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The Effect of Nighttime Consumption of Protein or Placebo on Morning Measures of Resting Metabolic Rate and Appetite in Pre- and Postmenopausal Women

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THE EFFECT OF NIGHTTIME CONSUMPTION OF PROTEIN OR PLACEBO ON MORNING MEASURES OF RESTING METABOLIC RATE AND APPETITE IN PRE- AND POSTMENOPAUSAL WOMEN

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ABSTRACT

PURPOSE: To determine the acute effects of nighttime pre-sleep consumption of casein protein and a placebo supplement on morning measures of RMR and appetite in pre- and postmenopausal women. **METHODS:** This study was a randomized crossover double-blind placebo-controlled trial. Fourteen pre- (n=7, age: 20 ± 2 years) and postmenopausal (n=7, age: 56 ± 5 years) women participated this study. On visit one subjects arrived to the laboratory between 8:00am and 12:00pm. Measurements of anthropometrics, body composition (DXA) and familiarization with RMR measurement were conducted. Visits two and three were used to assess the responses of appetite and RMR to nighttime pre-sleep consumption of a casein protein (35 g, 130 kcals) or placebo supplement 7.2g, 10 kcals). On both visits subjects arrived to the laboratory between 6:00am and 9:00am. Subjects completed a visual analogue scale (VAS) to assess hunger, satiety and desire to eat. This was followed by measurement of RMR via indirect calorimetry. After the completion of visit two subjects returned for visit three and the protocol was repeated. Dependent variables were analyzed by one-way analysis of variance (ANOVA) to determine differences between pre- and postmenopausal women. RMR and measures of hunger, desire to eat, and satiety was analyzed using 2x2 ANOVA (menopause status by supplement). Significance was accepted at $p \leq 0.05$ and data were reported as means ± standard deviations. **RESULTS:** There were no differences in subject characteristic of body composition and caloric intake between pre- and postmenopausal women except for age (pre:20 ± 2; post: 56 ± 5 yrs, p=0.001). There were significant group (pre- vs. postmenopausal) by supplement (casein protein vs. placebo) interactions for RMR expressed as total calories per day [$F(1,12)=14.474$, $p=0.003$, effect size (ES)=0.547] and oxygen consumption (VO_2)

(F(1,12)=7.633, p=0.017, ES=0.389). After consuming casein protein, total caloric expenditure (placebo: 1426 ± 260; casein protein: 1304 ± 269 kcals/day) and relative VO_2 (placebo: 3.46 ± 0.40; casein protein: 3.14 ± 0.28 ml/kg/min) were significantly lower in premenopausal women. There were no effects of the supplements in postmenopausal women. No group by supplement interactions or main effects were found on measures of appetite. **CONCLUSION:** Casein protein did not benefit RMR and appetite in pre- and postmenopausal women. In premenopausal women RMR was lower after consumption of casein protein. This seems unlikely since VO_2 measures were extremely high under the placebo condition in premenopausal women. Although casein protein showed no metabolic or appetite effects it is conceivable that an increase of protein in the diet could lead to other advantageous health outcomes over time. Overall the findings support the growing evidence that snack sized portions (150-200 kcal) are not harmful to metabolism or appetite when consumed before sleep.

CHAPTER ONE

INTRODUCTION

In 2012 the number of Americans age 65 years and older was approximately 44.1 million and this number is expected to nearly double to approximately 83.7 million by the year 2050, with women accounting for approximately 55.1% of this population (U.S. Census Bureau 2012c; U.S. Census Bureau 2012b). The average age of menopause onset in the United States is approximately 50 years and with, the average life expectancy of women being 81 years, a woman can expect to spend more than a third of her life in the postmenopausal stage (Notelovitz et al. 1989). Aging alone is associated with many poor health outcomes such as a reduction in lean mass and total daily energy expenditure (TDEE). It has been shown that postmenopausal women have a greater reduction in lean mass, reduced total daily energy expenditure (TDEE), lower protein and overall energy intake, and increased visceral body fat (Lovejoy et al. 2008; Wang et al. 1994; Hodson et al. 2014). These outcomes can lead to an increase in body mass index (BMI) and an increased risk for cardiometabolic disorders. Due to a growing number of women over the age of 60 years, it will be important to find an inexpensive method to combat the development of these postmenopausal-related health outcomes.

TDEE can be divided into the three components: resting metabolic rate (RMR), diet induced thermogenesis (DIT), and physical activity thermogenesis (PAT), which can be further divided into non-exercise activity: thermogenesis (NEAT) and exercise thermogenesis (ET) (Donahoo et al. 2004; Speakman et al. 2003; Donnelly et al. 2004; Lemmer et al. 2001). RMR accounts for approximately 60-75% of TDEE, DIT accounts for approximately 10% of TDEE, while

PAT accounts for the remaining 15-30% (MacLean et al. 2011). RMR and PAT are of major concern in postmenopausal women due to their noted decline. It is known that the reduction in RMR is due to a loss of lean mass following menopause while PAT declines for unexplained reasons across postmenopausal populations (Lovejoy et al. 2008; Hodson et al. 2014). Although some postmenopausal women unknowingly compensate for reductions in RMR and PAT by lowering caloric intake, the failure to decrease caloric intake will likely result in an increased BMI and elevated risk for cardiometabolic disorders (Lovejoy et al. 2008; Hodson et al. 2014).

Recently, a proposed strategy to combat the decline lean mass, RMR and rise in BMI has been to increase protein intake to enhance DIT, muscle protein synthesis (MPS), and satiety. DIT can be defined as the increase in energy expenditure above baseline following food consumption. Of the four macronutrients, protein elicits the greatest impact on DIT, causing a 20-30% increase (Tappy et al. 1996). Consuming a high protein diet, defined as protein accounting for 25% of caloric intake or higher, causes an increase in DIT and ultimately TDEE (Skov et al. 1999; Arciero et al. 2008; Arciero et al. 2013). Additionally, the application of more protein to the diet has been shown to aid in lean mass preservation and increases the feeling of satiety throughout the day (Arciero et al. 2013; Astrup et al. 2005).

A recent topic of debate has been the health impacts caused by nighttime feeding. Research suggests that meals of identical macronutrient composition can have different effects on DIT depending on the time of day that they are consumed. It has been shown that when consuming two meals of identical macronutrient composition, DIT was lower when the meal was consumed at night (Romon et al. 1993). In addition to a blunted DIT response, it has been shown that satiety is reduced when meal consumption occurs at night, thus leading to larger

caloric consumption during the daytime (De Castro 2004; De Castro 2007). This topic is important in the postmenopausal population due to the reduction in RMR. It would be expected that a postmenopausal woman, with absence of reduced caloric intake, would be at an elevated risk for weight gain when consuming calories at night. Recent research contradicts this thought and shows that nighttime consumption of 140-150 kcal of protein, specifically casein protein, results in increased morning RMR and satiety (Madzima et al. 2014; Ormsbee et al. 2015; Kinsey et al. 2014: Ormsbee et al. 2016). However, it has also been shown that nighttime consumption of 140-150kcal of casein protein may have negative side effects, causing increased morning levels of insulin in the absence of exercise (Kinsey et al. 2014). Excluding heightened insulin levels, nighttime consumption of casein protein can contribute to higher TDEE and a reduction in excess caloric consumption, equating to the maintenance or reduction of BMI. In addition, it has been found that protein intake at night can promote an increase in overnight MPS, thus helping to preserve lean mass and maintain RMR throughout the night (Groen et al. 2012; Res et al. 2012). It remains unknown as to how nighttime consumption of protein effects morning measures of RMR and appetite in postmenopausal women. Additionally, it is not known if these responses are different from that of premenopausal women.

Purpose of the Study

The purpose of this study was to determine the acute effects of nighttime consumption of casein protein and a placebo supplement on morning measures of RMR and appetite in pre- and postmenopausal women.

Research Hypotheses

Aim 1: To determine the acute effect of nighttime consumption of casein protein and a placebo supplement on morning RMR using indirect calorimetry in pre- and postmenopausal women.

Hypothesis 1: Casein protein will result in elevated morning RMR measured by oxygen uptake (VO_2) and total calories (kcals/day) compared to placebo in both pre- and postmenopausal women. Postmenopausal women will have lower RMR and a blunted response to casein protein compared to premenopausal women.

Aim 2: To determine the acute effect of nighttime consumption of casein protein and placebo on morning appetite, as measured by completion of a visual analogue scale to subjectively assess hunger, satiety and desire to eat.

Hypothesis 2: Casein protein will result in lower subjective feelings of hunger and desire to eat and higher feelings of satiety compared to placebo in pre- and postmenopausal women. There will be no differences in subjective feelings of hunger, desire to eat, and feelings of satiety between pre- and postmenopausal women.

Assumptions

The following assumptions for this study included:

1. All subjects accurately reported their past and current exercise histories.
2. All subjects accurately reported their current nutritional intakes.
3. All subjects followed instructions given to them regarding maintenance of current lifestyle (e.g., diet and daily physical activity) outside of the prescribed program.

4. All Laboratory equipment accurately recorded measurements over the course of repeated testing.

Delimitations

1. Seven sedentary healthy, premenopausal women and 7 sedentary, healthy postmenopausal women between the ages of 18-30 years and 45-65 years were recruited and completed the study from Florida State University and the local Tallahassee, FL area.
2. Subjects were healthy, pre- and postmenopausal women, and did not have any underlying diseases that prevented them from consuming a bolus of casein protein or from participating in metabolic testing.
3. Subjects were non-smokers, were not taking any weight control or stimulant type supplements (including coffee), and refrained from use of any nutritional supplements (vitamins, creatine, protein supplements other than those assigned, etc.) for an appropriate time (depending on the supplement) prior to the baseline visit and throughout testing.
4. Subjects were educated on how to record dietary intakes using food logs prior to the beginning of data collection.
5. Subjects did not have a BMI greater than 35 kg/m^2.
6. During the study, subjects were asked not to change their diets or exercise habits outside of the laboratory.
7. Subjects were familiarized with the ventilated hood and process of RMR measurement before their two visits.

8. Subjects had their morning RMR measured on two separate occasions: after administration of a casein protein supplement and after administration of a placebo supplement.

9. Administration of the protein or placebo supplement was completed in a randomized, double blind, placebo-controlled fashion.

Limitations

The major limiting factors in this study included the following:

1. Subjects were recruited from Florida State University and from the city of Tallahassee, FL. The subjects were from a sedentary population. The subjects that participated in this study were volunteers and may have been more motivated and looking for a nutritional and body composition benefit and therefore may not have been representative of the entire population.

2. Bias existed in this sample due to geographical location since all subjects were from the Tallahassee, FL area. Responses of these subjects to casein protein consumption may have been different than that of an individual randomly selected from the population.

3. Diet, sleep, and exercise outside of the laboratory were not controllable throughout the duration of the study. However, subjects were asked to follow a similar diet 24 hrs prior to testing and not to be physically active.

Definition of Terms

Basal Metabolic Rate (BMR): The integration of minimal activity of all the tissues in the body under steady state conditions, measured after an overnight sleep in a laboratory under optimal conditions of quiet, rest, and relaxation (Henry 2005; Wilmore et al. 1994).

Cardiometabolic Disorders: Conditions that include cardiovascular disease, Type II Diabetes, and metabolic syndrome (Parker et al. 2010).

Diet Induced Thermogenesis (DIT): The increase in energy expenditure above basal fasting level divided by the energy content of food ingested (Westerterp 2004).

Exercise Thermogenesis (ET): The increase in energy expenditure above baseline during "big muscle" exercise such as swimming, jogging, and cycling (McArdle et al. 2001).

Lean mass: Total amount of non-sex specific skeletal muscle contained in the body (McArdle et al. 2001).

Non-Exercise Activity Thermogenesis (NEAT): The energy expended for everything that is not sleeping, eating, or sports-like exercise (Levine 2004).

Physical Activity Thermogenesis (PAT): The combination of energy expended from NEAT and ET (Donahoo et al. 2004).

Postmenopausal: The absence of menses for ≥12 months (Hodson et al. 2014).

Premenopausal: The presence of regular menses for ≥12 months (Hodson et al. 2014). .

Respiratory Exchange Ratio (RER): CO_2production/O_2 consumption (VO_2) (Bergman and Brooks 1999).

Resting Energy Expenditure (REE): The sum of the metabolic rates of the tissues in the body while at rest (Nelson et al. 1992).

Resting Metabolic Rate (RMR): The lowest rate of energy expenditure that can sustain life also measured in a laboratory but under conditions less strict than BMR (Wilmore et al., 1994).

Total Daily Energy Expenditure (TDEE): The sum of energy expended by RMR, DIT, and PAT (McArdle et al. 2001).

CHAPTER TWO

REVIEW OF LITERATURE

By the year 2035 an estimated 89.2 million women over the age of 60 years living in the United States will likely be postmenopausal as the average age of menopause occurs a decade earlier (Bureau of the Census; Notelovitz 1989). Postmenopausal women have a greater reduction in total daily energy expenditure (TDEE), lower protein and overall energy intake, lower lean mass, and increased visceral body fat storage (Lovejoy et al. 2008; Wang et al. 1994; Hodson et al. 2014). One proposed intervention to help attenuate these postmenopausal side effects is to increase protein in the diet to enhance diet induced thermogenesis (DIT), muscle protein synthesis (MPS), and satiety. Of the four macronutrients, protein has the greatest impact on DIT and when protein is consumed in higher amounts (≥25% caloric intake) DIT is increased ultimately causing a rise in TDEE (Skov et al. 1999; Arciero et al. 2008; Arciero et al. 2013). Further, the addition of increased protein to the diet has been shown to aid in lean mass preservation and increase satiety throughout the day.

A recent topic of debate has been the health impacts caused by nighttime feeding. DIT is reduced at night and satiety is therefore reduced during the day when meal consumption occurs the night before (De Castro 2004; De Castro 2007). Because postmenopausal women have a reduction in TDEE, a heightened risk for weight gain would be expected when consuming excess calories, especially at night. Present data suggest nighttime consumption of 140-150 kcal of casein protein can have positive and negative effects on morning measures of RMR, satiety, and insulin (Madzima et al. 2014; Kinsey et al. 2014; Ormsbee et al. 2014). Research has shown that nighttime consumption of casein protein leads to heightened morning measures of RMR

and satiety, but also heightened morning concentrations of insulin, unless exercise is included as part of the weekly regiment (Ormsbee et al, 2014). It remains unknown as to how nighttime consumption of casein protein effects morning measures of RMR and appetite in postmenopausal women and if these effects are different from premenopausal women. The following chapter will provide further explanations on the components of TDEE and how they are impacted by menopause, exercise, and diet. The chapter will also discuss the changes in body composition and RMR that occur following menopause and how increased protein intake could aid in counteracting these changes. Finally, this chapter will examine the health impacts of nighttime feeding and if it can be used as a method to help regulate body composition.

Total Daily Energy Expenditure

Total daily energy expenditure (TDEE) is the total amount of energy expended throughout the day by the combination of RMR, AT and DIT (McArdle et al. 2001). RMR is the lowest rate of energy expenditure that can sustain life, measured after an overnight sleep in a laboratory under optimal conditions of quiet, rest, and relaxation (Wilmore et al. 1994). PAT is the sum of energy expended from NEAT, and ET, and DIT is the increase in energy expenditure above basal fasting level divided by the energy content of food ingested (Westerterp 2004; Donahoo et al. 2004). Each component of TDEE accounts for the total amount of energy expended during the day. It will be important to understand how each of these components of TDEE can be influenced, but more importantly, how variations in the components of TDEE relate to postmenopausal women.

Resting Metabolic Rate (RMR)

RMR accounts for the largest part of TDEE, being responsible for approximately 60-75% of daily energy expended (Donahoo et al. 2004). RMR is the lowest rate of energy expenditure that can sustain life (Wilmore et al. 1994). Due to the robust influence of RMR on TDEE, it can serve as a crucial determinant of body composition, and if reduced, a potential risk factor for weight gain (Ravussin et al. 1988). Before establishing how TDEE can be raised or maintained, it will be important to understand the factors causing differences and reductions in TDEE components beginning with the largest component, RMR.

It is known that women have higher obesity rates than men in the United States (Ogden et al. 2015). Because of RMR's large contribution to TDEE, an obvious question would be is there a gender difference in RMR? Arciero et al. (1993) studied 194 women, between the ages of 18-81 years, and reported the women to have a 3% lower (1,613 ± 127 vs. 1,563 ± 153 kcal/d; $p < 0.01$) RMR than a group of 328 men when differences in fat-free mass, fat mass, and peak oxygen uptake (VO2) were controlled. Similarly, Ferraro et al. (1992) support these findings and found after adjusting for age, fat-mass, and fat-free-mass, sedentary women exhibited a 44 kcal/d lower RMR than men. Conflicting data exist as, Cunningham et al. (1980) found no difference in RMR between genders. Arciero et al. (1993) noted that Cunningham et al. (1980) failed to directly measure lean mass and used the Harris Benedict equation for calculating RMR. The precise mechanism causing lower RMR in females remains unknown; however, possible mechanisms have been proposed. Two suggested mechanisms that may explain lower RMR in women are a reduction in muscular Na^+ K^- ATP-ase activity and lower sympathetic nervous system activity (Ferraro et al.1992; Simat et al. 1984).

Arciero et al. (1993) and Ferraro et al. (1992) established that women have a lower RMR when compared to men, but only the RMR of premenopausal women can fluctuate during the menstrual cycle. The menstrual cycle can cause variations in RMR based on if the follicular or luteal phase is occurring (Solomon et al. 1982; Snell et al. 1920; Wakeham et al. 1923). As explained by Nelson (2014) the menstrual cycle is the repeated process of ovum (egg) maturation and excretion to achieve pregnancy. On average the menstrual cycle lasts 28 days but can range from 21-35 days. The follicular phase accounts for the first 14 days of the 28-day menstrual cycle and is the time when the follicle and ovum is maturing. It is at the start of the follicular phase (days 1-5) that menstruation will occur. This occurs due to a drop in progesterone and estrogen causing the release of accumulated blood and tissue along the uterus. The luteal phase accounts for the second half of the menstrual cycle (days 14-28). This phase is triggered by a sudden rise in luteinizing hormone causing the ejection of the mature ovum from the follicle also known as ovulation. It is during this time that the uterine lining will thicken to provide nutrients if the ovum meets sperm and implants. If Implantation does not occur levels of progesterone and estrogen will decline and the egg and thickened layer of the uterus will be excreted and the menstrual cycle will repeat.

Solomon et al. (1992) recruited 6 women between the ages of 19-33 years and monitored RMR over a 92-day period. RMR was measured every day during the first week of the study and then every other day for the remainder. The subjects recorded the days of their menstrual cycle to determine cycle length. Morning measures of body temperature were also taken to determine the day of ovulation. Diets of the subjects were controlled and set at 1.65 times RMR, then adjusted to maintain bodyweight. Physical activity of the subjects was limited

to two walking sessions per day at 3.0 mph for 30 minutes on a treadmill. Results showed that 5 of the 6 women measured had a similar variation in RMR during the menstrual cycle. Subjects showed a decrease in RMR during the follicular phase and an increase in RMR during the luteal phase. On average, there was difference in RMR of 8.75 kcal/min from the lowest RMR of the follicular phase to the highest RMR of the luteal phase. Each subject had a different time span for her menstrual cycle causing the lowest days of follicular phase RMR and highest days of luteal phase RMR to vary. On average the lowest RMR in the follicular phase occurred during days 6-10 and the highest RMR in the luteal phase occurred during days 20-30. Because diet and exercise were controlled, it was concluded that menstruation caused variations in RMR. However, it has been debated whether the menstrual cycle causes significant variations in RMR (Smith and Doolittle 1923; Blunt and Dye 1921; Wiltshire 1921). There have been arguments that differences may exist because of the different methods of data analysis that have been used (Solomon et al. 1992). Blunt and Dye (1921*)* reported that RMR was only 1.6% lower during the follicular phase and concluded that this difference was too small to be considered not physiologically significant. Wakeham et al. (1923) utilized a method of measurement in which the change in RMR was plotted on the "normal" ordinate and the day relative to menstruation on the abscissa. The normal value was assigned based on body surface area and energy intake. Results showed a premenstrual rise in RMR beginning 1 week to 10 days before menstruation, a sudden drop in RMR at the beginning of the menstrual period, followed by a gradual return to the baseline value within 7 to 10 days. When this measurement was applied to the data reported by Blunt and Dye (1921) the same fluctuations in RMR occurred. Overall it appears that the different phases of the menstrual cycle have a significant impact on RMR.

Outside of the phase differences that may occur across a menstrual cycle, it is also suggested that postmenopausal women have a reduced RMR when compared to premenopausal women (Hodson et al. 2014; Lovejoy et al. 2008). Hodson et al. (2014) studied 51 women between the ages of 35-45 and 55-65 years with BMI ranging from 18.5-35.0 kg/m^2. The 35-45 years represented the premenopausal group and the women 55-65 years represented the postmenopausal group. Other than age, premenopausal was further confirmed by having regular menses over the past 12 months and blood Follicle Stimulating Hormone (FSH) <30 IU/l, while postmenopausal status was defined as the absence of menses for at least 12 months and blood FSH>30 IU/l. Following the measurement of RMR, results showed that the postmenopausal women had a significantly lower RMR than that of the premenopausal women (RMR: 1485 ± 41 vs. 1368 ± 31 kcal/day, p = 0.029) and presumably due to RMR reduction, a significantly lower TDEE (3034 ± 158 vs. 2599 ± 111 kcal/day, p=0.029). TDEE was calculated as RMR plus measured activity energy expenditure and calculated DIT. The differences in RMR remained even after differences in lean mass were accounted for. It was concluded that lower lean mass in the postmenopausal women (lean mass: 42.3 ± 1.0 vs. 39.3 ± 0.9 kg) accounted for the reduction in RMR, which equated to an overall reduction in TDEE. Findings by Lovejoy et al. (2008) also found a reduction of TDEE in a group of postmenopausal women and, interestingly, reported a reduction in sleeping metabolic rate.

Mechanisms Explaining Postmenopausal RMR Reduction

As confirmed by Illner et al. (2000) skeletal muscle has the largest impact on energy expenditure. This information provides a likely explanation as to why postmenopausal women have a significantly lower RMR than that of premenopausal women. Aloia et al. (1991) examined the rate of total body potassium loss (TBK), a primary marker of muscle mass, by counting the isotope ^{40}K in the Brookhaven whole-body counter, in relation to menopause. Increased levels of TBK loss were used as an indicator of muscle atrophy. 181 women were divided into pre- and postmenopausal groups with premenopausal women averaging 40.5 ± 8.8 years of age and postmenopausal averaging 56.6 ± 6.4 years of age. To be classified as postmenopausal, women had to be over the age of 45 years and have the absence of menstruation for over 12 months. After 964 measurements of TBK it was found that the rate of TBK loss for premenopausal women was significantly different compared to postmenopausal women (%TBK = -0.267 ± 0.401%/year vs. %TBK = -0.610 ± 0.108%/year). Interestingly, it was found that the rate of TBK loss was greatest in the 3 years immediately after menopause. Rate of TBK loss 3 years after menopause was significantly greater when compared to the rate of TBK 6 years after menopause (- 1.26 ± 0.19%/year vs. -0.49 ± 0. 12%/year). There was also a significant correlation between the closeness to menopause and rate of TBK loss ($F = 9.39$, $p < 0.0001$). These findings led to the conclusion that menopause had a significant impact on the loss of muscle mass. Overall, these findings provide a mechanism for the decrease in RMR with menopause, this being the decline in lean mass, and more specifically muscle mass. The previously mentioned study by Hodson et al. (2014) agrees with Aloia et al. (1991) in that each study found a reduced lean mass in postmenopausal women accompanied by a reduction in

RMR. Contradicting these findings, Lovejoy et al. (2008) did find a reduction in 24-hour energy expenditure in postmenopausal women, but there was no reduction in lean mass between pre- and postmenopausal women. A possible explanation for this could be due to the underreporting of energy intake. Tomoyasu et al. (1999) demonstrated that older men and women (age: 70±1 years) significantly underreport energy intake by an average of 20.2 ± 2.1%. If the underreported energy intake consisted of protein it is plausible to assume that this could contribute to the preservation of lean mass (Arciero et al. 2013). Taken together, it appears the conclusion made by Illner et al. (2000) is a plausible explanation for reduced RMR in the postmenopausal population.

As previously reported, reduced lean mass has been identified as one of the reasons for the reduction in RMR in postmenopausal women. The mechanism behind the drop in lean mass appears to be a reduction in physical activity following menopause, exposing muscle to greater time of disuse, and ultimately causing muscle atrophy. Indeed, Hodson et al. (2014), reported a reduction in physical activity in postmenopausal women. More specifically, premenopausal women spent significantly more time performing moderate intensity exercise (3–6 METs; P=0.03) than postmenopausal women who tended to perform more light intensity exercises (0-3 METs). There was no significant difference in respiratory quotient (RQ) between pre- and postmenopausal groups (PRE, RQ = .77; POST, RQ= .79; P=0.434).

A possible second mechanism to explain the reduction in lean mass with menopause is a reduction in protein intake. Protein intake plays an important role in the maintenance of lean mass, especially in elderly men and women (Houston et al. 2008; Meng et al. 2008; Gaffney et al. 2009; Breen and Phillips 2011; Phillips et al. 2016). Lovejoy et al. (2008) found a significant

reduction of kcal/d of protein consumed in women from four years prior to menopause to two years postmenopause (156.0 ± 2.1 kcal/d vs. 93.7 ± 52.5 kcal/d). This significant reduction could lead to a loss of lean mass, specifically muscle mass, and consequently a reduction in RMR and TDEE.

Physical Activity Thermogenesis (PAT)

PAT is defined as the additional energy that is expended above RMR by physical activity and muscular activity (Donahoo et al. 2004). The physical activity component of PAT is more commonly referred to as exercise thermogenesis (ET) and, as suggested by the name, accounts for all energy expended by purposeful exercise throughout the day. The muscular activity component of PAT is more commonly referred to as NEAT and encompasses all energy expended by activities such as fidgeting and shivering (Donahoo et al. 2004). PAT accounts for a large amount of TDEE, varying from 15-30% of TDEE. There is a noted decline in physical activity following menopause, leading to a reduction in PAT (Duval et al. 2013; Lovejoy et al. 2008; Hodson et al. 2014). Because PAT has a large contribution to TDEE, it will be necessary to understand how PAT can influence TDEE, particularly by changing RMR.

The Impact of Exercise on RMR

Two common modalities of exercise are resistance training and aerobic training. Each of these forms of exercise has an impact on RMR and TDEE and it will be necessary to understand the effects of each. Beginning with resistance training, it has been established that lean mass, specifically muscle mass, plays the largest role on RMR compared to all other bodily tissues.

Therefore, it can be extrapolated that muscle hypertrophy, induced by resistance training, would have a significant impact on RMR. Two studies have shown that resistance training can impact RMR in postmenopausal women. Hunter et al. (2000) studied 7 men and 8 postmenopausal women who participated in a 26-week resistance training program. Subjects ranged from 61-77 years of age. Training sessions were conducted 3 times per week and were composed of exercises to target all the major muscle groups. The subjects trained at 65-80% of 1 repetition maximal (1RM) for two sets of 10 repetitions. When compared to baseline measurements, it was found that both RMR and TDEE increased due to resistance training (REE, PRE: 1288 ± 124, POST: 1371 ± 134 kcal/day, $P<0.01$; TEE, PRE: 1872 ± 52 POST: 2102 ± 150 kcal/day, $P<0.01$). Similarly, Campbell et al. (1994) studied 8 men and 4 postmenopausal women. The subjects participated in a 12-week resistance training protocol that conducted training sessions three times per week. Exercises consisted of chest press, front pull-down, knee flexion, and knee extension. Subjects trained at 80% of 1RM for two sets of 8 repetitions and a third set to volitional fatigue or 12 repetitions, whichever came first. When compared to baseline measurements, the mean RMR increased by 6.8% (69.00 ± 3.30 vs. 73.00 ± 3.11 kcal/h) after resistance training ($P < 0.02$). Contradicting these two studies is work conducted by Lemmer et al. (2001) that showed 24 weeks of resistance training did not change RMR in both younger and older women. Like the previous two studies, Lemmer et al. (2001) utilized a small pool of postmenopausal women (N=10); however, Lemmer et al. (2001) did not restrict the use of hormone replacement therapy (HRT), thus it was reported that two of the women were currently taking HRT. There were also differences in the resistance training protocol in that repetition ranges changed halfway through the study. For the first 12 weeks of the study

subjects performed a 5-RM, followed by a reduction of weight so that one or two more repetitions could be completed. Each exercise was performed for one set of ~15 repetitions were completed. During the second 12 weeks of the protocol resistance began at 50% of one-repetition max and was gradually increased until failure to complete a repetition occurred. Additionally, subjects completed one set of each upper body exercise but up to two or three sets of each leg exercise depending on the day of the week.

Like resistance training, aerobic training can also have a positive effect on RMR. However, aerobic training works to increase RMR via different mechanisms than the preservation of lean mass and muscle hypertrophy. Adaptations to aerobic training are achieved via energy demand inducing the action of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). PGC-1α is a member of a family of transcription coactivators that play a central role in the regulation of cellular oxidative energy metabolism. PGC-1 α stimulates mitochondrial synthesis and is found in tissues where oxidative metabolism is active, such as skeletal muscle. As an adaptation to aerobic training Liang and Ward (2006) explained that the expression of PGC-1 α is induced in skeletal muscle causing a remodeling of fiber type. This remodeling causes a shift in the ratio of oxidative to glycolytic fibers meaning that there is a greater ratio of oxidative type I and IIa fibers compared to type IIb glycolytic fibers. These I and IIa fiber types have higher rates of oxidative metabolism due to a higher concentration of mitochondria created by the exercise induced action of PGC-1 α.

It could be hypothesized that the greater amount of mitochondrial oxidative metabolism would lead to increased energy expenditure. Canto and Auwerx (2009) illustrated this idea by showing that the overexpression of PGC-1α caused an increased energy

expenditure in cultured cells. This was accomplished by PGC-1α coordinately increasing mitochondrial biogenesis and respiration rates, as well as the uptake and utilization of substrates for energy production. Overall, it is seen that the shifting of fiber types and a coordinate increase in mitochondrial density can lead to an increase in oxidative metabolism. This rise in oxidative metabolism causes an increase in energy expenditure leading to an increase in RMR and TDEE.

Van Pelt et al. (1997) demonstrated the impact that aerobic training can have on RMR in postmenopausal women. Sixty-five healthy women ages 21-35 years and 50-72 years were divided into 5 groups consisting of 12 premenopausal and 15 postmenopausal sedentary women, 13 premenopausal and 15 postmenopausal distance runners, and 10 endurance trained postmenopausal swimmers. Endurance swimmers were used to establish that a higher RMR in postmenopausal runners vs. sedentary women, if observed, was related to endurance training. All women that were premenopausal were tested during the follicular phase (days 1-10) of their menstrual cycle. Five women in each postmenopausal group reported taking HRT and were matched to elucidate any difference between those not taking HRT. Results showed that sedentary postmenopausal women had a significantly lower RMR than that of sedentary premenopausal women (52 ± 2 vs. 57 ± 2 kcal/h; P = 0.001). However, when comparing the RMR of premenopausal endurance runners to postmenopausal endurance runners there was no significant difference found (59 ± 2 vs. 57 ± 1 kcal/h). The same non-significant difference was found when comparing RMR between postmenopausal runners and swimmers (57 ± 2 vs. 57 ± 2 kcal/h). These findings led to the conclusion that chronic aerobic exercise can help to attenuate the decline in RMR displayed by sedentary postmenopausal women. Likewise,

Froehle et al. (2013) support these findings by also demonstrating an elevated RMR in postmenopausal women participating in aerobic training. Thirty-one healthy, postmenopausal women were split into two groups, one participating in aerobic and resistance training and another participating in only aerobic training. Exercise was defined by the ACSM guidelines of "…planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness". Subjects were asked to record all daily exercise activities for four weeks along with details about type and duration of exercise, distance covered if applicable, and perceived level of exercise intensity. RMR was measured in each group following the conclusion of the four-week period. It was found that both groups had a positive correlation between RMR and exercise energy expenditure (r_s = 0.37, P = 0.04), and this partial correlation between exercise energy expenditure and RMR remained significant when controlling for fat free mass (r_s = 0.40, P = 0.03). This lead to the conclusion that aerobic training alone can serve to preserve RMR and prevent the decline in RMR following menopause. These findings are not supported by Bingham et al. (1988) who reported no significant difference in RMR following 9 weeks of aerobic training. It is important to note that this study utilized an extremely small sample of subjects (N=6) and only 3 of these subjects were women. The age of the women in this study was also well below that of the postmenopausal range (21-33 years). The presented evidence suggests that in a postmenopausal population, aerobic training has a positive impact on the retention of RMR, and consequently TDEE.

The Measurement of RMR

The measurement of RMR can be impacted by many factors therefore it will be necessary to discuss the relevant factors that can influence RMR measurement. The first consideration in measuring RMR is whether the subject slept in the laboratory the night before or slept at home and commuted to the laboratory the following day. Borsheim et al. (2003) suggested that an overnight stay in the laboratory is vital to reduce excess movement in the morning and to help ensure that exercise is not performed by the subject before testing. However, Turley et al. (1993) found no significant difference in morning RMR measurement in subjects staying home vs. those who stayed the night in the laboratory. Subjects were 10 healthy males (n=4) and females (n=6) with a mean age 21.6 years. Subjects were asked to fast for 12 hours prior to measurement of RMR and to standardize the last meal they ate prior to bed. Subjects were also asked to not exercise for 24 hours prior to measurement and had at least 7 hours of sleep. Each subject had 6 randomized RMR measurements three were conducted after a stay at home and commute to the laboratory while another three were conducted after an overnight stay in the laboratory. When subjects stayed at home they were advised to wake slowly, minimize movement, and promptly drive to the laboratory between 5:30 and 7:30am. Subjects that slept at the clinic in hospital beds were awakened between 5:30 and 7:30am. The morning movement by these subjects consisted of a 50-meter walk down the hallway to reach the metabolic cart. All subjects sat for 30 minutes in the semi-recumbent position before measurement of RMR began. Results showed no significant difference between groups (Home Total: 1.05 ± 0.20 kcal/min, vs. Laboratory Total: 1.05 ± 0.22 kcal/min). It was concluded that under most circumstances, it is not necessary that subjects spend the night in

the same facility that the measurement occurs. Bullough et al. (1993) supported these findings by showing no difference in RMR between a group that was fed a controlled meal and slept in the laboratory, a group that was fed a controlled meal in the laboratory and slept at home, and a group that was not fed a controlled meal at the laboratory and slept at home. The group that was not fed at the laboratory was asked to fast for 12 hours prior to RMR measurement. Results showed no significant difference in RMR among groups (1895 ± 86 vs. 1916 ± 79 vs. 1909 ± 75 kcal/day).

Time spent fasting is another important consideration in the measurement of RMR. Reed and Hill (1996) studied 77 female and 54 male subjects for 5 years to understand how long RMR remains elevated after meal consumption. The subjects had a mean age of 38 years (range: 18-65 years) and mean percent body fat of 35.4% (range: 8.7-51.3%). All subjects arrived after an overnight fast between 7:00 and 8:00am. The subjects rested quietly in a supine position for 45 minutes before their RMR was assessed for 30 minutes using a ventilated hood. The subjects then consumed a test meal within 15 minutes. Each subject consumed a different test meal and the size and composition of the meal varied. For some, a fixed meal size and composition were given (e.g., 999 kcal and 40% of energy as fat) and in others the size of the meal was related to the subject's usual intake based on diet records (25% of usual intake). All meals contained 15% of energy as protein and averaged 995 kcal (range: 650-1394 kcal). RMR was then measured for 10 minutes every 30 minutes for the subsequent 6 h, during which time the subject remained lying down but awake. Results showed that measurements concluding at hours 3, 4, or 5 of the 6-hour measurement period still had a significant elevation in RMR caused by the consumption of the meal (also known as the thermic effect of food (TEF))

(TEF Hour 3: 40.0% increase TEF Hour 4: 22.5% increase TEF Hour 5: 9.1% increase). Therefore, the authors concluded that if an accurate measurement of RMR is desired a fast of at least 6 hours should be utilized. Expanding on these results, Ormsbee et al. (2016) found elevated RMR in trained female runners for 8+ hours after the nighttime consumption of 335ml of chocolate milk. Overall, a fast of ≥ 8 hour is necessary to ensure no or minimal effects of the thermic effect of food on RMR measurement.

Diet Induced Thermogenesis

The final component of TDEE is the thermic effect of food, better known as diet induced thermogenesis (DIT). DIT can be defined as the increase in energy expenditure above basal fasting level divided by the energy content of food ingested (Westerterp 2004). DIT accounts for the smallest portion of TDEE, comprising approximately 10% of TDEE (Westerterp 2004). Although it has the smallest contribution to TDEE, it will be important to understand the factors influencing the variance of DIT and how they can affect TDEE. There is no apparent evidence to suggest if there is an influence of menopausal status on DIT, however variables such as obesity and advancing age do have an influence on DIT. These attributes can appear as a side effect of or are paired with postmenopausal status. Postmenopausal women have also been shown to have a reduction in the intake of protein, one of DIT's primary determinants (Lovejoy et al. 2008).

Factors Influencing Diet Induced Thermogenesis

The primary mechanism that affects the response of DIT is the total energy content of a meal followed by the protein content of the meal (Westerterp 2004). Protein has been shown

to have a significantly higher impact on DIT when compared to carbohydrates and fats (Tappy 1996). Karst et al. (1984) took 12 healthy men and divided them into two groups. Group 1 consumed 1MJ egg white, casein, gelatin, and starch. Group 2 consumed casein protein in the doses of 1 and 2MJ, and consumed hydrolyzed starch in the doses of 1, 2, and 4MJ. Each group consumed 2MJ sunflower oil and butter. The purpose of the carbohydrate, fat, and protein sources utilized was to 1) determine the influence of nutrient quality on DIT with varying macronutrients including 3 protein sources, 1 carbohydrate source, and 2 fat sources, and 2) determine the influence of nutrient quality on DIT with varying doses of protein (a single and double dose) and carbohydrate (a single, double, and quadruple doses). Following measures of DIT it was found that casein protein had the greatest impact on DIT, but was not significantly different from egg whites (62.14 ± 7.17 vs. 45.20 ± 5.26 kcal/6h). Sunflower oil and butter were shown to elicit the smallest rise in DIT (8.13 ± 8.84 and 15.80 ± 3.82 kcal/h). When comparing carbohydrate and protein it was found that at least 2 MJ of hydrolyzed starch had to be ingested to stimulate a statistically significant response in DIT. However, 1 MJ of casein protein was enough to significantly increase the response and it was found that 1 MJ of casein protein caused the same response in DIT as 4 MJ hydrolyzed starch (69.30 ± 5.50 vs. 53.30 ± 8.13 kcal/6h). This led to the conclusion that nutrient composition plays a significant role in the thermogenic response to food consumption.

The exact mechanism behind protein's robust increase in DIT remains uncertain. However, possible explanations include that the body has no storage capacity for protein and thus it needs to be metabolically processed immediately, high ATP costs of peptide bond synthesis, as well as other aspects of the increased protein turnover during increased protein

intake (Mikkelsen et al. 2000; Halton and Hu 2004). A review on the influence of dietary protein intake on whole-body protein turnover in humans conducted by Garlick et al. (1991) found that the longer-term adaptation to higher intakes of protein could readily be rationalized in terms of a modification of the fasting level of protein turnover and the immediate responses to meals. The former involves increased rates of both synthesis and degradation in the post-absorptive state. Therefore, protein synthesis and degradation are already elevated at a basal level. Further consumption of protein will cause an enhanced depression of degradation and strengthened increase in protein synthesis. The provided conclusions give credence to the possibility that high ATP costs during protein bond synthesis and protein turnover cause protein's enhanced thermic response, but the certain mechanism of this action remains unknown.

Several other factors can influence DIT response including the rate of gastric emptying, obesity, and age. Beginning with gastric emptying, Batemen (1982) recruited 8 healthy male subjects aged 21-29 years. Each subject received a bolus of orange cordial (an orange flavored drink that contained 20.0 g dextrose and 24.8 g fructose) that was given in two different sizes and at two different temperatures (200 mL 12^0C, 27^{o}C, 500 mL, 12^{o}C, 37^{o}C). The rate of gastric emptying was monitored using real-time ultrasound at 5, 10, 15, 20, 25, 30, 40, 50 and 60 min after drink administration. Following measurements, it was found that the volume of liquid in the stomach after consumption of 200 mL drinks was not affected by temperature (87.0 ± 16.4 ml at 12^{o}C; 98.0 ± 16.2 ml at 37^{o}C) and on average more than half of the meal had left the stomach after 5 minutes of consumption (12^{o}C; 56%; 37^{o}C; 51%). In the 500-mL drink temperature did play a factor showing a decreased rate of emptying in the colder drink (250 ±

33 ml at 12°C; 307 ± 26 ml at 37°C, $P < 0.05$). Additionally, the proportion of larger meals that had left the stomach was significantly less than that of the smaller 200 mL meals (12°C; 50%; 37^0C; 38%). The most important finding was that rates of gastric emptying varied across all subjects at each drink size and each temperature. It was concluded that: 1) the two phases of gastric emptying are related, the faster the first portion, the slower the half-life of the second portion. This meaning that a larger bolus will empty more slowly from the stomach than a smaller bolus; 2) the pattern of emptying of liquid from the stomach is a characteristic, which varies between individuals; and 3) warm liquid results in greater relaxation of gastric muscles as indicated by the reduction in the initial emptying, and the subsequent higher 5 min gastric volume. Not all agree that a larger bolus will empty more slowly and believe that the opposite is true. This could be because many of the gastric emptying studies utilize liquid to assess emptying rate. If this is the case, water will empty more rapidly with an increase in volume (Bowen 2006). However, if a liquid of high volume is consumed and it contains multiple nutrients it will empty more slowly due to the need for nutrient digestion and absorption (Bowen 2006). Applying these findings to DIT, larger boluses, drink temperature, and individual difference can lead to an increased or decreased time of transport to the small intestine. These differences in gastric emptying may result in different rates of nutrient absorption. Perhaps this explains the variation in DIT for each macronutrient across different individuals (Raben et al. 2003).

Obesity may play a role in the magnitude of DIT, however the data on this topic are conflicting. Bessard et al. (1983) demonstrated that obese women had a blunted DIT response before and after dieting for weight loss. The study comprised of 12 women averaging 27 ± 2

years of age who were separated into two groups. One group consisted of lean women (99 ± 2% ideal bodyweight and 24.2 ± 0.7% fat) while the other was comprised of obese women (152 ± 6% ideal bodyweight and 39.1 ± 1.1% fat) with group divisions based on height in relation to ideal bodyweight. Baseline measurements were taken on each group and then again following a weight loss period for the obese group. A mixed meal containing 17% protein, 54% carbohydrate, and 29% fat was administered before each measurement to determine DIT response. Following 300 minutes of postprandial measurement, both baseline and post weight loss measurements of DIT were found to be less in obese women than that of non-obese women (Before: 7.6 ± 0.4 vs. 9.5 ± 0.4%; After: 6.2 ± 0.8 vs. 9.5 ± 0.4%). Findings by Segal et al. (1990) support the previous findings, reporting a lower DIT in obese men vs. lean men. The study divided 14 men into lean (15.7 ± 1.5% body fat) and obese (37.3 ± 3.0% fat) groups ranging in age from 29-35 years. The men were fed a mixed, liquid meal of 24% protein, 21% fat, and 55% carbohydrate. Each group was monitored for 6 hours following the ingestion of the mixed meal. Results showed a significantly higher DIT response after 6 hours (100 ± 12 vs. 69 ± 5 kcal 6 hours; $P < 0.05$) and a significantly higher percent above RMR (28 ± 7% vs. 13 ± 3% above fasting; $P < 0.05$) for lean men vs. obese men.

On the other hand, D'Alessio et al. (1988) observed 5 lean (BMI < 25 kg/m^2) and 5 obese (BMI > 30 kg/m^2) men for 8 hours following the feeding of an Ensure drink that consisted of 53% carbohydrate, 32% fat, and 15% protein. Each subject consumed the Ensure at doses of 8, 16, 24, and 32 kcal/kg on separate occasions. This was done to show that DIT is based on energy intake and not body composition. If the given caloric load did not place a subject in positive energy balance, based on RMR, he was excluded. Reported results on 6 subjects

showed that there was no significant difference in DIT between the lean and obese subjects when in positive energy balance (lean subjects RMR (n=3) before caloric consumption: 466 kcal/8h, 517 kcal/8h, 503 kcal/8h; lean subjects RMR (n=3) after caloric consumption: 605 kcal/8h, 644 kcal/8h, 635 kcal/8h vs. obese subjects RMR (n=3) before caloric consumption: 835 kcal/8h, 595 kcal/8h, 545 kcal/8h; obese subjects RMR (n=3) after caloric consumption: 970 kcal/8h, 739 kcal/8h, 684 kcal/8h). The difference in RMR from baseline to post caloric consumption in lean subjects (139 kcal/8h, 127 kcal/8h, 132 kcal/8h) vs. the difference in RMR from baseline to post caloric consumption in obese subjects (135 kcal/8h, 144 kcal/8h, 139 kcal/8h) led to the conclusion that DIT response functions independently of leanness or fat mass. Similar findings were found in a study by Nair et al. (1983) that used two groups of 5 subjects, one group containing obese (age 37.8 ± 13.3 years, weight 100.6 ± 17.5 kg, BMI: 38.7 ± 6.4 kg/m^2 subjects and the other containing lean subjects (age 31.4 ± 9.6 years, weight 60.1 ± 4.9 kg, BMI: 21.3 ± 1.2 kg/m^2. Each group contained 4 women and one man and was fed 500 ml of a carbohydrate, protein, or fat-based liquid totaling 300 kcal. Following 6 hours of measurement, it was determined that oxygen consumption was greater for the obese group ($P<0.02$) than that of the lean group. However, when the response to the meal was calculated either as an absolute increase in oxygen consumption, or as a percentage increase over the baseline, there were no significant differences between the two groups.

A possible explanation for these conflicting findings is that many of these studies do not consider that the RMR in obese individuals is higher than that of lean individuals (D'Alessio et al. 1988). Therefore, administering an individual macronutrient, or mixed meal, to an obese individual may not cause as robust of an increase in DIT as it may for a lean individual. This

could cause the illusion that an obese individual has a blunted DIT response when compared to a lean counterpart. This explanation does seem plausible; however, the exact response is yet to be elucidated.

The final variable playing a role in the variation of DIT is age. Du et al. (2014) recruited 141 young males and females aged 18-35 years and 156 older males and females aged 60 to 88 years. Both groups had similar amounts of lean mass (Young: 54 ± 12 kg vs. Old: 54 ± 12 kg). Each age group consumed a mixed meal consisting of 57% carbohydrate, 27% fat (16% saturated fat, 27% monounsaturated fat, 57% polyunsaturated fat) and 15% protein, accounting for 33% of REE. RMR was measured for 4 hours following the consumption of the mixed meal and the magnitude of DIT increase was recorded. Results showed that both total DIT (kcal/4h, $P = 0.004$) and DIT expressed as a percentage of meal size ($P = 0.02$) were significantly less in the older adults than in the young. Peak DIT (kcal/min) was also significantly less in the older adults than the young ($P < 0.0001$) although meal size was the same. This led to the conclusion that regardless of meal size or lean mass there is a lowered DIT for older adults. Thorne and Wahren (1990) supported these findings showing that there was a reduction in DIT in older adults fed a mixed meal while Bloesch et al. (1988) and Golay et al. (1983) reported reduced DIT in older adults with the administration of 75 and 100 g of glucose. Du et al. (2014) also reported a reduction in RER for older vs. younger adults ($P < 0.01$), providing evidence that the reduction in DIT in all the previously mentioned studies could be due to a reduction in carbohydrate oxidation.

Changes in Body Composition with Menopause

As has been established, there is a reduction in RMR in postmenopausal women that is fueled by a reduction in lean mass, specifically skeletal muscle mass (Hodson et al. 2014; Aloia et al. 1991; Illner et al. 2000). This reduction in RMR occurs with a reduction in DIT, due to age, and a reduction in physical activity after menopause, leading to a reduction in PAT (Sinchun et al. 2014; Thoren and Wahren 2008; Bloesch et al. 1988; Golay et al. 1983; Duval et al. 2013; Lovejoy et al. 2008; Hodson et al. 2014). With the reduction of the three main components of TDEE, and TDEE overall, it would be expected that postmenopausal women may experience unfavorable changes in body composition (i.e. the accumulation of body fat). Toth et al. (2006), Lovejoy et al. (2008), Ley et al. (1992), and Tremollieres et al. (1996) all found higher percentages of fat mass in postmenopausal women, specifically an increased visceral storage of fat mass. In the work of Lovejoy et al. (2008), 129 women were followed for 4 years and measured annually for body composition and regional body fat. To be included, subjects had to be healthy, age 43 years or older, have had at least five menstrual periods in the 6 months prior to screening and have serum FSH <30 $mIU \cdot ml^{-1}$. The women were divided into three groups based on their menopausal status at the end of the four-year period. Premenopausal was classified as five menstrual periods in the 6 months prior to final measurements and serum FSH <30 mIU ml-1 at year four, perimenopausal was classified as irregular cycles and <5 cycles in the 6 months prior to final measurements at year four, and postmenopausal was classified as no menstrual cycles in the past year and FSH FSH >30 mIU ml-1 prior to final measurements at year four. DXA and an abdominal computed tomography scans were used to assess body composition and regional body fat accumulation. Results showed that there was a significant

increase in overall bodyweight from baseline to year four only in the postmenopausal group (Baseline: 70.8 ± 1.8 kg vs. Year 4: 73.1 ± 2.2 kg, P<0.0001). Also, increases in visceral adipose tissue were only significant in the postmenopausal group from baseline to year four (Baseline: 88.0 ± 7.7 kg vs. Year 4: 97.5 ± 6.7 kg, P<0.0002). The study concluded that menopausal transition years' place women at high risk for abdominal fat gain.

Despite the overwhelming evidence, there are studies that report there is no difference in body composition (Lindquist 1982; Wing et al. 1991; Akahoshi et al. 1996) or body fat distribution (den Tonkelaar et al. 1989; Pasquali et al. 1997; Lanska et al. 1985; Razay et al. 1992) when comparing pre- to postmenopausal women. A review by Tchernof and Poehlman (1998) provided possible explanations as to why these studies may not have shown a difference in postmenopausal body composition. It has been shown that many studies not utilizing computed tomography or DXA applied BMI as a determinant of total body fatness. Tchernof and Poehlman (1998) present a study where BMI was shown to be the greatest during perimenopause, before returning to premenopausal-like values after the conclusion of menopause (Pasquali et al. 1994). This led to the conclusion that the utilization of BMI on solely pre- and postmenopausal populations may be inaccurate due to the expected accumulation of body fat during the perimenopausal years. Other studies have also reported an absence of visceral adipose tissue accumulation in postmenopausal women (den Tonkelaar et al. 1989; Pasquali et al. 1997; Lanska et al. 1985; Razay et al. 1992). Tchernof and Poehlman (1998) noted that these studies utilized waist to hip ratios and waist circumference as their measure of body fat distribution. Interestingly, these studies did not find an increase in visceral adipose tissue, independent of age, in postmenopausal women. However, the studies utilizing DXA and

computed tomography scans showed that postmenopausal women had a greater concentration of visceral adipose tissue (Ley et al. 1992; Svendsen et al. 1995; Panotopoulos et al. 1996; Zamboni et al. 1992; Kotani et al. 1994) independent of age. Tchernof and Poehlman (1998) suggested that waist to hip ratio and waist circumference were not accurate methods for assessing visceral adipose tissue accumulation in postmenopausal women.

The Effect of Protein on Satiety

The previous findings suggest that the reduction of RMR, PAT, and DIT combine to facilitate an increase in body fat mass, specifically visceral adipose tissue, in postmenopausal women. With an increase in visceral adipose tissue, and overall fat mass, there is an increased risk for Type II Diabetes and cardiovascular disease, better known as cardiometabolic disorders (Kissebah and Krakower 1994; Depres et al. 2006). A proposed method to help combat the onset of obesity is the application of increased protein in the diet. High protein diets and meals (≥25% of caloric intake or 30 g per meal) have been shown to increase satiety, and therefore, induce weight loss, all the while preserving muscle mass, the main reason for decline in RMR (Westerterp-Plantenga et al. 2006; Weigle et al. 2005; Arciero et al. 2008; Arciero et al. 2013; Veldhorst et al. 2008; Smeets et al. 2008; Lejeune et al. 2006). Arciero et al. (2013) and Veldhorst et al. (2008) have shown the acute and long-term effects of protein on satiety and the long-term effects of protein on weight maintenance. Arciero et al. (2013) recruited physically inactive (<30 min, 2 d/wk of structured physical activity), overweight or obese (BMI 30.3 ± 5.9 kg/m^2; body fat 35.6 ± 6.6%), middle aged (45.9 ± 9.4 years) male (n=4) and female (n= 24) subjects. The subjects participated in a 62-day intervention consisting of a 5-day control

phase, a 28-day energy balance phase, and a 28-day negative energy balance phase. Blood samples were taken on the first day of the control phase and during the last day of the negative and balance phases. This was completed to assess biomarkers of cardiometabolic risk. DXA scans were taken during the same time frames to assess changes in body composition. During the 5-day control phase, subjects were asked to consume a standardized diet of 25% protein, 45% carbohydrate, and 30% fat. During the balance and negative energy balance phases subjects consumed 100% of estimated energy needs followed by a 25% caloric reduction in the negative energy balance phase. Upon conclusion of the control phase, the participants were divided into 3 groups for the balance and negative energy balance phases. Two groups consumed a high protein diet (35% protein, 45% carbohydrate, 20% fat) in either 3 meals or 6 meals per day. The third group consumed a more traditional diet (15% protein, 60% carbohydrate, 25% fat) in 3 meals per day. There were no caloric differences between the meals consumed, no differences in fat content, and no differences in glycemic index among all groups. Findings showed that both high protein groups significantly reduced total body fat and abdominal body fat compared to the traditional diet group ($P < 0.05$), during both balance and negative energy balance phases. Both high protein groups successfully decreased abdominal body fat, however the 6-meal group reduced abdominal body fat (Control: 3.7 ± 0.5 kg; Energy Balance: 3.3 ± 0.5 kg; Negative Energy Balance: 3.1 ± 0.5 kg) to a greater degree than that of the high protein 3 meal group (Control: 3.7 ± 0.3 kg; Energy Balance: 3.4 ± 0.3 kg; Negative Energy Balance: 3.3 ± 0.3 kg). Blood samples showed that plasma adiponectin increased from energy balance to negative energy balance in both high protein 3 meals and high protein 6 meals ($P < 0.05$) but remained unchanged at all test days in the normal diet group. Satiety measures were

constant in all phases other than the negative energy balance phase in which the high protein groups exhibited a significantly higher level of satiation ($P < 0.05$) when compared to the traditional diet group. Interestingly, the high protein group consuming 6 meals per day was the only group that did not decrease lean mass and gained lean mass. The results led to the conclusion that consuming higher protein diets in general, but more specifically, in 6 meals per day increments, appeared to elicit greater increases in lean mass, decreases in fat mass, and decreases in abdominal fat mass than that of a traditional diet.

Veldhorst et al. (2008) conducted a review on the acute effects of high protein meals on satiety and subsequent energy intake. Results showed that meals containing anywhere from 25-81% of energy from protein elicited a higher rating of satiety and lower subsequent energy intake throughout the remainder of the day (Hill et al. 1986; Stubbs et al. 1996; Johnson et al.1993; Latner and Schwartz 1999). Latner and Schwartz (1999*)* studied the acute effects of different macronutrient lunches on satiety and subsequent energy intake at dinner. Subjects were 12 women who averaged 20.8 years of age (range=18-37 years) and BMI of 22.5 kg/m^2 (range=19-29 kg/m^2). Subjects received 3 different liquid meals on 3 different occasions. The liquid meals consisted of 1) 111.3 g carbohydrate found in 118.4 g of Polycose (99% carbohydrate); 2) 84.8 g protein (71.5% energy), 9.6 g fat (19.2% energy), and 10.7 g carbohydrate (9.5% energy) coming from a protein supplement; or 3) a half and half mixture of both the protein supplement (53.0 g) and polycose (59.2 g). All liquid meals given to the subjects accounted for 450 kcal of energy regardless of varying macronutrient composition. Subjects arrived at 12, 1, or 2 pm, on 3 nonconsecutive days, and received their first liquid meal. Approximately 4.50-4.75 hours later, subjects received a buffet style dinner, ad libitum.

The buffet consisted of foods that were representative of all macronutrients and the subjects' food and drinks were measured before and after consumption. Results showed that intake following the protein meal was significantly less than that of the carbohydrate meal (Protein: 943.0 kcals vs. Carbohydrate: 1238.7 kcals, $F(1,11) = 5.42$, $P \leq 0.05$, $eta^2=0.33$) and the same held true when the mixed meal was compared to the carbohydrate meal (Mixed Meal: 1034.2 kcals vs. Carbohydrate: 1238.7 kcals, $F(1,11) = 4.26$, $P \leq 0.06$, $eta^2=0.280$). Likewise, there was a reported trend for highest carbohydrate consumption at dinner when previously consuming the carbohydrate meal and lowest when previously consuming the protein meal. Comparative hunger ratings showed that subjects felt the least satiated when they consumed the carbohydrate meal and the most satiated when they consumed the protein meal ($F(1,11) = 14.76$, $P \leq 0.005$, $eta^2=0.570$). These findings led to the conclusion that protein reduced later food intake and self-reported hunger measures compared to that of carbohydrates alone.

Evidence also exists that contradicts the previously presented findings, particularly in relation to protein and satiety (Barkeling et al. 1990; De Graaf et al. 1992; Geliebter et al. 1979). A review conducted by Halton and Hu (2004) explained possible reasons as to why increased satiety was not found following a high protein diet or the consumption of a high protein meal. Barkeling et al. (1990) fed 20 women lunch meals that consisted of either 10 or 43% protein and reported that there was no difference in satiety that followed the two meals. It was noted that the higher protein meal was significantly more palatable than that of the low protein meal. This could lead to potential bias, as it has been shown that greater palatability results in a reduced time of satiation (Stubbs et al. 1996). Similarly, De Graaf et al. (1992) and Geliebter et al. (1979) showed a lack of satiety following the feeding of varying protein pre-loads. Halton

and Hu (2004) noted that each of these studies utilized nose clips while feeding to negate scent from the protein pre-loads and liquid meals were utilized, making it difficult to generalize the findings. Overall, 11 out of the 14 studies reviewed by Halton and Hu (2004) showed that there was an increased satiety when a high protein meal was administered, further supporting the case that a high protein meal or diet can lead to increased measures of satiety.

Mechanisms Explaining Protein's Increase in Satiety

The mechanism(s) by which protein induces satiety is not known, however there are many proposed mechanisms as to how satiety is achieved (Halton and Hu 2004; Veldhorst et al. 2008). One proposed mechanism, termed the aminostatic hypothesis, states that since amino acid concentration is correlated with reduction in appetite, there must be a satiety center located in the brain (Mellinkoff 1956). It is believed that the satiety center is sensitive to serum amino acids levels and once the threshold is reached, hunger will cease (Mellinkoff 1956). A second mechanism, as investigated by Westerterp-Plantega (1999), could be the relation of DIT to satiety. It was shown that levels of satiety were positively correlated with levels of DIT over a 24-hour measurement period ($r = 0.80$, $p < 0.01$). Finally, the possibility that anorexigenic hormones, specifically GLP-1, may play a role in protein-induced satiety. The release of the hormones ghrelin and glucagon-like peptide 1 (GLP-1) are thought to influence post-ingestive satiety (Lejeune et al. 2006). Ghrelin is secreted from gut endocrine cells primarily located in the stomach and duodenum (Ariyasu et al. 2001). Ghrelin has been described as the "hunger hormone" causing changes in the profiles of hunger sensations and levels throughout the day increasing during fasting to stimulate hunger and decreasing after food intake to decrease

hunger (Gibbons et al. 2013). GLP-1 is secreted from gut endocrine cells in the distal region of the small and large intestine and is released into the circulation after a meal. Infusion of GLP-1 has been shown to reduce hunger levels and energy intake with the inverse occurring when levels are low (Gibbons et al. 2013). Lejeune et al. (2006) studied 12 healthy women ages 18-40 years with BMI of 20-25 kg/m^2. The subjects underwent two separate sessions of 36 hours in a metabolic chamber. The sessions were separated by 4 weeks to maximize potential for each subject to be at a similar phase of her menstrual cycle. Three days prior to each visit the subjects were fed either a high protein diet (30% protein, 40% carbohydrate, 30% fat) or an adequate protein diet (10% protein, 60% carbohydrate, 30% fat). The energy content of each diet was not significantly different (0.980 vs. 1.020 kcal/g) and divided into at 30% energy content at breakfast, 40% energy content at lunch, and 30% energy content at dinner. The diets were spread across 3 meals and were maintained during the time spent in the metabolic chamber. The 4th day marked the start of the 36-hour stay in the metabolic chamber where 9 blood samples were taken (at 08:45, 09:30, 10:15, 13:30, 14:15, 15:00, 19:15, 20:00, 20:45) to determine GLP-1 and ghrelin concentrations. Appetite profile was also recorded before and after every meal to assess satiety. Results showed that in the adequate protein condition the ghrelin concentrations decreased significantly after lunch and after dinner, whereas in the high protein condition the decrease in ghrelin was only seen after dinner. GLP-1 concentration was shown to be significantly higher 15 minutes after dinner for the high protein diet as opposed to the adequate protein diet, which increased to a lesser degree. Additionally, there was a trend for GLP-1 to remain higher with the high protein diet after breakfast compared to the adequate protein diet. It was found that both diets caused an increase in GLP-1 concentrations following

lunch and dinner. It was concluded that only in the high protein condition was satiety related to protein intake and incidentally to ghrelin and GLP-1 concentrations. Despite the presented hypotheses and findings, the precise mechanism for protein's ability to satiate remains unknown.

The Impacts of Nighttime Feeding

Of recent interest is the topic of nighttime feeding and how it can affect body composition. Until recently, nighttime feeding has been viewed as a negative practice. It is believed that nighttime consumption of food may lead to increased weight gain, consequently resulting in heightened risk for obesity and Type II Diabetes (Ormsbee et al. 2015). This idea is enforced by the fact that DIT appears to follow a circadian pattern, decreasing at night and allowing for fewer calories to be burned and more to be stored (Romon et al. 1993). It has been shown that caloric consumption at night is not as satiating as caloric consumption during the day, leading to increased daily caloric consumption (De Castro et al. 2004). Ormsbee et al. (2015) noted that there are many inconsistencies in the data showing negative health outcomes with nighttime feeding. However, it is apparent that consuming large quantities of food in the late evening may have adverse health implications. Nighttime feeding is of particular importance to postmenopausal women due to their noted reductions in RMR, DIT, and PAT. In the case that a postmenopausal woman decided to consistently consume large meals or most calories in the late evening or night, it would be hypothesized that reduced RMR, DIT, and PAT could increase the possibility of weight gain, and, due to higher levels of visceral adipose tissue concentration, increase cardiometabolic risk. However, recent research suggests

that a small consumption of carbohydrates or protein before bed (150-200kcal) may promote advantageous health outcomes in the acute and long-term settings (Waller et al. 2004; Groen et al. 2 012; Res et al. 2012; Madzima et al. 2014; Kinsey et al. 2014).

As previously mentioned, postmenopausal women have a reduced RMR when compared to premenopausal women. This alone could lead to weight gain however nighttime food consumption presents an opportunity to amplify weight gain, as it has been shown that RMR decreases from morning to night (Katayose et al. 2009). It would appear as though postmenopausal women are at a significantly heightened risk for weight gain with nighttime feeding however findings by Whitehead et al. (1996) contradict this.

Whitehead et al. (1996) recruited two male and six female overweight subjects. BMI ranged from 27.8 to 34.1 kg/m^2 and age ranged from 31 to 57 years. All subjects were in good health and did not take any medications. Three of the women that participated in this study were postmenopausal. The purpose of the study was to determine if protein intake influenced the daily decline in energy expenditure during caloric restriction. The investigators created four different diets to be administered to the subjects during the study. These diets consisted of a standardized diet (STD; 15% protein, 45% carbohydrate, 40% fat, 2389 kcal/d) and three low energy diets that included a high protein diet (36% protein, 32% carbohydrate, 32% fat, 1003 kcal/d), a high carbohydrate diet (HC; 15% protein, 53% carbohydrate, 32% fat, 1003 kcal/day), and a high fat diet (HF; 15% protein, 32% carbohydrate, 53% fat, 1003 kcal/day). The HP diet contained 87 g/d of protein while the HC and HF diets contained 38 g/d of protein. Before subjects began the 7-day consumption of one of the diets they consumed the STD diet the day before. Upon the conclusion of one diet a 7-day wash out period was observed before the

subjects started another. On days 0 and 7 of the diets the subjects consumed meals in a calorimeter chamber while days 1-6 were spent consuming the meals at home. All diets were administered in a random order over each 7-day period.

Two 24-hour whole body calorimetry sessions were conducted on day 0 (consumption of STD diet) and day 7 (final consumption day of HC, HF, or HP diet). Each subject spent 26 hours in the chamber beginning at 9:00 am and concluding at 11:00 am the following morning. Physical activity was tightly controlled by a program of set activities. Activity within the chamber was monitored by an ultrasound detector based on the Doppler principle. The sleeping metabolic rate (SMR) of each subject was monitored between midnight and 6:00 am during each stay. As expected, results showed a decline in SMR during the consumption of all three diets. However, the decline in SMR was significantly less when the HP diet was consumed as opposed to HC or HF (HP: 0.191 vs. HC: 0.931 vs. HF: 0.859 kcal/kg/d). The same held true over the entire 24-hour energy expenditure measurement (HP: 0.119 vs. HC: 0.931 vs. HF: 1.290 kcal/kg/d). The investigators concluded that maintaining protein intake during energy restriction could help to lessen the drop in RMR throughout the day. These findings are extremely pertinent to postmenopausal women due to their reduction in protein and overall energy intake (Lovejoy et al. 2008).

It is important to note that although the drop in RMR was reduced it still declined overall. This could cause the potential for weight gain due to nighttime food consumption to remain. Madzima et al. (2014) looked to determine if nighttime consumption of two different types of protein or a carbohydrate could negatively alter morning measures of RMR and appetite in an acute setting. Eleven recreationally trained men (age: 23.6 ± 1.0 years; body fat:

16.3 ± 2.3%) consumed two powdered protein sources, a powdered carbohydrate source, or a non-caloric placebo (Propel Zero) before bed on 4 separate occasions separated by 48-72 hours. The next morning the subjects were evaluated for RMR and satiety measures. Subjects did not consume any supplements for two weeks prior to testing and during the protocol, other than those that were given. Nutrient composition of administered supplements was as follows: 1) whey protein (38 g, 628 kJ (150 kcal), 30 g protein, 3 g carbohydrate, 2 g fat); 2) casein protein (38 g, 586 kJ (140 kcal), 30 g protein, 3 g carbohydrate, 1 g fat); 3) carbohydrate (maltodextrin; 38 g, 628 kJ (150 kcal), 0 g protein, 33 g carbohydrate, 2 g fat); and 4) placebo (2.9 g, 0 kJ (0 kcal); Propel Zero; PepsiCo Inc.). There were no significant differences in the energy composition of the two protein sources and the carbohydrate source. Results showed there were no statistical differences in morning measures of hunger, but, although not statistically significant, satiety was greater when whey protein (40.6 ± 5.4 mm) and casein protein (45.4 ± 0.4 mm) were consumed when compared to carbohydrate (36.1 ± 5.4 mm) and placebo (33.9 ± 5.4 mm). RMR was found to be higher in all groups compared to placebo with no statistical significance between casein, whey, or carbohydrate (whey: 8151 ± 65 kJ/d; 1947 ± 16 kcal/d, casein: 8126 ± 65 kJ/d; 1941 ± 16 kcal/d, carbohydrate: 7988 ± 65 kJ/d; 1908 ± 16 kcal/d, placebo: 7716 ± 65 kJ/d; 1843 ± 16 kcal/d; $P < 0.0001$). An interesting finding was that respiratory quotient (RQ) was similar when the subjects consumed casein protein and placebo. Specifically, RQ in the casein and placebo groups was lower compared to the carbohydrate and whey protein groups, potentially indicating that nighttime consumption of casein protein could help maintain fat oxidation while increasing energy expenditure. It was concluded that 586 to

628 kJ (140–150 kcal) of whey protein, casein protein, or carbohydrate before sleep increased morning REE in healthy, physically active young men, while the placebo did not.

A 4-week study by Waller et al. (2004) helped to elucidate the effects of sustained nighttime eating. 44 overweight/obese women and 14 overweight/obese men with BMIs ≥25 kg/m^2 were split into two groups, one group averaging 52 ± 8 years of age and the other averaging 48 ± 12 years of age. One group was instructed to consume 1 cup of ready-to-eat-cereal along with 2/3 cup of low fat milk, at least 90 minutes after dinner. The cereals provided from 100–135 kcal, 2–6 g protein, > 0.5 g fat, 23–32 g carbohydrates, and 1–1.5 g dietary fiber per cup. Those not receiving cereal were asked to maintain their normal diet. Following baseline measurements, subjects had measures of weight loss and differences in caloric consumption recorded on weeks 2 and 4 of the study. Results showed that the cereal group also significantly reduced caloric intake compared to the non-cereal group (-396.50 ± 641.55 kcal/d, p = 0.02 vs. -23.22 ± 889.60 kcal/d, p = ns). Also, subjects who consumed cereal following dinner, and were compliant to the protocol, significantly reduced bodyweight compared to the non-cereal consuming group (-0.84 ± 1.61 kg, p=0.01 vs. -0.18 ± 1.43 kg, p=0.06). It was concluded that offering a structured post-dinner snack to overweight and obese individuals reduced daily caloric intake and resulted in weight loss.

A topic of debate is if there is any difference in morning measures of RMR and satiety if different types of protein are utilized. Madzima et al. (2014) showed there was no difference in morning RMR between 140-150 kcal whey protein, casein protein, and carbohydrate (Maltodextrin). Yet, when looking at differences in morning measures of satiety, Ormsbee et al. (2014) showed that casein protein elicited the greatest change. Over a 4-week intervention, 3

groups of overweight/obese female subjects (ages 18-45 years) ingested one of the following 30 g casein protein (140 kcal), 30 g whey protein (150 kcal), or 30 g carbohydrate (Maltodextrin, 150 kcal) 7 days a week. Supplements were consumed at least 2 hours after dinner but no more than 30 minutes prior to getting into bed. Results showed that only casein protein caused a significantly greater feeling of morning satiety after 4 weeks of the intervention (casein: pre-testing, 25 ± 5; post-testing, 41 ± 6 mm vs whey: pre-testing, 34 ± 5; post-testing, 35 ± 6 mm vs. carbohydrate: pre-testing, 40 ± 8; post-testing, 43 ± 7 mm). Although not statistically significant, Madzima et al. (2014) supported these findings in the acute setting showing that casein protein showed the highest feeling of satiety compared to whey protein, carbohydrate, and placebo (casein 45.4 ± 0.4 mm; whey: 40.6 ± 5.4 mm; carbohydrate 36.1 ± 5.4 mm; placebo: 33.9 ± 5.4 mm).

Varying doses of protein consumption at night have also been called into question on the impact of morning measures of RMR and satiety. The answer to this question remains unknown but a 30-g dose of protein is typically utilized. This dose has been used in many of the previous nighttime feeding studies (Kinsey et al. 2014; Madzima et al. 2014; Ormsbee et al. 2014; Figueroa et al. 2014). It is also suggested that a 25-30 g dose of high quality protein (≥10 g essential amino acids) is ideal for older and younger individuals to stimulate MPS and build or maintain lean mass (Paddon-Jones and Rasmussen 2009; Moore et al. 2015; Symons et al. 2009).

The effect of nighttime feeding on postmenopausal women is yet to be elucidated. Kinsey et al. (2014) suggested that nighttime feeding of protein or carbohydrates in overweight and obese females, ages 18-45 years, resulted in an increased feeling of satiety and a reduced

desire to eat the following morning. Kinsey et al. (2014) also Freported that morning insulin levels increased. The investigators hypothesized that this increase was due to existing elevated fasting insulin levels in overweight/obese individuals compared to lean individuals. It is important to note that Ormsbee et al. (2014) showed that heightened morning insulin levels were negated with 3x/week of exercise. Hibi et al. (2013) reported young women (average age 23 ± 1 years, BMI, 20.6 ± 2.6 kg/m^2) who consumed a 192.4 ± 18.3 kcal snack (mean protein:fat:carbohydrate ratio of 5:50:45) at night (11 pm) for 13 days saw a rise in total and low density lipoprotein cholesterol, despite an increase in fat oxidation. It should be noted that although low density lipoprotein cholesterol increased it was still within normal ranges (76 ± 6 vs. 83 ± 7 mg/dl). The results by Hibi et al. (2013) are opposed by Kinsey et al. (2016) who showed that nighttime consumption of 30 g of casein protein did not blunt lipolysis in obese men (overnight Interstitial glycerol concentrations: casein group: 177.4 ± 26.7; placebo: 183.8 ± 20.2 µmol/L; p = 0.83 vs. next morning interstitial glycerol concentrations: casein: 171.6 ± 19.1; placebo: 161.5 ± 18.6 µmol/L, p = 0.44) but it remains unknown if the same holds true for postmenopausal women. Because of the many discrepancies between pre- and postmenopausal women, one may hypothesize that postmenopausal women could be at an elevated risk for weight gain with nighttime feeding. Also, because of redistribution of fat accumulation to the viscera they may be at an increased risk for cardiometabolic disorders. It will be necessary to explore this population and compare their outcomes to premenopausal populations to evaluate potential differences in nighttime feeding response. Therefore, the purpose of the following study was to determine and explain differences in morning measures

of RMR and satiety between sedentary pre- and postmenopausal women after consumption of casein protein or placebo supplement before going to bed.

CHAPTER THREE

RESEARCH METHODS

The purpose of this study was to determine the acute effects of nighttime pre-sleep consumption of casein protein and a placebo supplement on morning measures of RMR and appetite in pre- and postmenopausal women. Present studies suggest that nighttime consumption of either carbohydrate or protein ranging from 140-200 kcal, show positive morning effects on RMR and satiety (Kinsey et al. 2014; Madzima et al. 2014). However, there is no evidence on how nighttime feeding affects postmenopausal women and if this effect is at all different from premenopausal women.

Experimental Design

The study was a double blind, randomized, placebo-controlled study comparing the effects of 30 g of casein protein with a flavor-matched placebo on RMR and satiety in pre- and postmenopausal women. Subjects came to the laboratory on three different occasions. After eligibility was determined the first visit at the Margaret Sandels building began between 8:00 am and 12:00 pm. Baseline measurements of blood pressure, height, weight, waist circumference, and body composition via dual energy x-ray absorptiometry (DXA; model DPX-IQ, GE healthcare Inc., Madison WI) were performed to measure lean mass and fat mass. Following baseline testing, subjects were familiarized with procedures for the metabolic testing of RMR. The visit concluded with subjects being given a 3-day dietary food log and a 24-hour food log that was completed and returned to the research staff on the second visit. Subjects

were also given either a protein or placebo supplement that was randomly assigned. Supplements were assigned by subjects selecting a letter from a hat that displayed “AB” or “BA”. The order of these letters demonstrated the order that the participants would consume the supplements. The supplement that corresponded with each letter (A= placebo, B= casein protein) were assigned by a third-party investigator not otherwise involved with the study.

The second visit at the ISSM began with collection of the 3-day and 24-hour food logs and completion of a VAS to assess hunger, satiety and desire to eat. Subjects then had their RMR measured. At the end of testing, subjects were given the opposite supplement to take home and consume the night before their third visit. Subjects were also given their 24-hour food log back so they could replicate their diet 24 hours prior to their third visit. The third visit followed the same procedures as the second visit. Each visit lasted approximately 1.5 to 2 hours.

Subjects

Seven premenopausal and seven postmenopausal women from Florida State University and the city of Tallahassee were recruited for this study by posting flyers (Appendix A) in buildings around campus, handing out flyers at events around campus and the city, social media posts and by word of mouth. Premenopausal subjects ranged from 18-30 years of age and postmenopausal women ranged from 45-65 years of age. All subjects were healthy and were excluded if they had uncontrolled hypertension (blood pressure (BP)>160/100 mmHg), diabetes, thyroid problems, kidney dysfunction, and/or had milk allergies. In addition, subjects were excluded if they were smokers and or participated in structured physical activity for >30

minutes 2x/week. Sedentary, otherwise healthy, pre- and postmenopausal women were selected due to the lack of data on pre-sleep protein consumption in these populations. No subjects were found to be taking nutritional supplements. In the event that they were they could participate as long as a wash-out occurred before testing. The classification of "postmenopausal" was applied to all women that had not had menses for ≥12 months and were between 45-65 years of age. All subjects signed informed consents (Appendix B) before participating in the study. The study was approved by the Institutional Review Board for Human Subjects at Florida State University before recruitment began (Appendix C).

Data Collection

Subjects were screened and told about the study over the phone (Appendix D). If the subject was interested in participation she was invited to the laboratory for her first day of testing and familiarized with the metabolic equipment. For the first visit subjects were asked to wear sport bras and shorts and arrive to the laboratory after a 3-hour fast. During their first visit subjects completed demographics, physical activity, and medical history questionnaires (Appendix E). On completion of the questionnaires, subjects had their anthropometrics and body composition measurement taken and were familiarized with the equipment used to measure RMR.

Height was measured using a wall mounted stadiometer and weight was measured using a digital scale (Seca Corporation, Hanover, MD, USA). Waist circumference was measured using a Gulick fiberglass measuring tape with a tension handle (Creative Health Products, Inc; Ann Arbor, MI). Measurements for waist circumferences were taken two times and additional measurements were made if the initial readings were not within 5 cm of each other. Waist

circumference was measured at the smallest area around the torso, above the umbilicus and below the xiphoid process.

All DXA scans were completed and evaluated in accordance to the manufacturer's instructions by the same certified x-ray technician. The subjects were asked to lie in a supine position while the scan was being completed. Body composition values of lean mass, fat mass, and the appendicular skeletal muscle index (ASMI) were determined. The ASMI was calculated by dividing the sum of the upper and lower limb muscle mass by the square of the height (total kg of upper and lower limb muscle /m^2).

After completing the body composition measures subjects were taken to the ISSM and familiarized with the metabolic testing of RMR. Subjects were asked to rest in a semi-recumbent position with a ventilated hood covering their head and torso for 40 minutes.

Three-day food logs were recorded on two weekdays and one weekend of the subject's choosing. One of the investigators instructed the subjects on how to properly record their food intake. All dietary log data were analyzed by the same research personnel using the United States Agriculture Super Tracker (https://supertracker.usda.gov/). If USDA SuperTracker could not provide nutrition information for a food the official website of the food's name brand was utilized. Values for macronutrients and kilocalories were expressed as a gram per kilogram per day (g/kg/d) and kilocalories per day (kcal/d) respectively. Twenty four-hour food logs (Appendix G) were analyzed using the same protocol following the completion of the study. In addition to food consumption subjects were asked to record the estimated time they fell asleep and woke up before the second and third visit to determine hours slept before consumption of each supplement.

Composition of nutritional drinks was either Dymatize Nutrition Casein Protein: 35 g, 130 Calories, 25 g protein, 1,5 g fat, 4 g carbohydrate, 500 mg Calcium, 210 mg Sodium, 370 mg Potassium) or a flavor-matched Dymatize Nutrition placebo: 10 Calories, 7.2 g, Gum Arabic 5 g, Ultrasmooth 500 mg, N&A Vanilla Flavor 960 mg, Sucralose 180 mg, Potassium Chloride 80 mg, White Blend Opacifier Sensient 500 mg). Drinks were delivered to subjects pre-mixed in an opaque container. Subjects were instructed to take the supplement 30 minutes before going to sleep and at least two hours after their last meal. A phone call or text message was made to each subject in the evening on the day she was to consume the supplement to serve as a reminder.

On the second visit subjects reported to the ISSM in a fasted state (no food or drink, except for water, for at least 8 hours) between 6:00 and 9:00 am. Subjects were asked to rate their feelings of hunger, satiety and desire to eat using a VAS (Appendix H). The VAS was a 10-cm horizontal scale with opposing extremes ('not at all' to 'extremely') of each appetite sensation located at each end of the 10-cm line. The subjects indicated their subjective feelings by placing a vertical line along the 10-cm scale. Each rating was converted to a score in centimeters using a standard ruler. Higher scores indicated greater feelings of each sensation.

RMR (respiratory quotient (RQ), kcals/day and VO_2) was measured using an open-circuit indirect calorimeter (ParvoMedics TrueOne 2400 metabolic cart, Sandy, UT) with the ventilated hood. Subjects rested in a semi-recumbent position within a dark, quiet, and climate-controlled isolated room (20-23°C) with a ventilated hood covering their head and torso. Prior to testing, calibrations were performed on the flow meter and gas analyzers using standard gases of known concentrations. Subject's RMR was measured continuously for 40 minutes and the most

stable 10-minute segment was used for analysis of RMR. For premenopausal women, the second and third visit was completed during the follicular phase. All premenopausal women reported menses lasting for 5 days therefore the second and third visit occurred over a 5-day span. Figure 1 and 2 presents the outline of data collection for pre- and postmenopausal women, respectively.

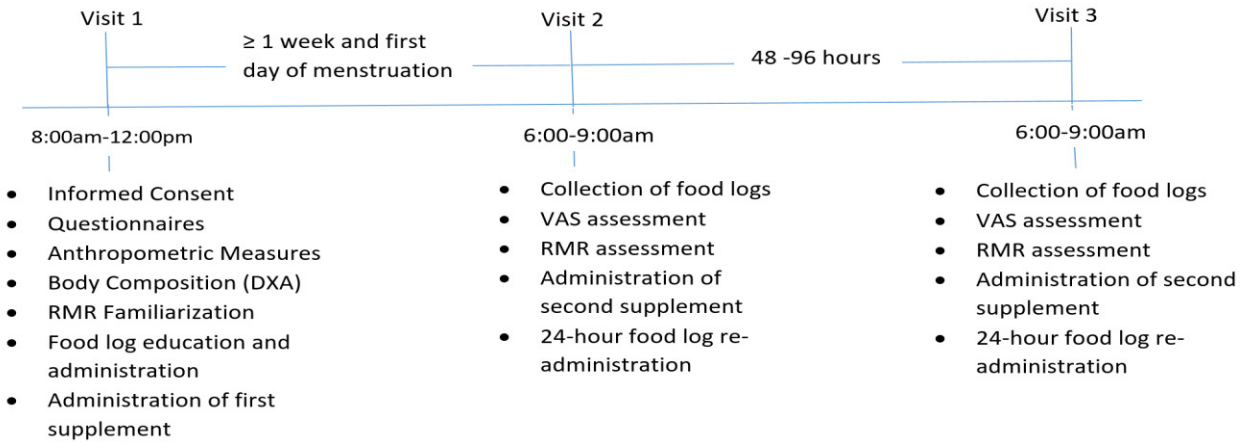


Figure 1: Timeline for visits by Premenopausal Women; VAS= Visual analogue scale; RMR= Resting metabolic rate

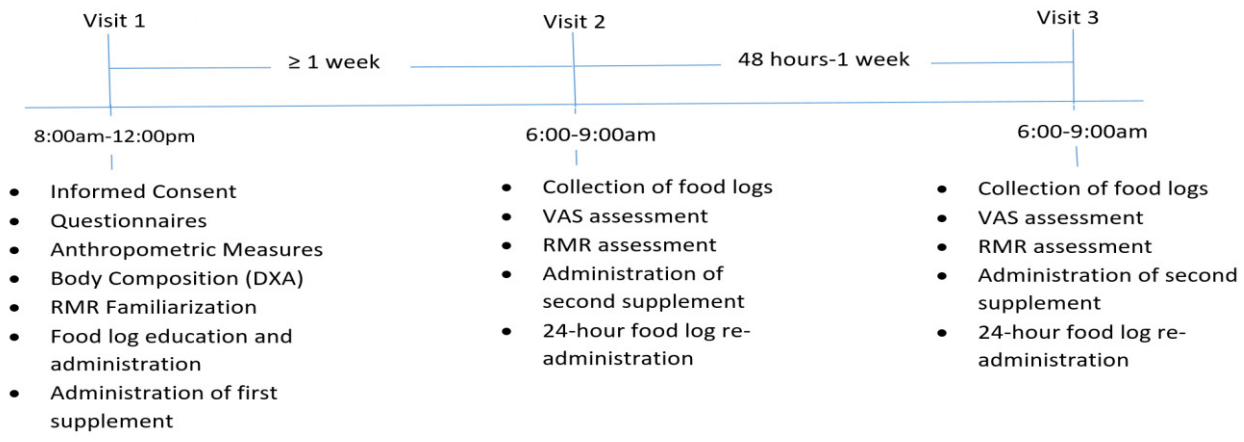


Figure 2: Timeline for Visits by Postmenopausal Women; VAS= Visual analogue scale; RMR= Resting metabolic rate

Statistical Analysis

Sample size calculation was based on the data collected by Madzima et al. (2014) who found a significant difference in RMR between a placebo and casein protein supplement. Based an alpha of 0.05, a power of 80% and an effect size of 1.1, seven subjects per group were needed for the study. Statistical analysis was performed using SPSS. Dependent variables of subject characteristics (age, anthropometrics, body composition by DXA, 3-day food logs) were analyzed by one-way analysis of variance (ANOVA) to determine differences between pre- and postmenopausal women. The most stable 10-minute period of the RMR measurement (VO_2, total calories, RQ) and measures of hunger, desire to eat, and satiety was analyzed using a 2 X 2 [group (pre- vs. postmenopausal) x supplement (placebo vs. casein protein)] ANOVA. If there were group X supplement interactions independent and paired t-tests were used to analyze group and time differences. Significance was set at $p \leq 0.05$ and data were reported as the mean ± standard deviation.

CHAPTER FOUR

RESULTS

Nineteen women were originally recruited for the study (n=10 postmenopausal; n=9 premenopausal). Three postmenopausal women were unable to complete the study (n=1 scheduling conflict; n=1 due to claustrophobia caused by the ventilated hood; n=1 BMI over 35.0 kg/m^2) and two premenopausal were unable to complete the study (n=1 due to modification of physical activity habits; n=1 due to scheduling conflicts). Thus, 14 sedentary women completed the study. Subject characteristics are displayed in Table 1. Excluding age there were no significant differences in characteristics between groups.

Table 1: Subject Characteristics (N=14).

Variables	Premenopausal (n=7)	Postmenopausal (n=7)
Age (yr)	20 ± 2	56 ± 5*
Height (m)	1.60 ± 0.10	1.59 ± 0.05
Weight (kg)	60.8 ± 13.2	67.1 ± 8.7
Waist (cm)	75.7 ± 10.4	82.5 ± 8.5
BMI (kg/m^2)	23.8 ± 5.3	26.3 ± 3.5

Values are means ± standard deviations; BMI= Body Mass Index

***P≤0.05, significantly different between groups**

Measures of subjects' body composition by DXA are displayed in Table 2. There were no significant differences found between groups in body composition measures.

Table 2: Body Composition Measures (N=14)

Variables	Premenopausal (n=7)	Postmenopausal (n=7)
Fat Mass (kg)	20.1 ± 7.8	27.4 ± 6.8
Lean mass (kg)	38.0 ± 6.4	37.1 ± 3.2
Android LM (kg)	2.52 ± 0.46	2.72 ± 0.31
Android FM (kg)	1.39 ± 0.84	2.30 ± 0.83
Gynoid LM (kg)	5.91 ± 1.08	5.76 ± 0.57
Gynoid FM (kg)	3.76 ± 1.40	4.84 ± 1.40
ASMI (kg/m^2)	6.62 ± 1.33	6.33 ± 0.80

Values are means ± standard deviations; FM= Fat Mass; LM=Lean Mass; ASMI=Appendicular Skeletal Muscle Index

Results from the three-day food logs are displayed in Table 3. There were no significant differences found between groups in terms of average daily protein, carbohydrate, fat and total caloric intake.

Table 3: Macronutrient and Kilocalorie Intakes Based on 3-Day Food Logs (N=14).

Variables	Premenopausal (n=7)	Postmenopausal (n=7)
Protein (g/kg/d)	1.13 ± 0.20	1.02 ± 0.23
Carbohydrate (g/kg/d)	3.51 ± 1.06	2.50 ± 0.74
Fat (g/kg/d)	1.20 ± 0.43	0.96 ± 0.31
Kcals/d	1768 ± 152	1592 ± 300

Values are means ± standard deviations. Supplement(s) were not included in these results.

Hours of sleep and the metabolic responses of the subjects to the consumption of placebo and casein protein are displayed in Table 4. There was a significant group (pre- vs. postmenopausal) by supplement (placebo vs. casein) interaction in RMR (kcals/day)

[F(1,12)=14.474, p≤0.05, Effect Size (ES)=0.547]. When evaluating main effects of the supplement, RMR (kcals/day) was shown to be significantly lower the morning after casein protein (1304 ± 269 kcals/day, p=0.007) compared to placebo (1426 ± 260 kcals/day) in premenopausal subjects. In the placebo condition, premenopausal women had a measurement of RMR (kcals/day) that was approaching significance to be higher than postmenopausal women (p=0.07). There was not a main effect of the supplement on kcals/day in postmenopausal women (1222 ± 135 kcals/day vs. 1241 ± 128 kcals/day, p=0.401).

There was a significant group (pre- vs. postmenopausal) by supplement (placebo vs casein) interaction for absolute VO_2 (F(1,12)=4.645, p=0.05, ES=.279). When analyzing main effects of supplement, absolute VO_2 was significantly lower (p=0.05) in casein protein (0.19 ± 0.04 L/min) compared to placebo (0.21 ± 0.04 L/min) in premenopausal women. There was no main effect of supplement for absolute VO_2 in postmenopausal women. There was also a significant group (pre- vs. postmenopausal) by supplement (placebo vs. casein) interaction for relative VO_2 [F(1,12)=7.633, p=0.017, ES=0.389]. The main effects of the supplement were similar to the measures of absolute VO_2. Relative VO_2 was significantly lower (p=0.03) after consumption of casein protein (3.14 ± 0.28 ml/kg/min) compared to placebo (3.46 ± 0.40 ml/kg/min) in premenopausal women. No main effect of the supplement was found in postmenopausal women. Under the placebo condition relative VO_2 was significantly higher in premenopausal compared to postmenopausal women (Placebo: 3.46 ± 0.40 ml/kg/min vs. 2.72 ± 0.23 ml/kg/min, p=0.05).

There was no significant group by supplement interaction for RQ [F(1,12)= 1.437, p=0.254, ES=0.107] or for hours of sleep [F(1,12)= 1.047, p=0.325, ES=0.075].

Table 4: Hours of Sleep and Metabolic Responses to Placebo and Protein Supplements in Premenopausal vs. Postmenopausal Women (N=14).

Variables	Placebo Premenopausal	Protein Premenopausal	Placebo Postmenopausal	Protein Postmenopausal
RMR (kcals/day) [a]	1426 ± 260[ŧ]	1304 ± 269*	1222 ± 135	1241 ± 128
VO_2 (L/min) [a]	0.21 ± 0.04	0.19 ± 0.04*	0.18 ± 0.02	0.18 ± 0.02
VO_2 (ml/kg/min) [a]	3.46 ± 0.40[#]	3.14 ± 0.28*[#]	2.72 ± 0.23	2.73 ± 0.28
RQ	0.78 ± 0.05	0.78 ± 0.05	0.72 ± 0.05	0.75 ± 0.05
Sleep (hours)	6.5 ± 1.0	6.6 ± 1.3	6.5 ± 1.1	6.6 ± 0.8

Values are means ± standard deviations; RMR=Resting Metabolic Rate; RQ=Respiratory quotient; VO_2=Oxygen Uptake

[a] P≤0.05, significant group by supplement interaction

[ŧ] *P*=0.07, different from placebo condition of postmenopausal women

****P≤0.05, significantly different from placebo within premenopausal group***

#P≤0.05, significantly different from placebo and protein of postmenopausal group

Subjects' perceived feelings of hunger, satiety and desire to eat are shown in Table 5. There were no significant group (pre- vs. postmenopausal) by supplement (placebo vs. casein) interactions found for hunger [F(1,12)=.573, p=0.464, ES=0.046], satiety [F(1,12)=.228, p=0.642, ES=0.019] or desire to eat [F(1,12)=.019, p=0.893, ES=0.002].

Table 5: Visual Analogue Scale (VAS) Results for Premenopausal vs. Postmenopausal Women (N=14).

Variables	Placebo Premenopausal	Protein Premenopausal	Placebo Postmenopausal	Protein Postmenopausal
Hunger (cm)	2.4 ± 3.6	3.8 ± 4.3	3.4 ± 3.1	3.7 ± 3.1
Satiety (cm)	5.8 ± 2.8	5.2 ± 3.1	4.9 ± 3.3	3.6 ± 1.6
Desire to Eat (cm)	2.9 ± 3.1	3.9 ± 4.1	3.7 ± 3.2	4.9 ± 3.4

Values are means ± standard deviations

CHAPTER FIVE

DISCUSSION

To our knowledge this is the first study to compare the responses of appetite and metabolism to nighttime pre-sleep casein protein feeding in sedentary pre- and postmenopausal women. The purpose of this study was to determine the acute effects of nighttime pre-sleep consumption of casein protein and a placebo supplement on morning measures of RMR and appetite in pre- and postmenopausal women. Because of noted impact of protein on satiety, this study also sought to determine the effect of nighttime pre-sleep protein consumption on measures of appetite in pre- and postmenopausal women (Arciero et al. 2013; Veldhorst et al. 2008).

The results of this study showed that pre- and postmenopausal women did not have significantly different body composition or macronutrient intake. RMR (kcals/day) was approaching significance for postmenopausal women to be lower than premenopausal under the placebo condition. Casein protein did not significantly increase or change RMR (kcals/day or VO_2) in postmenopausal women. However, in premenopausal women RMR (kcals/day and VO_2) was significantly lower after protein consumption compared to placebo. Hunger, satiety and desire to eat were not significantly different after consumption of casein protein. Therefore, we reject both hypotheses that there would be differences in RMR between pre- and postmenopausal women and that casein protein consumed pre-sleep would increase next-morning RMR and there would be a blunted effect in postmenopausal women. We also reject

the hypothesis that casein protein consumed at night pre-sleep would decrease morning measures of appetite and hunger in pre- and postmenopausal women.

Body Composition in Pre- and Postmenopausal Women

Postmenopausal women are known to have significantly less lean mass and significantly more fat mass than premenopausal women (Lovejoy 2008; Hodson et al. 2014). Results from the present study indicated that postmenopausal women did not have significantly different lean mass or ASMI when compared to the premenopausal women nor was there a significant difference in fat mass. Lovejoy et al. (2008) found that there was no significant change in lean mass from premenopause to postmenopause. However, they did report a difference in fat mass in postmenopausal women. Tremollieres et al. (1996) elucidated a possible explanation for these findings. They found that women who were postmenopausal and <60 years of age did not have significantly different lean mass when compared to premenopausal women who were 45-56 years of age. However, postmenopausal women >60 years of age had significantly less lean mass when compared to premenopausal women. It would appear as though this loss of lean mass is dependent upon age as opposed to the time removed from menopause onset, as suggested by Aloia et al. (1991). The postmenopausal women in the present study averaged 56.4 ± 4.7 years of age, which would place them in the same age range as the subjects in the study by Tremollieres et al. (1996). Therefore, it is possible that the group of postmenopausal subjects were not old enough to have experienced a decline in lean mass. Tremollieres et al. (1996) also noted that the loss of lean mass in those >60 years of age was predominately from the lower limbs suggesting that a decline in physical activity could be the cause for this loss of lean mass as opposed to menopause. Many postmenopausal subjects in the present study had

jobs that required walking or excessive time spent on their feet. It is possible that the postmenopausal women, although sedentary, spent more time on their feet, walking, or performing more tasks of daily living throughout the day which contributed to the retention of lean mass (Raguso et al. 2006). In terms of fat mass the results found by Tremollieres et al. (1996) support the present findings and are also illustrated by Lindsay et al. (1992) who reported no difference in fat mass between pre- and postmenopausal women. It should be noted that a potential explanation for this lack of difference in total body fat is our small sample size. Wang et al. (1994) noted that the previously cited study by Lindsay et al. (1992) had a small sample size compared to their study that did find a difference in body fat between premenopausal and postmenopausal women. A small population size of 14 could have contributed to the lack of difference.

Dietary Intake Between Pre- and Postmenopausal Women

Caloric and macronutrient data from the 3-day food logs revealed that there were no significant differences in averages for kcals/day nor grams of protein, carbohydrates, and fat consumed per kilogram of bodyweight per day. Again, Hodson et al. (2014) reported similar findings to the present study and reported no differences in average daily caloric intake (Premenopausal: 1944.0 ± 87.0 kcal/day vs. Postmenopausal: 1748.0 ± 14.6 kcal/day, p= 0.07), carbohydrates (Premenopausal: 3.28 g/day vs. Postmenopausal: 3.29 g/day), fat (Premenopausal: 1.11 g/day vs. Postmenopausal: 1.02 g/day) and protein (Premenopausal: 1.10 g/day vs. Postmenopausal: 1.03 g/day). These values are similar to values in the present study. Bopp et al. (2008) recruited 70 overweight and obese postmenopausal women (Age: 50-70 years) and subjected them to a hypocaloric diet that averaged a 350-calorie deficit for 20

weeks. The maximum amount of protein that was consumed during the study was 0.8 g/kg. However, there was still a significant effect on the retention of lean mass with subjects who consumed 0.8 g/kg, losing approximately 2.5 kg lean mass and 0.9 kg of appendicular muscle mass. More importantly, Bopp et al. (2008) found that for every 0.1 g/kg protein consumed there was a 0.62 kg reduction in lean mass loss. The postmenopausal women in our study averaged 1.02 ± 0.23 g/kg/d protein and, although not significant, consumed 176.5 kcal/day less than that of the premenopausal women. Lovejoy et al. (2008) reported a significant decline in caloric intake and protein intake from four years before menopause to two years after. It is possible that our group of postmenopausal women reduced their caloric intake but due to their protein intake remaining above 0.8 g/kg they maintained lean mass and therefore were not significantly different from the premenopausal women. The findings of Lovejoy et al. (2008) also provide a nutritional explanation for lack of difference in fat mass. Because women have been shown to decrease their caloric intake following menopause it is possible that the energy deficit could lead to a reduction in fat mass (Farnsworth et al. 2003). This potentially produced the lack of difference in fat mass between groups in the present study.

Metabolic Measures Between Placebo and Casein Protein

Resting metabolic rate was not significantly different between pre- and postmenopausal women in the current study. This is in contrast with the studies of Lovejoy et al. (2008) and Hodson et al. (2014) who both found a decline in RMR following menopause and have contributed this decline to the loss of lean mass. In the present study this decline in lean mass was not found. Therefore, it is possible that this absence of decline equated to the maintenance of RMR following menopause. Although not significantly different it is also possible that the

larger BMI in the postmenopausal group helped to blunt a drop in RMR as body surface area positively correlates with RMR (BMI Premenopausal: 23.8 ± 5.3 kg/m^2 vs. BMI Postmenopausal 26.3 ± 3.5 kg/m^2, P=0.319) (White and Seymour 2003).

In terms of casein, it was hypothesized that postmenopausal women would exhibit a blunted metabolic response after consumption of casein protein. When comparing the response to casein protein between groups this blunted response did not occur. However, when the response to placebo and casein protein in postmenopausal women was compared no significant difference was found. This finding could be potentially explained by the effect of age on gastric emptying which ultimately influences DIT. Kao et al. (1994), Madsen et al. (1992), and Moore et al. (1982) all reported a decline in the rate of gastric emptying with age. Conceivably a slower rate of gastric emptying would allow fewer nutrients to be absorbed over a set amount of time when compared to a faster rate. This would amount to a lower DIT response after food consumption. Du et al. (2014) supported this showing that adult males and females ages 60-88 years had a significantly reduced response of DIT after the consumption of a meal when compared to younger adult males and females aged 18-35 years. Ormsbee et al. (2016) demonstrated that DIT can remain elevated for over 8 hours in college aged female runners after the nighttime consumption of chocolate milk which is high in casein protein. It would be expected that the group of postmenopausal women would show a response because their average sleep the night of casein protein consumption was 6.5 ± 1.1 hours. However, aging could have slowed gastric emptying and made fewer nutrients available for absorption during this time span. It is possible that this caused DIT to be significantly impaired to the point of showing little to no change in RMR.

It should be noted that greater amounts of protein are required to stimulate MPS with age (Katsanos et al. 2006). If too small a dose of protein was consumed there would be no subsequent rise in MPS. Conceivably an increase in MPS could augment RMR.

RMR (kcals/day) and absolute and relative VO_2 were not significantly different when placebo was compared to casein protein in postmenopausal women. Of most interest are the findings from the group of premenopausal women. This group showed significant differences in total calories/day, absolute and relative VO_2 when placebo was compared to casein protein but no significant difference in RQ. Before providing potential explanations as to why RMR was lower under the casein protein condition it should be mentioned that the weight of each subject was recorded only during the first visit but not immediately before RMR measurements on the second and third visit. This could have affected the relative VO_2 measurements but should not have affected the calculation for total calories/day or absolute VO_2.

The fact that subjects were not weighed immediately before RMR measurement on the second and third visit could affect the relative VO_2. Due to the need to wait for the onset of menses before measurement of RMR almost all the subjects had two to four weeks between their first and second visits. This is a sharp contrast with the group of postmenopausal women who had a maximum of 7 to 10 days between their first and second visits. This excessive time between visits left room for the potential of weight fluctuations via changes in diet and activity levels. Leibel et al. (1995) reported that a 10% increase or decrease in bodyweight resulted in a 16% increase or a 15% decrease in RMR even after correction for body composition. A change in bodyweight without adjustment before measurement could skew results and this may have influenced our findings.

Three subjects in the premenopausal group displayed excessively high relative VO_2 after consumption of placebo (3.88, 3.95 and 3.74 ml/kg/min) and the group average for relative VO_2 after consumption of placebo was 3.46 ± 0.40 ml/kg/min. Byrne et al. (2005) discovered that out of a pool of 769 subjects (638 women and 131 men; age: 38 ± 9 years) only 14 showed a resting relative VO_2 that was ≥3.5ml/kg/min while three of the seven premenopausal women in the present study had a relative VO_2 well over 3.5 ml/kg/min. The authors noted that this characteristic was homogenous to those with a BMI of 18-22 kg/m^2 but this does not apply to our group of premenopausal women as their BMI averaged 23.8 ± 5.3 kg/m^2. Additionally, male subjects in the study by Madzima et al. (2014) and female subjects in the study by Ormsbee et al. (2014) had average relative VO_2's of 3.16 ml/kg/min and 3.20 ml/kg/min, respectively. Ormsbee et al. (2014) used 150 kcal of maltodextrin as placebo and still did not equal the relative VO_2 found in premenopausal women under a 10 kcal placebo condition.

Predictably total calories/day and absolute VO_2 were similar to the results of the relative VO_2 and were significantly higher in the placebo condition (placebo: 1426 ± 260 kcal/day vs. casein protein: 1304 ± 269 kcal/day; Absolute VO_2 placebo: 0.21 ± 0.04 L/min vs. casein protein: 0.19 ± 0.04 L/min, p=0.05). It is possible that the three outliers in the premenopausal group had weight fluctuations between their first and second visit, which caused inaccurate metabolic measurements. Even with the exclusion of the three outlier's main effects of the supplement remained for RMR and relative VO_2 in premenopausal women. It is possible that with a larger sample size these differences would be attenuated. However, because a larger population was not attained it was necessary to retain outliers to satisfy the required population size determined by the power calculation.

If it was not weight change that impacted the results it is possible that the placebo supplement contained a substance that caused an increase in metabolism. The composition of the placebo supplement was as follows: Gum Arabic (5000 mg), Ultrasmooth (500 mg), N&A Vanilla Flavor (960 mg), Sucralose(180mg), Potassium Chloride (80 mg), White Blend Opacifier Sensient CSL42885 (500 mg). Gum Arabic and other food gums have been shown to play a role in the reduction of serum cholesterol and regulation of blood glucose and insulin levels but have not been shown to increase energy expenditure (Ali et al. 1994). Food gums have been shown to slow gastric emptying implying a reduction in energy expenditure not an increase (Leclere et al. 1994). All other ingredients either had no caloric value or were in too small of a concentration to impact RMR therefore it is unlikely that the ingredients in the placebo supplement caused a change in RMR. Based on these previous findings, barring a major weight fluctuation, we cannot explain why premenopausal women under the placebo condition had high VO_2 measures and more research will be necessary.

While these findings are statistically significant it comes into question if they are clinically significant. If the protocol was followed for an extended period of time RMR (kcals/day) would result in 854 less kilocalories burned per week. Katan and Ludwig (2010) reported that women in the 1970's had a mean BMI of 23 kg/m^2 but this average rose to 29 kg/m^2 30 years later. The authors reported that an increase of approximately 370 kcals/day would produce this change in BMI. The difference we reported was approximately three times less than this and thus a gain of approximately 2 kg/m^2 could be predicted over 30 years. However, this would only hold true if the postmenopausal subjects maintained the same macronutrient intake and activity level. In the case that a larger meal was consumed at night

and ambulation was reduced weight gain could easily exceed 2 kg/m^2. Any macronutrient intake that appears to cause reductions in RMR should be regarded as significant as the smallest modulation can lead to a significant change. Katan and Ludwig (2010) noted that the addition of one ounce of sugar sweetened beverage to the diet and a reduction of one minute of walking per day would be enough to create the 370 kcals/day addition over 28 years to cause a gain of 6 kg/m^2. It should be kept in mind that the reduction in RMR (kcals/day) by casein protein is due to the high VO_2 under the placebo condition. However, in the event that casein protein truly caused a reduction in RMR (kcals/day) it appears that the found statistical significance could produce clinically significant outcomes.

There was no significant group (pre- vs. postmenopausal) by supplement (placebo vs. casein) interaction found for RQ. Previously it has been reported that nighttime pre-sleep consumption of casein protein has no effect on RQ (Ormsbee et al. 2014; Kinsey et al. 2014). Our results support this finding, which is further supported by Kinsey et al. (2016) who reported no changes in lipolysis with nighttime consumption of casein protein. Overall this indicates that fat oxidation was neither negatively nor positively affected by nighttime consumption of casein protein in pre- and postmenopausal women.

Measures of appetite (hunger, satiety and desire to eat) were not significantly different between and within groups for placebo and casein protein. These results are supported by Kinsey et al. (2014) and Madzima et al. (2014). A potential explanation for this finding relates to the dosing of protein utilized in this study. Crovetti et al. (1997) demonstrated that a high protein meal (96.6 grams of protein) significantly increased satiety compared to a high carbohydrate meal (14.0 grams of protein). This is not to say that greater than 90 grams of

protein were needed to elicit changes in appetite. However, different levels of dosing are an area that is yet to be explored in nighttime feeding. It appears that the traditional dose of 30 g casein protein used by Madzima et al. (2014), Kinsey et al. (2014) and in the present study was not effective in changing appetite. It is interesting to note that Trommelen et al. (2017) demonstrated no augmentation in MPS after the consumption of 30 g casein protein but did find a difference after consumption of 40 g. Although not an effect on appetite, this does show that greater doses of protein can cause changes in appetite. Potentially a dose of 40 or 50 grams of casein protein could produce a significant change in appetite.

Limitations

This study has a few limitations that must be addressed. The first limitation is that subjects were not weighed immediately before RMR measurement. This is of concern for the group of premenopausal women because more time was spent between their first and second visit (2-4 weeks) when compared to the postmenopausal group (7-10 days). This additional time left a large timespan in which a change in nutritional or activity habits could have impacted bodyweight. Because bodyweight was not assessed immediately prior to RMR measurement error in RMR measurement could have occurred. This still does not explain the differences in total calories/day and absolute VO_2 measurements.

In the group of premenopausal women, it was necessary to schedule RMR measurements during menses. The time that menses occurred was semi-predictable at best therefore some visits for RMR measurement could occur on a weekend and a weekday. Because of this it is possible that activity habits, specifically time spent walking, varied between the two days of measurement and could have impacted RMR more significantly on one day

compared to another. However, the randomization of the supplements should have taken care of this but again the sample size may have been too small.

In both groups of subjects time slept was self-reported. This did not consider the quality of sleep that they received. Subjects may have gone to bed at 12:00 pm and gotten out of bed at 6:00 am thus causing them to record that they slept for 6 hours. However, these 6 hours could have involved sleep disturbances causing the subjects to awake numerous times throughout the night. This would amount to the subjects receiving fewer than 6 hours of sleep although it was recorded as so. Hours slept could also have been limited due to the early hours that RMR measurement occurred. Some women in the postmenopausal group had measurements before reporting to work therefore they had to wake earlier than usual. The same was seen in premenopausal women as many opted to come in for measurements hours before their first class of the day, potentially causing them to wake earlier than usual and sleep for a shorter duration. As noted by Knutson et al. (2007), loss of sleep can potentially lead to a reduction in RMR.

Future Research

To our knowledge this is the first study to compare the effects of nighttime consumption of casein protein on morning measures of RMR and appetite in sedentary premenopausal and postmenopausal women. Future studies should aim to utilize these two populations again; however, it should be ensured that assessment of bodyweight occurs before all RMR measurements.

It would also be interesting to see how different doses of casein protein effect morning measures of RMR and appetite. The previously mentioned study by Crovetti et al. (1997) gives

reason to believe that a higher dose of protein may elicit significant differences in measures of appetite. Trommelen et al. (2017) demonstrated that a 40 g dose of casein protein stimulated MPS while a 30 g dose did not. This rise in muscle metabolism could augment RMR. Additionally, the potential for reduction in the rate of gastric emptying in postmenopausal women makes it possible that a higher dose of protein could cause significant changes in RMR.

Based on previous work by Madzima et al. (2014) future research should look to explore how different types of protein and complex carbohydrates individually affect RMR and appetite. Madzima et al. (2014) found that responses in appetite and RMR did not vary among carbohydrate, whey protein and casein protein. However, it remains uncertain as to if the same applies to the sedentary pre- and postmenopausal populations. Also, it should be explored how mixed macronutrients effect these populations. It is more likely that a snack or meal would contain multiple macronutrients as opposed to one. Therefore, the morning response of RMR and appetite to a mixed meal versus an isocaloric individual macronutrient should be explored.

Conclusions

Nighttime pre-sleep consumption of casein protein was not beneficial or detrimental to pre- and postmenopausal women. Resting VO_2 in premenopausal women under the placebo condition was significantly higher than in the casein protein condition. This is unlikely and this point is further enforced by the fact that VO_2 was so much higher under the placebo condition and reasons for this cannot be explained. Although casein protein showed no metabolic or appetite benefits across all groups it is conceivable that an increase of protein in the diet could lead to other advantageous health outcomes over time. It will be necessary for methodological errors to be corrected and for the study of nighttime feeding in these populations to continue

before further inferences can be made. Overall the findings of our study support the growing evidence that snack sized portions of 150-200 kcals are not harmful to metabolism or appetite when consumed before sleep.

APPENDIX A

RECRUITMENT FLYER

SUBJECTS NEEDED

Do you want to know how many calories your body burns at rest and how eating food at night can impact this?

Female subjects are needed to participate in a 3-visit study that will investigate the effects of nighttime eating on morning metabolism.

Subjects must be premenopausal and of ages 18-30 years or postmenopausal and of ages 45-65 years. Subjects must participate in physical activity for <30 minutes 2 times per week and must be non-smokers.

FREE assessments include:

- Body Composition
- Resting Metabolic Rate
- Blood Pressure
- Waist and Hip Circumferences
- Fasting Blood Insulin and Glucose Levels

Contact Chris at [redacted] for more information.

APPENDIX B

INFORMED CONSENT

The Effect of Nighttime Consumption of Protein or Non-Energy Containing Placebo on Morning Measures of Resting Metabolic Rate, Appetite, and Insulin and Glucose Levels in Sedentary Pre- and Postmenopausal Women.

Informed Consent Form

Title of Project: The Effect of Nighttime Consumption of Protein or Non-Energy Containing Placebo on Morning Measures of Resting Metabolic Rate, Appetite, and Insulin and Glucose Levels in Pre- and Postmenopausal women.

Principal Investigator: Christopher Schattinger

Other Investigators: Dr. Lynn Panton and Dr. Michael Ormsbee (co-PIs)

Subject's Printed Name:_____

1. Voluntary Consent

I voluntarily and without element of force or coercion consent to be a subject in the research project entitled "The Effect of Nighttime Consumption of Protein or Non-Energy Containing Placebo on Morning Measures of Resting Metabolic Rate, Appetite, and Insulin and Glucose Levels in Sedentary Pre- and Postmenopausal Women." This study is being conducted by Mr. Christopher Schattinger and Drs. Lynn Panton and Michael Ormsbee of the Department of Nutrition, Food & Exercise Sciences at Florida State University.

2. Purpose of the Research

The primary purpose of this research is to examine the effects of overnight consumption of protein on morning measures of resting metabolic rate.

Ten sedentary (<30 min of structured physical activity 2x/week) premenopausal and ten sedentary postmenopausal (absence of menses for ≥12 months) will be recruited from Florida State University and the city of Tallahassee.

3. Procedures

Subjects in the study will be required to perform laboratory testing at the Margaret Sandels Building at Florida State University. All measurements and assessments to be completed are described in detail below.

I must meet the following criteria to be included in the study: Absence of (1) uncontrolled hypertension (blood pressure (BP)>160/100 mmHg) (2) diabetes (3) thyroid problems (4) kidney dysfunction (5) milk allergies (6) smoking, and/or (7) physical activity for >30 minutes 2x/week. In addition, all nutritional supplement use must cease 2 weeks prior to baseline measurements and for the duration of the study (multivitamins are allowed). I will also refrain from exercise, caffeine, and alcohol for 24 hours before my resting metabolic rate (RMR) measurements on visit 2 and 3.

Upon arrival to the Margaret Sandels Building written informed consent will be signed as well as questionnaires on demographics, health history, and physical activity levels. Baseline measurements will include blood pressure, height, weight, waist and hip circumferences, body

FSU Human Subjects Committee approved on 09/29/2016, void after 09/13/2017. HSC #2016.19098

composition via dual energy x-ray absorptiometry (DXA), and familiarization with metabolic testing. I will need to be fasted for 3 hours before I arrive for testing.

Height and weight will be assessed using a wall mounted stadiometer and a digital scale. Waist and hip circumferences will be measured using a Gulick fiberglass measuring tape with a tension handle. Measurements will be taken 2 times and additional measurements will be made if the initial readings are not within 5mm of each other. Waist circumferences will be measured at the smallest area around the torso, above the umbilicus and below the xiphoid process. Hip circumference will be measured at the largest protrusion of the buttocks with feet together.

Body composition will be assessed using dual energy x-ray absorptiometry. This involves some radiation of approximately 0.5 mREM for the total body scan. This is less radiation than a person receives from a chest X-ray (20-50 mREM), a full dental X-ray (300 mREM) or an abdominal X-ray (250 mREM). I will be asked to lie in a supine positon while the scan is being completed. The measurement of body composition using the DXA is non-invasive.

Upon completing the DXA scan I will be familiarized with the metabolic testing of RMR. I will be asked to rest in a semi-recumbent position with a ventilated hood covering my head and torso. I will rest in this position for approximately 5 minutes or until I feel comfortable with the procedures.

At the end of the first visit I will receive a 3-day dietary log and I will complete this log on two weekdays and one weekend before my second visit. I will also receive a 24-hour food log to complete before my second visit of all foods I consumed over the 24-hour period before my second visit. I will be given this food log back to replicate before my third visit to the laboratory.

I will also be given one of two supplements that are being tested over the course of the study. The supplements will include casein protein, and a non-energy containing placebo. The casein protein supplement will contain 30g of casein protein, 3g of carbohydrate, and 1g of fat for a total of 140 kcals per serving. The placebo supplement will be a non-energy, flavored, pre mixed drink. Other ingredients will include small amounts of sodium, potassium, and calcium for consistency and flavoring. I will not be told which supplement I will be given. I will take this supplement a half an hour before going to sleep and at least 1 hour after my last meal the night before my second and third visits.

My first visit should take about 1 hour to complete.

On my second visit (occurring at least a week following the first visit if I am a postmenopausal woman or during the start of my menstrual cycle if I am a premenopausal women) I will come to the laboratory in a fasted state (no food or drink, except water for at least 8 hours and I will also refrain from exercise, caffeine, and alcohol for 24 -hours) between 6 and 9am after consuming the supplement the night before. I will turn in the 3-day dietary and 24-hour food logs. I will fill out a visual analog scale (VAS) for hunger, satiety and mood. The visual analogue

scale is a 10cm horizontal scale with opposing extremes ('not at all' to 'extremely') of each appetite sensation and mood located at each end of the 10cm line. I will indicate my subjective feelings by placing a vertical line along the 10cm scale. Each rating will be converted to a score in millimeters using a standard ruler. Higher scores will indicate greater feelings of each sensation. I will have my RMR measured using indirect calorimetry. This is a non-invasive test that involves lying down on a padded table for a total of 60 minutes, comprised of 20 minutes at rest, then 10 minutes with a ventilated hood covering my head and torso, followed by 10 minutes without the ventilated hood, then 10 minutes with the hood back on, alternating every 10 minutes until 60 minutes is complete. During the RMR measurement my heart rate will be monitored using a heart rate monitor. On completion of the RMR measurement, I will have my blood sampled to assess my fasting insulin and glucose levels. The blood draw will be taken by a trained technician in a sterile setting. I will have approximately 20 milliliters of blood drawn from the antecubital vein. The blood will be stored for later analysis.

After finishing visit two I will be given the second supplement and scheduled at least 48 hours or one week later for my third visit depending whether I am in the pre- or postmenopausal group, respectively. I will be given my 24-hour food log to replicate 24-hours prior to visit three and will take the assigned supplement 30 minutes before going to sleep and at least 1 hour after my last meal the night before my third visit. The third visit will follow the same procedures as described for visit two.

Both visits should last approximately 90 minutes.

4. Discomforts and Risks

I understand there is a minimal level of risk involved if I agree to participate in this study. The risk will be minimized by using qualified investigators to supervise testing and ensure proper procedures.

The risks of DXA are small; there is exposure to a small amount of radiation (approximately.5 mREM for the total body scan). This amount is less than a person receives during a chest X-ray (20-50 mREM), a full dental X-ray (300 mREM) or an abdominal X-ray (250 mREM).

The risks of blood drawing are small; there may be some slight discomfort at the site of needle placement with possible bruising or swelling. The risk will be minimized by the use of skilled technicians using sterile techniques and equipment.

5. Possible Benefits

Possible Benefits of participating in this study will include gaining knowledge about my dietary intake, body composition and RMR. I will also learn how casein protein may affect my RMR.

6. Statement of Confidentiality

The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with my name and code number will be kept by the co-investigator, Dr. Lynn Panton, in a locked drawer in office 100 C. Data will be kept for 10 years and then destroyed. Results of the study will be given to me upon request once the study is completed. My own individual results will also be given to me upon request in a hardcopy format and only I can receive my results from the principal investigator.

7. Notice of Potential Injury

In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense.

8. Contact Information for Questions or Concerns

Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Christopher Schattinger a[redacted] ([redacted], Dr. Lynn Panton at [redacted], or Dr. Michael Ormsbee at [redacted] answers to questions about this research study or my rights. Group results will be sent to me upon my request.

If I have questions about my rights as a subject in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@magnet.fsu.edu).

10. Signature and Consent to Participate in Research

The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

_____ _____

Subject Date

APPENDIX C

INTERNAL REVIEW BOARD

HUMAN SUBJECTS APPLICATION

Florida State UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2742
(850) 644-8673 · FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 09/30/2016

To: Christopher Schattinger [redacted]

Address: 120 Convocation Way

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
The Effect of Nighttime Consumption of Protein or Non-Energy Containing Placebo on Morning Measures of Resting Metabolic Rate, Appetite, and Insulin and Glucose Levels in Pre- and Postmenopausal women.

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 09/14/2016 Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 09/13/2017 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.

Cc: Chester Ray <caray@fsu.edu>, Chair
HSC No. 2016.19098

APPENDIX D

PRESCREENING QUESTIONAIRRE

Pre Screening Questions

- **Do you use any medications that may alter RMR (Thyroid medications)? ____**
- **Do you have uncontrolled hypertension (blood pressure (BP)>160/100 mmHg)? ____**
- **Do you have Diabetes? ____**
- **Do you have Thyroid or Kidney dysfunction? ____**
- **Do you have milk allergies? ____**
- **Do you smoke? ____**
- **Do you participate in structured physical activity for >30 minutes 2x/week? ____**
- **Are you taking nutritional supplements other than multivitamins? ____**
- **Age?____**
- **Postmenopausal: Absence of menses for ≥12 months? ____ Premenopausal: Presence of menses? ____**
- **Presence of pregnancy? ____**

*****Arrive 3 hours fasted to the Margaret Sandels Building**

*****Arrive wearing sport bras and yoga pants/elastic waist clothing**

APPENDIX E

SUBJECT QUESTIONAIRRES

Florida State University
Dept. of Nutrition, Food & Exercise Sciences
Tallahassee, FL 32306

CARDIOVASCULAR HISTORY

ID# _____
DATE _____

Answer the following questions, indicating the month and year of the event or diagnosis where appropriate.

		Yes	No	Month/Year
1.	Has a doctor ever told you that you have heart disease?	___	___	____/____
2.	Have you ever had a heart attack?	___	___	____/____
3.	Have you ever had chest pain?	___	___	____/____
4.	Have you ever had cardiac catheterization?	___	___	____/____
5.	Have you ever had balloon angioplasty?	___	___	____/____
6.	Have you had coronary artery bypass graft surgery?	___	___	

If yes, list date and number of grafts:

____/____ # grafts: ___ 1 ___ 2 ___ 3 ___ 4^{+}
Mo. Yr.

7.	Have you ever had a stroke?	___	___	____/____
8.	Do you have hypertension (high blood pressure)?	___	___	____/____

If yes, how long have you had hypertension?

_____ less than 1 year
_____ 1-5 years
_____ 6-10 years
_____ more than 10 years

		Yes	No	Month/Year
9.	Do you have diabetes mellitus?	___	___	____/____
10.	Do you take insulin for diabetes?	___	___	
	If yes, how long have you taken insulin? _____ less than 1 year _____ 1-5 years _____ 6-10 years _____ more than 10 years			
11.	Do you take oral hypoglycemics for diabetes?	___	___	
12.	Do you have a cardiac pacemaker?	___	___	
	If yes, how long have you had a cardiac pacemaker? _____ less than 1 year _____ 1-5 years _____ 6-10 years _____ more than 10 years			
13.	Have you had a carotid endarterectomy?	___	___	____/____
14.	Has your doctor ever told you that you have a heart valve problem?	___	___	____/____
15.	Have you had heart valve replacement surgery?	___	___	____/____
	If yes, what heart valves were replaced?	___ mitral	___ aortic	
16.	Have you had cardiomyopathy?	___	___	____/____
17	Have you had a heart aneurysm?	___	___	____/____
18.	Have you had heart failure?	___	___	____/____
19.	Have you ever suffered cardiac arrest?	___	___	____/____

20. OTHER MEDICAL PROBLEMS: Indicate if you have had any of the following medical problems:

Past	Now	
____	____	Alcoholism
____	____	Allergies
____	____	Anemia
____	____	Arthritis
____	____	Asthma
____	____	Back injury or problem
____	____	Blood clots
____	____	Bronchitis
____	____	Cirrhosis
____	____	Claudication
____	____	Elbow or shoulder problems
____	____	Emotional disorder
____	____	Eye problems
____	____	Gall bladder disease
____	____	Glaucoma
____	____	Gout
____	____	Headaches
____	____	Hemorrhoids
____	____	Hernia
____	____	Hip, knee, or ankle problems
____	____	Intestinal disorders
____	____	Kidney disease
____	____	Liver disease
____	____	Lung disease
____	____	Mental illness
____	____	Neck injury or problem
____	____	Neuralgic disorder
____	____	OB/GYN problems
____	____	Obesity/overweight
____	____	Osteoporosis
____	____	Parkinson's disease
____	____	Phlebitis
____	____	Prostate trouble
____	____	Rheumatic fever
____	____	Seizure disorder
____	____	Stomach disease
____	____	Thyroid disease
____	____	Tumors or cancer - List type: _____
____	____	Ulcers
____	____	Other - specify: _____

List medications you are taking below:

Name of Drug	Dosage	Times/day	Duration of drug use

Florida State University
Dept. of Nutrition, Food & Exercise Sciences
Tallahassee, FL 32306
(850) 644-4685

DEMOGRAPHIC INFORMATION

ID#_____

Home address _____
Street

_____
City State ZIP County

Personal Information

Age _____ Date of birth _____/_____/_____
Month Day Year

Height _____ in. _____ cm

Weight _____ lb. _____ kg

Sex ____ Male
____ Female

Race ____ White
____ Black
____ Asian
____ Hispanic
____ Other: _____

Marital Status

____ Single
____ Married # years _____
____ Divorced or separated # years _____
____ Widowed # years _____

Religion (optional)

____ Catholic ____ Hindu
____ Protestant ____ Muslim
____ Jewish ____ None
____ Jehovah Witness ____ Other: _____

ID#_____

Education Completed

____ 1-8 years
____ 9-12 years
____ 13-16 years
____ 17-18 years
____ more than 18 years

____ High school graduate
____ Bachelor's degree
____ Master's degree
____ Doctoral degree

Occupation (list) _____

Present work status

____ Working full time
____ Working part time
____ Not employed - Reason: ____ Medical ____ Other
____ Retired

Physical Activity Questionnaire

Activity at Work

1. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate (like carrying or lifting heavy loads, digging or construction work) for at least 10 minutes continuously?

   Y___ N___

2. In a typical week, on how many days do you do vigorous-intensity activities as part of your work?

   Number of days___

3. How much time do you spend doing vigorous-intensity activities at work on a typical day?

   Hours___ Minutes___

4. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking (or carrying light loads) for at least 10 minutes continuously?

   Y___ N___

5. In a typical week, on how many days do you do moderate-intensity activities as part of your work?

   Number of days

6. How much time do you spend doing moderate-intensity activities at work on a typical day?

   Hours___ Minutes___

Travel to and from Places

The next questions exclude the physical activities at work that you have already mentioned. Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places?

Y___ N___

1. In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?

   Number of days____
2. How much time do you spend walking or bicycling for travel on a typical day?

   Hours___ Minutes___

Recreational Activities

The next questions exclude the work and transport activities that you have already mentioned.

1. Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate (like running or football), for at least 10 minutes continuously?

   Y___ N___
2. In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?

   Number of days____
3. How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?

   Hours___ Minutes___
4. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that causes a small increase in breathing or heart rate such as brisk walking, (cycling, swimming, and volleyball) for at least 10 minutes continuously?
5. In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?

   Number of days____
6. How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?

   Hours___ Minutes___

Sedentary Behavior

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent (sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television), but do not include time spent sleeping.

1. How much time do you usually spend sitting or reclining on a typical day?

   Hours____Minutes____

APPENDIX F

3-DAY FOOD LOG

Directions for 3-Day Food and Activity Record

1. Keep your 3-day food record on two weekdays and one weekend of your choice. Avoid one of these days being 24 hours before your first visit.

2. Please record and photograph each food you eat immediately after you eat it. E-mail all pictures to cms15j@my.fsu.edu.

3. Record only one food item per line.

3. Be as specific as possible when describing a food eaten: how it was cooked and the amount you ate. Don't forget to include all beverages you drink. For example: Coffee with 1 tsp. Cream, 12 oz. Regular Coke, or 8 oz. Sweetened Tea.

4. Include brand names or labels from food items whenever possible.

5. Record amounts eaten in household measures. For example: one cup nonfat milk, 3 ounces grilled chicken, 2 tablespoons ranch dressing, 1 medium fruit, 2 slices cheese.

6. Include the method used to prepare the food item. For example: fresh, frozen, stewed, fried, baked, canned, broiled, raw, braised.

7. For canned foods, include the liquid in which it was canned. For example: Sliced peaches in heavy syrup or Fruit cocktail in light syrup.

8. If you eat at a restaurant, do your best to estimate portion size and list the restaurant you ate at. List any visible fat, oil, or sauces added to your food.

9. List amount and type of oil or butter you use in the preparation of your food.

10. Do not alter your diet while you are keeping a food record.

11. Please indicate the activities you participated in during each of the days that you record your diet along with the duration of activity.

12. Please indicate the time you went to sleep and the time you awoke.

13. See the following page for an example of how to properly complete the 3-day food log.

Date __October 2, 2012__ Participant ID # ___035_____

Day of the Week __Wednesday_____

Time of day	Serving Size	Food Item	Specific Activity
7:30 am	1 cup	Cheerios	Sat still on couch
	½ cup	2% milk	
	1 cup	Apple juice	
10:00 am	1 medium	Banana	Chores in house
	1 cup	Water	
12:00 pm	2 slices	Bread – hamburger bun	Walked short
	1 slice	Cheddar cheese	Distances. To and from car
	1 patty	Hamburger	
	1 supersized	French fries	
	1 16 ounce	Regular coke	
3:30 pm	15	Crackers – Sociables	Worked at desk.
	2 Tbsp	Peanut butter	Seated.
	1 8 ounce	Juice box	
6:30 pm	5 ounces	Chicken –thigh – baked	Watched TV
	1 ½ cups	Rice	
	½ cup	Broccoli	
	1 cup	2% milk	
	½ cup	Mixed fruit – fruit cocktail with sauce	
7:45 pm	1 ½ cups	Vanilla ice cream	Watched TV
	3 Tbsp	Chocolate sauce	
10:00 pm			Fell Asleep
7:00 am			Woke up

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain).
__No, this was not a typical day's intake because I had a doctor's appointment and we went to McDonald's afterwards for lunch.

Date _____ **Participant ID # _____**

Day of the Week _____

Time of day	Serving Size	Food Item	Specific Activity

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain).

_____

_____

_____

Date _____ **Participant ID # _____**

Day of the Week _____

Time of day	Serving Size	Food Item	Specific Activity

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain).

_____

_____

_____

Date _____ **Participant ID # _____**

Day of the Week _____

Time of day	Serving Size	Food Item	Specific Activity

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain).

_____

_____

_____

APPENDIX G

24-HOUR FOOD LOG

Directions for 24-hour Food and Activity Record

1. Complete your 24-hour food record on the day before your second visit.

2. Please record and photograph each food you eat immediately after you eat it. E-mail all pictures to cms15j@my.fsu.edu.

3. Record only one food item per line.

4. Be as specific as possible when describing a food eaten: how was it cooked and the amount you ate. Don't forget to include all beverages you drink. For example: coffee with 1 tsp. cream, 12 oz. regular coke, or 8 oz. sweetened tea.

5. Include brand names or labels from food items whenever possible.

6. Record amounts eaten in household measures. For example: one cup nonfat milk, 3 ounces grilled chicken, 2 tablespoons ranch dressing, 1 medium fruit, 2 slices cheese.

7. Include the method used to prepare the food item. For example: fresh, frozen, stewed, fried, baked, canned, broiled, raw, braised.

8. For canned foods, include the liquid in which it was canned. For example: Sliced peaches in heavy syrup or fruit cocktail in light syrup.

9. If you eat at a restaurant, do your best to estimate portion size and list the restaurant you ate at. List any visible fat, oil, or sauces added to your food.

10. List amount and type of oil or butter you use in the preparation of your food.

11. Do not alter your diet while you are keeping a food record.

12. Please indicate the activities you participated in during each of the days that you record your diet along with the duration of activity.

13. Please indicate the time you went to sleep and the time you awoke.

14. See the following page for an example of how to properly complete the 24-hour food and activity record.

Date __October 2, 2012__ Participant ID # ____035_____

Day of the Week __Wednesday_____

Time of day	Serving Size	Food Item	Specific Activity
7:30 am	1 cup	Cheerios	Sat still on couch
	½ cup	2% milk	
	1 cup	Apple juice	
10:00 am	1 medium	Banana	Chores in house
	1 cup	Water	
12:00 pm	2 slices	Bread – hamburger bun	Walked short
	1 slice	Cheddar cheese	Distances. To and from car
	1 patty	Hamburger	
	1 supersized	French fries	
	1 16 ounce	Regular coke	
3:30 pm	15	Crackers – Sociables	Worked at desk.
	2 Tbsp	Peanut butter	Seated.
	1 8 ounce	Juice box	
6:30 pm	5 ounces	Chicken –thigh – baked	Watched TV
	1 ½ cups	Rice	
	½ cup	Broccoli	
	1 cup	2% milk	
	½ cup	Mixed fruit – fruit cocktail with sauce	
7:45 pm	1 ½ cups	Vanilla ice cream	Watched TV
	3 Tbsp	Chocolate sauce	
10:00 pm			Fell asleep
7:00 am			Woke up

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain). No, this was not a typical day's intake because I had a doctor's appointment and we went to McDonald's afterwards for lunch.

Date _____ **Participant ID # _____**

Day of the Week _____

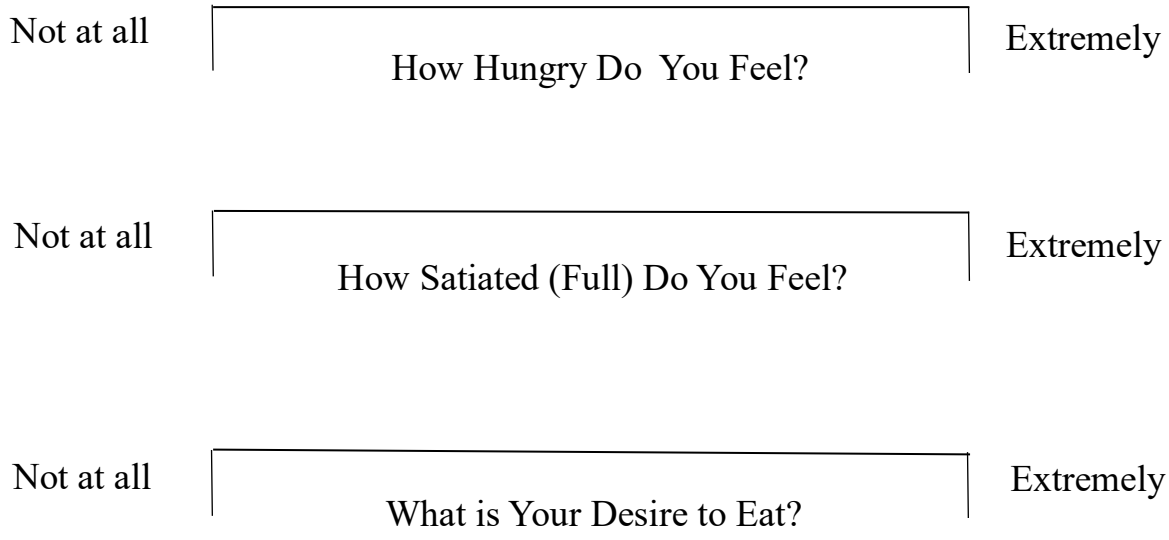
Time of day	Serving Size	Food Item	Specific Activity

Did you consume your supplement? (Y/N) _____

Was this a typical day's intake? (Y/N. If no, please explain).

APPENDIX H

VISUAL ANALOGUE SCALE



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BIOGRAPHICAL SKETCH

Christopher Schattinger received his Bachelor's degree in Exercise Science from the University of South Carolina in Columbia, South Carolina. After receiving his degree Chris was accepted to Florida State University and enrolled in the master's program. While collecting his research Chris taught lab classes in Human Anatomy and Physiology. After receiving his M.S. Chris will continue his education at Florida State University to earn his PhD in Exercise Physiology.