.9	7.2 ± 2.3	**Time**	**<0.0001**
Change	-3.2 ± 2.4	-4.0 ± 3.1	DxT	0.4474
Cholesterol (mg/d)				
Pre	286.4 ± 141.2	233.1 ± 107.0	Diet	0.3091
During	158.4 ± 120.4	148.6 ± 68.2	**Time**	**<0.0001**
Change	-128.1 ± 140.2	-84.5 ± 123.7	DxT	0.3361
Alcohol (%kcals/d)				
Pre	1.4 ± 2.6	2.0 ± 3.1	Diet	0.7560
During	0.6 ± 1.8	0.4 ± 1.3	**Time**	**0.0245**
Change	-0.8 ± 2.4	-1.5 ± 3.3	DxT	0.4364
Fiber (g/1000kcals/d)				
Pre	9.5 ± 5.2	9.2 ± 2.5	Diet	0.8982
During	14.3 ± 5.9	14.8 ± 6.8	**Time**	**<0.0001**
Change	4.8 ± 7.6	5.6 ± 5.7	DxT	0.7174

15PRO=15% energy from protein diet group
25PRO=25% energy from protein diet group
[a]Values are reported as daily means ± SD
[b]p-values are reported from repeated measures ANOVA

CHAPTER 3 – MANUSCRIPT #2: EFFECTS OF MODERATE AND HIGH-PROTEIN, ENERGY-RESTRICTED DIETS ON BODY COMPOSITION CHANGES IN OVERWEIGHT PREMENOPAUSAL WOMEN

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ABSTRACT

Weight loss through energy restriction (ER) can lead to loss of metabolically active lean body mass (LBM) in addition to body fat (BF). **Purpose:** To determine the effect of an ER diet with either 15% or 25% of energy from protein (PRO) combined with high-intensity interval training (HIT) on body composition in overweight, premenopausal women with abdominal obesity (AbOb). **Methods:** Sedentary, premenopausal women (age=35±10y) with AbOb were randomized to a 16-wk ER (-300kcals/d) diet with 15% (15PRO; n=17) or 25% (25PRO; n=18) of energy from PRO, plus 45min/d, 3d/wk HIT and 45min/d, 2d/wk moderate-intensity exercise (exercise=-200kcals/d). Diet and physical activity (PA) were assessed using 4-d weighed food and PA records, respectively; diet and exercise compliance were assessed with monthly multiple-pass 24-h recalls and weekly tracking logs. Body composition (BF [%], BF [kg], trunk fat [kg], and LBM [kg]) was assessed by dual energy X-ray absorptiometry pre/post-intervention. Repeated measures analysis of variance and two sample t-tests were used at analyze the date. Results are reported as means±standard deviations. **Results:** There were significant time, but not group, effects on changes in BW (-5.1kg±2.6, $p<0.0001$), BF (-3.3%±1.6, $p<0.0001$), and LBM (-0.6kg±1.5, $p=0.0283$). The 15PRO group lost more absolute whole BF (-5.2kg vs. -3.9kg, $p=0.0355$) and trunk fat (-3.1kg vs. -2.2kg) over time. **Conclusion:** Both diets significantly reduced BW, BF (%), and LBM (kg); however, the 15PRO diet reduced absolute BF (kg) and trunk fat (kg) greater than the 25PRO group. The impact of HIT/CME and the 1.3kg greater BW loss in the 15PRO group may have influenced the greater changes in absolute BF and trunk fat in this group. More research is needed to separate the impact of HIT/CME and weight loss from the impact of PRO during ER.

INTRODUCTION

Obesity and its associated chronic diseases are major public health priorities in the United States.[91] Excess abdominal fat (abdominal obesity [AbOb]), is considered a primary contributor to these chronic diseases and is independently associated with the development of metabolic syndrome (MetS).[6,118] Thus, the health consequences of AbOb and MetS are increased risk of cardiovascular disease and type 2 diabetes.[119]

Effects of energy restriction and exercise on body composition

Weight loss through dietary energy restriction (ER), with or without exercise, is effective for reducing obesity and MetS risk factors.[42,76] However, weight loss with ER alone can lead to upwards of 25% of total body mass lost as LBM.[120] Development of MetS is inversely related to muscle mass quantity.[121] The loss of metabolically active LBM reduces energy expenditure (EE) and increases the risk of weight loss plateau or weight regain after weight loss. Additionally, loss of LBM increases the risk of chronic hyperglycemia and diabetes, independent of obesity, due to a reduced ability to dispose of glucose into muscle tissue.[122] Finally, when coupled with aging, loss of LBM can increase the risk of sarcopenia, physical frailty, and likelihood of fails and impaired physical functioning.[123]

Combining ER with physical activity (PA), in the form of aerobic exercise, with or without resistance training, is the optimal approach for targeting fat mass reduction.[76,124] Research now shows that high-intensity aerobic interval training (HIT), characterized by short periods of high-intensity exercise separated by low/moderate-intensity recovery periods, is superior to continuous moderate-intensity aerobic exercise (CME) for improving fitness.[99] Further, HIT requires less time, yet is better than CME for improving insulin sensitivity and equally as effective for reducing body fat, AbOb and MetS risk factors.[99] The health benefits from HIT can be achieved in ~15-20% less time than CME[99] and as little as

1d/mo to 4d/wk of HIT is associated with reduced abdominal fat accumulation.[125] However, exercise increases whole body protein (PRO) breakdown while PRO synthesis remains unchanged or decreases.[57] The level of PRO breakdown during exercise is positively related to intensity;[16] thus, higher intensity exercise, such as HIT, could result in greater amino acid oxidation than low or CME. However, the addition of exercise (aerobic or resistance) to modest ER (~500-750 kcals/d) has been shown to attenuate LBM loss compared to ER alone.[126]

Effect of dietary PRO on body composition during ER and HIT

Though time efficient and beneficial for body weight (BW), fat, and MetS risk factor reductions, the combination of ER and HIT may be detrimental to LBM due to the higher intensity of HIT. To maintain or build LBM during modest ER and HIT compared to ER alone, increased dietary PRO intake may be needed.[76,101] Increased amino acid availability from higher PRO intake can ameliorate the negative nitrogen balance and reduce LBM loss.[101] Krieger et al[49] found greater LBM retention during ER (ER to no less than 60% of energy needs) when PRO intake is >1.05g/kg/d; whereas Westerterp-Plantenga et al[46] suggests at least 1.2g/kg/d is needed during ER. Taken together, when modest ER (reductions of -500 to -750 kcal/d) and HIT are combined for BW loss and MetS risk factor reductions, higher PRO intake may prevent LBM losses.

MetS and women

As women age and transition through menopause, body composition typically changes. Aging increases total body fat, including absolute and percent abdominal fat,[127] and is associated with reductions in PA.[9] Menopause is associated with decreases in total LBM.[127] Combined, these factors increase the risks for obesity, AbOb, MetS, and sarcopenia. Thus, for premenopausal women who are already overweight/obese and have AbOb, reducing BW and AbOb, while maintaining LBM, during ER is critical to attenuate the effects of aging and menopause.

To date, no study has incorporated an ER-diet with higher dietary PRO intake, while including HIT, for reducing body fat, AbOb, and preserving LBM in sedentary, premenopausal, overweight/obese women. Thus, the purpose of this study was to examine the effect of an ER-diet, providing two levels of dietary PRO (15 and 25% of energy), with an exercise (HIT and CME) program on changes in total and regional body composition in premenopausal women with AbOb. We hypothesize that higher PRO intake would increase loss of body fat (whole body and abdominal) while preserving LBM over a more typical protein diet.

METHODS

Participants

Sedentary (<1h/wk CME for ≥6mo), overweight and obese (body mass index [BMI] between 25-33kg/m^2), premenopausal (18-50y) women with AbOb (Waist Circumference [WC] ≥80cm) were recruited, screened (n=261) for eligibility by based on their WC, BMI, and a health and PA questionnaire. All participants were apparently healthy, weight stable (±2kg) for >4-6mo, and provided medical clearance to participate in HIT prior to initiation of exercise. Participants were excluded from the study if they were diabetic, hypertensive, pregnant or lactating, smokers, unable to consume lactose-containing products, or taking medication to alter metabolism. Overall, 52 women were randomized into the study; seven participants withdrew from the study prior to the intervention due to discontent with group assignment, seven withdrew during the study due to time conflicts, one withdrew due to an unrelated illness, and three were excluded due to non-compliance. Data from the remaining 35 women were used for data analysis. The Oregon State University Institutional Review Board approved this study and all participants provided written informed consent to participant in the study and received monetary compensation for participation.

Study Design

The study was a 16-wk non-blinded randomized control trial with two treatment groups (see Figure 2.1). After completing eligibility screening and baseline assessments, participants were randomized to one of two diets differing in PRO intake (15% [15PRO] or 25% [25PRO] of energy). All participants consumed an ER diet (~-2100kcals/wk) and participated in an exercise program with 3d/wk of HIT and 2d/wk of CME (~-1400kcals/wk). The 15PRO diet served as the control diet with a PRO level representing typical PRO intake in the United States;[12] inclusion of the same exercise program in both groups served as a control for exercise. Budget limitations restricted our ability to include diet- and exercise-only groups. All assessments were completed pre/post-intervention; diet and exercise compliance were monitored weekly with participant-maintained diet and PA tracking logs and investigator-maintained attendance records.

Intervention

After randomization, each participant met with investigators, including a registered dietitian (RD) and exercise specialist, to review group assignment, intervention protocol, and their individual diet and exercise plans. The 16-wk intervention was divided into two phases: Phase I=wk 1-4, Phase II=wk 5-16. During Phase I, participants attended weekly nutrition education classes and engaged in progressive exercise training, including Zumba® (explained below). Nutrition classes emphasized portion size estimation, following a low energy-dense eating plan and use of the diet exchange system (described below).

Energy-restricted diet. Energy needs were assessed using the 2005 Institute of Medicine Dietary Reference Intake equation for estimating Energy requirements (EER) based on total EE equations for women ages ≥19y. At baseline, a PA coefficient of 1.0 was used to represent a sedentary PA level, and was increased as the PA level increased during the study. After energy needs were determined, an ER (EER - 300kcals/d) diet plan was created for each participant using the

Exchange List from the Academy of Nutrition and Dietetics (AND) and the American Diabetes Association (ADA).[104] In general, participants were encouraged to follow the Dietary Guidelines for Americans, including increasing consumption of low energy-dense foods, such as whole fruits and vegetables, whole grains, and low-fat dairy and lean meats, and reducing/eliminating intake of high-energy drinks. The 15PRO diet had an energy distribution of 15% PRO (~0.85g/kg/d), 60% carbohydrate (CHO), and 25% fat; the 25PRO diet had an energy distribution of 25% PRO (~1.26g/kg/d), including 18g of supplemented whey protein (WP; Whey Pro Complete®, Standard Process Inc., Palmyra, WI), 50% CHO, and 25% fat. Participants in the 25PRO group were instructed to consume the supplemental WP daily at breakfast (9g) and immediately following exercise (9g); on non-exercise days, the WP was to be consumed at breakfast and during any snack/meal of their choosing. The WP was a non-fat, high quality PRO source that was easy to incorporate into the diet.

Exercise Program. During Phase I, participants engaged in a moderate-intensity (65-80% Heart Rate $[HR]_{max}$; RPE=13-14) Zumba® dance-style fitness class, led by a certified instructor, 2d/wk for 30-45min/d. During Phase II, attendance and duration increased to 3d/wk, 45-60min/d and the intensity of the Zumba® class increased to meet our requirements of HIT (alternating bouts of high [85-90% HR_{max}] and low/moderate-intensity [50-80% HR_{max}]). Additionally, participants were instructed to increase their participation in CME to ≥2d/wk (total PA min/wk ≥150). The overall goal was for ~200 kcal/d expended through structured PA. Zumba® exercise intensity was confirmed using HR monitors (Polar Xtrainer Plus, Polar Electro Inc., Lake Success, NY), during Zumba classes.

Estimates of EE were based on BW (kg) and estimated metabolic equivalents (MET) for the exercise sessions. Using the 2011 Compendium of Physical Activities, the HIT sessions were assigned 6.5 MET, which is listed as the MET level for general aerobic dancing; the moderate-intensity exercise

sessions were assigned 3.5 MET, which is equivalent to walking 3.0mph on a level, firm surface.[90] Table 2.3 shows the exercise routine for both groups and the estimated EE for a 70kg female. Together, the diet and PA during Phase II was designed to provide an energy deficit of ~3300-3600 kcal/wk.

Assessments

Diet and Physical Activity. To assess pre/post-intervention energy and nutrient intakes, participants were given a calibrated food scale (Cuisinart® WeightMate™, model KS-55, East Windsor, NJ) to complete 4-d weighed food intake. Concurrently, they kept 4-d PA records. Verbal and written instructions were provided to each participant. To monitor diet and PA adherence during the intervention, participants completed weekly tracking logs and monthly multiple-pass 24-h recalls. Logs were analyzed for energy and nutrient intake and total daily activity, including PA, using The Food Processor SQL (ESHA, version 10.3.0, Salem, OR). Food labels and recipes were entered into the software program, as necessary, to obtain the most accurate results. If an activity was not listed in the database, staff selected the closest activity matching the movement, the muscle group(s) used, and the MET level.

Anthropometrics. Height, BW, and body composition were measured with participants wearing minimal clothing without shoes. Height was measured to the nearest 0.5cm using a wall-mounted stadiometer. Weekly BW was measured using a Seca mechanical scale (model 761, Chino, CA). Pre/post-intervention body composition (body fat and LBM [excludes bone mineral content]) and BW were measured using a Hologic QDR Discovery Dual Energy X-Ray Absorptiometry (DXA) scanner (Hologic, Inc., Bedford, MA) and analyzed using Hologic whole body and sub-region analysis modes (APEX System software version 3.1.1). Manufacturer-defined regions of interest (ROI) were used to analyze body composition of the trunk and android regions. The trunk analysis

includes the chest, abdomen, and pelvis, while the android analysis is defined as 20% of the distance from the iliac crest to the neck cut line (Figure 3.1).

Statistical Analysis

Repeated measures analysis of variance (ANOVA) was used to analyze changes overtime (pre/post) for the treatment groups (15PRO/25PRO) for changes in body composition and diet variables (pre/during) using SAS statistical software (version 9.3; SAS Institute Inc., Cary, NC, USA). Based on a power analysis, a sample size of 16/group was needed to provide 80% power to detect a WC difference of >3cm (1.2in). Significance was set at $p \leq 0.05$. All values are reported as means ± standard deviations (SD), unless otherwise noted. Analyses were conducted on data from participants who completed all phases of the study.

RESULTS

Baseline Characteristics, Retention, & Attendance

Overall, 35 participants (15PRO: n=17; 25PRO: n=18) completed the study. At baseline, groups did not differ for age (35.3±10.2y), BW (80.5±8.4kg), BMI (28.8±2.1kg/m^2), total body fat (40.8±3.1%), or LBM (45.2±4.5kg) (Table 3.1). Class attendance did not different between groups for HIT/Zumba® class ($p=0.2073$) or total (nutrition education + HIT/Zumba® classes; $p=0.1249$) attendance; however, nutrition education class attendance was lower in the 15PRO group ($p=0.0002$).

Diet and Physical Activity

Both groups reported similar dietary intake for energy and macronutrients at baseline (Table 2.6). During the intervention, groups significantly reduced total energy intake by -550±370kcals/d ($p<0.0001$) and energy from fat from 33% to 23% ($p<0.0001$), with no differences between groups. By design, the energy intake from PRO ($p<0.0001$) and CHO ($p=0.0396$) differed. PRO intake in the 25PRO group increased from 0.94g/kg/d to 1.26g/kg/d, while PRO intake in the 15PRO group decreased from 0.94g/kg/d to 0.85g/kg/d.

At baseline, the groups did not differ in the time spent in CME (4-6 METs) (15PRO=7.4±12.7min/wk, 25PRO=10.7±25.4; p=0.6246) or high-intensity PA (>6 METs) (15PRO=4.4±13.9min/wk, 25PRO=1.3±2.7; p=0.3781). During the intervention, the groups spent similar time in HIT/Zumba® (15PRO=88.6±25.2 min/wk, 25PRO=80.7±21.7; p=0.3347) and self-selected, non-supervised CME (15PRO=94.3±62.3min/wk, 25PRO=98.3±54.3; p=0.8436). Overall, participants spent ~181min/wk exercising at intensities greater than 4 METs, including ~96±58min/wk at 4-6 METs and ~85±24min/wk at >6 METs (e.g. HIT).

Body Composition

Body composition data are presented in Table 3.2. Overall, participants lost 5.1kg±2.6 ($p<0.0001$) representing a 6.3% reduction in BW, with no differences between groups. Changes in BW, fat, and LBM, can be seen in Figures 3.2, 3.3, and 3.4. There were no differences in the body fat percentage losses between groups (-3.3±1.6% lost; $p<0.0001$), but there was a significant group x time effect for total body fat mass (kg) loss ($p=0.0355$). The change in body fat mass was -5.2±1.8kg and -3.9±1.9kg in the 15PRO and 25PRO groups, respectively. Due to the intervention, LBM decreased 0.6±1.5kg ($p=0.0283$) with no differences between groups, representing ~12% loss of BW from LBM. At baseline, trunk fat was similar between groups (combined mean=5.5±2.5kg, $p=0.5504$); however, there was a diet x time effect for trunk fat loss ($p=0.0243$). The 15PRO group lost 3.1±1.2kg of trunk fat, while the 25PRO group lost 2.2±0.9kg. Android fat, a component of trunk fat (see Figure 3.1), was also similar between groups at baseline (combined mean=2.8±0.6kg, $p=0.7097$), with no differences between groups due to the intervention (combined mean=0.05±0.2kg; $p<0.0001$). Participant changes in trunk fat (kg) and total body mass (kg) can be seen in Figures 3.5 and 3.6.

DISCUSSION

Summary of Key Findings

There is growing interest in high-PRO, ER diets for weight loss and improved health outcomes.[13,49] To our knowledge, this study is the first study to examine the effects of a high-PRO, ER diet on changes in body composition during a 16-wk weight loss program with HIT/CME in overweight, premenopausal women with AbOb. We hypothesize that a higher PRO intake would results in greater losses of body fat (kg and %), and better retention of LBM than more typical, moderate PRO intake (15% of energy). We found, however, that while both ER diets (15% energy from PRO=0.85g/kg/d and CHO:PRO=3.5:1; 25% energy from PRO=1.26g/kg/d and CHO:PRO=2:1) resulted in similar reductions in BW (kg), body fat percent, android fat (kg), and LBM (kg). The 15PRO diet produced greater body fat mass (kg) and trunk fat mass (kg) losses than the 25PRO diet. This greater reduction in the 15PRO group may be reflective of their slightly higher BW loss (1.3kg greater BW loss) overall, although this difference was not significant. Our groups had similar participation in HIT/CME, reductions in energy and fat intake, and increases in fiber intake. Thus, total BW loss may have a stronger effect than PRO intake on whole body and abdominal (trunk) fat loss during ER plus HIT/CME.

Body Weight

A recent meta-analysis (n=54) examining the impact of high-PRO ER diets on weight loss concluded that higher PRO diets increase BW loss over more typical PRO intakes.[108] However, this analysis included studies with and without exercise as part of the weight loss program. The authors assumed no masking effect of exercise would occur if all diet treatments within the study included the same exercise program. Conversely, when researchers include 90-180min/wk of exercise, within the context of a weight loss program varying in PRO intake,[41,111,113,116] they find no effect of PRO level on BW loss during ER. For

example, two studies by Kerksick et al[111,113] used a supervised circuit-style resistance exercise (3d/wk; 30min/wk) and ER (1200kcal/d) program and found comparable weight loss across groups, regardless of PRO intake (15%, 50%, and 63% of energy from PRO). Josse et al[110] conducted a study that was similar to ours in population, duration, and energy deficit from intake (-500kcals/d), and exercise (-250kcals/d from 5-7d/wk aerobic exercise plus 2d/wk resistance training) and they found no effect of PRO (15% and 30% energy from PRO) on weight loss. We found no effect of PRO level on changes in BW when ER (~-550kcals/d) and EE (~-200kcal/d) were similar between groups. Our participants lost ~6.4% (5.1kg) of their baseline BW over 16wks, which is similar to the BW losses (~5%) observed by Josse et al.[110] Thus, these studies support our findings.

Total Body and Trunk Fat (kg)

The 15PRO group had significantly greater losses in absolute whole body fat (1.3kg greater) and absolute trunk fat (0.9kg greater) compared to the 25PRO group. Josse et al[110] reported no significant effect of PRO intake (30% vs. 15% of energy from PRO) on absolute whole body or trunk fat following 16wks of ER and exercise. However, others report greater absolute whole body[109,116,128] and absolute trunk fat[109,128] reductions with high-PRO intakes (30%; CHO:PRO<2:1; 1.6g/kg), compared to low-PRO intakes (15% energy from PRO; CHO:PRO>2:1; <1.05g/kg/d), during ER with and without exercise. In a 16-wk study with a similar population to ours and using an ER diet with or without exercise, Layman et al[109] found a 2.0kg greater reduction in absolute whole body fat in the high-PRO (1.6g/kg/d) ER diet groups compared to low-PRO (0.8g/kg/d) ER diet groups. Further, absolute trunk fat loss was 1.2kg greater in the high-PRO groups compared to the low-PRO groups. However, absolute whole body fat loss was also greater when separating groups by exercise; the exercising groups had a 1.7kg greater reduction than the non-exercising groups, regardless of PRO intake

level. In a 12-wk study by Lee et al,[128] high-PRO (30% vs. 15% of energy from intake) intake among participants with high dietary compliance (≥70%) resulted in a 1.2kg and 1.1kg greater reduction in absolute whole body and trunk fat, respectively. The Lee et al[128] study did not include exercise. Thus, the inclusion of HIT/CME, combined with the greater BW loss (1.3kg greater) in the 15PRO group, may be influencing the greater changes in absolute whole body and trunk fat in the 15PRO group, rather than PRO intake.

Total Body Fat (%)

We found no differences in percent of total body fat losses with differing protein intakes. These findings are supported by others who also report no effect of PRO on changes in percent body fat during ER, with[111,113] or without exercise.[49] Conversely, Layman et al[109] found main effects of PRO level (high-PRO diet only and high-PRO diet + exercise) and main effects of exercise (high-PRO diet + exercise and high-CHO diet + exercise) during ER, but no interactive effect of PRO level and exercise. Thus, taken together, the impact of PRO intake on percent body fat loss is not as evident when the ER is combined with exercise.

Lean Body Mass (kg)

Increasing PRO intake during ER is recommended to minimize losses of LBM.[13,46,49] The meta-regression by Krieger et al[49] found that during ER, PRO intakes >1.05g/kg/d are beneficial for LBM retention. The meta-analysis by Wycherley et al[13] found that PRO intakes between 1.07-1.60g/kg/d were superior to 0.55-0.88g/kg/d. Finally, the review by Westerterp-Plantenga[46] recommends PRO intakes of 1.2g/kg/d for reducing LBM loss during ER. However, these systematic reviews did not include studies incorporating exercise. In the current study, participants on the 15PRO diet had mean PRO intakes of 0.85g/kg/d, while those on the 25PRO diet had 1.26g/kg/d. We found similar reductions in LBM loss (0.6±1.5kg) in both groups. These results are similar to those of Weinheimer, Sands, and Campbell,[126] who conducted as systematic review of

ER plus exercise studies. They found that the addition of exercise (aerobic and/or resistance) to ER, compared to ER alone, reduced LBM loss from ~24% to 11% of weight lost. Consist with these findings, our participants lost ~12% BW from LBM. Thus, it is possible that the impact of our HIT/CME masked the impact of PRO intake level on LBM changes in our overweight, premenopausal participants.

Strengths and Limitations

Our study intervention was designed for easy integration into the lives of our participants. The diets were personalized within the parameters of the diet treatment and each participant was able to choose foods from the Exchange list, a commonly used diet tool for people with diabetes. Additionally, the use of Zumba® to meet our HIT requirements was very engaging for the participants opposed to more traditional use of cycle ergometry or treadmills.

Our intervention was effective for reducing BW and improving body composition in all participants, but there are many factors that impact the amount and type of weight loss that occurs in response to a given level of ER and increases in EE.[129,130] Further, as with all free-living weight loss studies, there are inherent limitations with regard to intervention adherence, data reporting, and collection. Our outcomes rely on EE that was predicted, rather than measured, and dietary intake that was estimated from baseline 4-d food records (pre/post) and monthly 24-h recalls. To better assess diet intake and compliance, more frequent 24-h recalls could have been used, but would have increased participant burden. Urea nitrogen assessment would have provided a more accurate assessment of compliance with assigned PRO intake. To reduce errors in estimation of dietary intake, participants were provided with foods scales and trained to weigh and measure food and estimate portion sizes. Daily tracking logs were also used to verify accuracy of 24-h recalls.

DXA-assessed abdominal (trunk and android) fat is similar to computed tomography scans for detecting metabolic risk factors,[97] however, it cannot distinguish be between visceral and subcutaneous abdominal fat. Additionally, DXA-assessed lean tissue correlates well with magnetic resonance imaging-derived skeletal muscle mass,[131] but it is unable to distinguish between muscle protein and other sources of LBM, such as body water. Lastly, we were unable to separate the effects of HIT/CME without a control group.

CONCLUSION

For overweight, premenopausal women with AbOb, an ER diet with 15% or 25% energy from PRO results in similar changes in BW, body fat percent, and LBM while participating in HIT/CME. We found no additional benefit of increasing PRO intake above the RDA during modest ER and HIT/CME. The impact of HIT/CME and the greater (-1.3kg) changes in BW in the 15PRO group may have influenced the greater changes in absolute BF and trunk fat. More research is needed to separate the impact of HIT/CME and weight loss from the impact of PRO during ER.

Table 3.1. Baseline characteristics and intervention class attendance by group and with groups combined[a]

Variable	Combined (n=35)	15PRO (n=17)	25PRO (n=18)	p-value[b]
Age (y)	35.3 ± 10.2	34.7 ± 10.9	35.8 ± 9.8	0.7622
Body Weight (kg)	80.5 ± 8.4	80.4 ± 8.6	80.6 ± 8.5	0.9348
BMI (kg/m^2)	28.8 ± 2.1	29.0 ± 2.3	28.7 ± 1.9	0.5832
Body Fat (%)	40.8 ± 3.1	41.2 ± 3.4	40.4 ± 3.0	0.4697
LBM (kg)	45.2 ± 4.5	44.8 ± 4.5	45.7 ± 4.6	0.5517
Class Attendance (%)				
Nutrition Education	87.9 ± 16.5	77.9 ± 17.4	97.2 ± 8.1	**0.0002**
HIT/Zumba® Exercise	82.3 ± 13.9	79.2 ± 15.4	85.2 ± 11.9	0.2073
Total Attendance	82.9 ± 13.1	79.4 ± 14.6	86.2 ± 10.9	0.1249

[a]Values are reported as means ± SD; Significant difference = $p<0.05$
[b]p-values for baseline group differences using two-sample t-tests
BMI = body mass index (kg/m^2)
LBM = Lean body mass (excluding bone)
HIT = High-intensity interval training

Table 3.2. Body composition measures by group and with groups combined[a,b]

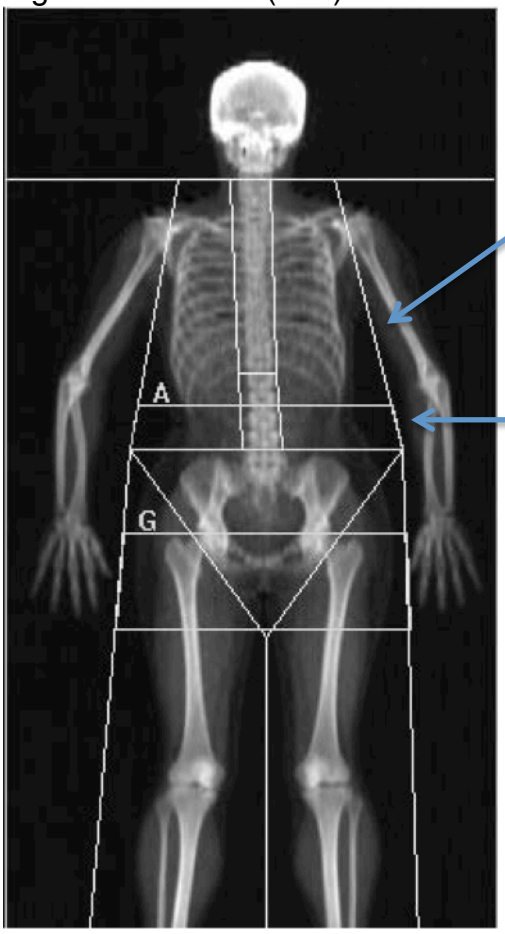
Variable	15PRO (n=17)	25PRO (n=18)	Combined (n=35)	Effect[b]	p-value
Body Weight (kg)					
Pre	80.4 ± 8.6	80.6 ± 8.5	80.5 ± 8.4	Diet	0.7492
Post	74.5 ± 8.4	76.1 ± 8.6	75.4 ± 8.4	**Time**	**<0.0001**
Change	-5.8 ± 2.7	-4.5 ± 2.4	-5.1 ± 2.6	DxT	0.1279
Body Fat Mass (%)					
Pre	41.2 ± 3.4	40.4 ± 3.0	40.8 ± 3.1	Diet	0.7707
Post	37.4 ± 3.8	37.5 ± 3.9	37.4 ± 3.8	**Time**	**<0.0001**
Change	-3.8 ± 1.3	-2.9 ± 1.8	-3.3 ± 1.6	DxT	0.1023
Body Fat Mass (kg)					
Pre	33.2 ± 5.3	32.6 ± 4.8	32.9 ± 5.0	Diet	0.9786
Post	28.0 ± 5.2	28.7 ± 5.6	28.4 ± 5.3	Time	<0.0001
Change	-5.2 ± 1.8	-3.9 ± 1.7	-4.5 ± 1.8	**DxT**	**0.0355**
Lean Body Mass[c] (kg)					
Pre	44.8 ± 4.5	45.7 ± 4.6	45.2 ± 4.5	Diet	0.5156
Post	44.1 ± 4.3	45.1 ± 4.2	44.7 ± 4.2	**Time**	**0.0283**
Change	0.6 ± 1.5	0.6 ± 1.6	0.6 ± 1.5	DxT	0.8806
Trunk Fat Mass (kg)					
Pre	15.7 ± 2.5	15.2 ± 2.6	15.5 ± 2.5	Diet	0.9257
Post	12.7 ± 2.3	13.0 ± 3.1	12.9 ± 2.7	Time	<0.0001
Change	-3.1 ± 1.2	-2.2 ± 0.9	-2.6 ± 1.2	**DxT**	**0.0243**
Android Fat Mass (kg)					
Pre	2.8 ± 0.6	2.7 ± 0.6	2.8 ± 0.6	Diet	0.9844
Post	2.2 ± 0.5	2.3 ± 0.6	2.2 ± 0.6	**Time**	**<0.0001**
Change	-0.6 ± 0.3	-0.5 ± 0.2	-0.5 ± 0.2	DxT	0.0811

[a] Values are means ± SD; Significant change=$p<0.05$

[b] Repeated measures ANOVA analyses

[c] Lean body mass excludes bone mass

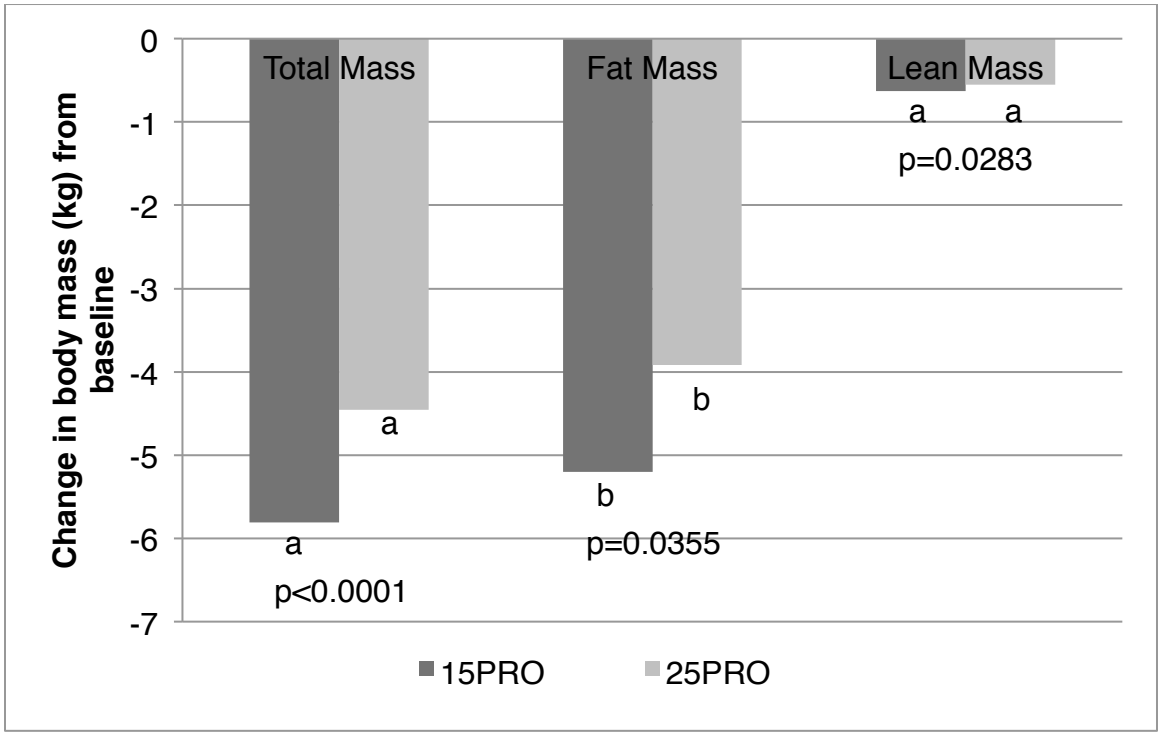
Figure 3.1. Hologic dual energy x-ray absorptiometry scan with Hologic-defined regions of interest (ROI) for trunk and android body composition analysis.



Trunk ROI – begins at the neck cut line and extends down to the angled cut lines position in neck of the femurs.

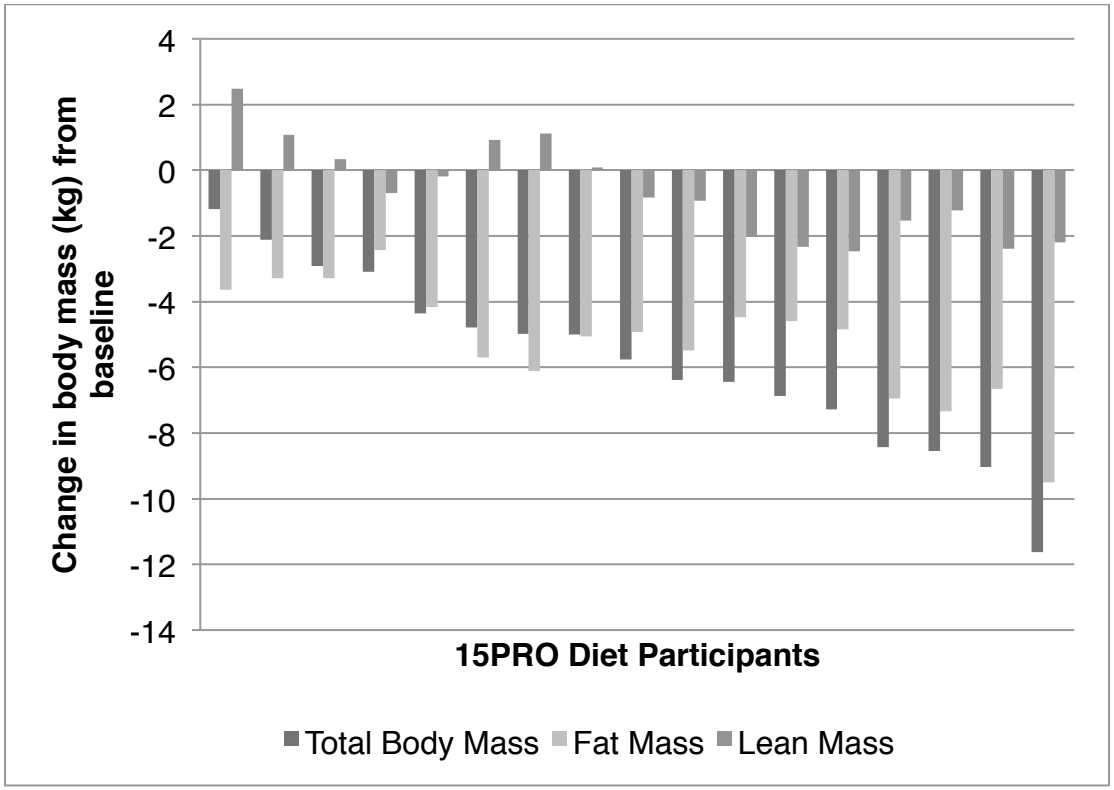
Android ROI – 20% of the distance from the neck cut line to the iliac crest.

Figure 3.2. Group (15% or 25% energy from protein) mean changes in body mass (kg) (total, fat, and lean) from baseline (pre) to post-intervention.



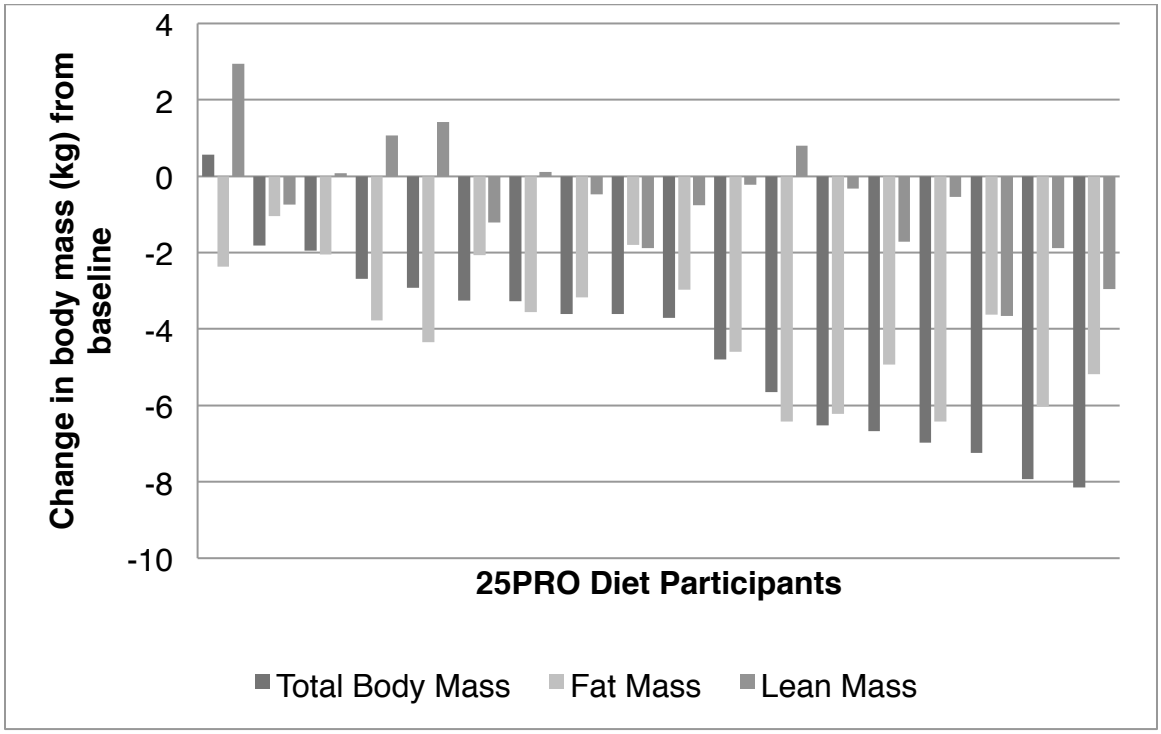
a=significant time effect
b=significant diet x time effect
15PRO=15% energy from protein; 25PRO=25% energy from PRO.

Figure 3.3. Participant changes in body mass (kg) (total, fat, and lean) from baseline in the 15PRO group.



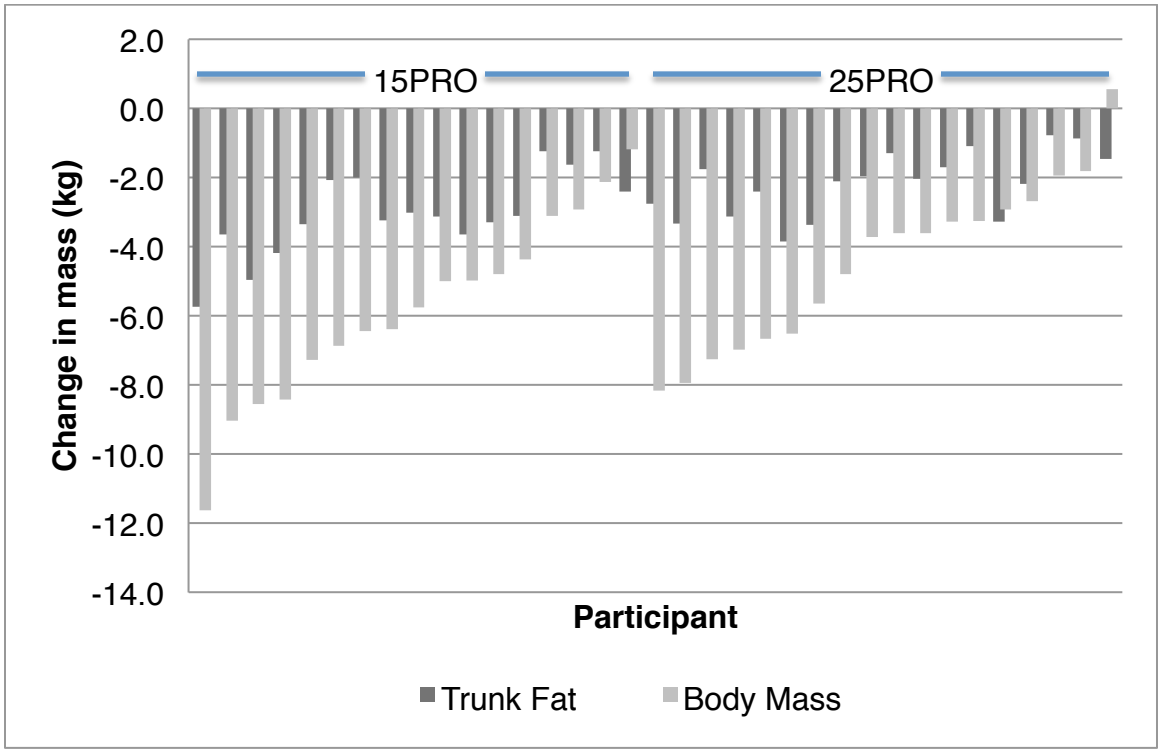
15PRO=15% energy from protein.

Figure 3.4. Participant changes in body mass (kg) (total, fat, and lean) from baseline in the 25PRO group.



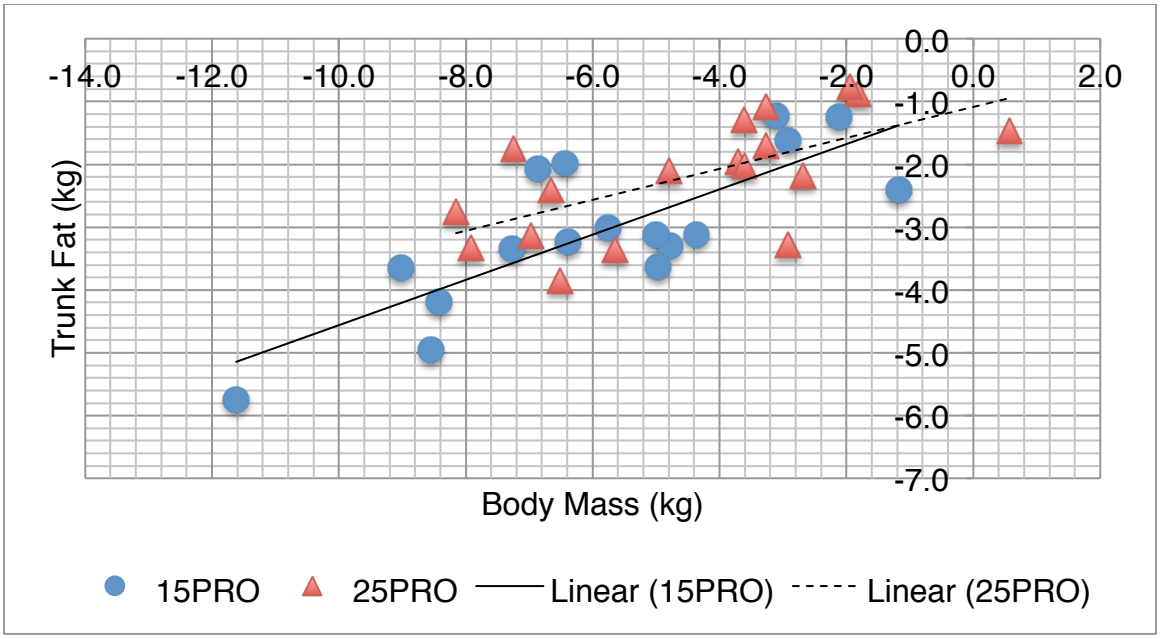
25PRO=25% energy from protein

Figure 3.5. Changes by group (15% or 25% energy from protein) in total body mass (kg) and trunk fat (kg) from baseline.



15PRO=15% energy from protein; 25PRO=25% energy from protein

Figure 3.6. Linear regression scatterplot of change in body mass (kg) and trunk fat mass (kg) by group (15% 0r 25% energy from protein).



15PRO=15% energy from protein; 25PRO=25% energy from protein

CHAPTER 4: CONCLUSION

The lifestyle and hormonal changes unique to women as they age and transition through menopause increase the risk for metabolic consequences, including unhealthy changes in body composition and the development of metabolic syndrome (MetS). The purpose of this research was to determine if higher protein (PRO) intake (25% of energy), compared to more typical PRO (15% of energy) intake, during modest energy restriction (ER) plus aerobic high intensity interval training (HIT) would more significantly improve MetS risk factors and body composition in sedentary, overweight, premenopausal women. A brief summary of key findings of this research are summarized and presented in this chapter, along with the strengths and limitations of this research project, lessons learned, and future research.

Key findings

MetS and chronic disease risk factors. We hypothesized that body weight (BW), waist circumference (WC), and MetS risk factors would improve greater with higher PRO (25% vs. 15% of energy) intake during modest ER diet (-300kcals/d) combined with HIT and continuous moderate-intensity exercise (CME) (-200kcals/d). However, improvements in BW, WC, MetS and chronic disease risk factors, including fasting blood lipids and markers of glycemic control, were found from both 15% and 25% energy from PRO, with no differences between groups.

Body composition. We hypothesized that higher PRO (25% vs. 15% energy) intake during modest ER (-300kcal/d) and HIT/CME participation (-200kcal/d) would result in greater reductions in body fat (whole body and abdominal [trunk]) and retention of LBM. Contrary to our hypothesis, the 15% energy from PRO diet group lost more absolute whole body and trunk fat than the 25% energy from PRO diet. However, similar reductions in body fat percent and LBM were found in both diet groups.

Strengths and Limitations

Our study intervention was designed for easy integration into the lives of our participants. The diets were personalized within the parameters of the diet treatment and each participant was able to choose foods from the Exchange list, a commonly used diet tool for people with diabetes. Additionally, the use of Zumba® was an engaging mode of exercise to meet our HIT requirements.

Our intervention was effective for reducing BW and improving body composition in our participants, but there are many factors that impact the amount and type of weight loss that occurs in response to a given level of ER and increases in energy expenditure (EE).[129,130] Further, as with all free-living weight loss studies, there are inherent limitations with regard to intervention adherence, data reporting, and collection. Our outcomes rely on EE that was predicted, rather than measured, and dietary intake that was estimated from pre/post-intervention 4-d weighed food records and monthly 24-h recalls. To better assess diet intake and compliance, more frequent 24-h recalls could have been used, but would have increased participant burden. Urea nitrogen assessment would have also provided a more accurate assessment of compliance with assigned PRO intake. To reduce errors in estimation of dietary intake, participants were provided with foods scales and trained to weigh and measure food and estimate portion sizes. Daily tracking logs were also used to verify accuracy of 24-h recalls.

DXA-assessed abdominal (trunk and android) fat is similar to computed tomography scans for detecting metabolic risk factors,[97] but it cannot distinguish be between visceral and subcutaneous abdominal fat. Additionally, DXA-assessed lean tissue correlates well with magnetic resonance imaging-derived skeletal muscle mass,[131] but it is unable to distinguish between muscle protein

and other sources of LBM, such as body water. Lastly, we were unable to separate the effects of HIT/CME without a control group.

Lessons Learned

Online screening. The use of the Internet as a medium for our screening process was highly efficient. Once a screening form was completed online it was forwarded to researchers. All potentially eligible screening forms were reviewed and followed-up with an e-mail or phone call, depending on the participants preference. The review of the screening form and time spent contacting the potentially eligible participant was ~2-5min, compared to screening a participant over the phone, which took ~10-20min and often required multiple attempts to reach the person over the phone at a convenient time. Over 230 participants were screened through the Internet compared to approximately 30 who chose to be screened over the phone or in-person. Thus, the Internet was both effective and time efficient for screening potential study participants.

College student participants. When using a college population, term-by-term schedules can be prohibitive for continuous participation with interventions lasting longer than the academic term length. This needs to be considered when recruiting college students.

Mid-life women. Schedules for mid-life women are often full of work and family obligations. Time away from the study often included family vacations and childcare issues. Though these obligations are expected with the population targeted, flexible scheduling, and possibly childcare options, must to be considered to assure participation in assessments and intervention requirements. For example, an early morning assessment appointment for a single mother may be difficult. Thus, if the assessment must be done in the morning, special arrangements may need to occur to fit the participant's needs.

Research team. In order to implement a weight loss study including a diet and exercise program, a large team of qualified researchers is necessary. Participant

assessments and intervention classes required a minimum of two staff members assisting at all times; three were often needed. If two classes were being held simultaneously, the number doubled. Further, same sex researchers should be available at all times.

Duration of study. The duration of our intervention was similar to other studies in the literature. However, participants in our study were showing less motivation as the weeks passed, particularly in the last month of the intervention. Though researchers attended all exercise classes and met with participants monthly, peer-reinforcement from group discussion sessions may have been beneficial.

Future Research

This is the first study to examine the effects of two levels of PRO (15% and 25% of energy) during ER combined with HIT/CME on changes in MetS risk factors and body composition. Additional research replicating the study and incorporating a HIT/CME control group is needed to validate the findings of this study and explore in the impact of HIT/CME on outcomes. Future research is also warranted to elucidate the effects of age, during mid-life/premenopause, on outcomes from PRO intake during ER combined with HIT/CME. This would help determine if interventions targeting MetS and changes in body composition should be adjusted as women age during their mid-life years to optimize risk reduction prior to menopause.

Summary

MetS risk factors and body composition were significantly improved with both 15% and 25% of energy from PRO during modest ER, combined with HIT/CME in premenopausal women with AbOb. Though consuming 15% energy from PRO resulted in greater reductions in absolute body fat and trunk fat, the impact of HIT/CME and the 1.3kg greater BW loss in the 15PRO group may have influenced the greater changes in absolute BF and trunk fat.

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APPENDICES

WEBSITE HOMEPAGE SCRIPT

Welcome to the LITEN Up! study website. This study is being led by Dr. Melinda Manore of the Nutrition and Exercise Sciences Department at Oregon State University.

The purpose of the study is to test strategies for preventing and reducing abdominal obesity and metabolic syndrome, a health condition characterized by high blood glucose, abnormal blood lipids, high blood pressure, and being overweight. In particular we are looking at the effects of an exercise and nutrition program. Participation in this study could last up to 20 weeks (5 months), including FREE exercise classes, a personalized weight loss diet and sessions with a Registered Dietitian, and health tests (blood tests, body composition analysis, fitness testing, and diet assessment). At the start of the study, participants are randomly assigned to one of two weight loss diets, both requiring adherence to an exercise program. The exercise program for both groups includes regularly scheduled exercise classes 3 days/week for 30-60 minutes and exercising outside of class on 2 other days of the week. Participants will also attend a total of 4 nutrition education classes lasting about 1 hour each at the start of the study.

If you have any questions or would like to speak with a researcher, please contact us directly at (541) 737-8516. An OSU Researcher will return your call within 2-3 business days.

If you are still interested in participating in this study, please click here to determine if you are eligible. Please note that completion of the eligibility questionnaire does not require you to participate in the study. Additionally, you may choose to discontinue any level of participation at anytime."

Continue to Eligibility Questionnaire

WEBSITE ELIGIBILITY SCREENING QUESTIONNAIRE

Introductory Script

Thank you for your interest in the Losing Inches Through Exercise and Nutrition (LITEN Up!) study for weight loss and waist reduction. In order to best determine your eligibility, please answer the following questions to the best of your ability. If you are unsure of any answer, or would rather speak to a researcher, please contact us directly at (541) 737-8516. An OSU researcher will return your call within 2-3 business days.

Privacy Statement: Your privacy is very important to us. Any information you provide will be kept confidential and used only for the purpose of this study. To further safeguard your privacy, we ask that you provide only your first name and a telephone number for contact purposes.

Contact Information

First Name:_____Phone Number: (____)_____

Best time to reach you by telephone: _____

Eligibility Criteria

1. Are you between the age of 18 and 50 years? YES **NO – Exclude**
2. Are you premenopausal (still having menstrual cycles)?
   YES **NO – Exclude**
3. Have you had a hysterectomy? **YES – Exclude** NO
4. Are you pregnant or do you plan to become pregnant within the next 4-6 months? **YES – Exclude** NO
5. Are you lactating? **YES – Exclude** NO
6. Is your activity level less than 1-2 hours per week? YES **NO – Exclude**
7. Have you been weight stable (<5 lbs weight change) for 4-6 months?
   YES **NO – Exclude**
8. Are you currently taking a weight loss supplement?
   If NO: Proceed to question 9.
   If YES: Are you willing to stop taking the supplement for the duration of the study? YES **NO – Exclude**
9. Using the following calculator, determine your Body Mass Index (BMI):
   $\frac{\text{Weight (pounds)}}{\text{Height (inches)}^2} \times 703 = \boxed{\text{BMI}}$ ***If BMI is <25 or >32, Exclude**
10. Is your waist circumference greater than 31.5 inches? YES **NO – Exclude**
11. Have you ever been diagnosed with cardiovascular disease, including cardiac, peripheral vascular or cerebrovascular disease? **YES – Exclude** NO
12. Have you been diagnosed with high blood pressure? **YES – Exclude** NO
13. Have you ever been diagnosed with asthma or pulmonary disease including chronic obstructive pulmonary disease (COPD), interstitial lung disease, or cystic fibrosis? **YES – Exclude** NO

14. Have you ever been diagnosed with metabolic disease, including diabetes mellitus (types 1 or 2), thyroid disorders, renal or liver disease?
YES – Exclude NO
15. Are you currently receiving treatment for diagnosed cancer?
YES – Exclude NO
16. Are you able to exercise through walking and weight bearing activities?
YES **NO – Exclude**
17. Would you be able to participate for 3d/wk for 4 months in a diet and exercise program for the purpose of weight loss? YES **NO – Exclude**

Concluding Script

The following scripts will appear after the website eligibility screening questionnaire has been completed

If NOT Eligible: "Thank you for taking the time to answer these questions. At this time it does not appear that you are eligible to participate in this study. If you have any questions, or believe that an error has occurred, please contact us directly via telephone at 541-737-8516. An OSU Researcher will return your call within 2-3 business days.

Again, thank you for your interest in the study and for taking the time to complete our eligibility questionnaire."

If Eligible: "Thank you for taking the time to answer these questions. It appears that you fit our initial eligibility criteria. We would like to invite you to visit the Nutrition and Exercise Sciences Department at the Oregon State University campus to confirm your eligibility and to begin the study assessment process. Your information will be forwarded to an OSU Researcher who will contact you within the next 2-3 business days to set up an appointment. If you wish you may also try to contact us directly via telephone at 541-737-8516.

Again, thank you for your time and we look forward to talking to you very soon!"

TELEPHONE INTRODUCTION SCRIPTS

Volunteers calling or e-mailing in response to the Losing Inches Through Exercise and Nutrition (LITEN Up!) study will complete this process before proceeding to the eligibility screening questionnaire:

Direct call from potential participant:
"Thank you for calling about the LITEN Up! study led by Dr. Melinda Manore of the Nutrition and Exercise Sciences Department at Oregon State University. My name is [staff name]; I am a team member on the study. Would you mind if I briefly give you a little information about the study?

If YES, proceed; If NO, thank them for their time.

The purpose of the study is to test strategies for preventing and reducing abdominal obesity and metabolic syndrome, a health condition characterized by high blood glucose, abnormal blood lipids, high blood pressure, and being overweight. In particular we are looking at the effects of an exercise and nutrition program. Participation in this study could last up to 20 weeks (~5 months), and involves exercise classes, a personalized weight loss diet, sessions with a Registered Dietitian, and health tests (including blood tests, body composition analysis, fitness testing, and diet assessment), all FREE to you. If chosen to participate in this study, you will be randomly assigned to one of two weight loss diets, both requiring adherence to an exercise program. The exercise program for both groups includes regularly scheduled exercise classes 3 days/week for 30-60 minutes and exercising outside of class on 2 other days of the week. Participants will also attend a total of 4 nutrition education classes lasting about 1 hour each at the start of the study.
Do you have any questions at this time? (The staff member should answer questions to the best of their ability or ask to re-contact them with the answers after speaking with the PI.)

Does this study sound interesting and potentially valuable to you?"
Study staff to circle response: YES NO

If YES, proceed to the <u>eligibility screening questionnaire</u>; If NO, thank them for their time.

Returned call to potential participant:
"Hello [name], my name is [staff name] and I am calling on behalf of the LITEN Up! study led by Dr. Melinda Manore of the Nutrition and Exercise Sciences Department at Oregon State University. I would like to briefly give you a little information about the study. The purpose of the study is to test strategies for preventing and reducing

abdominal obesity and metabolic syndrome, a health condition characterized by high blood glucose, abnormal blood lipids, high blood pressure, and being overweight. In particular we are looking at the effects of an exercise and nutrition program. Participation in this study could last up to 20 weeks (~5 months), and involves exercise classes, a personalized weight loss diet, sessions with a Registered Dietitian, and health tests (including blood tests, body composition analysis, fitness testing, and diet assessment), all FREE to you. If chosen to participate in this study, you will be randomly assigned to one of two weight loss diets, both requiring adherence to an exercise program. The exercise program for both groups includes regularly scheduled exercise classes 3 days/week for 30-60 minutes and exercising outside of class on 2 other days of the week. Participants will also attend a total of 4 nutrition education classes lasting about 1 hour each at the start of the study.
Do you have any questions at this time? (The staff member should answer questions to the best of their ability or ask to re-contact them with the answers after speaking with the PI.)

Does this study sound interesting and potentially valuable to you?"
Study staff to circle response: YES NO

If YES, proceed to the eligibility screening questionnaire; If NO, thank them for their time.

TELEPHONE ELIGIBILITY SCREENING QUESTIONNAIRE

Demographics

Name: _____ Age: _____ Birth date: _____

Race (optional): _____Ethnicity (optional):_____

Contact Information

Mailing Address: _____

Phone Number: (___)_____E-mail: _____

Best time to reach you by telephone: _____

Eligibility Criteria

18. Are you between the age of 18 and 50 years? YES **NO – Exclude**
19. Are you premenopausal (still having menstrual cycles)?
    YES **NO – Exclude**
20. Have you had a hysterectomy? **YES – Exclude** NO
21. Are you pregnant or do you plan to become pregnant within the next 4-6 months? **YES – Exclude** NO
22. Are you lactating? **YES – Exclude** NO
23. Is your activity level less than 1-2 hours per week? YES **NO – Exclude**
24. Have you been weight stable (<5 lbs weight change) for 4-6 months?
    YES **NO – Exclude**
25. Are you currently taking a weight loss supplement?
    If NO: Proceed to question 9.
    If YES: Are you willing to stop taking the supplement for the duration of the study? YES **NO – Exclude**
26. Using the following calculator, determine your Body Mass Index (BMI):
    $\frac{\text{Weight (pounds)}}{\text{Height (inches)}^2} \times 703 = \boxed{\text{BMI}}$ ***If BMI is <25 or >32, Exclude**
27. Is your waist circumference greater than 31.5 inches?
    YES **NO – Exclude**
28. Have you ever been diagnosed with cardiovascular disease, including cardiac, peripheral vascular or cerebrovascular disease? **YES - Exclude** NO
29. Have you been diagnosed with high blood pressure?
    YES – Exclude NO
30. Have you ever been diagnosed with asthma or pulmonary disease including chronic obstructive pulmonary disease (COPD), interstitial lung disease, or cystic fibrosis? **YES – Exclude** NO
31. Have you ever been diagnosed with metabolic disease, including diabetes mellitus (types 1 or 2), thyroid disorders, renal or liver disease?
    YES – Exclude NO
32. Are you currently receiving treatment for diagnosed cancer?
    YES – Exclude NO
33. Are you able to exercise through walking and weight bearing activities?
    YES **NO – Exclude**

34. Would you be able to participate for 3d/wk for 4 months in a diet and exercise program for the purpose of weight loss? YES **NO – Exclude**

Concluding Script

The following scripts should be read at the conclusion of the questionnaire, as appropriate.

If <u>NOT</u> Eligible: "Thank you for taking the time to answer these questions. At this time it does not appear that you are eligible to participate in this study. However, we would like to retain your contact information incase our criteria change. Would this be okay?

Do you have any questions at this time? *(The staff member should answer questions to the best of their ability or ask to re-contact them with the answers after speaking with the PI.)*

Again, thank you for your interest in the study and for taking the time to complete our eligibility questionnaire."

If Eligible: "Thank you for taking the time to answer these questions. It appears that you fit our initial eligibility criteria. We would like to invite you to visit the Nutrition and Exercise Sciences Department at the Oregon State University campus to confirm your eligibility and to begin the study assessment process. This first visit will include a fasted blood draw, measurements of your height, weight, waist circumference, resting heart rate and blood pressure, questionnaires about your health and eating and exercise patterns, and a whole-body Dual X-ray Absorptiometry scan to determine your body composition.

Because we are using Dual X-ray Absorptiometry you will be exposed to a small dose of radiation, equivalent to about $1/12^{th}$ the radiation of a chest x-ray. Because of the nature of the scan, calcium pills on the day of the scan may affect the accuracy of the results. Therefore, we encourage you to not take calcium pills the day of the scan. In addition, if you must wait at least one week before have a scan if you have had a procedure involving:

1. Iodine
2. Barium
3. Isovue
4. Nuclear medicine isotope*
5. Technetium 99 bone scan

Additionally, y**ou should know that there are risks associated with being scanned if you are pregnant or suspect that you may be pregnant.** The possible risks associated with participating in this research project are as follows: There is a risk of fetal or embryonic radiation exposure from the body composition scan. You may not receive the body composition scan if you are pregnant or suspect that you may be pregnant. The body composition scan must be performed within the first 10 days of the beginning of you last menstrual cycle if you are not on an effective form of birth control. This will reduce the risk of performing the scan on a developing

embryo. You must inform the researchers if there is a chance that you may be pregnant.

At the end of this visit we will train you to complete food intake and physical activity records to be returned to us during your second visit. We will also give you more information about participating in the study and answer any relevant questions you may have. The visit will take approximately 2.5 hours and you will need to fast for at least 8 hours (no food or drink other than water) prior to the visit; we will provide you with a snack after the necessary assessments are complete. If you would like, we can also mail you copies of the questionnaires to review prior to the visit.

Should you fit within our eligibility criteria after the first visit, and are willing to continue, we will invite you back for a second assessment visit. The second visit will include a second measurement of your resting heart rate and blood pressure, review of your food and physical activity records and an exercise test. The exercise test will require you to walk on a treadmill for up to 30 minutes while having your heart-rate and pulmonary function monitored. The second visit will take approximately 1.5 hours. At the end of the second visit, you will be assigned to one of the two diet and exercise programs and give you further instructions regarding your participation within your assigned group.

All of the assessments we conduct, including the blood tests and body composition assessment, are available to you free of charge.

Do you have any questions? If YES, answer questions; If NO, proceed.

If you are still interested in the study I'd like to set up your first visit"

Would you like me to mail copies of the informed consent and questionnaires to you prior to your first visit?" YES NO

Baseline Visit #1 scheduled for:
Date:_____
Time:_____
Entered into calendar by:___

DEPARTMENT OF NUTRITION AND EXERCISE SCIENCES
Oregon State University, 103 Milam Hall, Corvallis, Oregon 97331
Tel 541-737-8516 | Fax 541-737-6914| http://www.hhs.oregonstate.edu/nes/

INFORMED CONSENT DOCUMENT

Project Title:	**Losing Inches Through Exercise and Nutrition (LITEN Up!)**
Principal Investigator:	**Melinda M. Manore, PhD, RD, CSSD, Nutrition and Exercise Sciences**
Co-Investigator(s):	**Gianni Maddalozzo, PhD, Nutrition and Exercise Sciences**
	Kari D. Pilolla, MS, Nutrition and Exercise Sciences

WHAT IS THE PURPOSE OF THIS STUDY?

You are being invited to take part in a research study designed to evaluate the effects of exercise and nutrition programming on the reduction of body weight, waist circumference, and other chronic disease risk factors. We are examining the effects of diet composition combined with an exercise program incorporating both low-moderate and high intensity exercise on these risk factors. This study specifically aims to reduce waist circumference and the risks associated with the development of chronic diseases in premenopausal women aged 18-50 years who are overweight and have waist circumferences greater than 31.5 inches. The outcomes from this study will be used for a doctoral dissertation and for publication in a scientific journal. We are studying this because waist circumference has been hypothesized to be a primary factor contributing to chronic diseases such as diabetes and heart disease. Women are especially at risk for increases in waist circumference with advancing age and the transition through menopause.

WHAT IS THE PURPOSE OF THIS FORM?

This consent form gives you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask any questions about the research, the possible risks and benefits, your rights as a volunteer, and anything else that is not clear. When all of your questions have been answered, you can decide if you want to be in this study or not.

WHY AM I BEING INVITED TO TAKE PART IN THIS STUDY?

You are being invited to take part in this study because you meet the following criterion to participate in the LITEN Up! study:

- Female
- Premenopausal (i.e. still having periods)
- Aged 18-50 years
- Waist circumference >31.5in (80cm)
- Body Mass Index (BMI) range of 25-32 kg/m^2
- No significant negative health history as identified in the pre-screening criteria.

WHAT WILL HAPPEN DURING THIS STUDY AND HOW LONG WILL IT TAKE?

During this 5-month study you will participant in series of health assessment visits, adhere to a healthy diet prescribed by the study dietitian, and attend regularly scheduled exercise classes. The first and last 2 weeks of the study will involve health assessments on the OSU campus once each week. These

assessments include height, weight, body composition, questionnaires, resting blood pressure, resting metabolic rate, a fitness test on a treadmill and a fasted blood draw.

In order to participate in this study, you will be required to provide the researchers with proof of medical clearance to exercise at high intensities with a form signed by your primary care physician. You may incur a cost associated with obtaining a signature on the medical clearance to exercise form (e.g. insurance co-pay). You will be responsible for this cost; Oregon State University and the researchers of this study will not be responsible for the cost associated with acquiring medical clearance to exercise.

During the course of this study, a total of 80 ml, which is just over 1/3 of a cup of your blood, will be taken from you. At each session only 40 ml, which is just less than 3 tablespoons, will be taken. The blood will be drawn by a licensed phlebotomist. Your blood will be analyzed for cholesterol, triglycerides, glucose, insulin, and other cardiac risk factors by the Good Samaritan Regional Medical Center in Corvallis, OR. You will receive copies of your blood analyses at the end of the study to discuss the results with your physician.

At the end of the first 2 assessment visits you will be randomly assigned to one of two diet that vary in composition. Both diets are restricted by 300 kcals to elicit weight loss. You may be assigned to a diet that includes consuming a protein powder supplement.

After you have been assigned to a diet and exercise program you will begin the 16-week intervention phase of the study. During the first 4 weeks of the intervention phase you will attend 4 nutrition classes (1 day/week for 4 weeks) to learn about the study diet and healthy eating habits. You will also attend 8 physical conditioning exercise classes (2 days/week for 4 weeks) to gradually increase your fitness levels. During the last 12 weeks of the intervention you will maintain your prescribed diet and attend regular low-moderate and high intensity interval exercise classes 3 days/week on the OSU campus or at a community facility (fitness, dance, or other recreational facility). The intervention phase will also require you to exercise on your own (e.g. walking) 2 additional days each week. To help assess your exercise sessions, you will be given small fitness monitoring devices (pedometer, heart rate monitor, accelerometer) to use periodically throughout the study.

Throughout the intervention phase you will interact regularly with study staff members and take part in brief weekly and monthly health assessments. On a weekly basis you will report your physical activities using a written log and discuss diet and nutrition compliance issues with a research staff member. On a monthly basis, you will visit the OSU campus for no more than 30 minutes to have your body weight, waist circumference, and blood pressure checked. You will also be asked to recall the last 24 hours of food intake and physical activity.

At the end of the intervention phase you will complete 2 more health assessment visits. These visits will be similar to the first 2 visits, but do not require you to complete as many questionnaires. You will also be asked to participate in brief follow-up assessments (height, body weight, waist circumference, resting blood pressure, blood draw, and an interview about your diet and exercise patterns) 6 and 12 months later.

Aside from the possible cost to acquire medical clearance to exercise, you will incur no costs associated with the intervention or assessments. All parking and memberships costs related to this study will be paid for by the study.

In all, if you agree to take part in this study, your involvement will last for approximately 5 months (20 weeks) for a total of approximately 58 hours. During the first and last 2 weeks you will visit the OSU campus for health assessments once each week (1.5-2.5 hrs each visit); during the middle 16 weeks, you will attend nutrition and exercise classes 3 days each week (30-60 minutes each day). You will also be asked to participate in 6 and 12 month follow-up visits lasting about 45 minutes for each.

Oregon State University • IRB Study #:4376 Approval Date: 09/15/09 Expiration Date:

WHAT ARE THE RISKS OF THIS STUDY?

The possible risks and/or discomforts associated with the procedures described in this study include:

- **Women of Childbearing Potential**: If you are a woman of childbearing potential, a pregnancy test may be required before having your body composition analyzed using Dual X-ray Absorptiometry (DXA). This procedure involves radiation that may cause birth defects. Although this DXA scan only exposes you to about 1/30th the radiation of a chest x-ray, there are **risks of harm to an unborn child** associated with radiation exposure if you are pregnant or may be pregnant. It is recommended that you use an effective form of pregnancy prevention while you are enrolled in this study. If you are not currently using an effect form of pregnancy prevention, the DXA scan must be performed within the first 10 days of the beginning of you last period. If you are not on an effective form of birth control and are not within the first 10 days of your menstrual cycle, you will be required to take a urine test for pregnancy at the OSU lab, at no cost to you, immediately before your scheduled DXA scan. If you test positive for pregnancy, you will not receive a DXA scan.**You MUST inform us if you are pregnant, may be pregnant, or plan to become pregnant during the course of this research study.**
- **Cumulative Effects of Radiation Exposure:** At high doses there are cumulative effects of radiation exposure; currently there are no established guidelines regarding the potential risk for individuals who have been exposed to low doses of radiation (for example, x-rays, cross country flights). If you participated in a previous study or studies involving radiation exposure, the doses you have been exposed to are too low to have established guidelines regarding any potential risk in regards to the cumulative effects.
- **Blood Draws:** There are a few potential risks associated with blood draws. Among them are: temporary discomfort from where the needle is inserted into your arm, bruising around the site where the needle was inserted, and rarely infection. To minimize these risks, you will be instructed to keep pressure on the site where the blood was drawn for 1 minute and keep a bandage over the area for at least 1 hour.
- **Submaximal Exercise Testing:** There are minor risks associated with submaximal exercise testing. Among them are: increased blood pressure and heart rate, and shortness of breath during the test, physical discomfort (soreness, muscle fatigue, and dizziness). However, the exercise test will be conducted by a skilled and experienced technician trained in First Aid and CPR. In the event of research related injury, compensation for medical treatment is not provided by Oregon State University or the researchers.
- **Exercise Training:** There are some risks associated with the exercise training. High intensity exercise is associated with increased demands on your heart, lungs, and circulatory system. However, these demands vary widely and depend on the person's usually frequency of exercise. You may experience abnormal heart rate, increase blood pressure, shortness of breath, and physical discomfort (soreness, muscle fatigue, and dizziness). To minimize the risks, we will gradually increase your fitness levels during the first 4 weeks of the intervention to prepare you for the higher intensity exercise. The exercise classes will alternate between high and low-moderate intensity exercise every couple of minutes, and you will warm-up before, and cool-down after each exercise session. Finally, a study staff member trained in First Aid and CPR will be present at each exercise session. In the event of research related injury, compensation for medical treatment is not provided by Oregon State University or the researchers.

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WHAT ARE THE BENEFITS OF THIS STUDY?

Although we do not know if you will benefit from being in this study, we anticipate that you will lose weight and improve your fitness level. You will also get expert diet and exercise guidance and support to help reduce your risk factors related to chronic diseases. All health assessments, nutrition classes, and exercise classes will be provided at no cost for participants. We also hope that, in the future, other people might benefit from this study because this information will help determine effective strategies to reduce the risks associated with chronic diseases in women prior to the onset of menopause.

WILL I BE PAID FOR PARTICIPATING?

You will be paid for being in this research study. There is a potential to receive $50.00 for participating in the full 5 months of the study. Upon the completion of post-intervention testing, you will receive $50.00 if you completed all assessments and at least 80% of the intervention requirements. There is no compensation for participating in the 6- and 12-month follow-up assessments at this time.

WHO WILL SEE THE INFORMATION I GIVE?

The information you provide during this research study will be kept private to the extent permitted by law. To help protect your privacy, we will replace your name with a code on all documents and biological samples (blood). Neither your name nor any identifying information will be used in any data summaries or publications. All data will be assessed on a group basis only. Additionally, data will be locked in a filing cabinet or on a password protected computer in a locked office on the OSU campus.

If the results of this project are published your identity will not be made public.

WHO IS PAYING FOR THIS STUDY?

This study is being sponsored by Standard Process, Inc. a whole food supplement company. The sponsor is the manufacturer of the protein supplement being used in this study. If you are randomly assigned to the protein powder supplement, the sponsor will provide the supplement at no cost to you.

DO I HAVE A CHOICE TO BE IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study and still keep the benefits and rights you had before volunteering. You will not be treated differently if you decide to stop taking part in the study. You may skip any questions, verbal or written, if you prefer not to answer. If you choose to withdraw from this project before it ends, the researchers may keep information collected about you and this information may be included in study reports.

WHAT IF I HAVE QUESTIONS?

If you have any questions about this research project, please contact:

Melinda M. Manore, PhD, RD, CSSD
(541) 737-8701
Melinda.manore@oregonstate.edu

Kari D. Pilolla, MS, MS, Doctoral Candidate
(541) 737-8516
pilollak@onid.orst.edu

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If you have questions about your rights as a participant, please contact the Oregon State University Institutional Review Board (IRB) Office, at (541) 737-8008 or by email at IRB@oregonstate.edu.

There is a chance you may be contacted in the future to participate in an additional study related to this project, which will require the researchers to retain your contact information after this study has been completed. If you would prefer not to be contacted, please let the researchers know, at any time. **If you are contacted, you can choose whether or not to participate.**

Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Participant's Name (printed): _____

_____ _____

(Signature of Participant) (Date)

Oregon State University • IRB Study #:4376 Approval Date: 09/15/09 Expiration Date:

HEALTH HISTORY QUESTIONNAIRE

ACROSTIC

Date

Age:_____ Date of Birth:_____(mm/dd/yyyy)

Menstrual History

1. Do you have regular menstrual bleeding? YES NO

If YES:

a. How many cycles per year do you have? _____

b. When was the start date of your last cycle? _____

If NO:

a. When was the last time you had any menstrual bleeding or spotting?

€ Within the last 6 months

€ 7-12 months ago

€ Over 12 months ago

b. Have you completed menopause? YES NO

c. Have you had a hysterectomy? YES NO

2. Are you on an effective form of pregnancy prevention? YES NO

If YES, what form:_____

3. Have you given birth to any children? YES NO

If YES, please list the year(s):

_____

HEALTH HISTORY QUESTIONNAIRE

ACROSTIC

Date

Health Assessment

1. How would you rate your overall health at the present time? (Circle One)

Excellent Good Fair Poor

Please complete the following health history table based on diagnoses by a physician:

		Circle YES or NO	Year of Diagnosis	Comments (Resolved, On-going)
I.	**Cardiovascular**			
a	Heart attack	YES NO		
b	Hypertension (high blood pressure)	YES NO		
c	Hypotension (low blood pressure)	YES NO		
d	Angina (chest pain)	YES NO		
e	Heart murmur	YES NO		
f	Mitral Valve Prolapsed	YES NO		
g	Congestive Heart Failure	YES NO		
h	Rheumatic Fever	YES NO		
i	Other heart problems:	YES NO		
II.	**Respiratory**			
a	Shortness of breath	YES NO		
b	Valley Fever	YES NO		
c	Pneumonia	YES NO		
d	Collapsed lung	YES NO		
e	Emphysema	YES NO		
f	Tuberculosis	YES NO		
g	Chronic bronchitis	YES NO		
h	Asthma	YES NO		
i	Allergies	YES NO		
j	Other respiratory problems:	YES NO		
III.	**Endocrine/Metabolic**			
a	Type I Diabetes Mellitus	YES NO		
b	Type II Diabetes Mellitus	YES NO		
c	High cholesterol	YES NO		
d	Hyperthyroid (overactive thyroid)	YES NO		
e	Hypothryoid (underactive thyroid)	YES NO		
f	Gout	YES NO		
g	Other endocrine/metabolic problems:	YES NO		

HEALTH HISTORY QUESTIONNAIRE

ACROSTIC

Date

		Circle YES or NO	Year of Diagnosis	Comments (Resolved, On-going)
IV.	**Musculoskeletal**			
a	Rheumatoid arthritis	YES NO		
b	Osteoarthritis	YES NO		
c	Osteoporosis	YES NO		
d	Fibromyalgia	YES NO		
e	Fractures/dislocations of bones/joints Please list:	YES NO		
f	Bursitis	YES NO		
g	Other musculoskeletal problems:	YES NO		
V.	**Neurological**			
a	Paralysis	YES NO		
b	Multiple Sclerosis	YES NO		
c	Seizures	YES NO		
d	Epilepsy	YES NO		
e	Stroke	YES NO		
f	Parkinson's Disease	YES NO		
g	Transient Ischemic Attack	YES NO		
h	Other neurological problems:	YES NO		
VI.	**Cancers**			
a	Breast	YES NO		
b	Ovary/Uterus	YES NO		
c	Melanoma (Skin Cancer)	YES NO		
d	Lung	YES NO		
e	Leukemia/Lymphoma	YES NO		
f	Colorectal	YES NO		
g	Other Gastrointestinal Cancer:	YES NO		
h	Other Cancer: (Please specify)	YES NO		

Do you have a family history of any of the following health problems? (Circle YES or NO)

Cardiovascular disease	YES	NO
High Blood Pressure	YES	NO
Diabetes	YES	NO
High Blood Lipids	YES	NO
Obesity	YES	NO

WEIGHT, PHYSICAL ACTIVITY, & DIET HISTORY QUESTIONNAIRE

ACROSTIC

Date

Weight History

1. Current weight:_____lbs Current height:_____inches
2. How long have you been at your current weight?_____
3. Have you had any weight fluctuations (up or down) of more than 5 pounds in the past 6 months? YES NO
4. What is your highest non-pregnant weight since age 18?_____
5. What is your lowest weight since age 18?_____
6. Please check the category which best describes you during:

	Underweight	Normal	Overweight
a) Childhood	_____	_____	_____
b) Teens	_____	_____	_____
c) 20's	_____	_____	_____
d) 30's	_____	_____	_____
e) 40's	_____	_____	_____

Physical Activity History

1. Do you engage in any regular physical activity? YES NO

If YES, please complete the following information:

Activity	Time Per Occasion	Times Per Week	Intensity
Ex: Walking	30 min	3	Low, Moderate, or High
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

2. Is the total activity you have recorded your USUAL level of activity? YES NO

If NO, why not:

_____ a. Illness/injury of self or close relative

_____ b. Out of town

_____ c. Extra time commitments

_____ d. Other: _____

WEIGHT, PHYSICAL ACTIVITY, & DIET HISTORY QUESTIONNAIRE

3. Are you unable participate in physical activity for any reason? YES NO

If YES, please explain:_____

4. Are you unable to participate in strenuous physical activity for any reason? YES NO

If YES, please explain:_____

5. How active were you 5 years ago compared with today?

_____ a. Much more active

_____ b. More active

_____ c. Same level of activity

_____ d. Less active

_____ e. Much less active

Diet History

7. Are you on a special diet? (Check all that apply)
   - € NO
   - € YES, weight loss
   - € YES, weight gain
   - € YES, diabetic
   - € YES, vegetarian. If yes, please specify type:_____
   - € YES, low salt/sodium
   - € YES, low fat
   - € YES, low cholesterol
   - € YES, for a medical condition. If yes, please specify:_____
   - € YES, for other reasons. If yes, please specify:_____

   Have any of the above diets been recommended to you by a health care professional?
   YES NO

   If YES, which diets and who recommended them?_____

_____

8. Do you consciously limit food choices (e.g. no "bad" foods) to control your weight?
   YES NO

WEIGHT, PHYSICAL ACTIVITY, & DIET HISTORY QUESTIONNAIRE

9. Do you consciously restrict food intake (calories) in order to control your weight?
   YES NO
   IF YES, do you: (please check the one that best applies)
   _____Continuously or chronically diet OR _____Go on and off diets regularly
10. How often do you choose the reduced-fat or non-fat versions of a particular food? (Circle one) NEVER RARELY SOMETIMES OFTEN ALWAYS
11. Are there any foods that you do not eat or eat very frequently?
    _____
12. What 2 beverages do you currently drink most often? Give amount and frequency for each.
    1._____
    2._____
13. Do you drink beverages containing alcohol? YES NO

If YES, how many times per week? _____

If YES, please check the types of drinks and list how many you consume each time.

Beer _____ fluid ounces (12 fl oz is 1 can of beer)
Wine _____fluid ounces (5 fl oz is 1 glass of wine)
Liquor _____fluid ounces (1.5 fl oz is 1 shot)
Other _____

14. Do you drink beverages containing caffeine? YES NO

If YES, please check the types of drinks and list how many you consume each time:
(8 oz = 1 cup; 12 oz = 1.5 cups; 16 oz = 16 oz, etc)

Coffee _____cups/day

Tea _____cups/day

Soda _____cups/day (12 oz = 1 can of soda)

15. Are you taking a vitamin or mineral supplement?
    YES NO

If YES, please specify type(s), brand(s), amount(s), and frequency with which you use these supplements:_____
_____

If NO, have you used them within the past month?_____ past year?_____

WEIGHT, PHYSICAL ACTIVITY, & DIET HISTORY QUESTIONNAIRE

16. Are you currently taking any other type of nutritional or weight loss supplement?

YES NO

If YES, please specify type(s), brand(s), amount(s), and frequency with which you use these supplements:_____

_____

If NO, have you used them within the past month?_____ past year?_____

17. Who is the primary food preparer and shopper in your household?

Food Shopper: Self Spouse/Significant Other Other_____

Food Preparer: Self Spouse/Significant Other Other_____

INSTRUCTIONS FOR RECORDING 4-DAY DIETARY RECORDS

1. Please record your food & beverage intake over three (3) week days & one (1) weekend day. Each day recorded should correspond with your 4-day physical activity records.

2. Please record each food & beverage item you consume on a separate line. Be sure to include all snacks & all beverages (including water).

3. Please record the time the food/beverage was consumed.

4. Record each item after weighing in exact amounts:
   - liquids in cups or **fluid** ounces
   - vegetables and fruits in cups, grams, or ounces
   - beans, grains, and pasta in cups **dry** or cups **cooked** (please be specific)
   - bread in slices, indicate what kind of bread (brand name and type)
   - meats, fish, poultry and cheeses in ounces
   - nuts in cups, ounces, or grams
   - chips or other snack type foods in cups, ounces, or grams
   - Spread (butter, cream cheese, margarine, etc.) in tsp or Tbs

5. Please specify if food is consumed raw. Also indicate if it was prepared from fresh, frozen, or canned products.

6. Indicate how the foods were prepared, such as fried, baked, boiled, etc.

7. If a food has a mixture of ingredients (sandwich or casserole), list the major ingredients separately in their proportions or amounts.

8. Use brand names whenever possible, or mention comparable brand.

9. For fruits and vegetables, please indicate if the skin was removed.

10. Indicate if dairy products are whole, 2%, 1%, or skim.

11. Be sure to include sauces, gravies, marinades, milk/sugar in coffee, etc.

12. Check food labels for weights, etc. Candy bars, cheeses, cookies, juices are all labeled with their weights -----Write this information down!

13. Provide any other information you feel might be helpful, such as food labels and/or recipes.

14. Record EVERYTHING edible that goes in your mouth.

15. MOST IMPORTANTLY, eat as you normally would -- please don't change your usual eating habits or modify your portion size.

For Staff Use Only:
Height _____
Weight _____
Age _____
Gender _____
Activity Level _____

4-DAY FOOD/BEVERAGE INTAKE RECORD

ACROSTIC

Date

Please measure and weigh all food and beverages you eat throughout the day and write them down as you eat them. Remember to give as many details as possible and keep any food labels if you think it will help describe the food better than you are able to. Providing us with recipes for homemade foods is helpful for us, too. Please list any vitamin or mineral supplements or any other supplements taken on the backside of this form and attach these labels if possible. It's best to be as descriptive as possible!

Meal or Snack	Time	Food or Beverage item	Brand/source (manufacturer)	Type of preparation (bake, boil, fry, etc.)	Amount/Wt (ounces, grams, fluid ounces, cups, tsp, TBS)
Meal	8:15 am	Total Cereal	General Mills	NA	1 oz/1 cup
M	8:15 am	Light Soy milk, vanilla	Silk	NA	4 fl oz / .5 cup

4-DAY FOOD/BEVERAGE INTAKE RECORD

Meal or Snack	Time	Food or Beverage item	Brand/source (manufacturer)	Type of preparation (bake, boil, fry, etc.)	Amount/Wt (ounces, grams, fluid ounces, cups, tsp, TBS)

List any vitamin/mineral pills or other supplements here:

Add any additional comments here:

INSTRUCTIONS FOR RECORDING 4-DAY PHYSICAL ACTIVITY RECORDS

1. Please maintain your normal activity level -- do NOT increase your activity level or change your normal intensity (how difficult) or duration (how long) of activities.

2. Record all your daily activities for three (3) week days and one (1) weekend day.

3. Please record all activity for the same 24-hour periods as your food intake records, starting at 5am each day and continuing until 5am the next day. Estimate as closely as possible the length of time sleeping as well as length of time for each activity.

   Example:

   Wednesday 5am - Thursday 5am = day 1
   Thursday 5am - Friday 5am = day 2
   Friday 5am - Saturday 5am = day 3
   Saturday 5am - Sunday 5am = day 4

4. Be as prompt as possible when recording your activities. Try to record all daily physical activities on your activity log as soon as you have completed them in **minutes**. Also, be as specific and accurate as possible when recording intensity and length of time the activity was performed.

5. How to estimate intensity:
   Resting = sleeping, watching tv, reading
   Very light = desk work or activities that still allows you to sing a song
   Light = Activity allows you to converse freely and breathing fine (full sentences)
   Moderate = Activity allows you to converse, but you find yourself needing to take a breath every few words (partial sentences)
   Heavy = unable to converse due to exertion level (minimal words)

Example of how to record in log:

Clock Time	Total Minutes	Activity Description	Intensity of Activity (record minutes)				
			Resting	Very Light	Light	Moderate	Heavy
5:00am - 7:15am	135	sleeping	135				
7:16am - 8:30am	74	Eat, shower, dress		64	10		
8:31am - 8:54am	23	House chores		4	6	10	3
8:55am - 10:59pm	848	walk to work & sit		793	50	5	
11:00pm - 5:00am	360	sleeping	360				
TOTAL = 1440 minutes **Total the minutes for each level of intensity:**			495	861	66	15	3

For Staff Use only:
Height _____
Weight _____
Age _____
Gender _____

4-DAY PHYSICAL ACTIVITY RECORD

_____
ACROSTIC

_____
Date

Clock Time	Total Minutes	Activity Description	Intensity of Activity (allocate minutes)				
			Resting	Very Light	Light	Moderate	Heavy
5:00 AM -							

4-DAY PHYSICAL ACTIVITY RECORD

Clock Time	Total Minutes	Activity Description	Intensity of Activity (allocate minutes)				
			Resting	Very Light	Light	Moderate	Heavy
TOTAL =	**1440 minutes**	**Total the minutes for each level of intensity:**					