Analyzing Bone, Muscle and Adipose Tissue Biomarkers to Identify Osteosarcopenic Obesity Syndrome in Older Women

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ANALYZING BONE, MUSCLE AND ADIPOSE TISSUE BIOMARKERS TO IDENTIFY
OSTEOSARCOPENIC OBESITY SYNDROME IN OLDER WOMEN

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Dedicated to my beloved family: Peyman Faizian, Hossein JafariNasabian, Farrin Gharabaghi, Payam JafariNasabian. Your love and support made me who I am.
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DEFINITION OF TERMS

**Appendicular Skeletal Muscle Index:** Appendicular skeletal muscle mass (arm and leg muscle mass) adjusted by height (ASMI; kg/m²).

**Body Composition:** The relative amounts of protein, fat, water, and mineral components present in the body.

**Bone Mineral Density (BMD):** The amount of the mineral content (particularly calcium and phosphorous) in a volume of bone. BMD assessments are used to diagnose osteoporosis and osteopenia.

**Dual-energy X-ray Absorptiometry (DXA):** A manner of measuring bone density by conducting x-rays at two different energy levels across the bone.

**Frailty:** A geriatric syndrome in which deterioration in physiologic reserve escalates the risk of adverse health and physical outcomes along with physical dependence, falls, fractures, and mortality.

**Isotonic Strength:** The maximum load that can be moved through the complete range of motion accurately for no more than one entire repetition in a given exercise. Isotonic strength is measured by one repetition maximum (1RM).

**Lean Body Mass:** The non-fat and non-bone mineral content compartments of total tissue mass as assessed by DXA.

**Osteopenia:** A less severe form of bone loss, labeled as having a BMD laying between 1 and 2.5 standard deviation (SD) below average value for young healthy individuals.

**Osteoporosis:** A more severe form of bone loss generally diagnosed when an individual’s BMD being 2.5 SD or more below the average value for young healthy individuals.

**Osteosarcopenic Obesity:** The concurrent occurrence of osteoporosis/osteopenia, sarcopenia and increased adiposity (obesity).

**Quality of life:** An individual’s sense of well-being, capacity to perform various activities and overall enjoyment of life.

**Sarcopenia:** The age-related loss of skeletal muscle mass and strength, by approximately 0.5–1% loss per year after the age of 50. Sarcopenia is described as having an appendicular skeletal muscle mass (arm + leg muscle mass) adjusted by height of <7.26 kg/m² in men and <5.45 kg/m² in women.

**Sarcopenic obesity:** The coexisting oappearance of low muscle mass (sarcopenia) and high adiposity (obesity).

**Total Body Mass:** The sum of total tissue mass as determined by DXA.

**Total Body Weight:** The scale weight of a person.
ABSTRACT

Background: Osteosarcopenic obesity (OSO) is a recently identified geriatric syndrome characterized by the simultaneous presence of osteopenia/osteoporosis, sarcopenia, and increased adiposity either as overt overweight or fat infiltration into bone and muscle. The diagnostic criteria for OSO are just being established, but there are no data on biomarkers that might characterize this syndrome. Our objective was to examine possible biomarkers associated with OSO syndrome, including serum sclerostin (as a hinder of bone formation), skeletal muscle-specific troponin T (sTnT) (as an indicator of muscle turnover/damage) and serum leptin and adiponectin (as indicators of fat metabolism). C-reactive protein (CRP) and serum lipids were analyzed to evaluate the level of inflammation and lipid profile, respectively.

Methods: A total of N=59 healthy Caucasian women ≥65 years were classified into 4 groups based on bone and body composition profile identified by dual-energy x-ray absorptiometry (DXA) measurements: 1) Osteopenic obese (N=35); 2) Obese-only (N=10); 3) OSO (N=10); 4) Osteopenic/sarcopenic non-obese (N=4). Osteopenia/osteoporosis was determined by T-score of L1-L4 and/or femoral neck ≤-1. Sarcopenia was identified from appendicular lean mass adjusted for both height and fat mass, using linear regression with the residual value of ≤-1.43. Obesity cut-off was set at body fat of ≥32%. Serum samples were analyzed using ELISA kits for sclerostin, sTnT, leptin and adiponectin. CRP and lipid profile were analyzed by contracted laboratories. In addition, diet and habitual physical activity were evaluated by 3-day dietary record and the Allied Dunbar National Fitness Survey, respectively. For data analysis, Pearson’s correlations and ANOVA followed by Tukey’s tests were used with p≤0.05.

Results/Discussion: Serum sclerostin concentrations were significantly higher in the OSO and osteopenic obese group in comparison to the obese-only group (1.10±0.2; 1.09±0.2; 0.7±0.4 ng/mL, respectively). The sTnT concentrations were significantly higher in the OSO group in comparison to osteopenic obese and obese-only groups (3.2±2.9; 1.1±2.2; 0.6±0.7 pg/mL, respectively). Sclerostin was negatively correlated with bone mineral density/content (BMD/BMC) of all skeletal sites, but statistically significant only with femoral neck BMD/BMC. Moreover, there was a significant positive correlation between serum sclerostin and sTnT concentrations indicating their simultaneous mediation in bone and muscle loss. The OSO group had the highest concentrations of serum leptin but statistical significance was reached only with osteopenic/sarcopenic non-obese group (17.3±4.5; 8.7±1.1, respectively). Overall, serum leptin
had a significant negative correlation with total and femoral neck BMD/BMC and T-scores, after adjusting for weight or BMI. The osteopenic/sarcopenic non-obese group had the highest concentration of adiponectin (9.2±7.0 µg/mL) in comparison to other groups. Overall, a statistically significant negative correlation of serum adiponectin with body fat percentage was noted, as well as with the BMD and T-scores of total femur and femoral neck. The CRP concentrations for all participants ranged from 0.01 to 1.43 mg/dL. None of the CRP concentrations were above the high threshold (3.0 mg/dL). Although the highest concentrations of CRP were observed in the OSO group, there was no significant difference among groups. The highest concentrations of cholesterol and low-density lipoprotein were observed in the OSO group. The highest concentrations of triglyceride were observed in the osteopenic obese group vs. the lowest in osteopenic/sarcopenic non-obese group. Although, the lowest amount of energy and protein intake was reported in the OSO group, there was no significant difference among groups. Moreover, there was no significant difference in amount of vitamin D and calcium intake among the groups. Overall, a significant positive correlation was reported between total calcium intake, and lean/fat ratio and negative between protein intake and waist/hip ratio. Total hours of physical activity and average number of steps climbed were negatively correlated with total body fat, total body fat percentage, weight, BMI, hip circumference and abdominal circumference.

**Conclusion:** Women identified with OSO syndrome have presented with the poorest outcomes for each serum and dietary variable, although the differences were not always statistically significant. The combination of high concentrations of sclerostin, sTnT and leptin can be used to better identify the metabolic profile of OSO syndrome and can possibly be applied as measurements for its diagnostic criteria. Adequate protein, calcium and vitamin D, along with habitual physical activity should be recommended as a standard-of-care to manage bone and body composition in this population.
CHAPTER 1
INTRODUCTION

1.1 Background

Aging is accompanied by changes in body composition, where an individual experiences gradual bone and muscle mass loss and, at the same time, gaining in fat or redistribution of fat in the abdominal region and its infiltration into bone and muscle (JafariNasabian et al.; Frontera et al. 2000; Ilich et al. 2014a, 2016a). When bone loss (osteoporosis or osteopenia) is accompanied with an increase in the fat mass (obesity) or the fat’s infiltration into the bone, osteopenic obesity may occur (Ilich et al. 2014a). Similarly, with a decrease in lean/muscle tissue (sarcopenia) and an increase in infiltration of fat into muscle, the sarcopenic obesity may arise (Ilich et al. 2014a; Lee et al. 2016). The combination of all three conditions (osteoporosis/osteopenia, sarcopenia and obesity) may result in a syndrome named osteosarcopenic obesity (OSO) (Figure 1), which is associated with greater medical complications and decreased quality of life, compared to that of the each individual condition. Older individuals or those suffering from chronic conditions (e.g. cancer, diabetes) may be at a particular risk for OSO syndrome (Frontera et al. 2000; Ilich et al. 2014a, 2015, 2016a; Hita-Contreras et al. 2015). One of the most detrimental consequences to such changes is the increased risk of falling and subsequent fractures (Robinson et al. 2011). Besides aging and chronic conditions, environmental, nutritional, and lifestyle factors may affect the deterioration of all three tissues (Clark & Manini 2008; Laurent et al. 2015; JafariNasabian et al. 2017a).

Bone, muscle and fat are interconnected endocrine organs which secrete different biomarkers that each may affect bone, muscle and fat tissues (Mantzoros et al. 2011; Karsenty & Ferron 2012; Pedersen & Febbraio 2012; Ilich et al. 2014a; JafariNasabian et al. 2015, 2017b). Some of these biomarkers have been linked with osteoporosis, sarcopenia, obesity, or combination of the three conditions. For example, serum sclerostin concentration seems to be a useful biomarker for the rate of bone resorption. Sclerostin inhibits the differentiation of osteoblasts, reducing bone formation (Wijenayaka et al. 2011). Moreover, sclerostin could increase the urinary excretion of calcium and phosphorus, thereby reducing the availability of these ions for bone mineralization (refer to Specific Aim 1) (Ryan et al. 2013).
The biomarkers for muscle tissue are less known and/or investigated compared to those for bones; however, the family of troponins have shown some promising results. Troponins include a family of key regulatory proteins associated with the contractility processes of cardiac and skeletal muscle tissues. They are not normally found in the blood, except in cases of muscle turnover or muscle damage, therefore, they can serve as indicators of muscle breakdown. Skeletal muscle contraction is regulated by calcium through the troponin complex, in particular, the skeletal muscle-specific troponin T (sTnT). This complex is needed for the repetitive cycles of contraction and relaxation. Skeletal muscles are protected by several layers of connective tissue, which maintain the muscle integrity. As a result of muscle loss or muscle injury, internal components of muscle, particularly sTnT, can leak out to the blood, enabling the use of sTnT as a novel biomarker of sarcopenia (refer to Specific Aim 2) (Chase et al. 2013).

Leptin and adiponectin as adipocyte-secreted hormones have been speculated to be the connection between adipose tissue and bone (Gordeladze et al. 2002; JafariNasabian et al. 2015). Leptin can act on bone both through the peripheral and central nervous system. The peripheral effect of leptin is displayed on osteoblasts and osteoclasts to enhance bone formation and inhibit bone resorption (Gordeladze et al. 2002). Moreover, its central effect through the central nervous system can be reflected in either positive or negative modulation of bone (Karsenty 2001; Gordeladze et al. 2002). Adiponectin mediates multiple functions, such as systemic insulin sensitivity, glucose homeostasis, anti-inflammation, and bone regulation. These regulations of adiponectin target multiple organs and systems, including the liver, pancreas, and the adipose tissue itself. Decreased adiponectin levels were reported in insulin-resistant states, such as obesity (refer to Specific Aim 3) (Fasshauer et al. 2004; Nedvídková et al. 2005) and type 2 diabetes (Hotta et al. 2000).

It is important to emphasize that obese individuals have abnormal circulating levels of pro-inflammatory cytokines. It has been shown that excess adiposity can enhance inflammatory state and adipogenic signals. The net result is bone loss and muscle loss along with the maintenance of low-grade chronic inflammation (Endres 1993; Ilich et al. 2014b). In humans, C-reactive protein (CRP) is the main acute phase reactant protein secreted by various cells such as vascular, peripheral blood mononuclear, liver, and kidney cells in response to tissue injury and is indicative of inflammation (Moshage et al. 1988; Jabs et al. 2003; Haider et al. 2006). Chronic inflammation is one of the major contributors to the age-related decrements in bone and muscle
mass and increase in adiposity (refer to **Specific Aim 4**) (Cesari *et al.* 2004; Ilich *et al.* 2014b; Wåhlin-Larsson *et al.* 2014). Moreover, it has been reported that an increase in weight is associated with an increase in systemic inflammation (Fogarty *et al.* 2008).

Excess adipose tissue can be distributed commonly around the abdominal subcutaneous regions (Coutinho *et al.* 2011). Central obesity has a strong association with an increased risk of metabolic dysfunction including hyperlipidemia (Cnop *et al.* 2002; Karelis *et al.* 2004). Previous studies have revealed that greater amount of visceral fat has a significant association with higher triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and decreased high density lipoprotein (HDL) concentrations (Brochu *et al.* 2001; Palacios *et al.* 2011). This study aims to evaluate the lipid profile of the participants, in order to better investigate the OSO syndrome characteristics (refer to **Specific Aim 5**).

Since bone, muscle and fat tissues are interrelated; serum biomarkers associated with each tissue should be considered when investigating the OSO syndrome. Therefore, in this study serum biomarkers were analyzed for sclerostin as a measure of bone turnover, sTnT as a marker of sarcopenia, and leptin and adiponectin as markers of fat metabolism. Since chronic inflammation can impair each of the tissues, it is important to evaluate the inflammatory state in the context of OSO syndrome. In this study, serum CRP was chosen as a reliable marker of increased inflammation (Haider *et al.* 2006; Shrivastava *et al.* 2015). Additionally, participants’ lipid profile was evaluated to better understand the OSO syndrome effect on fat metabolism.

Nutrition plays an important role in body composition and overall health status. Poor and inadequate diet may contribute to the development of chronic diseases, including osteoporosis, sarcopenia and obesity and perpetuate low-grade chronic inflammation (Ilich *et al.* 2014b; JafariNasabian *et al.* 2017a). The important role of calcium and vitamin D on bone health has been thoroughly investigated and reported previously (Ilich & Kerstetter 2000; Ilich *et al.* 2003). Likewise, energy, protein, and calcium intake have been shown to be independently related to bone mineral density (BMD) on several skeletal sites (Ilich *et al.* 2003; JafariNasabian *et al.* 2017a). While low calcium and vitamin D status have been known to be detrimental to bone, vitamin D status is also associated with muscle mass and strength, as well as with the functionality measures (Tieland *et al.* 2013). Furthermore, a study in the Netherlands investigating older women and men showed that low vitamin D and high parathyroid hormone can increase the risk of sarcopenia as reflected in lower muscle mass and hand grip strength.
(Visser et al. 2003). It has been reported that vitamin D supplements may help improve muscle function and strength, and reduce the risk of falls and mortality (Morley et al. 2010; Post & Ilich 2013). In addition, 25-hydroxy vitamin D has been shown to be positively correlated with muscle mass and negatively correlated with fat mass in overweight and obese postmenopausal women (Truesdell et al. 2011; Wierzbicka et al. 2016). Regarding the protein intake, it has been reported that approximately 10%–25% of older adults eat less than the recommended daily allowance (RDA) and 5%–9% consume less than the Estimated Average Requirement (EAR) (Fulgoni 2008). Inadequate protein intake may further exacerbate the decline in muscle protein synthesis associated with aging (Campbell et al. 2001). Therefore, this study aims to evaluate the nutritional status of the participants, particularly in respect to calcium, vitamin D, and protein intake, in order to better investigate the OSO syndrome characteristics (refer to Specific Aim 6).

Physical activity also plays a critical role in bone and muscle strength/health, as well as in reducing the risk of adiposity (refer to Specific Aim 7). Particularly, weight bearing exercise has been shown to improve BMD (Guadalupe-Grau et al. 2009), while resistance training has been reported as the most appropriate for preventing sarcopenia and muscle loss (Rolland et al. 2011). Similarly, physical activity has an important role in reduction/maintenance of body weight (Jakicic 2003; JafariNasabian et al. 2017a). Unfortunately, the levels of physical activity decrease with age. Even healthy, physically active participants (≥ 70 years old) are still 30% less physically active than young adults (19–35 years) (Morse et al. 2004). This reduced activity can lead to exacerbated bone loss and muscle wasting (Booth et al. 2012). It has been shown that muscle wasting with aging results in decrements of muscular strength, thereby, impacting physical function and the ability to live independently (Rosenberg 1997; JafariNasabian et al. 2017a).

Although osteopenia/osteoporosis, sarcopenia, and obesity have each been recognized and assessed previously, the simultaneous appearance of derangement of each tissue is a new concept. Despite common belief, increased body fat by aging may be detrimental for both bone and muscle (Liu et al. 2010, 2014; Tang et al. 2010; Ilich et al. 2014a). Aging is accompanied by a redistribution of fat to the intra-abdominal area (visceral fat) and fat infiltration in bone and muscle, contributing to the conditions known as osteopenic obesity (Ilich et al. 2014a, 2016a; JafariNasabian et al. 2017a) and sarcopenic obesity, respectively (Baumgartner 2000). Osteopenic obesity is a condition characterized by excess body fat and low BMD (Ilich et al.
Sarcopenic obesity is an evolving health problem characterized by the concurrent manifestation of excess body fat and low muscle mass and strength (Baumgartner 2000; Roubenoff 2004). Different studies have found that sarcopenic obesity is a better predictor of impaired health and worse clinical outcomes than sarcopenia or obesity alone (Roubenoff 2004; Zamboni et al. 2008; Prado et al. 2012). As the prevalence of elderly individuals increases in the United States (U.S.), so does the prevalence of health problems related to aging such as osteoporosis and sarcopenia. The addition of obesity to these existing conditions aggravates the metabolic abnormalities, which could lead to reduced physical function and increased mortality. Due to the lack of studies measuring all of the relevant variables, precise criteria are yet to be identified for diagnosing OSO syndrome since specific cut-off points are needed to accurately evaluate fat, lean, and bone tissue compartments and the metabolic profile for this syndrome.

In this study, we analyzed the serum samples of the participants to better understand the metabolic profile of the OSO syndrome. These samples were analyzed for bone and muscle biomarkers, indicative of bone and/or muscle health. Moreover, serum samples were analyzed to evaluate the effect of adiposity and inflammation on OSO syndrome. Serum samples were also analyzed to evaluate the lipid profile of the population. Additionally, we evaluated the participants’ nutritional status as well as their involvement in habitual physical activity. These parameters were combined with other bone and body composition measurements (using dual-energy x-ray absorptiometry (DXA)) to better define diagnostic criteria for OSO syndrome.

1.2 Objectives

The main objective of this study was to determine the metabolic profile of the OSO syndrome in the cohort of postmenopausal women and combine it with other physical parameters for a more complete OSO diagnosis. Specific serum markers (sclerostin as a measure of bone turnover, sTnT as a marker of sarcopenia, leptin and adiponectin as a measurement of fat metabolism and CRP as a pro-inflammatory biomarker) were analyzed to establish a better portrayal of OSO syndrome. Our secondary objective was to evaluate the lipid profile, the nutrient intake (particularly calcium, vitamin D, protein, and energy intake of the cohort) and habitual physical activity and examine the relationship with bone, muscle, and fat metabolism.

1.3 Significance

To the best of our knowledge there have been no studies investigating the metabolic profile of OSO syndrome considering biomarkers for each tissue (bone, muscle and fat) and
other aspects of their lifestyle including diet, physical activity, alcohol intake, smoking, supplements and medications. The proposed investigation addresses important issues pertinent to the newly identified OSO syndrome in older women and aims to investigate the association of different serum biomarkers with this syndrome. This type of research is fundamental for identifying the individuals with OSO and establishing the diagnostic criteria upon which possible prevention strategies and treatments can be developed.

1.4 Specific Aims and Hypotheses

Specific Aim 1: To assess bone status, by measuring serum sclerostin in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

Hypothesis 1: Women identified with OSO will have higher sclerostin concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

Specific Aim 2: To assess the muscle tissue by measuring serum sTnT concentrations in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

Hypothesis 2: Women identified with OSO will have higher serum sTnT concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

Specific Aim 3: To assess the adipose tissue status by measuring serum leptin and adiponectin concentrations in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

Hypothesis 3: Women identified with OSO will have higher serum leptin and lower adiponectin concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

Specific Aim 4: To assess the level of inflammation by analyzing the pro-inflammatory biomarker, CRP, in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

Hypothesis 4: Women identified with OSO will have higher CRP concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

Specific Aim 5: To assess the overall serum lipid profile in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

Hypothesis 5: Women identified with osteopenic/sarcopenic non-obese will have lower concentrations of cholesterol, TG, LDL, VLDL and higher concentrations of HDL in comparison to osteopenic obese, obese-only and OSO.
**Specific Aim 6:** To assess the role of nutrient intake of calcium, vitamin D, protein and energy on bone, muscle and adipose tissue status in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

**Hypothesis 6:** Women identified with OSO will have lower calcium, vitamin D, protein and energy intake in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

**Specific Aim 7:** To assess the overall fitness level by evaluating the level of habitual physical activity in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

**Hypothesis 7:** Women identified with OSO will have lower level of habitual physical activity in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

### 1.5 Assumptions

The assumptions for the present investigation are as follows:

1. Study participants correctly reported their age, menopausal and health status, and medication use, to comply with the inclusion/exclusion criteria of the protocol.
2. To the best of our knowledge, the classification of women into osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese groups was accurate.
3. All laboratory equipment and performed techniques yielded accurate measurements.
4. All participants accurately reported their current dietary intake and current habitual physical activity.
   a. All participants followed the instructions given to them regarding the maintenance of their current dietary and habitual physical activity.
   b. All self-recorded data were of accurate reporting from each participant.
Figure 1. Changes in muscle, fat, and bone tissues with aging leading to osteosarcopenic obesity syndrome (Adapted from the article by Ilich, et al., 2014 with modifications).
CHAPTER 2

REVIEW OF LITERATURE

In this review of the literature we explain each aspect of OSO syndrome separately by focusing on the classifications, epidemiology, medical costs, health complications, and underlying mechanism affecting osteoporosis/osteopenia, sarcopenia, and obesity. Bone, muscle, and fat are integrated organs that act as endocrine organs involved in secreting different biomarkers. While nutrition and physical activity might not have as powerful impact on each of the tissues as some genetic factors, they are, nevertheless, becoming increasingly important in management of geriatric diseases. We conclude this chapter by reviewing different serum biomarkers associated with bone loss, muscle mass loss, and increase fat mass and their association with OSO syndrome.

2.1 Osteoporosis/Osteopenia

2.1.1 Definition and Classification

Osteoporosis is a well-known disease of aging and is manifested by fragile bones and low BMD. It is defined as impaired bone strength and architecture, in addition with decreased BMD, leading to increased fracture risk. Osteoporosis is influenced by bone mineral density, which is the amount of bone mineral in a given area, and bone quality, the latter including the bone structure, composition and turnover rate (‘Osteoporosis Prevention, Diagnosis, and Therapy: National Institutes of Health Consensus Development Statement’ 2000). Aging increases the risk for low BMD, which can severely increase the risk of fractures and disabilities in the elderly, specifically in postmenopausal women (Eastell et al. 1989).

The gold standard test for diagnosing osteoporosis is the DXA. The World Health Organization has established the T-score value of bone mineral density for accurate identification of osteoporosis. A T-score value of BMD ≤ to 2.5 standard deviations below the normal young adult reference population is defined as osteoporosis, whereas a T-score between -2.5 to -1 is defined as osteopenia and a T-score higher than -1 is defined as normal bone density (Kanis et al. 1994).

2.1.2 Epidemiology

According to the National Osteoporosis Foundation, approximately 54 million Americans have osteoporosis/osteopenia, putting them at risk for bone fracture. This decreased bone mass is
responsible for two million broken bones per year in the U.S. (‘Osteoporosis Prevention, Diagnosis, and Therapy: National Institutes of Health Consensus Development Statement’ 2000). Based on data published from the National Center for Health Statistics, between 2005 and 2008, 52% of adults in the U.S. over the age of 50 had low BMD at either the femoral neck or lumbar spine (Looker et al. 2012).

2.1.3 Medical Costs

The cost of treating osteoporosis and its fractures has been estimated to be $19 billion annually (‘National Osteoporosis Foundation prevalence report’). Due to a constantly growing elderly population, the costs associated with osteoporosis are likely to increase significantly in the future. In 2014, one third of fall-related deaths were attributable to low BMD (Sánchez-Riera et al. 2014). Bone fractures, especially in the elderly, can lead to long-term disability and even mortality (Kanis et al. 2000).

2.1.4 Bone Fractures

Physical activity levels, BMI, race, gender and age all contribute to fracture risk (Johnell et al. 1992; Elffors et al. 1994; O’Neill et al. 1996). Bone fractures result from decreased bone mass, but may also be related to decreased bone quality and even decreased trabecular bone volume (Garnero et al. 2000; Zebaze et al. 2010). Fractures occur most commonly in the hip, distal forearm bones, and vertebrae, with hip and vertebral fractures being the most severe ones among the elderly (Chrischilles et al. 1991; Wilson et al. 2015). Hip fractures increase significantly with age, especially in women, who are two times more likely than men to fracture their hips after age fifty (Melton et al. 1988). Survival rates five years after hip and vertebral fractures are approximately eighty percent; however, these rates are heavily influenced by gender, age, health status and functionality before fracture (Cooper et al. 1993). The fractures themselves do not usually cause death, but complications associated with the fractures, including infection and trauma, can decrease the survival rates (Sernbo & Johnell 1993).

2.1.5 Bone Metabolism

Bone is an active endocrine organ continuously being reshaped in a process called remodeling or bone turnover. The turnover of bone begins with osteoclasts’ excavation on the surface of bone, thus the area becomes bone resorption pits. Subsequently, osteoblasts fill the pit with matrices and the areas of bone ossify with minerals such as calcium. Under high loading conditions, osteoblasts increase bone mass, whereas under low loading conditions, osteoclasts
remove bone tissue. Osteoporosis occurs when imbalanced bone remolding happens which favors resorption and results in decreased BMD and increased fragility (Buckwalter et al. 1996). Several factors play a role in bone metabolism as discussed in section 2.1.6.

2.1.6 Hormonal Changes

Hormonal changes in the elderly influence BMD in both men and women. Changes in trabecular bone volume are related to decline in sex steroid hormones, primarily estrogen and testosterone (Khosla et al. 2005a, b). Particularly, postmenopausal women are most at risk for developing osteoporosis and osteopenia, due to decrease in their estrogen level (Specht 1980). Research has shown that estrogen deprivation upregulates production of osteoclasts that reabsorb bone and increases expression of inflammatory cytokines (Cenci et al. 2000). After menopause, with declining estrogen concentrations, elderly women have a considerable increase in overall bone turnover rate. The high bone turnover rate results in low BMD (Garnero et al. 1996a). Estrogen increases production of growth factors and procollagen synthesis; it has an important role in regulating osteoblasts, thus, it has a protective effect on bone. Estrogen also influences receptor activator or nuclear factor-KB ligand (RANKL) and osteoprotegerin (OPG) ratios which is known as osteoclastogenesis inhibitory factor (Ernst et al. 1989; Oursler et al. 1991; Eghbali-Fatourechi et al. 2003). As estrogen plays an important role in protecting bone against osteoporosis, its lack in older age may cause detrimental consequences in bone health. OPG/RANKL Pathway has been discussed in more details in section 2.1.8.

Another hormone involved in bone metabolism is growth hormone. Its effects are mediated by insulin-like growth factor 1 (IGF-1), which plays a role in enhancing the differentiation of osteoblasts. Growth hormone and IGF-1 (both anabolic hormones) are important regulators of bone homeostasis by regulating bone modeling and remodeling on trabecular and cortical bone (Seeman 2003; Giustina et al. 2008). Growth hormone deficiency may lead to low bone turnover and osteoporosis (Giustina et al. 2008).

2.1.7 Bone Biomarkers

While BMD provides an overview of skeletal status assessing various skeletal sites and is used as a proxy to diagnose osteoporosis in women (Weissman 1987), bone turnover markers provide more information about the dynamic processes in bone remodeling. Hence, testing bone turnover markers enables researchers to gain a better understanding of changes in bone
metabolism and allows for quicker assessment of changes in bone (Hauschka et al. 1989; Kalaiselvi et al. 2013).

Osteocalcin is the most abundant non-collagen protein in bone. Osteocalcin, also referred to as bone γ-carboxyglutamic acid (Gla) protein and is produced predominantly by osteoblasts. Smaller amounts of osteocalcin are produced in teeth by odontoblasts and hypertrophic chondrocytes (Hauschka et al. 1989). Once synthesized, it is largely combined into the extracellular bone matrix and thus presents an important component of bone extracellular matrix. Serum osteocalcin secretion occurs in a small portion in blood circulation and is regarded as one of the bone formation markers. Osteocalcin is synthesized as a pre-pro-osteocalcin that is cleaved after translocating into the endoplasmic reticulum. After translocation, the majority of osteocalcin is completely carboxylated. Osteocalcin will be deposited into the bone extracellular matrix with calcium ion or released into blood circulation. Due to decarboxylation or low activity of vitamin-K-dependent γ-glutamyl carboxylase, some osteocalcin is partially carboxylated (undercarboxylated osteocalcin) (Lee et al. 2000). Undercarboxylated osteocalcin does not readily bind to hydroxyapatite which prompts its leakage into the blood (Price & Nishimoto 1980). Increased serum osteocalcin, particularly the undercarboxilated fraction, is associated with an increasing rate of bone formation. Osteocalcin is thought to play an important role in skeletal development and is considered a marker of bone formation and bone remodeling (Kruse & Kracht 1986; Seibel et al. 1997). However, conflicting results of other studies have reported that osteocalcin may work as a negative regulator of bone formation. For example, in a study conducted by Ducy et al, osteocalcin-deficient mice (osteocalcin knockout mutants) have increased BMD (Ducy et al. 1996). Moreover, it has been demonstrated that osteocalcin is negatively associated with total body, hip and forearm BMD in healthy postmenopausal women (Kalaiselvi et al. 2013).

There are other markers of bone turnover (formation/resorption), namely, bone specific alkaline phosphatase (BALP), serum amino- (N-) terminal cross-linking telopeptides of type I collagen (NTx) and urine Carboxyl- (C-) terminal cross-linking telopeptides of type I collagen (CTx), tartrate-resistant acid phosphatase (TRAP) and the newest one, sclerostin, that have been used with more or less success to assess bone metabolism (Garnero et al. 1996a; Taga & Minaguchi 1997; Kawana et al. 2002; Čepelak & Čvorišće 2009; Roforth et al. 2014).
BALP is a protein secreted by osteoblasts, and some cells in liver, kidney and some other tissues (Ma et al. 2008). It is considered to play a role in osteoid formation and mineralization and it is considered a marker of bone turnover (Singer & Eyre 2008). In women with osteoporosis, BALP was found to be elevated but the concentration goes down with treatment (Kress et al. 1999).

NTx and CTx are both amino acid sequences (telopeptides) that have been associated with bone turnover. Garnero et al. (Garnero et al. 1996b) indicated that NTx and CTx concentrations are significantly higher in postmenopausal women, and they are linked with increase rate of bone turnover and risk of hip fracture. Moreover, serum CTx, as well as urinary CTx, have been shown to be associated with increased rate of bone resorption in patients with vertebral fractures and hip fractures (Kawana et al. 2002).

TRAP is an iron-containing enzyme that is secreted from diverse tissues including bone and cartilage (Hayman et al. 2000). TRAP is expressed highly in osteoclasts and, therefore, used as a specific marker for osteoclasts (Burstone 1959). TRAP, as a marker of bone resorption, can be used to measure osteoclast activity directly. Increased levels of TRAP occur during bone resorption. It is a useful indicator of anti-resorptive treatment for osteoporosis as well as an early detection marker for risk of osteoporosis (Habermann et al. 2007). However, TRAP activity measurement in serum has many disadvantages including relatively low activity, presence of inhibitors, instability by alkaline pH, interference by hemolysis, therefore, it is not recommended to be used for diagnosing bone resorption (Čepelak & Čvorišćec 2009).

Sclerostin is a protein that is mainly secreted by osteocytes and its concentrations increase with aging (Roforth et al. 2014). Sclerostin inhibits the differentiation of osteoblasts and reduces bone formation. In addition to the anti-anabolic action, sclerostin is capable of stimulating osteoclast differentiation through OPG/RANKL pathway (refer to section 2.1.8) and therefore can indirectly promote bone resorption (Wijenayaka et al. 2011). Moreover, sclerostin negatively regulates bone mineralization and can increase urinary excretion of calcium and phosphorus, thereby reducing the availability of these ions for bone mineralization (Ryan et al. 2013). Since this is one of the newer bone markers and since it showed promising results in evaluating activity of osteoblasts, osteoclasts and it’s relation with BMD we chose to analyze this marker to evaluate bone status.
2.1.8 OPG/RANKL Pathway

Osteoblasts play a role in modulating osteoclast activation and differentiation by secreting the receptor activator of RANKL and OPG. RANKL, a protein expressed by osteoblasts, plays a key role in osteoclast formation, function, and survival through interactions with the receptor activator of nuclear factor κ B (RANK), expressed on the surface of osteoclasts. RANKL binds to RANK on osteoclast’s precursor (preosteoclast) which initiates multinucleated osteoclast formation, thereby promoting bone resorption (Hofbauer & Schoppet 2004; Rosen & Bouxsein 2006). OPG is another protein secreted by osteoblast that acts as a natural inhibitor of RANKL, thus, it plays a role in regulating the bone resorption. When OPG binds to RANKL, it inhibits mature osteoclast formation and the survival of pre-existing osteoclasts, thereby inhibiting bone resorption and bone loss (Rosen & Bouxsein 2006). An imbalanced ratio of OPG/RANKL may lead to accelerated bone resorption, and subsequently osteoporosis (Horwood et al. 1998; Nagai & Sato 1999).

2.1.9 Obesity and Bone

Along with osteoporosis, obesity is also a major public health issue in the U.S., particularly in postmenopausal women. As noted by the World Health Organization (WHO) factsheet in 2013, the prevalence of obesity has doubled since 1980 (‘Obesity and overweight’ 2013). Because of the progressively rising prevalence of obesity and its related clinical and socioeconomic consequences, the necessity of effective treatment strategies have been one of the primary focuses of the medical community.

The effect of obesity on osteoporosis has been widely investigated and the results are controversial (Felson et al. 1993; Rosen & Bouxsein 2006; Zhao et al. 2008; Liu et al. 2014). Evidence in epidemiological studies indicates that obesity may or may not have a protective factor against osteoporosis (Rico et al. 2002; Hsu et al. 2006; Compston et al. 2011). While Rico et al. 2002 (Rico et al. 2002) showed that obese individuals had higher BMD in comparison to non-obese, Hsu et al. 2006 and Compston et al. 2011 (Hsu et al. 2006; Compston et al. 2011) showed that obesity and higher body fat percentage has a correlation with increased risk of fracture and is not protective against bone fracture in postmenopausal women. The complex mechanism of the relationship between bone, fat, and muscle include multiple factors. Some studies have shown that obesity is correlated with greater bone mass, bone strength and even bone quality throughout the lifespan, mostly as a result of increased mechanical loading (Castro
Mechanical loading from increased fat and/or lean mass, altered hormonal status and serum levels of cytokines and adipokines (Shapses & Sukumar 2012). Mechanical loading stimulates bone formation by decreased apoptosis and also increases proliferation and differentiation of osteoblasts and osteocytes (Ehrlich & Lanyon 2002; Cao & Picklo 2015). In addition to providing added mechanical loading, obesity also promotes higher levels of estrogen and leptin, which are generally believed to preserve bone mass and decrease the susceptibility to fracture (Berg et al. 2015). Osteoblasts and adipocytes share common mesenchymal stem cell lineages in the bone microenvironment, which when deregulated via inflammation, increase adipogenesis and suppress osteoblastogenesis (Gimble et al. 2006; Rosen & Bouxsein 2006; Deshpande et al. 2013; Ilich et al. 2014b).

Adipose tissue was traditionally considered as energy storage (Flynn et al. 1990). It is now considered as an active endocrine organ (Dodds et al. 1994; Fernández-Real & Ricart 2003; Wellen & Hotamisligil 2005; Cao 2011). Excess adipose tissue, specifically the one distributed around visceral organs, secretes cytokines including interleukin 6 (IL-6), interleukin 1 (IL-1), and tumor necrosis factor-alpha (TNF-α), which may be detrimental to bone (Dodds et al. 1994; Fernández-Real & Ricart 2003; Wellen & Hotamisligil 2005; Cao 2011; Mantzoros et al. 2011; Ilich et al. 2014b). Excessive adiposity creates a type of pro-inflammatory state that appears to favor fat accrual at the expense of bone. Elevated pro-inflammatory cytokines such as TNF-α and IL-1, activate osteoclasts as they promote a chronic inflammatory state and results in bone loss. IL-6 as a pro-inflammatory cytokine is a known bone-resorbing factor, stimulating osteoclastogenesis (Dodds et al. 1994; Ilich et al. 2014b; Krisher & Bar-Shavit 2014).

Besides these metabolic and physiologic changes, obese individuals also have a higher risk for falls than non-obese adults albeit a higher risk of fracture. Elevated adiposity also contributes to greater force during a fall, which impacts fracture risk (Berg et al. 2015; Scott et al. 2015). However, adipose tissue is also a source of extra-glandular estrogen synthesis, and as stated previously, estrogen helps reduce the risk of bone weakening (Douchi et al. 2000). There is a very fine line at which adiposity stops being beneficial for bone and starts to have adverse effects. In a cross-sectional study conducted by Liu et al. (Liu et al. 2014) on 471 healthy women in 2014, it was reported that there was a negative correlation between body fat mass and BMD as long as the percentage of body fat was higher than 33%. In conclusion, overweight or obesity after a certain amount of fat may not be a protective factor against osteoporosis in females.
Overall, obesity does not appear to be as protective to bone health as once thought, especially in older women (Ilich et al. 2014a; Shin et al. 2014).

2.2 Sarcopenia

2.2.1 Definition, Epidemiology and Medical Costs

Sarcopenia is an age-related loss of muscle mass, which was first defined in the late 1980s by Irwin Rosenberg (Rosenberg 1997). The European Working Group on Sarcopenia in Older People has defined sarcopenia as a generalized and progressive loss of skeletal muscle mass and strength, with increased risk of adverse outcomes such as diminished physical function, physical disability, and poor quality of life (Cruz-Jentoft et al. 2010). Sarcopenia can coincide with other complications prevalent in elders such as anorexia, frailty, osteoporosis, obesity, and falls (Cruz-Jentoft et al. 2010; Rolland et al. 2011). Sarcopenia is diagnosed by measuring appendicular skeletal muscle mass (ASM), the sum of arm and leg muscle mass, adjusted by squared height (ASM; kg/m²) of two standard deviations or more below the young adult mean of the reference population (Morley et al. 2001). It is also defined as having an ASM index (ASMI) of <7.26 kg/m² in men and < 5.45 kg/m² in women (Baumgartner et al. 1999).

As per some estimates, sarcopenia affects 5 to 13% and 11 to 50% of adults between the age of 60-70 years old, and over the age of 80 years, respectively (Morley 2008). The WHO estimates more than 50 million people worldwide are sarcopenic, with the prevalence expected to increase to 1.2 billion people by 2025 (Cruz-Jentoft et al. 2010).

The estimated economic costs associated with sarcopenia were $18.5 billion dollars in the U.S. in 2000. 10% reduction in the prevalence of sarcopenia is estimated to save $1.1 billion dollars per year in healthcare costs (Janssen et al. 2004).

2.2.2 Underlying Mechanism of Muscle Loss by Aging

Sarcopenia has a complex multifactorial etiology, including decrease in physical activity, malnutrition, changes in neuromuscular function, changes in hormonal levels, decrease in protein synthesis, decrease in mitochondrial content and function, increase in inflammation and oxidative stress, and increase in adiposity (Rosenberg 1997). All of these factors together and independently contribute to the loss of muscle mass, function, and muscle quality (Buford et al. 2010). Aging is thought to be associated with decrease in muscle mitochondrial content and function, decrease in hormonal levels including androgens, growth hormone and IGF-1, and increase in inflammation (increased pro-inflammatory cytokines) that contribute to sarcopenia.
(Doran et al. 2009). Insufficient energy intake, especially protein intake, is common in the elderly and can increase muscle wasting (Morley 2001). Anorexia from aging, caused by loss of appetite, change in gastrointestinal hormones, and social and economic limitations deprive the skeletal muscles of amino acids required for protein synthesis, thereby causing protein degradation (Morley 2001; Bales & Ritchie 2002; Waters et al. 2008). A positive association between amount of protein intake and lean mass in older adults has been reported. In a study conducted by Houston et al., (Houston et al. 2008) men and women (over the age of 70) who consumed greater than the RDA recommendation (1.1 g/kg/day) had smaller decrements in lean mass over three years than those that consumed protein at or below the RDA (0.8 g/kg/day).

In addition to the aforementioned mechanisms, hormonal changes in androgens, insulin, growth hormone and IGF-1 contribute to loss of muscle mass with aging (McIntire & Hoffman 2011; Sakuma & Yamaguchi 2012). Insulin inhibits muscle protein breakdown and therefore shifts the balance of protein turnover towards protein synthesis (Rasmussen & Phillips 2003). Growth hormone acting via IGF-1 has an important role in the regulation of skeletal muscle mass. IGF-1 produced in skeletal muscle declines with aging and plays a crucial role in muscle hypertrophy and repair (Philippou et al.).

Even though estrogen has beta receptors on skeletal muscle membranes (Brown 2008), the direct effect of estrogen on muscle mass is controversial with some studies showing a positive correlation with muscle mass (Iannuzzi-Sucich et al. 2002) and others reporting no association in women ≥65 years of age (Baumgartner et al. 1999).

2.2.3 Chronic Inflammation and Muscle Loss

Research has also supported the role of chronic inflammation and oxidative stress on age-related muscle loss (Moylan & Reid 2007; Siu et al. 2008; Chung et al. 2009). Sarcopenic obesity is a condition associated with increased adiposity, which in turn can increase the inflammatory environment. This increase in the inflammation may accelerate muscle loss (Ferrante 2007; Shrager et al. 2007; Thornell 2011; Ilich et al. 2014a, b). Aging is associated with increased reactive oxygen species and elevated pro-inflammatory mediators (such as TNF-α, IL-6, and Interferon gamma (IFN-γ)) (Chung et al. 2009; Meng & Yu 2010). The continuous activation of reactive oxygen species and the inflammatory mediators (TNF-α, IL-6) causes a chronic inflammatory state that increases protein degradation (Wyke & Tisdale 2005; Clavel et al. 2006; Sishi et al. 2011). Evidence suggests that TNF-α triggers apoptosis in muscle tissue via
mitochondrial dysfunction (Marzetti et al. 2008). When TNF-α binds to the its receptor, also known as the death receptor, muscle atrophy occurs as a result of apoptosis (Jo et al. 2012) (Pistilli et al. 2006). In addition, research has shown the negative association between CRP (pro-inflammatory cytokine) and fat-adjusted appendicular lean mass and positive association with BMI and fat percentage (Cesari et al. 2005). Therefore, obesity-associated inflammation may play an important role in the age-related process that leads to sarcopenia (refer to Specific Aim 4).

2.2.5 Protein Needs of Aging Adults

The RDA for protein is 0.8 g/kg/day for adults over the age of 19 years. However, nitrogen balance studies have suggested older adults need to consume a higher amount of protein (1.4-1.6 g/kg/day) (Campbell et al. 1994; Breen & Phillips 2013; Churchward-Venne et al. 2014). Approximately 10%-25% of older adults eat less protein than the RDA and 5%-9% of consume less than the EAR of protein (Fulgoni 2008). Inadequate protein intake combined with physical inactivity may further exacerbate the decline in muscle protein synthesis associated with aging adults. In a 14-week study conducted by Campbell and colleagues, men and women age 54-78 were provided with eucaloric diets of 0.8 g/kg protein per day, 21% reduction in nitrogen excretion was reported and was significantly correlated (r=0.83) with a \(-1.7 \pm 0.6\) cm\(^2\) decrease in thigh muscle cross-sectional area (Campbell et al. 2001). A decrease in nitrogen excretion may indicate the compromise of muscle protein synthesis in order to conserve total body protein in response to decreased protein intake. These findings indicate that the RDA for protein (0.8 g/kg/day) may not be adequate for the maintenance of skeletal muscle in aging adults.

Age-related muscle loss may involve a reduced response to anabolic factors of muscle protein synthesis. Anabolic resistance to amino acid feeding may cause a diminished post-absorptive muscle protein synthesis which leads to muscle loss (Guillet et al. 2004; Katsanos et al. 2005). Several pathways have been associated with anabolic resistance of muscle protein synthesis with aging, including impaired digestion and absorption of protein (Boirie et al. 1997), reduced amino acid uptake (Dickinson et al. 2013), as well as insulin-regulated tissue perfusion, (Rasmussen et al. 2006) and diminished signaling protein activity (Fry & Rasmussen 2011; Burd et al. 2013). Nevertheless, physical activity performed prior to ingestion of protein can reverse the anabolic resistance to muscle protein synthesis (Pennings et al. 2011). Resistance training has been shown to be the most appropriate for preventing sarcopenia and muscle loss (Rolland et al. 2006).
2011). It has been reported that muscle wasting by aging will result in decrements in muscular strength thereby impacting physical function and the ability to live independently (Rosenberg 1997). The benefits of physical activity are well documented, the physiological benefits of physical activity in skeletal muscle include improvements in mitochondrial content and function (Melov et al. 2007), satellite cell signaling (Snijders et al. 2009), muscle protein synthesis and reduced long-term inflammation (Grewe et al. 2001). The levels of physical activity decrease with age. A study by Morse et al., reported that even healthy physically active participants (≥ 70 years old) were still 30% less physically active than young adults (19–35 years) (Morse et al. 2004). This emphasizes on the importance of regular physical activity in older adults.

The aforementioned mechanisms of the inability of aging adults to fully utilize protein for muscle protein synthesis indicate the need for increased dietary protein in this population. Further, increased protein intake to 1.4–1.6 g/kg/day may be most optimal in older adults with or at risk of severe muscle wasting (Breen & Phillips 2013; Churchward-Venne et al. 2014). In this study we assessed the amount of protein intake in OSO women and compared it with osteoporotic obese, obese-only and osteoporotic non-obese (Specific Aim 6). We hypothesized that women identified with OSO will have significantly lower protein intake compared to other women.

2.2.6 Biomarkers of Sarcopenia

Skeletal muscle has been identified as an endocrine organ and is involved in secretion of different biomarkers including IL-6, IL-7, and myostin (Pedersen & Febbraio 2012). IL-6 as a pro-inflammatory cytokine has also been recognized as a myokine, since it is secreted by contracting skeletal myocytes. Following exercise, IL-6 plasma concentration may increase gradually up to 100 times of the basal concentration (Pedersen & Febbraio 2012; Pedersen 2013; Catoire & Kersten 2015). Recent research has supported the role of human skeletal muscle cells in secreting IL-6 and increase in its concentration by muscle contraction (Lambernd et al. 2012; Raschke & Eckel 2013). IL-6 will increase during exercise without any sign of muscle damage (Fischer 2006). In addition, exercise promotes secretion of anti-inflammatory cytokines, including IL-1 receptor antagonist, IL-10, and soluble tumor necrosis factor receptor (TNFR) which is the inhibitor of TNF-α (Pedersen 2013). On the other hand, elevated circulating levels of IL-6 are known to be associated with a reduction in IL-10 as an anti-inflammatory cytokine (Michaud et al. 2013), therefore, high levels of IL-6 may promote inflammation. Beneficial
effects of IL-6 are associated with its transient production and short-term action by exercise. On the contrary, persistent inflammatory conditions, as a result of chronic diseases, are associated with long-lasting elevated IL-6 levels. In the latter situation, IL-6 has shown to be associated with muscle damage (Barbieri et al. 2003).

The research on myostatin as a growth and differentiation factor 8 (GDF-8) has yielded conflicting results. McPherron et al. (1997) conducted a study on mice carrying a targeted inactivation GDF-8 (myostatin) revealed an increase in muscle cell hyperplasia and hypertrophy. These results suggest that myostatin functions specifically as a negative regulator of skeletal muscle growth (McPherron et al. 1997). Myostatin may have a strong capacity to inhibit skeletal muscle growth. In this context it could be associated with sarcopenia. Elevated serum and mRNA myostatin levels were reported in old individuals as compared with their young counterparts (Yarasheski et al. 2002; Léger et al. 2008). Myostatin levels in skeletal muscle may be significantly modulated by the content of adipose tissue, especially in patients suffering from sarcopenic obesity. Elevated myostatin secretion and expression have been found in skeletal muscle and adipose tissue samples derived from obese women (Hittel et al. 2009). However, other human reports did not demonstrate age-related differences in either circulating protein or skeletal muscle myostatin mRNA levels (Welle et al. 2002; Ratkevicius et al. 2011). These controversial findings suggest that myostatin may not be a primary trigger of sarcopenia or the best choice for diagnosing it.

Troponins include a family of key regulatory proteins associated with the contractility process of cardiac and skeletal muscle tissues and are not normally found in the blood, except for muscle turnover or muscle damage. Skeletal muscle contraction is regulated by calcium ion over the troponin complex and the sTnT. This complex is needed for the repetitive cycles of contraction and relaxation. Skeletal muscles are protected by several layers of connective tissue, which maintain the muscle integrity. If this barrier is injured, internal components of muscle, particularly sTnT, can leak out to the blood, thus, the presence of sTnT in blood could be interpreted as pathological condition, suggesting the use of sTnT as a novel biomarker of sarcopenia (Specific Aim 2) (Chase et al. 2013).

2.2.7 Sarcopenia and Obesity

The coexistence of diminished muscle mass/strength and increased fat mass is referred to as sarcopenic obesity, first identified by Baugartner (Baumgartner 2000). Current definitions of
sarcopenia, however, include not only diminished muscle mass but also measures of weakness and physical function (Baumgartner 2000; Zamboni et al. 2008). Muscle tissue peaks between 30-40 years of age and then gradually declines as some individuals may lose up to 40% of their muscle mass by the time they reach 70–80 years of age (Cruz-Jentoft et al. 2010). By aging, intramuscular and visceral fat increases which contributes to muscle fatigue, fragility, and lifestyle-related diseases (Cree et al. 2004; Lang et al. 2010). Therefore, muscle mass/quality and adipose tissue are interrelated. This will be discussed more under osteosarcopenic obesity section (2.4).

2.3 Obesity

2.3.1 Definition and Classifications

Obesity is a chronic condition generally defined as a disease in which body fat has accumulated to an extent where health is adversely affected by comorbidities and increased risk for premature death (Haslam & James 2005). A more clinically relevant definition of obesity relies on the basis of BMI which is calculated by dividing body mass by the square of body height (kg/m\(^2\)), serves as an experimental proxy for adiposity given its positive correlation to both body fat percentage and total body fat (Keys et al. 1972; Gray & Fujioka 1991). Even though BMI is a widely applied utility for weight-status classification for most epidemiologic and clinical investigations, it is controversial in its use as a diagnostic criterion (Keys et al. 1972; ‘Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults.’ 1998). Based on BMI-referenced classifications provided by the WHO, it identifies BMI of less than 18.5 kg/m\(^2\) as underweight which may be indicative of health problems linked to malnutrition and eating disorders (‘Obesity: preventing and managing the global epidemic. Report of a WHO consultation.’ 2000). Normal BMI ranges from 18.5 to 24.9 kg/m\(^2\), while overweight is associated with a BMI between 25.0 and 29.9 kg/m\(^2\) (‘Obesity: preventing and managing the global epidemic. Report of a WHO consultation.’ 2000). Adults with a BMI ≥ 30.0 kg/m\(^2\) are considered obese which can be further subdivided into several classes that correspond to the severity of disease manifestation. These classes include class I (moderate with BMI ranges of 30.0-34.9 kg/m\(^2\) ), II (severe, 35.0-39.9 kg/m\(^2\) ), and III (very severe, ≥ 40.0 kg/m\(^2\) ) (‘Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults.’ 1998, ‘Obesity: preventing and managing the global epidemic. Report of a WHO consultation.’ 2000; Berrington de Gonzalez et
al. 2010). With respect to clinical application of BMI as a diagnostic and prognostic utility, these values remain disputed (‘Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults.’ 1998; Berrington de Gonzalez et al. 2010).

2.3.2 Central Obesity

While BMI is widely used to monitor the prevalence of obesity, it provides no information about the distribution of body fat. One common fat distribution pattern occurs when the excess adipose tissue is more pronounced in the abdominal subcutaneous regions (Coutinho et al. 2011). Individuals with this pattern of obesity tend to have more health complications than individuals with more excess adipose tissue in their legs and buttocks regions. Therefore, abdominal obesity is more closely related to coronary heart disease (CHD) morbidity and mortality than is BMI (Lee et al. 2008). Moreover, central obesity is progressively related to lower HDL and higher TG which are both closely related to an increased risk of CHD and metabolic syndrome (Arthur et al. 2012).

2.3.3 Relationship of Body Composition with Lipid Profile

BMI have been used in combination with serum lipid profile as a useful screening tool for health risk in clinical settings (Müller et al. 2012; Ilich et al. 2016b). However, the diagnostic value of these measures would be limited as inability of BMI to evaluate fat distribution and infiltration into the bone and muscle.

Excess adipose tissue can be distributed throughout the body in a variety of ways, however, one common distribution pattern is central obesity (Coutinho et al. 2011). Central obesity has stronger associations with risk of metabolic dysfunction than the overall excess body fat (Cnop et al. 2002; Karelis et al. 2004). Brochu et al., (Brochu et al. 2001) revealed a significant association between higher amount of visceral fat and higher TG and lower HDL concentrations. In another study, central obesity was significantly associated with high LDL and low HDL in adults (Palacios et al. 2011). In addition to high fat, recent studies have revealed that low muscle mass is also associated with dyslipidemia (Specific Aim 5) (Messier et al. 2009; Hwang et al. 2012).
2.3.4 Excess Adiposity and Body Fat Percentage

Aging results in 5-25% decrease in basal (resting) metabolic rate which leads to gain in body fat and weight (St-Onge & Gallagher 2010). The distribution of fat in the body will change as we get older by increasing the amount of fat mass in the abdominal area and visceral organs, as well as its increased fat infiltration into bone and muscle (Hunter et al. 2010; Lang et al. 2010; Kawai et al. 2012; Ilich et al. 2014a).

As it has been mentioned, all three tissues are closely interrelated, thus, osteopenia/osteoporosis, sarcopenia and increased adiposity need to be evaluated concurrently (JafariNasabian et al.; Ilich et al. 2014a). Although very important, fat tissue is still kept out of the picture in most cases, and not evaluated fully in the scope of its interrelation with the bone and muscle. As it has been discussed in in section 2.3.1, obesity assessments in the clinical setting are often based on BMI; however, this will not reflect the impact of fat mass on bone, muscle and its outcome on physical ability. The lack of precision of BMI for the classification of individuals has been addressed (Coutinho et al. 2011). More appropriate assessment, especially in older individuals, would be to assess the percent body fat using the DXA (Shea et al. 2012). However, there is still a disagreement as to what level of body fat percentage should define obesity in women or men. Based on the most current recommendation for women by the American Society of Bariatric Physicians body fat mass $\geq$ 32% (obtained by DXA) will count as excess adiposity (‘Obesity Algorithm: Clinical Guidelines for Obesity Treatment’ 2015). In addition, recently 32% body fat has been used as the cutoff for overweight/obesity in women (Ilich et al. 2016a; Wanner et al. 2016; Inglis et al. 2017; JafariNasabian et al. 2017b). In other studies, 35% body fat was used as a cutoff for classification of obesity (Ilich et al. 2015) and body fat above 33% showed adverse influence on various skeletal sites (Liu et al. 2014).

2.3.5 Epidemiology

Despite numerous interventions, overweight and obesity are increasing at an epidemic rate within the past three decades. As noted by the WHO factsheet, the prevalence of obesity has doubled since 1980 and it is becoming a widespread multifactorial disease (‘Obesity and overweight’ 2013). The most comprehensive data on the prevalence of obesity in the U.S. can be derived from the National Health and Nutrition Examination Survey (NHANES). Epidemiological data reveals that 68.8% of U.S. adults are overweight or obese (BMI $\geq$ 25
kg/m$^2$). Moreover, the prevalence of severe obesity (class II) and very severe obesity (class III) in the U.S. is 9.1% and 6.3%, respectively (Flegal et al. 2012).

In terms of epidemiological trends, NHANES data reported a steady growth in the obese population since the late 1980s, with the age-adjusted prevalence increasing from approximately 23% in NHANES III (1988-1994) to 36% in 2009-2010 (‘Prevalence of obesity in the United States, 2009–2010’; Flegal et al. 2012). Recent projections based on prior NHANES reports predict that 51.1% of U.S. adults will be obese by 2030 if the epidemic trend remains unresolved (Wang et al. 2008). Because of these projections and concern for imminent socioeconomic instability, national health initiatives, such as Healthy People 2020, have been established in part to reduce the prevalence of adulthood obesity by 10%. Achieving this agenda would be expected to alleviate the growing public health and socioeconomic burdens directly attributable to the obesity epidemic and perhaps initiate restoration of the current nationwide health status.

2.3.6 Medical Costs

The economic impact of obesity is especially manifested in costs associated with healthcare burden and loss of productivity (Colditz 1999; Cawley & Meyerhoefer 2012). The annual medical costs for people who are obese are $1,429 higher than that in comparison with normal weight population. Because of the broad spectrum of acute and long-term complications related to obesity, it is imposing substantial economic costs. In 2008, medical costs associated with obesity in U.S. was estimated to be around $147 billion which nearly one-half was paid by government or tax payers while the other half was financed by private insurers (i.e. Medicare and Medicaid) (Finkelstein et al. 2009). This suggests that the economic burden of obesity is shared in society as taxes and insurance premiums finance the growing burden of medical costs associated with the management of obesity and related complications. This rise in the prevalence of obesity is associated with comorbidities such as type II diabetes mellitus, hypertension, hyperlipidemia, CHD, stroke, asthma, obstructive sleep apnea, osteoarthritis, renal failure, cancer and so on (Reilly & Kelly 2011). Aside from all of these complications, depending on age and race, obesity has proven to be linked with a 6 to 20 year loss in life-expectancy (LeBlanc et al. 2011). The methodological approaches for the analyses of healthcare cost are uniquely complex for obesity in that estimations are not solely based on obesity but rather on complications and comorbid conditions related to obesity (Finkelstein et al. 2009).
2.3.7 Disease Burden

The risk of developing medical conditions including type II diabetes, CHD, and cancer increase exponentially with excessive adiposity (Peeters et al. 2004; Haslam & James 2005; Rodríguez et al. 2011). The range of comorbidities associated with obesity is reflected in the metabolic syndrome which is defined by a complex interrelated risk factors for CHD and type II diabetes (‘Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III).’ 2001; Rodríguez et al. 2011). The current diagnostic criteria for diagnosing metabolic syndrome was established by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII), particularly focuses on waist circumference as a surrogate measure for central or abdominal obesity. At least three of the five following criteria are required for diagnosing metabolic syndrome: 1) hypertriglyceridemia: ≥ 150mg/dl; 2) dyslipidemia: HDL < 40mg/dl for male and < 50mg/dl for female; 3) hypertension: ≥ 130/85mmHg; 4) elevated fasting glucose: ≥ 110mg/dl; and 5) waist circumference: > 102cm for male and > 88cm for female (‘Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III).’ 2001). Central obesity remains a core pathophysiological feature of metabolic syndrome and is considered a primary category of causation (Kassi et al. 2011).

Obesity is strongly correlated with increased risk of type II diabetes regardless of gender or ethnicity, specifically when body fat is localized to the viscera (Chan et al. 1994; Colditz et al. 1995). Based on the data obtained from 114,281 female registered nurses ages 30 to 55 (Colditz et al. 1995), the risk of type II diabetes mellitus was strongly correlated with higher BMI. In fact, a BMI of 35 kg/m² significantly increased the risk for type II diabetes by 4000%. Reduction in weight has shown to be advantageous in terms of reducing risk of type II diabetes and improving insulin sensitivity. A 5-11 kg weight-loss may reduce risk for diabetes mellitus by 50%, whereas a loss of 20 kg or more could eradicate the risk entirely (Chan et al. 1994).

Another major comorbidity closely associated with obesity is heart disease. Liu et al., reported that (Liu et al. 2012) abdominal fat mass and waist circumference could be used as a predictor of blood lipid profiles and glucose in overweight or obese African American women (age, 48.7 ± 5.6 years). Manson et al., (Manson et al. 1995) reported a 3.3 fold increased risk for
CHD in women, with a BMI exceeding 29 kg/m\(^2\). Available data point towards a significant improvement in several clinical parameters related to reduced risk of CHD with weight-loss. For instance, Sjostrom and colleagues (Sjöström et al. 1997) reported a sustained change in blood pressure and triglycerides following a 5-10% weight-loss.

### 2.3.8 Mortality Risk

Based on comprehensive analysis of existing epidemiological and clinical studies and data collected from Centers for Disease Control and Prevention (1980-2002), tobacco smoking continued to be the leading cause of preventable death (Mokdad et al. 2004, 2005). Followed by, poor diet and physical inactivity, otherwise termed overweightness (National Institutes Of Health 1998), which was estimated to have caused 365,000 deaths in 2000, with nearly a one-third increase from a prior estimation in 1990 (McGinnis & Foege 1993; Mokdad et al. 2004). Jia et al. (Jia & Lubetkin 2010) suggesting that obesity has overtaken smoking as the primary U.S. health threat in terms of mortality and disease causation.

A collaborative analyses of 57 prospective studies, evaluated the relationship between BMI and mortality in 900,000 individuals revealed that all-cause mortality was lowest within an ideal BMI range of 22.5-25.0 kg/m\(^2\) (Whitlock et al. 2009). In a more current study, controlled for smoking and preexisting cancer, suggested a BMI range of 20.0-24.9 kg/m\(^2\) as optimum for survival. Findings also indicated that a 5 kg/m\(^2\) rise in the BMI corresponded with a 30% greater overall mortality rate. Additionally, with a BMI of 30-35 kg/m\(^2\), median survival declined by 2-4 years while an 8-10 year-loss was associated with a BMI of 40-45 kg/m\(^2\) (Berrington de Gonzalez et al. 2010). Accordingly, Peeters et al., (Peeters et al. 2003) discovered similar survival patterns in 3,457 participants of The Framingham Heart Study, a longitudinal research program which provided 40 years of follow-up data for mortality. Participants were 30-49 years of age at the time baseline values were collected. Results indicated that overweight, nonsmoking males lost 3.1 years while females lost 3.3 years in life-expectancy. With obesity, however, these values increased to 5.8 years-lost for males and 7.1 years-lost for females (Dawer et al. 1951; Hubert et al. 1983). Nevertheless, since BMI is an imperfect measure of total body fat and neglects to account for all anatomical partitions of adipose tissue, the mortality attributable to obesity and adiposity-related factors is likely greater than what these findings suggest.

It is evident that obesity has a negative effect on life-expectancy. In fact, the present obesity trends strongly suggests that the current U.S. generation will have a shorter life-
expectancy than their parents provided this obesity epidemic persists without resolution (Olshansky et al. 2005).

2.3.9 Weight Loss in Relation to Bone

Weight loss has been used as a medical strategy for health maintenance caused by obesity and its comorbidities in obese individuals. However, another health concern accompanying weight reduction is bone loss. Several studies have shown decreased BMD in overweight or obese women during and after weight loss (Compston et al. 1992; Pritchard et al. 1996; Chao et al. 2000; Ensrud et al. 2003). Either intentional or unintentional weight loss is accompanied by bone loss and thus can raise the risk for fractures in older women (Ensrud et al. 2003). It has been suggested that diet-induced weight loss promotes bone loss due to decreased mechanical load on the skeleton (Jensen et al. 2001)

2.3.10 Adipose Tissue and Related Biomarkers

Adipose tissue was traditionally considered as energy storage. It is now regarded as an active endocrine organ involved in the secretion of adiponectin, leptin, TNF-α, IL-6 and others (Mantzoros et al. 2011).

Leptin as an adipocyte-secreted hormone has been speculated to be a possible link between fat and bone (Thomas & Burguera 2002). Leptin can act on bone both peripherally and centrally. The peripheral effect of leptin is manifested on osteoblasts and osteoclasts to enhance bone formation and inhibit bone resorption (Gordeladze et al. 2002) and its central effect through the central nervous system can be reflected in either positive or negative modulation of bone (Karsenty 2001; Gordeladze et al. 2002). Although a positive relationship was observed earlier, conflicting results of the relationship between leptin and bone were also reported in both men and women (Iwamoto et al. 2000; Dennison et al. 2004; Weiss et al. 2006; Koroglu et al. 2011; Barbour et al. 2012). An important receptor in relation to leptin is β2-adrenergic receptors located on the surface of osteoblast and is responsible for receiving signals from the sympathetic nervous system (SNS). Previous, evidence from mice β2-adrenergic receptors-knockout showed increased bone mass and reduced receptor activator of RANKL expressions compared with the wild-type (Elefteriou et al. 2005). Leptin passes through the blood brain barrier and binds to the leptin receptor in the hypothalamus which, in turn, suppresses serotonin production in the brainstem. In the absence of serotonin, the SNS successfully sends signals to osteoblasts via norepinephrine. The binding between norepinephrine and β2-adrenergic receptors on osteoblasts increases
RANKL gene expression which activates osteoclast differentiation and the process eventually decreases bone formation and increases bone resorption (Motyl & Rosen 2012). In addition to SNS regulation, leptin also directly mediated bone marrow stromal cells, osteoblasts, and osteoclasts in a systemic manner (Mantzoros et al. 2011). Leptin favors osteoblastic differentiation from bone marrow stromal cells (Thomas et al. 1999; Hamrick et al. 2005, 2007) and it is capable of upregulating osteoblast proliferation and differentiation (Gordeladze et al. 2002) as well as increasing osteoblastic cell growth and mineralization (Reseland & Gordeladze 2002). In vivo studies demonstrated that the bone-protective effect of leptin occurs through inhibiting osteoclast formation (Steppan et al. 2000; Burguera et al. 2001).

Adiponectin is another adipocyte-derived hormone. Low concentrations of adiponectin have been associated with obesity, insulin resistance, type II diabetes, and cardiovascular disease (Arita et al. 1999; Lindsay et al. 2002). Moreover, based on in vitro studies, adiponectin promotes osteoblastogenesis, and affects osteoclastogenesis in dual way, both positively and negatively (Luo et al. 2006; Williams et al. 2009). Clinical evidence demonstrated that adiponectin is either inversely or not related to BMD (Richards et al. 2007; Kanazawa et al. 2009; Ozkurt et al. 2009; Miazgowski et al. 2012; Tenta et al. 2012). It is evident that adiponectin increased bone mass by decreasing osteoclast numbers and NTx concentrations, as well as increasing osteoblast activity and ALP mRNA expression (Oshima et al. 2005). The possible mechanism of adiponectin in suppressing osteoclast activity appears to be via OPG/RANKL pathway. However, in vitro study also indicated an opposite effect of adiponectin on osteoclasts. Luo et al. (Luo et al. 2006) discovered that osteoclast activity significantly increased in the presence of adiponectin by reducing OPG and increasing RANKL expressions in osteoblasts. Although they also found adiponectin had no direct effects on osteoclast differentiation, this study suggested that adiponectin indirectly affected osteoclast formation. Overall, adiponectin production may negatively or positively affect osteoclasts through (OPG/RANKL) pathway. In conclusion, even though adiponectin might not be the best choice to evaluate the effect of adiposity on bone due to its dual effects, it is the best biomarker of adiposity alone.

2.4 Osteosarcopenic Obesity

Body composition consists of bone, muscle, and fat tissues and each of these tissues changes with aging. Specifically, in older women there is an age-related loss of bone, loss of lean
mass and muscle strength, and gain in adiposity. This triad of bone, muscle, and adipose tissue deterioration was identified recently as an OSO syndrome (Ilich et al. 2014a). Aging may result not just in increased adiposity, but also in fat redistribution and infiltration into bone and muscle tissues (JafariNasabian et al.; Ilich et al. 2014a). Moreover, as discussed before, excessive adiposity creates a type of pro-inflammatory state which appears to favor fat accumulation at the expense of bone and muscle (Ilich et al. 2014b).

There is a lack of diagnostic criteria and cut-off points for OSO syndrome. Since OSO syndrome is a complex condition, the criteria for its definition must be based on the combination of diagnostic criteria reflecting bone, muscle, and fat impairment. Based on the results of a recent study, Ilich et al., (Ilich et al. 2016a) recommended a combination of several criteria for OSO diagnosis. These include: BMD assessed using T-scores ≤ −1.0 SD of the lumbar spine (L₁-L₄) and/or femoral neck (Kanis et al. 1994), appendicular lean mass measured by DXA and adjusted for both height (m) and fat mass (kg) (Newman et al. 2003). The 20th percentile of the residual distribution is used as the cut-off point for diagnosing sarcopenia (appendicular lean mass residual value of ≤ -1.43) and body fat is ≥32% using DXA (‘Obesity Algorithm: Clinical Guidelines for Obesity Treatment’ 2015). Refer to the Table 1 and Figure 2 for diagnostic criteria of OSO syndrome based on bone density and body composition.
<table>
<thead>
<tr>
<th>Condition</th>
<th>T-score for BMD ≤ −1.0 SD at the femoral neck, proximal femur, or lumbar spine</th>
<th>&lt;20th percentile of ALM for women</th>
<th>Fat mass ≥ 32% for women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopenia/osteoporosis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Obesity</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Osteopenic sarcopenia</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Osteopenic obesity</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sarcopenic obesity</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>OSO</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

BMD: bone mineral density; ALM: appendicular lean mass; OSO: osteosarcopenic obesity

\[
\text{ALM} = -17.4 + 18.3 \times \text{height (m)} + 0.16 \times \text{body fat (kg)}
\]

(Adapted from the article by Ilich, et al., 2016 with modifications)
CHAPTER 3
RESEARCH DESIGN AND METHODS

3.1 Target Population

This study included postmenopausal women, 65 years and older recruited from several senior facilities located in Tallahassee. These included, but are not limited to: Westminster Oaks (4449 Meandering Way, Tallahassee, FL 32308), Tallahassee Senior Center (1400 N Monroe St, Tallahassee, FL 32303) and Cherry Laurel (1009 Concord Rd, Tallahassee, FL 32308). This specific age and gender group was more likely to have diseases of aging such as osteoporosis, sarcopenia and obesity. This study was designed to establish the metabolic profile (serum biomarkers) for OSO syndrome and, together with other physical measurements, help in setting up the diagnostic criteria. Additional outcomes that were examined to strengthen the diagnostic criteria are the nutritional profile of the cohort, with emphasis on calcium, vitamin D, protein and energy intake in relation to each or combination of conditions, as well as habitual physical activity. The habitual physical activity was assessed as well to get more insight into bone, muscle, and fat and their biomarkers. The Florida State University Institutional Review Board approved this study (Appendix A).

3.2 Inclusion Criteria

Caucasian postmenopausal women, ages 65 and over, were recruited from Tallahassee, FL and surrounding areas. Women needed to be ambulatory and able to come to Florida State University Nutrition and Exercise Lab for the measurements. Those who were taking calcium and/or vitamin D supplements were also eligible to participate, as those parameters were carefully recorded and taken into account during analyses. There were no restrictions to level of education or socioeconomic status.

3.3 Exclusion Criteria

By working with only one gender (women, otherwise much more prone to both osteoporosis and sarcopenia) we were provided with a more homogeneous sample, therefore, men were not included in the study. Women diagnosed with cognitive impairment disorder, Parkinson’s disease, multiple sclerosis, systemic lupus, thyroid disease, severe rheumatoid arthritis, osteoarthritis, chronic fatigue, vertigo, cancer, kidney disease or insufficiency and hyperparathyroidism were excluded. Additionally, women taking any of the following
medications: corticosteroids, diuretic, hormone replacement therapy (HRT), anti-resorptive medications such as bisphosphonate and calcitonin or parathyroid hormone, were also excluded.

3.4 Participant Recruitment and Screening

There was an initial screening in the senior facilities where the potential participants resided (Appendix B). The participant was screened and given an orientation to the study; this was followed by telephone-interview (Appendix C) to review the study and schedule a 2-3 hour appointment at Florida State University. The study coordinator gave each participant a reminder phone call with specific instructions the night before the appointment (discussed in the following section). During the laboratory visit, the study coordinator explained the study protocol; the time commitment involved for participation and addressed all questions or concerns. If the participant expressed interest in participating, she was given the informed consent form to read and raise any additional questions and concerns before signing the agreement. A copy of the signed consent form was provided to the participant at the end of the study (Informed Consent-Appendix D).

3.5 Data Collection

3.5.1 Anthropometric and Body Composition Measurements (to assess physical parameters of participants)

Height and weight were assessed using standardized methods and recorded in cm and kg, respectively. Waist and hip circumference were taken at least two times at the narrowest part of the waist and largest protrusion around the buttocks while participant was exhaling and the average number was recorded. Waist-to-Hip Ratio and BMI were recorded. Blood pressure and heart rate were assessed in a quiet room on the brachial artery of non-dominant arm in a sitting position using an automated blood pressure device (HEM-907XL, Omron Healthcare, Inc., Bannockburn, IL), after the participants were seated for a period of five minutes. Blood pressure was taken two times within the same morning that the participant was in the lab, and the average of the two measurements was calculated (Appendix E).

Body composition measurements of total body, android, gynoid regions, and appendicular regions were measured using the DXA to assess BMD and kg and % of lean and fat tissues (Lunar iDXA, GE Healthcare, Madison, WI), as described previously (Brownbill & Ilich 2005). Moreover, BMD in hip and lumbar spine was assessed with the participant laying supine on the DXA machine, as described previously (Brownbill & Ilich 2005). ASM was calculated by
sum of the muscle mass of the four limbs assessed by DXA and then ASMI as ASM/height$^2$ (kg/m$^2$).

### 3.5.2 Blood Draw and Analysis

Prior to the laboratory testing, the participant was asked to refrain from alcohol or exercise for 24 hours. After an overnight fasting (between 8-12 hours), participant met the study coordinator at Florida State University. Fasting blood draws were taken from a forearm vein in the amount of 20 milliliters by the study coordinator. The collected blood was centrifuged for 15 minutes (3500 revolutions per minute (RPM) at 4°C) using an IEC CL31R multi-speed centrifuge (Thermo Electron Corporation, Waltham, MA). Serum was separated into individual aliquots (500μL), stored in cryotubes and frozen at -80°C until further analysis. All serum samples were analyzed for the designated biomarkers at the conclusion of the study using commercially available ELISA kits. Specific biomarkers were chosen in order to identify the diagnostic metabolic profile for the OSO syndrome. Serum biomarkers were analyzed as outlined below:

1. **Sclerostin (Specific Aim 1 and Hypothesis 1)** was analyzed with commercially available ELISA kits (RayBiotech, Norcross, GA, Code: ELH-SOST-1) according to the manufacturer’s instructions. All samples were tested at room temperature and in duplicate. 100 μL of standards and diluted samples (4 fold) were added into appropriate wells. The plate was incubated for 2.5 hours at room temperature with gentle shaking. The solution was discarded and washed 4 times with 300 μL wash buffer. 100 μL of prepared biotin antibody was added to each well and it was incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washed 4 times with wash buffer. 100μL of prepared streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and washed 4 times with wash buffer. 100μL of prepared streptavidin solution was added to each well and incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and washed 4 times with wash buffer. 100μL of tetramethylbenzidine (TMB) substrate was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. The plate was read at a wavelength of 450 nm. The coefficients of variation for the test were <10% according to the manufacturer.

2. **sTnT (Specific Aim 2 and Hypothesis 2)** was analyzed with commercially available ELISA kits (RayBiotech, Norcross, GA, Code: ELH-TroponinI-1). All reagents were prepared and steps performed at room temperature and according to the manufacturer’s
instructions. All samples were tested in duplicate. 100 µl of standards or samples (diluted 2 fold) were added to each well. The plate was incubated for 2.5 hours at room temperature with gentle shaking. 100 µl of prepared biotin antibody was added to each well and it was incubated this time for 1 hour at room temperature with gentle shaking. 100 µl of streptavidin solution was added to each well and it was incubated 45 minutes at room temperature. 100 µl of TMB was added to each well and it was incubated for 30 minutes at room temperature in the dark with gentle shaking. At the end, 50 µl of stop solution was added to each well and it was read at 450 nm immediately. The coefficients of variation for the test were <10% according to the manufacturer.

3. **Leptin** (Specific Aim 3 and Hypothesis 3) was analyzed with commercially available ELISA kits (RayBiotech, Norcross, GA, Code: ELH-Leptin-1). All reagents were prepared and steps performed at room temperature and according to the manufacturer’s instructions. All samples tested in duplicate. Each serum sample was diluted 40 fold with serum assay diluent. 100 µL of serum controls, diluted serum samples, and prepared standards were added to the 96 wells, which were then incubated at room temperature for 2.5 hours with gentle shaking. The wells were washed 3 times with 300 µL of 1× wash solution. 1X prepared biotin antibody (100 µL) was added to each well, and the plate was incubated for 1 hour with gentle shaking and washed as above. Prepared Streptavidin solution (100 µL) was added to each well, and the wells were incubated for 45 minutes at room temperature with gentle shaking. The plate was decanted and washed as above, and 100 µL of the substrate solution was added to each well. The plate was incubated and protected from light for 30 minutes with gentle shaking. 50 µL stop solution was added to each well. The plate was read on a microwell plate reader at 450 nm. The coefficients of variation for the test were <10% according to the manufacturer.

4. **Adiponectin** (Specific Aim 3 and Hypothesis 3) was analyzed with commercially available ELISA kits (RayBiotech, Norcross, GA, Code: ELH-Adiponectin-1). All reagents were prepared and steps performed at room temperature and according to the manufacturer’s instructions. All samples tested in duplicate. Each serum sample (2 µL) was diluted 30,000 fold with serum assay diluent. 100 µL of serum controls, diluted serum samples, and prepared standards were added to the 96 wells, which were then incubated at room temperature for 2.5 hours with gentle shaking. The wells were washed
3 times with 300 μL of 1× wash solution. 1X prepared biotin antibody (100 μL) was added to each well, and the plate was incubated for 1 hour with gentle shaking and washed as above. Prepared streptavidin solution (100 μL) was added to each well, and the wells were incubated for 45 minutes at room temperature with gentle shaking. The plate was decanted and washed as above, and 100 μL of the substrate solution was added to each well. The plate was incubated and protected from light for 30 minutes with gentle shaking. 50 μL stop solution was added to each well. The plate was read on a microwell plate reader at 450 nm. The coefficients of variation for the test were <10% according to the manufacturer.

5. **CRP (Specific Aim 4 and Hypothesis 4)** concentrations were analyzed using latex amino assay with commercially available clinical chemistry laboratory machine (Abbott Clinical Chemistry, Abbott Park, Illinois) at Tallahassee Memorial Hospital clinical laboratory.

6. **Lipid Profile (Specific Aim 5 and Hypothesis 5)**: Cholesterol, TG and HDL concentrations were analyzed by Abbott Clinical Chemistry, Abbott Park, Illinois at Tallahassee Memorial Hospital clinical laboratory. Enzymatic method was used to evaluate cholesterol concentrations. Glycerol phosphate oxidase was used to measure TG concentration and accelerator selective detergent was used to analyze HDL concentrations. The coefficients of variation for the cholesterol, TG and HDL were <3%, ≤ 5% and ≤ 4% according to the manufacturer. LDL concentrations were calculated using the Friedewald Formula (LDL Cholesterol = Total cholesterol – HDL cholesterol – VLDL as VLDL=Triglycerides/5).

### 3.5.3 Dietary Assessment (Specific Aim 6 and Hypothesis 6)

Each participant completed a detailed 3-day dietary record (2 weekdays and one weekend-day). A dietitian gave participants instructions on how to complete the records and how to choose typical days for reporting. The dietary record was analyzed by Food Processor, version 10.11.0 (ESHA Research, Salem, OR) for all macro and micro nutrients and energy consumption (Appendix G).

### 3.5.4 Habitual Physical Activity Assessment (Specific Aim 7 and Hypothesis 7)

In order to assess habitual physical activity, each participant completed the Allied Dunbar National Fitness Survey for older adults (Dunbar 1992). The activities examined included: heavy housework (vacuuming, mopping, scrubbing floors); gardening (heavy and light); do-it-yourself
activities (wall-papering, wall-painting); walking (distances of at least 1 mile or 20 minutes in duration); and recreational and sport activities (aerobics, bicycling, swimming, tennis) (Ilich & Brownbill 2008). Data collected included frequency and duration of each activity as hours per week based on the previous four weeks. Stair climbing was reported as the average number of steps climbed per day in and outside of the home. Walking pace was self-reported as slow, average, brisk, or fast. (Appendix H).

3.5.5 Medication and Supplement Evaluation

Each participant filled a detailed questionnaire, which included a complete record of prescribed and over the counter medication, as well as the supplements that were being used. (Appendix J). These were accounted for in the final analyses.

3.6 Anticipated Risks and Solutions

Potential physical risks to participants in this study were minimized by using skilled personnel in testing and intervention procedures. If participants expressed significant fatigue or did not feel comfortable with testing procedures, the test was not completed at that time. If at any time a participant experienced chest pain, displayed any signs or symptoms of dizziness, had any abnormal blood pressure or heart rate response to testing, emergency personnel was contacted (none of the above situation were happened during the study). In case of emergency, emergency personnel were available within five minutes on the university campus. There was some risk associated with the blood draw, particularly local bruising, tenderness, and infection. This was minimized by using only trained phlebotomists and sterile techniques/supplies. All data and samples were coded numerically and results were kept confidential throughout the course of the study. The master sheet of codes and questionnaires were retained by the study coordinator and were kept in a locked cabinet in a locked room in the Department of Nutrition, Food and Exercise Sciences. Results of this study were published and/or presented at meetings but no names, initials, or other identifying characteristics were reported. Medical records were maintained in the strictest confidence according to current legal requirements and were not revealed unless required by law.

3.7 Statistical Methods

Descriptive statistics were calculated for all variables and means and standard deviations or standard errors were reported. Distributions of outcome variables were examined for symmetry and for outliers. Extreme outliers were investigated to determine whether there were
any technical or clerical errors and if such outliers were not attributed to technical or clerical error, they were included in the analysis. Pearson correlation coefficients were calculated among nutrient intake, habitual physical activity, serum biomarkers and anthropometrics, body fat percentage, BMD, BMC and T-score. Data were evaluated among the experimental groups by One-Way Analysis of Variance (ANOVA) followed by Tukey’s multiple comparison tests to test each hypothesis. Kruskal-Wallis test was performed for non-parametric data (non-normal distribution). All significance was accepted at $p \leq 0.05$. All analyses were performed using the SPSS (v.21) statistical package (IBM®, Armonk, New York).
CHAPTER 4

RESULTS

4.1 Participants Characteristics

A total of 73 women responded to recruitment efforts for the study. Of 73 women, 4 declined to participate upon receiving a detailed description of the commitments of the study and 9 did not meet the eligibility requirements for the study for the following reasons: Parkinson’s disease (n=1), multiple sclerosis (n=1), currently undergoing treatment with specific medications (diuretic (n=3), hormone replacement therapy (n=3), or corticosteroids (n=1)) and one participant was excluded as she did not fall under the four classifications (sarcopenic obese) (discussed in Figure 2). Of the 59 participants that gave consent, all completed the study. The 59 participants were stratified into four groups based on body fat percentage (obese ≥32%), appendicular lean mass adjusted for both height and body fat (sarcopenia ≤-1.43 or below 20th percentile of the residual distribution), T-score of L₁-L₄ and/or femoral neck ≤-1:

1) 35 women were identified as osteopenic obese
2) Ten were classified as obese-only
3) Ten were classified as OSO
4) The remaining four women were osteopenic/sarcopenic non-obese

An outline of the participant flow-chart from recruitment to final analysis is displayed in Figure 2. It needs to be noted though, that classification used in our study was done according to the percentage body fat, due to numerous disadvantages of BMI, as discussed in Literature Review chapter, section 2.3.4.

All 59 women in this study were Caucasian, with average age of 76 ± 7.3 years, ranging from 65-93 years. The average BMI was 27 ± 5.2 kg/m². Principal characteristics of study population are presented in Table 2.

There were no significant differences in age, height, age of menopause, waist circumference, abdominal circumference, waist/hip ratio, blood pressure and heart rate among the women in the four groups. The average weight for all groups was 70.9 ± 14.7 kg. The average weight for non-obese group was significantly lower than the other three groups. The average waist circumference for all women was 83.9 ± 12.7 cm and ranged from 60 to 114 cm.
The average waist to hip ratio for all participants was 0.8 ± 0.1 and ranged from 0.6 to 1.0. Principal characteristics of each group are presented in Table 3.

Among all 59 participants only one was currently a smoker; 31.6% of the participants were former smokers with the history of smoking ranged from 1 to 40 years. The range of consumption of caffeinated coffee was between 0 to 12 cup/day with average of 1.1 ± 1.7 cup/day. The average alcohol drinking for all participants were one drink/day; 20% of the participants didn’t drink at all.

With regard to occupational status, 85% of the participants were retired and living in one of the retirement centers or senior centers in Tallahassee area including Westminster Oaks (4449 Meandering Way, Tallahassee, FL 32308) or Cherry Laurel (1009 Concord Rd, Tallahassee, FL 32308). The other 15% of participants were living independently and were recruited through the Tallahassee Senior Center (1400 N Monroe St, Tallahassee, FL 32303).

4.2 Body Composition and Bone Mineral Density

Total body lean mass and total body fat mass ranged from 31.4 to 49.6 kg and 9.4 to 58.5 kg, respectively with average body fat of 41.8%. The average lean to fat ratio for all participants was 1.4 ± 0.4 and ranged from 0.8 to 3.6. In addition, the ASMI ranged from 5.03 to 8.3 kg/m² with average of 6.3 ± 0.8 kg/m². The average BMD for all participants was 1.056 ± 0.112 g/m². The summary of body composition measurements and BMD are detailed in Table 4. The average BMD for each group are presented in Table 5. The obese-only groups had significantly higher BMD for all skeletal sites (1.177 ± 0.099 g/m²) in comparison to osteopenic obese, OSO and osteopenic/sarcopenic non-obese groups.

4.3 Serum Biomarkers

The comparisons among the groups using the blood biomarkers of bone (sclerostin), muscle (sTnT) and fat (Leptin, adiponectin and L/A ratio) metabolism as well as inflammation (CRP) are displayed in Table 16. From 59 women that enrolled in the study, all 59 samples were analyzed by ELISA. All samples were run in duplicate and the averages of the duplicate samples were used for analysis. Furthermore, only samples with coefficient of variation less than 10% were used for analysis and all samples with coefficient of variation higher than 10% were reanalyzed. Overall, the average values for sclerostin, sTnT, leptin, adiponectin, L/A ratio and CRP were 1.0 ± 0.3 ng/mL, 1.3 ± 2.2 pg/mL, 13.6 ± 4.7 ng/mL, 4.7 ± 3.2 µg/mL, 5.1 ± 5.9 and 0.2 ± 0.3 mg/dL, respectively (Table 16).
4.3.1 Bone Biomarker (Sclerostin)

Serum sclerostin concentrations ranged from 0.03 to 2.49 ng/mL. The concentrations of serum sclerostin were significantly higher ($p \leq 0.05$) in OSO and osteopenic obese group ($1.1 \pm 0.2; 1.1 \pm 0.09$ ng/mL, respectively) in comparison to obese-only group (Table 16). There was a statistically significant negative correlation between sclerostin and right femoral neck BMD, T-score and BMC ($r=-0.308, r=-0.303, r=-0.336$, respectively; $p \leq 0.05$) and positive correlation with years since menopause ($r=0.018; p=0.89$) (Tables 17 and 18). A positive significant correlation between serum sclerostin and troponin T concentrations was observed among all groups ($r=0.3; p \leq 0.05$). Sclerostin concentrations were positively correlated, although not significantly, with serum adiponectin ($r=0.164; p=0.214$) and negatively with CRP ($r=-0.224; p=0.117$), leptin ($r=-0.106; p=0.424$) and L/A ratio ($r=-0.243; p=0.064$) (Table 19).

4.3.2 Muscle Biomarker (sTnT)

The values for the sTnT concentrations ranged from 0.02 to 12.06 pg/mL. The concentrations of serum sTnT were significantly higher ($p \leq 0.05$) in the OSO group ($3.2 \pm 2.9$ pg/mL) in comparison to osteopenic obese and obese-only group as it has been illustrated in Table 16. In addition, there was a trend for negative correlations with lean mass ($r=-0.078; p=0.556$), lean/fat ratio ($r=-0.161; p=0.223$), ALM ($r=-0.057; p=0.666$) and ASMI ($r=-0.162; p=0.22$) and for positive correlations with total body weight ($r=0.025; p=0.851$), total body fat percentage ($r=0.169; p=0.202$) and age ($r=0.119; p=0.368$) in whole population (Table 17). No significant correlations were observed between sTnT concentration and bone variables of different skeletal sites (Table 18). Serum sTnT concentration was negatively correlated with adiponectin and CRP ($r=-0.112$ and -0.116, respectively). There was a positive significant correlation between serum sTnT and sclerostin ($r=0.3; p \leq 0.05$). There was a trend for positive correlations with leptin as well as L/A ratio ($r=0.16; p=0.228$ and $r=0.118; p=0.375$) (Table 19).

4.3.3 Adipose Tissue Biomarkers (Leptin and Adiponectin)

The values for the leptin concentrations in whole population ranged from 7.4 to 26.7 ng/mL with average of $13.6 \pm 4.7$ ng/mL. The highest concentrations of serum leptin were observed in the OSO group. Women in OSO group had significantly greater leptin concentration than those in osteopenic/sarcopenic non-obese group (Table 16). There was a significant positive correlation between leptin and total body fat ($r=0.429; p \leq 0.01$), age ($r=0.265; p \leq 0.05$), BMI ($r=0.385; p \leq 0.01$), waist ($r=0.415; p \leq 0.01$), hip ($r=0.367; p \leq 0.01$) and abdominal circumference
A significant negative correlation was observed between leptin and total vitamin D intake ($r=-0.424; p\leq0.01$). Overall, serum leptin had a significant negative correlation with left femoral neck BMD and T-score, total BMC and left femur BMC after adjusting for weight or BMI (Table 22). A significant negative correlation between serum leptin and ALM was observed after adjusting for weight ($r=-0.378; p\leq0.01$) and BMI ($r=-0.262; p\leq0.01$).

The values for the adiponectin concentrations ranged from 0.5 to 19.43 µg/mL. The osteopenic/sarcopenic non-obese group had the highest adiponectin concentrations (µg/mL) compared to obese-only, osteopenic obese; and OSO group (Table 16). In the whole population, statistically significant negative correlation of serum adiponectin with body fat percentage was noted, as well as with the BMD, BMC and T-scores of several skeletal sites, including total femur and femoral neck (as noted in Table 21). After adjusting for weight or BMI, the same correlations remained (Table 22). Moreover, serum adiponectin concentrations were negatively correlated with serum sTnT and CRP concentrations ($r=-0.112$ and -0.078, respectively) (Table 19).

The average L/A ratio was 5.08 ± 5.9. Lowest ratio of L/A was observed among osteopenic/sarcopenic non-obese group (1.3 ± 0.8) as noted in Table 16. L/A had a significant positive and negative correlation with total body fat percentage ($r=0.279; p\leq0.05$) and lean/fat ratio ($r=-0.260; p\leq0.05$), respectively (Table 20). A significant positive correlation was observed between L/A ratio and right/ left femur BMD and T-score (Table 21).

### 4.3.4 Inflammatory Biomarker (CRP)

The CRP concentrations for all participants ranged from 0.01 to 1.43 mg/dL. None of the CRP concentrations were above 3.0 mg/dL, which is considered to be a high threshold. Although the highest concentrations of CRP were observed in the OSO group, there was no significant difference between groups. Furthermore, CRP was negatively correlated with sclerostin ($r=-0.224; p=0.117$) adiponectin ($r=-0.078; p=0.588$), troponin T ($r=-0.116; p=0.424$) and age ($r=-0.05; p=0.723$) and was positively correlated with leptin ($r=0.244; p=0.088$), L/A ratio ($r=0.085; p=0.556$) total body weight ($r=0.208; p=0.147$), BMI ($r=0.217; p=0.13$) total body fat % ($r=0.204; p=0.156$), lean mass ($r=0.146; p=0.31$) and ASMI ($r=0.228; p=0.112$), although none of these relationships were significantly different. CRP concentrations had a significant negative correlation with right femoral neck BMD ($r=-0.303; p\leq0.05$) and T-score ($r=-0.301; p\leq0.05$) (Tables 16, 17, 18 and 19).
4.3.5 Serum Lipid Profile

Serum samples were analyzed for cholesterol, TG, LDL, HDL and VLDL. The results are presented in Table 23. The values for cholesterol concentration ranged from 107.0 to 296.0 mg/dL, with average of 195.1 ± 37.2 mg/dL. The highest average cholesterol concentration was observed in the OSO group (215.3 ± 49.6 mg/dL). The values for triglyceride concentration ranged from 37.0 to 200.0 mg/dL, with an average of 97.8 ± 41.7 mg/dL. The highest average triglyceride concentration was observed in the osteopenic obese group (105.03 ± 42.9 mg/dL) vs lowest (49 ± 10.8 mg/dL) for osteopenic/sarcopenic non-obese group. The values for LDL concentrations ranged from 35.4 to 224.4 mg/dL, with average of 110.8 ± 35.1 mg/dL. The highest concentrations of LDL were observed in the OSO group (129.8 ± 55.0 mg/dL) vs lowest (93.2 ± 21.6 mg/dL) for osteopenic/sarcopenic non-obese group. HDL concentrations ranged between 38.0 to 95.0 mg/dL, with average of 64.6 ± 15.07 mg/dL. The lowest concentrations of HDL were observed in the obese group (59.0 ± 11.5 mg/dL). The value for VLDL concentrations ranged from 7.4 to 199.8 mg/dL, with average of 23.1 ± 26.5 mg/dL. The highest concentrations of VLDL were observed in the OSO group (35.7 ± 57.9 mg/dL) vs lowest (9.8 ± 2.2 mg/dL) for osteopenic/sarcopenic non-obese group. Non-HDL cholesterol and chol/HDL ratio were calculated to better understand the fat metabolism of the population. Non-HDL cholesterol concentrations ranged between 54.0 to 247.0 mg/dL and highest concentrations were reported in the OSO group (147.3 ± 31.2 mg/dL). The total average of chol/HDL ratio was 3.1 ± 0.8 and the highest concentrations were reported in the OSO group (3.3 ± 1.4) (Table 23 and 24). A significant difference was observed in TG and VLDL concentration when comparing the osteopenic/sarcopenic non-obese group with the rest of the population. No significant correlation were observed between lipid biomarkers and sclerostin, leptin, adiponectin, sTnT, total body weight, BMI, total body fat %, lean mass or bone variables.

4.4 Dietary Intake from Food & Supplements

Food Processor SQL 10.11.0 (ESHA Research, Salem, OR) was used to analyze self-reported three day dietary records in order to compare the average energy and other nutrients intake of the participants. The average total energy intake/day was 1851.7 ± 582.3 kcal/day ranging from 873.0 to 3,216.0 kcal/day (Table 8). Although, the lowest amount of energy intake was observed in the OSO group, there was no significant difference among groups (Table 9). Total vitamin D and calcium intake from diet and supplements was 1762.2 ± 1480.6 IU/day and
1381.8 ± 674.6 mg/day, respectively. The average amount of vitamin D and calcium intake was higher than the RDA (RDA: 600 IU vitamin D and 1200 mg calcium per day for women of 50 years and above). At the time of study, 86.6% and 71.6% of participants were receiving vitamin D and calcium, respectively. The highest amounts of calcium intake were observed in the obese-only group. Overall vitamin D and calcium intake are presented in Table 8. The lowest amount of calcium and vitamin D intake were observed among the OSO group. There was no significant difference in amount of vitamin D and calcium intake among groups (Table 9).

The average total protein intake was 82.4 ± 35.9 g/day with 21.6% of participants consuming less than the RDA for protein (RDA: 0.8 g/kg/day). The average protein intake did not meet the recommendation of 1.4-1.6 g/kg/day (Campbell et al. 1994; Breen & Phillips 2013; Churchward-Venne et al. 2014) and only 26.7% and 18.3% of participants consumed more than 1.4 and 1.6 protein g/kg/day, respectively. The lowest amount of protein intake was observed in the OSO group; however, there was no significant difference among groups. All groups were similar in amount of nutrient intake except for vitamin B₆ and B₁₂. The osteopenic/sarcopenic non-obese group had significantly higher amount of vitamin B₆ and B₁₂ consumption in comparison to the other three groups (p≤0.05). The summary of the dietary factors is presented in Tables 6, 7, 8 and 9.

Pearson correlation analysis was used to analyze the relationship among energy, calcium, vitamin D, protein intake and anthropometric, body composition and bone variables. There was a significant positive correlation between total calcium intake and lean/fat ratio (r=0.308; p≤0.05). Total vitamin D intake was significantly negatively correlated with years since menopause onset (r=-0.29; p≤0.05). Moreover, there was a significant negative correlation between amount of protein intake and waist/hip ratio (r=-0.289; p≤0.05) (Table 10). Total protein intake was significantly positively correlated with right femoral neck BMD and T-score (r=0.291 and r=0.290, respectively; p≤0.05). Positive correlations were observed among total energy, calcium, vitamin D, protein and BMD, T-score and BMC of different skeletal sites (Table 11).

4.5 Habitual Physical Activity

Allied Dunbar National Fitness Survey for older adults was used to evaluate habitual physical activity of participants. Activities examined included: heavy housework (vacuuming, mopping, scrubbing floors); gardening (heavy and light); do-it-yourself activities (wall-papering, wall-painting); walking (distances of at least 1 mile or 20 minutes in duration); and recreational
and sport activities (aerobics, bicycling, swimming, tennis). Stair climbing was reported as the average number of steps climbed per day in and outside of the home. The total hours of physical activity per week ranged from 0.6 to 49 hours per week with average of 10.2 ± 8.1. The average number of steps climbed per day was 23 ± 41.6. There was no significant difference among groups in total hours of physical activity or number of steps climbed (Table 12).

Pearson correlation analyses were used to analyze the correlation among total hours of physical activity, average number of steps climbed per day and anthropometric, body composition, and bone variables. There was a significant negative correlation among age and total hours of physical activity and average number of steps climbed per day (r= -0.278; p≤0.05 and r=-0.385; p≤0.01, respectively). There was a positive correlation between lean/fat ratio and total hours of physical activity and average number of steps climbed (r=0.021, p= 0.876 and r=0.039, p= 0.774). Total hours of physical activity per week were significantly negatively correlated with waist circumference (r=-0.29; p≤0.05). In addition, total hours of physical activity and average number of steps climbed were negatively correlated with total body fat, total body fat percentage, weight, BMI, hip circumference, abdominal circumference and energy intake (Table 14). Average numbers of steps climbed per day were significantly negatively correlated with years since menopause onset (r=-0.393; p≤0.01). Pearson’s correlation coefficients among total hours of physical activity per week, average number of steps climbed per day and variables of bone-mineral density, bone-mineral content and T-score are presented in Table 15.

**4.6 Specific Aims and Hypotheses Testing**

**Specific Aim 1:** To assess bone status, by measuring serum sclerostin in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

We hypothesized that women diagnosed with OSO will have higher sclerostin concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

The concentrations of sclerostin were significantly higher (p≤0.05) in OSO and osteopenic obese group (1.1 ± 0.2 and 1.09 ± 0.2 ng/mL, respectively) in comparison to obese-only group (0.7 ± 0.4 ng/mL). Although the highest concentrations of sclerostin were observed in OSO group, there was no significant difference between OSO and osteopenic obese population. Therefore, the hypothesis was partially accepted as the concentration of sclerostin was higher in
the OSO group in comparison to the rest of population but it did not reach the significant level when comparing all groups (Table 16 and Figure 3).

**Specific Aim 2:** To assess the muscle tissue status by measuring serum sTnT concentrations in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

We hypothesized that women identified with OSO will have higher serum sTnT concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

The serum sTnT concentration was significantly higher ($p \leq 0.05$) in the OSO group ($3.2 \pm 2.9$ pg/mL) in comparison to osteopenic obese ($1.1 \pm 2.2$ pg/mL) and obese-only groups ($0.6 \pm 0.7$ pg/mL). There was no significant different between the OSO and non-obese group. Therefore, the hypothesis was partially accepted.

**Specific Aim 3:** To assess the adipose tissue status by measuring serum leptin and adiponectin concentrations in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

We hypothesized that women identified with OSO will have higher serum leptin and lower adiponectin concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

The OSO group had significantly greater leptin values than the osteopenic/sarcopenic non-obese group ($17.3 \pm 4.5$ vs. $8.7 \pm 1.1$). There was no significant difference among osteopenic obese, obese-only and OSO group. Therefore, our hypothesis was partially accepted because the leptin concentration was higher in the OSO group in comparison to the rest of population. Women in the osteopenic/sarcopenic non-obese group had significantly greater adiponectin values than the women in the other three groups ($p \leq 0.05$). The lowest concentrations of adiponectin were observed in obese-only group (Figure 4, 5 and 6). Therefore, the hypothesis was rejected because the OSO group did not have the lowest adiponectin concentration.

**Specific Aim 4:** To assess the level of inflammation by analyzing the pro-inflammatory biomarker, CRP, in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.
We hypothesized that women identified with OSO will have significantly higher CRP concentrations in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

The highest CRP concentration was observed in the OSO group (0.3 ± 0.4 mg/dL); however, there was no significant difference among groups (0.2 ± 0.2, 0.2 ± 0.3 and 0.2 ± 0.2 for osteopenic obese, obese-only group and osteopenic/sarcopenic non-obese respectively) (Table 16 and Figure 7). Therefore our hypothesis is partially accepted.

**Specific Aim 5:** To assess the overall serum lipid profile in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

We hypothesized that women identified with osteopenic/sarcopenic non-obese will have significantly lower concentrations of cholesterol, TG, LDL, and VLDL and higher concentrations of HDL in comparison to osteopenic obese, obese-only and OSO.

Osteopenic/sarcopenic non-obese group had the lowest concentrations of cholesterol, TG, LDL, VLDL and highest HDL concentration. There was no significant difference in concentration of cholesterol and LDL among groups. Therefore our hypothesis is partially accepted.

**Specific Aim 6:** To assess the nutritional status with respect to bone, muscle and adipose tissue by evaluating calcium, vitamin D, protein and energy intake in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.

We hypothesized that women identified with OSO will have significantly lower calcium, vitamin D, protein and energy intake in comparison to osteopenic obese, obese-only and osteopenic/sarcopenic non-obese women.

The lowest amounts of energy calcium and vitamin D intake were observed in the OSO group (1679.3 ± 457.1 kcal/day, 1151.4 ± 511.9 mg/day and 1445.8 ± 1298.2 IU/day, respectively) (Table 7 and 9); however, there was no significant difference among the groups. The least amounts of protein intake were observed in the OSO group (69.8 ± 15.6 g). However, there was no significant difference between the groups. Therefore our hypothesis is partially accepted.

**Specific Aim 7:** To assess the overall fitness level by evaluating the level of habitual physical activity in osteopenic obese, obese-only, OSO and osteopenic/sarcopenic non-obese women.
We hypothesized that women identified with OSO will have a significantly lower level of habitual physical activity in comparison to women in the osteopenic obese, obese-only and osteopenic/sarcopenic non-obese groups.

The obese-only group had the lowest amount of physical activity per week in comparison to other groups (6.3 ± 3.7 hours/week). There was no significant difference between groups in total hours of physical activity or number of steps climbed (Table 13). Therefore our hypothesis is rejected.
Table 2. Principal characteristics of all participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum/Maximum</th>
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<tr>
<td>Age (years)</td>
<td>76.0 ± 7.2</td>
<td>65.0/93.0</td>
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<tr>
<td>Height (cm)</td>
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<td>143.0/175.8</td>
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<td>Weight (kg)</td>
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<td>45.0/111.0</td>
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<td>15.5/41.7</td>
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<td>25.0/60.0</td>
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<tr>
<td>Years since menopause</td>
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<td>10.9/57.9</td>
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<td>Waist Circumference (cm)</td>
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<td>60.0/114.0</td>
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<tr>
<td>Hip Circumference (cm)</td>
<td>105.0 ± 13.3</td>
<td>68.0/140.0</td>
</tr>
<tr>
<td>Abdominal Circumference (cm)</td>
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<td>66.0/145.0</td>
</tr>
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<td>Waist to Hip Ratio</td>
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<td>0.6/1.0</td>
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<td>Systolic Blood Pressure (mmHg)</td>
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<td>Diastolic Blood Pressure (mmHg)</td>
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<td>Heart Rate (BPM)</td>
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BMI = Body mass index; BPM = Beats per minute
Table 3. Participants’ characteristics per each groups (N=59)

<table>
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<tr>
<th>Variables</th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
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<td>Age (years)</td>
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<tr>
<td>Weight (kg)</td>
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<td>77.3 ± 13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.5 ± 14.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.2 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>27.1 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.2 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>19.7 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Age of Menopause (years)</td>
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<td>Waist Circumference (cm)</td>
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<td>80.4 ± 13.9</td>
<td>69.0 ± 10.0</td>
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<td>Hip Circumference (cm)</td>
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<td>110.9 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>91.8 ± 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Waist to Hip Ratio</td>
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<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
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<td>Systolic Blood Pressure (mmHg)</td>
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<td>139.4 ± 21.1</td>
<td>158.6 ± 31.0</td>
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<td>Diastolic Blood Pressure (mmHg)</td>
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<td>70.9 ± 9.2</td>
<td>69.0 ±10.1</td>
<td>62.6 ± 6.1</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation (SD)
BMI = Body mass index; OSO= Osteosarcopenic obesity
Values within a row with different upper script letters indicate that they are significantly different from each other (p≤0.05)
Table 4. Body composition and bone mineral density for the all participants (N=59)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum/Maximum</th>
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<tr>
<td>Total BMD (g/m$^2$)</td>
<td>1.056 ± 0.112</td>
<td>0.797/1.341</td>
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<td>Right femur BMD (g/m$^2$)</td>
<td>1.148 ± 2.093</td>
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<td>Left femur BMD (g/m$^2$)</td>
<td>0.875 ± 0.116</td>
<td>0.611/1.149</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/m$^2$)</td>
<td>0.817 ± 0.111</td>
<td>0.617/1.229</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/m$^2$)</td>
<td>0.812 ± 0.091</td>
<td>0.607/1.033</td>
</tr>
<tr>
<td>L1-L4 BMD (g/m$^2$)</td>
<td>1.194 ± 0.194</td>
<td>0.914/1.665</td>
</tr>
<tr>
<td>Non-dominant arm radius BMD (g/cm$^2$)</td>
<td>0.428 ± 0.077</td>
<td>0.267/0.605</td>
</tr>
<tr>
<td>Non-dominant arm 33% radius BMD (g/cm$^2$)</td>
<td>0.564 ± 0.191</td>
<td>0.359/0.781</td>
</tr>
<tr>
<td>Total T-score</td>
<td>-0.2 ± 1.1</td>
<td>-2.8/2.6</td>
</tr>
<tr>
<td>L1-L4 T-score</td>
<td>-0.05 ± 1.4</td>
<td>-2.2/3.8</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-1.0 ± 0.9</td>
<td>-2.6/1.1</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-1.0 ± 0.9</td>
<td>-3.2/1.1</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-1.5 ± 0.7</td>
<td>-3.1/1.4</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-1.6 ± 0.6</td>
<td>-3.1/0.0</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>38.8 ± 4.6</td>
<td>31.4/49.6</td>
</tr>
<tr>
<td>Lean/fat mass ratio</td>
<td>1.4 ± 0.4</td>
<td>0.8/3.6</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>16.7 ± 2.5</td>
<td>12.3/23.09</td>
</tr>
<tr>
<td>ASMI (kg/m$^2$)</td>
<td>6.3 ± 0.8</td>
<td>5.03/8.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.2 ± 9.7</td>
<td>9.4/58.5</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>41.8 ± 6.4</td>
<td>21.6/54.9</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; ALM= Appendicular lean mass; ASMI= Appendicular skeletal muscle mass index
Table 5. Body composition and bone mineral density in subjects divided by groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMD (g/cm²)</td>
<td>1.039 ± 0.099</td>
<td>1.177 ± 0.099</td>
<td>1.017 ± 0.105</td>
<td>0.999 ± 0.096</td>
</tr>
<tr>
<td>Right femur BMD (g/cm²)</td>
<td>0.849 ± 0.093</td>
<td>1.054 ± 0.082</td>
<td>0.805 ± 0.111</td>
<td>0.797 ± 0.056</td>
</tr>
<tr>
<td>Left femur BMD (g/cm²)</td>
<td>0.867 ± 0.095</td>
<td>1.032 ± 0.091</td>
<td>0.809 ± 0.104</td>
<td>0.781 ± 0.042</td>
</tr>
<tr>
<td>Right femoral neck BMD</td>
<td>0.793 ± 0.083</td>
<td>1.012 ± 0.102</td>
<td>0.778 ± 0.080</td>
<td>0.772 ± 0.048</td>
</tr>
<tr>
<td>Left femoral neck BMD</td>
<td>0.801 ± 0.074</td>
<td>0.956 ± 0.047</td>
<td>0.759 ± 0.065</td>
<td>0.747 ± 0.017</td>
</tr>
<tr>
<td>L₁-L₄ BMD (g/m²)</td>
<td>1.181 ± 0.183</td>
<td>1.393 ± 0.210</td>
<td>1.181 ± 0.183</td>
<td>1.064 ± 0.136</td>
</tr>
<tr>
<td>Non-dominant arm total</td>
<td>0.423 ± 0.071</td>
<td>0.499 ± 0.073</td>
<td>0.391 ± 0.075</td>
<td>0.388 ± 0.012</td>
</tr>
<tr>
<td>Non-dominant arm 33%</td>
<td>0.562 ± 0.088</td>
<td>0.631 ± 0.086</td>
<td>0.512 ± 0.094</td>
<td>0.539 ± 0.033</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.4 ± 0.9</td>
<td>0.9 ± 0.9</td>
<td>-0.6 ± 1.0</td>
<td>-0.8 ± 0.9</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-1.2 ± 0.7</td>
<td>0.3 ± 0.6</td>
<td>-1.4 ± 0.7</td>
<td>-1.6 ± 0.4</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-1.1 ± 0.7</td>
<td>-0.2 ± 0.7</td>
<td>-1.5 ± 0.8</td>
<td>-1.7 ± 0.3</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-1.7 ± 0.5</td>
<td>-0.1 ± 0.7</td>
<td>-1.8 ± 0.5</td>
<td>-1.9 ± 0.3</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-1.7 ± 0.5</td>
<td>-0.5 ± 0.3</td>
<td>-2.0 ± 0.4</td>
<td>-2.1 ± 0.1</td>
</tr>
<tr>
<td>L₁-L₄ T-score</td>
<td>0.02 ± 1.5</td>
<td>1.7 ± 1.7</td>
<td>-0.3 ± 1.1</td>
<td>-0.9 ± 1.1</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>2123.7 ± 259.7</td>
<td>2412.6 ± 309.9</td>
<td>2014.2 ± 205.5</td>
<td>1936 ± 223.8</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>27.8 ± 3.4</td>
<td>33.7 ± 3.2</td>
<td>26.3 ± 3.7</td>
<td>26.1 ± 2.3</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>28.4 ± 4.1</td>
<td>33.2 ± 3.8</td>
<td>26.1 ± 3.2</td>
<td>25.4 ± 2.2</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>3.9 ± 0.5</td>
<td>5.1 ± 0.8</td>
<td>3.9 ± 0.5</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>4.0 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>3.8 ± 0.5</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>L₁-L₄ BMC (g)</td>
<td>65.4 ± 16.2</td>
<td>81.1 ± 16.3</td>
<td>64.3 ± 9.6</td>
<td>58.8 ± 7.2</td>
</tr>
<tr>
<td>Lean/fat mass ratio</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>ALM</td>
<td>17.0 ± 2.3</td>
<td>18.5 ± 2.6</td>
<td>15.1 ± 1.6</td>
<td>13.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Osteopenic obese (N=35)</td>
<td>Obese-only (N=10)</td>
<td>OSO (N=10)</td>
<td>Osteopenic/sarcopenic non-obese (N=4)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------</td>
<td>-------------------</td>
<td>------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>ASMI</td>
<td>6.4 ± 0.7</td>
<td>6.9 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.6 ± 8.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.9 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.0 ± 10.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>42.2 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.6 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.0 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.1 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

BMD= Bone mineral density; BMC= Bone mineral content; ALM= Appendicular lean mass; ASMI= Appendicular skeletal mass index; OSO= Osteosarcopenic obesity

Values within a row with different upper script letter indicate that they are significantly different from each other ($p \leq 0.05$)
Table 6. Dietary intake of selected nutrients for all participants (N=59)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum/Maximum</th>
<th>Daily Recommendation / Dietary Reference Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1851.7 ± 582.3</td>
<td>872.7/3215.9</td>
<td>1600-2000 based on level of activity (‘Estimated Calorie Needs per Day by Age, Gender, and Physical Activity Level’ 2002)</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>71.5 ± 32.1</td>
<td>25.6/174.7</td>
<td>20-35% of total energy intake (‘Know Your Fats, American Heart Association’)</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>244.0 ± 137.9</td>
<td>38.8/591.7</td>
<td>The previous recommendation for cholesterol intake was &lt;300 mg/day, however, since there is no relationship between serum cholesterol and its dietary consumption, cholesterol is not a nutrient of concern for overconsumption anymore (‘Scientific report of the 2015 dietary guidelines advisory committee, USDA’)</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>20.8 ± 8.5</td>
<td>5.9/47.2</td>
<td>&lt;5-6% of total energy intake (‘Know Your Fats, American Heart Association’)</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>14.8 ± 9.5</td>
<td>2.3/42.5</td>
<td>The majority of fats in diet (20-35% of total energy intake) should be monounsaturated or polyunsaturated (‘Know Your Fats, American Heart Association’)</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>11.7 ± 15.6</td>
<td>0.7/112.5</td>
<td>The majority of fats in diet (20-35% of total energy intake) should be monounsaturated or polyunsaturated (‘Know Your Fats, American Heart Association’)</td>
</tr>
<tr>
<td>Trans fat (g)</td>
<td>1.4 ± 4.5</td>
<td>0/30.6</td>
<td>&lt;1% of total energy intake (‘Know Your Fats, American Heart Association’)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>82.43 ± 35.9</td>
<td>33.1/213.36</td>
<td>0.8 g/kg/day 10-35% of total energy intake (‘Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids : Health and Medicine Division’ 2002)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>224.4 ± 79.8</td>
<td>90.2/427.4</td>
<td>45-65% of total energy intake (‘Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids : Health and Medicine Division’ 2002)</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>24.9 ± 13.1</td>
<td>8.3/74.4</td>
<td>21.0 (‘Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids : Health and Medicine Division’ 2002)</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Mean ± SD</td>
<td>25th/75th Percentile</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------</td>
<td>----------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>948.9 ± 635.7</td>
<td>179.9/3930.5</td>
<td>700.0 ('Phosphorus in diet: MedlinePlus Medical Encyclopedia')</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>231.4 ± 130.8</td>
<td>25.3/560.9</td>
<td>320.0 ('Magnesium — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>7.6 ± 8.0</td>
<td>0.8/55.9</td>
<td>8.0 ('Zinc — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>14.5 ± 11.1</td>
<td>2.5/64.3</td>
<td>8.0 ('Iron — Fact Sheet for Consumers')</td>
</tr>
<tr>
<td>Selenium (mcg)</td>
<td>64.1 ± 49.2</td>
<td>5.7/309.5</td>
<td>55.0 ('Selenium — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.1 ± 1.1</td>
<td>0.16/7.3</td>
<td>N/A ('Determinants of Copper Needs Across the Life Span')</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2265.5 ± 1494.3</td>
<td>166.5/8700.7</td>
<td>4700.0 ('Dietary guideline for American 2010')</td>
</tr>
<tr>
<td>Folate (mcg)</td>
<td>309.5 ± 288.3</td>
<td>7.8/1428.5</td>
<td>400.0 ('Folate — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>9271.8 ± 9017.2</td>
<td>348.4/53807.2</td>
<td>75.0 ('Vitamin A — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>156.7 ± 248.5</td>
<td>6.29/1300.1</td>
<td>75.0 ('Vitamin C — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Vitamin K (mcg)</td>
<td>185.5 ± 310.1</td>
<td>0.42/1681.9</td>
<td>90.0 ('Vitamin K — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Vitamin B12 (mcg)</td>
<td>5.5 ± 13.9</td>
<td>0.06/101.0</td>
<td>2.4 ('Vitamin B12 — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>2.5 ± 7.6</td>
<td>0.14/56.8</td>
<td>1.5 ('Vitamin B6 — Health Professional Fact Sheet')</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Osteopenic obese (N=35)</td>
<td>Obese-only (N=10)</td>
<td>OSO (N=10)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------</td>
<td>-------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1916.2 ± 629.2</td>
<td>1838.9 ± 628.1</td>
<td>1679.3 ± 457.1</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>78.6 ± 37.2</td>
<td>65.4 ± 23.3</td>
<td>55.1 ± 16.4</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>254.6 ± 141.1</td>
<td>287.2 ± 120.6</td>
<td>190.9 ± 74.3</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>21.9 ± 9.1</td>
<td>20.3 ± 6.9</td>
<td>17.3 ± 5.8</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>15.2 ± 9.8</td>
<td>15.8 ± 9.1</td>
<td>14.1 ± 9.3</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>14.2 ± 19.7</td>
<td>9.7 ± 7.0</td>
<td>7.6 ± 6.3</td>
</tr>
<tr>
<td>Trans fat (g)</td>
<td>2.1 ± 5.9</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>84.1 ± 41.3</td>
<td>89.7 ± 28.5</td>
<td>69.8 ± 15.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>222.5 ± 76.1</td>
<td>217.7 ± 98.4</td>
<td>233.1 ± 95.5</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>25.5 ± 15.1</td>
<td>20.6 ± 4.2</td>
<td>23.2 ± 10.6</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>975.7 ± 735.2</td>
<td>1104.9 ± 516.2</td>
<td>802.2 ± 302.2</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>228.4 ± 124.7</td>
<td>252.4 ± 135.5</td>
<td>221.9 ± 119.8</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>8.4 ± 9.9</td>
<td>7.2 ± 4.4</td>
<td>6.8 ± 3.6</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>16.2 ± 13.5</td>
<td>14.6 ± 7.8</td>
<td>10.2 ± 4.9</td>
</tr>
<tr>
<td>Selenium (mcg)</td>
<td>64.7 ± 55.5</td>
<td>74.5 ± 42.4</td>
<td>58.1 ± 36.0</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.1 ± 1.4</td>
<td>1.1 ± 0.9</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2171.0 ± 1334.5</td>
<td>2810.6 ± 2286.4</td>
<td>2177.1 ± 1001.0</td>
</tr>
<tr>
<td>Folate (mcg)</td>
<td>327.4 ± 313.2</td>
<td>280.6 ± 124.2</td>
<td>234.8 ± 126.1</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>9441.1 ± 7900.5</td>
<td>7280.8 ± 4050.2</td>
<td>6813.8 ± 4052.1</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>159.5 ± 276.5</td>
<td>95.3 ± 41.8</td>
<td>201.8 ± 310.6</td>
</tr>
</tbody>
</table>
Table 7 Continued

<table>
<thead>
<tr>
<th></th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin K (mcg)</td>
<td>180.7 ± 281.4</td>
<td>89.9 ± 79.3</td>
<td>142.4 ± 141.4</td>
<td>545.4 ± 789.8</td>
</tr>
<tr>
<td>Vitamin B₁₂ (mcg)</td>
<td>4.3 ± 5.4ᵃ</td>
<td>4.1 ± 3.6ᵃ</td>
<td>2.6 ± 1.3ᵃ</td>
<td>25.8 ± 50.1ᵇ</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.6 ± 1.5ᵃ</td>
<td>1.5 ± 0.8ᵃ</td>
<td>1.1 ± 0.4ᵃ</td>
<td>14.6 ± 28.1ᵇ</td>
</tr>
</tbody>
</table>

OSO= Osteosarcopenic obesity
Data are presented as mean ± standard deviation
Values within a row with different upper script letters indicate that they are significantly different from each other (p≤0.05)
Table 8. Calcium and vitamin D intake for all participants (N=59)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum/Max</th>
<th>Recommended Dietary Allowances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium intake from diet (mg)</td>
<td>754.2 ± 358.2</td>
<td>136.5/1809.0</td>
<td>51≤years: 1,200 ('Calcium Fact Sheet for Health Professionals')</td>
</tr>
<tr>
<td>Calcium supplement (mg)</td>
<td>584.2 ± 521.1</td>
<td>0.0/2000.0</td>
<td></td>
</tr>
<tr>
<td>Total calcium intake (mg)</td>
<td>1382.0 ± 674.6</td>
<td>136.5/3809.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake from diet (IU)</td>
<td>160.0 ± 339.6</td>
<td>0.0/2002.0</td>
<td>51-70 years: 600 &gt;70 years: 800 ('Vitamin D Fact Sheet for Health Professionals')</td>
</tr>
<tr>
<td>Vitamin D supplement (IU)</td>
<td>1543.0 ± 1395.0</td>
<td>0.0/5800.0</td>
<td></td>
</tr>
<tr>
<td>Total vitamin D intake (IU)</td>
<td>1762.0 ± 1481.0</td>
<td>0.0/5800.0</td>
<td></td>
</tr>
</tbody>
</table>
### Table 9. Calcium and vitamin D intake by groups (mean ± SD)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium intake from diet (mg/day)</td>
<td>805.9 ± 310.2</td>
<td>818.2 ± 388.7</td>
<td>612.2 ± 291.5</td>
<td>803.6 ± 672.3</td>
</tr>
<tr>
<td>Calcium supplement (mg/day)</td>
<td>595.2 ± 487.1</td>
<td>644.0 ± 522.1</td>
<td>539.1 ± 502.1</td>
<td>555.0 ± 964.7</td>
</tr>
<tr>
<td>Total calcium intake (mg/day)</td>
<td>1420.8 ± 576.3</td>
<td>1513.6 ± 617.8</td>
<td>1151.4 ± 511.9</td>
<td>1358.6 ± 1633.6</td>
</tr>
<tr>
<td>Vitamin D intake from diet (IU/day)</td>
<td>190.31 ± 410.05</td>
<td>113.8 ± 154.18</td>
<td>68.6 ± 47.1</td>
<td>257.5 ± 479.6</td>
</tr>
<tr>
<td>Vitamin D supplement (IU/day)</td>
<td>1404.2 ± 1370.6</td>
<td>2050.0 ± 1369.9</td>
<td>1377.1 ± 1302.1</td>
<td>1940.0 ± 1593.8</td>
</tr>
<tr>
<td>Total vitamin D intake (IU/day)</td>
<td>1646.7 ± 1523.8</td>
<td>2269.3 ± 1593.6</td>
<td>1445.8 ± 1298.2</td>
<td>2307.5 ± 1429.3</td>
</tr>
</tbody>
</table>

OSO= Osteosarcopenic obesity
Data are presented as mean ± standard deviation (SD)
Values within a row with different superscript sign indicate that they are significantly different from each other (p≤0.05)
<table>
<thead>
<tr>
<th>Variables</th>
<th>Energy intake (kcal/day) r</th>
<th>Total calcium intake (mg/day) r</th>
<th>Total vitamin D intake (IU/day) r</th>
<th>Protein intake (g) r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.042</td>
<td>-0.095</td>
<td>-0.191</td>
<td>-0.046</td>
</tr>
<tr>
<td>Age at menopause onset (years)</td>
<td>-0.123</td>
<td>-0.029</td>
<td>0.272</td>
<td>-0.071</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>0.102</td>
<td>-0.052</td>
<td>-0.290*</td>
<td>0.011</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.015</td>
<td>-0.021</td>
<td>-0.097</td>
<td>-0.113</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.277*</td>
<td>0.356**</td>
<td>0.162</td>
<td>0.307*</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>-0.122</td>
<td>-0.162</td>
<td>-0.158</td>
<td>-0.236</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.072</td>
<td>-0.148</td>
<td>-0.161</td>
<td>-0.215</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.057</td>
<td>-0.089</td>
<td>-0.078</td>
<td>-0.057</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>-0.142</td>
<td>-0.179</td>
<td>-0.186</td>
<td>-0.163</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>-0.204</td>
<td>-0.125</td>
<td>-0.167</td>
<td>-0.289*</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>-0.053</td>
<td>-0.250</td>
<td>-0.179</td>
<td>-0.163</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>-0.042</td>
<td>-0.110</td>
<td>-0.151</td>
<td>-0.142</td>
</tr>
<tr>
<td>Total lean (kg)</td>
<td>0.032</td>
<td>0.152</td>
<td>0.004</td>
<td>-0.043</td>
</tr>
<tr>
<td>Lean/fat ratio</td>
<td>0.055</td>
<td>0.308*</td>
<td>0.163</td>
<td>0.182</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>0.044</td>
<td>0.146</td>
<td>0.031</td>
<td>-0.013</td>
</tr>
<tr>
<td>ASMI (kg/m^2)</td>
<td>-0.088</td>
<td>-0.086</td>
<td>-0.050</td>
<td>-0.155</td>
</tr>
</tbody>
</table>

ALM= Appendicular lean mass; ASMI= Appendicular skeletal muscle mass index; BMI = Body mass index
*p≤0.05
**p≤0.01
Table 11. Pearson’s correlation coefficients between total energy, calcium, vitamin D, protein intake and variables of bone-mineral density, bone-mineral content and T-score

<table>
<thead>
<tr>
<th>Variables</th>
<th>Energy intake (kcal/day)</th>
<th>Total calcium intake (mg/day)</th>
<th>Total vitamin D intake (IU/day)</th>
<th>Protein intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMD (g/cm²)</td>
<td>0.174</td>
<td>0.027</td>
<td>0.214</td>
<td>0.147</td>
</tr>
<tr>
<td>Right femur BMD (g/cm²)</td>
<td>0.170</td>
<td>0.080</td>
<td>0.193</td>
<td>0.196</td>
</tr>
<tr>
<td>Left femur BMD (g/cm²)</td>
<td>0.159</td>
<td>0.002</td>
<td>0.240</td>
<td>0.108</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/cm²)</td>
<td>0.127</td>
<td>0.207</td>
<td>0.153</td>
<td>0.291*</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/cm²)</td>
<td>0.156</td>
<td>0.092</td>
<td>0.273</td>
<td>0.208</td>
</tr>
<tr>
<td>L₁-L₄ BMD (g/m²)</td>
<td>0.066</td>
<td>-0.020</td>
<td>-0.154</td>
<td>0.090</td>
</tr>
<tr>
<td>Non-dominant arm total radius BMD (g/cm²)</td>
<td>0.042</td>
<td>0.119</td>
<td>0.132</td>
<td>0.137</td>
</tr>
<tr>
<td>Non-dominant arm 33% radius BMD (g/cm²)</td>
<td>0.119</td>
<td>0.145</td>
<td>0.163</td>
<td>0.214</td>
</tr>
<tr>
<td>Total T-score</td>
<td>0.181</td>
<td>0.010</td>
<td>0.216</td>
<td>0.143</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>0.151</td>
<td>0.075</td>
<td>0.256</td>
<td>0.198</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>0.154</td>
<td>-0.010</td>
<td>0.234</td>
<td>0.101</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>0.131</td>
<td>0.200</td>
<td>0.162</td>
<td>0.290*</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>0.163</td>
<td>0.106</td>
<td>0.27</td>
<td>0.209</td>
</tr>
<tr>
<td>L₁-L₄ T-score</td>
<td>0.066</td>
<td>-0.019</td>
<td>-0.159</td>
<td>0.088</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>0.222</td>
<td>0.162</td>
<td>0.169</td>
<td>0.255</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>0.251</td>
<td>0.130</td>
<td>0.148</td>
<td>0.220</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>0.253</td>
<td>0.072</td>
<td>0.233</td>
<td>0.152</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>0.067</td>
<td>0.163</td>
<td>0.035</td>
<td>0.229</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>0.122</td>
<td>0.073</td>
<td>0.071</td>
<td>0.171</td>
</tr>
<tr>
<td>L₁-L₄ BMC (g)</td>
<td>0.186</td>
<td>0.132</td>
<td>-0.113</td>
<td>0.102</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; BMC= Bone mineral content

*p≤0.05
Table 12. Descriptive statistics of habitual physical activity for all participants (N=59)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Min/Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hours of physical activity per week</td>
<td>10.2 ± 8.1</td>
<td>0.6/49.1</td>
</tr>
<tr>
<td>Average number of steps climbed per day</td>
<td>23.0 ± 41.6</td>
<td>0.0/173.0</td>
</tr>
</tbody>
</table>

Table 13. Total hours of habitual physical activity and average number of steps climbed for each group (mean ± SD)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hours of physical activity per week</td>
<td>12.0 ± 9.1^a</td>
<td>6.3 ± 3.7^a</td>
<td>9.4 ± 8.8^a</td>
<td>7.2 ± 2.1^a</td>
</tr>
<tr>
<td>Average number of steps climbed per day</td>
<td>23.1 ± 44.2^a</td>
<td>47.2 ± 70.7^a</td>
<td>22.8 ± 41.4^a</td>
<td>8.6 ± 9.7^a</td>
</tr>
</tbody>
</table>

OSO=Osteosarcopenic obesity
Data are presented as mean ± standard deviation
Values within a row with different superscript sign indicate that they are significantly different from each other (p≤0.05)
Table 14. Pearson’s correlation coefficients between total hours of habitual physical activity per week, average number of steps climbed per day and anthropometrics, body fat, dietary intake of calcium and vitamin D, energy intake total hours of physical activity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total hours of physical activity per week</th>
<th>Average number of steps climbed per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.278*</td>
<td>-0.385**</td>
</tr>
<tr>
<td>Age at menopause onset (years)</td>
<td>0.030</td>
<td>0.163</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>-0.239</td>
<td>-0.393**</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.160</td>
<td>-0.091</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.045</td>
<td>0.061</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>-0.178</td>
<td>-0.127</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.290*</td>
<td>-0.162</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>-0.247</td>
<td>-0.090</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>-0.163</td>
<td>-0.078</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>-0.127</td>
<td>-0.134</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>-0.058</td>
<td>-0.105</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>-0.131</td>
<td>-0.113</td>
</tr>
<tr>
<td>Total lean (kg)</td>
<td>-0.181</td>
<td>0.024</td>
</tr>
<tr>
<td>Lean/fat ratio</td>
<td>0.021</td>
<td>0.039</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>-0.131</td>
<td>0.096</td>
</tr>
<tr>
<td>ASMI (kg/m$^2$)</td>
<td>-0.160</td>
<td>0.056</td>
</tr>
<tr>
<td>Energy intake (kcal/day)</td>
<td>-0.036</td>
<td>-0.142</td>
</tr>
<tr>
<td>Total calcium intake (mg/day)</td>
<td>0.089</td>
<td>0.010</td>
</tr>
<tr>
<td>Total vitamin D intake (IU/day)</td>
<td>-0.024</td>
<td>0.030</td>
</tr>
</tbody>
</table>

BMI=Body mass index; ALM= Appendicular lean mass; ASMI= Appendicular skeletal mass index

*p≤0.05

**p≤0.01
Table 15. Pearson’s correlation coefficients between total hours of physical activity per week, average number of steps climbed per day and variables of bone-mineral density, bone-mineral content and T-score

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total hours of physical activity per week</th>
<th>Average number of steps climbed per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMD (g/cm²)</td>
<td>-0.183</td>
<td>-0.007</td>
</tr>
<tr>
<td>Right femur BMD (g/cm²)</td>
<td>-0.127</td>
<td>-0.057</td>
</tr>
<tr>
<td>Left femur BMD (g/cm²)</td>
<td>-0.159</td>
<td>0.081</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/cm²)</td>
<td>-0.140</td>
<td>0.179</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/cm²)</td>
<td>-0.099</td>
<td>0.114</td>
</tr>
<tr>
<td>L1-L4 BMD (g/cm²)</td>
<td>-0.241</td>
<td>-0.253</td>
</tr>
<tr>
<td>Non-dominant arm total radius BMD (g/cm²)</td>
<td>0.045</td>
<td>0.222</td>
</tr>
<tr>
<td>Non-dominant arm 33% radius BMD (g/cm²)</td>
<td>0.055</td>
<td>0.073</td>
</tr>
<tr>
<td>Total T-score</td>
<td>-0.198</td>
<td>0.003</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-0.115</td>
<td>0.175</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-0.169</td>
<td>0.083</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-0.135</td>
<td>0.147</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-0.099</td>
<td>0.116</td>
</tr>
<tr>
<td>L1-L4 T-score</td>
<td>-0.239</td>
<td>-0.254</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>-0.108</td>
<td>-0.048</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>-0.238</td>
<td>0.043</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>-0.312</td>
<td>-0.052</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>-0.187</td>
<td>-0.007</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>-0.202</td>
<td>-0.132</td>
</tr>
<tr>
<td>L1-L4 BMC (g)</td>
<td>-0.259</td>
<td>-0.312</td>
</tr>
</tbody>
</table>

BMD=Bone mineral density; BMC= Bone mineral content
Table 16. Serum biomarkers concentrations per each group

<table>
<thead>
<tr>
<th></th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/sarcopenic non-obese (N=4)</th>
<th>Total average (N=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin (ng/mL)</td>
<td>1.1 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>sTnT (pg/mL)</td>
<td>1.1 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.3 ± 2.2</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>13.4 ± 4.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.4 ± 4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.3 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.6 ± 4.7</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>4.8 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2 ± 7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7 ± 3.2</td>
</tr>
<tr>
<td>*L/A ratio</td>
<td>3.9 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5 ± 6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.1 ± 5.9</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.3</td>
<td>0.3 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.3</td>
</tr>
</tbody>
</table>

OSO = Osteosarcopenic obesity; CRP = C-reactive protein
Data are presented as mean ± standard deviation
Values within a row with different upper script letters indicate that they are significantly different from each other (p≤0.05)
*There was no significant difference among groups using the ANOVA test. Kruskal-Wallis test was performed on the non-parametrical data which are presented in the table.
Table 17. Pearson’s correlation coefficients among sclerostin, sTnT, CRP and anthropometrics, body fat, dietary intake of calcium and vitamin D, energy intake total hours of physical activity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sclerostin (ng/mL)</th>
<th>sTnT (pg/mL)</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.024</td>
<td>0.119</td>
<td>-0.050</td>
</tr>
<tr>
<td>Age at menopause onset (years)</td>
<td>-0.052</td>
<td>-0.029</td>
<td>-0.054</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>0.018</td>
<td>0.116</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.145</td>
<td>0.025</td>
<td>0.208</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.088</td>
<td>0.140</td>
<td>-0.031</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.101</td>
<td>-0.038</td>
<td>0.217</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-0.023</td>
<td>0.077</td>
<td>0.122</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>-0.174</td>
<td>0.059</td>
<td>0.032</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>-0.141</td>
<td>0.072</td>
<td>0.020</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.169</td>
<td>0.040</td>
<td>0.156</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>-0.002</td>
<td>0.169</td>
<td>0.204</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>-0.077</td>
<td>0.084</td>
<td>0.226</td>
</tr>
<tr>
<td>Total lean (kg)</td>
<td>-0.208</td>
<td>-0.078</td>
<td>0.146</td>
</tr>
<tr>
<td>Lean/fat ratio</td>
<td>0.006</td>
<td>-0.161</td>
<td>-0.188</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>-0.223</td>
<td>-0.057</td>
<td>0.162</td>
</tr>
<tr>
<td>ASMI (kg/m²)</td>
<td>-0.174</td>
<td>-0.162</td>
<td>0.228</td>
</tr>
<tr>
<td>Energy intake (kcal/day)</td>
<td>0.037</td>
<td>0.146</td>
<td>0.137</td>
</tr>
<tr>
<td>Total calcium intake (mg/day)</td>
<td>-0.205</td>
<td>-0.052</td>
<td>-0.123</td>
</tr>
<tr>
<td>Total vitamin D intake (IU/day)</td>
<td>0.054</td>
<td>-0.111</td>
<td>-0.273</td>
</tr>
<tr>
<td>Total hours of physical activity per week</td>
<td>0.171</td>
<td>0.116</td>
<td>-0.078</td>
</tr>
<tr>
<td>Number of steps climbed per day</td>
<td>-0.005</td>
<td>0.060</td>
<td>-0.233</td>
</tr>
</tbody>
</table>

ALM= Appendicular lean mass, ASMI= Appendicular skeletal muscle mass index
Table 18. Pearson’s correlation coefficients between sclerostin, sTnT, CRP and variables of bone-mineral density, bone-mineral content and T-score

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sclerostin (ng/mL)</th>
<th>sTnT (pg/mL)</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMD (g/cm²)</td>
<td>-0.134</td>
<td>-0.080</td>
<td>0.052</td>
</tr>
<tr>
<td>Right femur BMD (g/cm²)</td>
<td>-0.162</td>
<td>0.049</td>
<td>-0.046</td>
</tr>
<tr>
<td>Left femur BMD (g/cm²)</td>
<td>-0.096</td>
<td>-0.010</td>
<td>0.143</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/cm²)</td>
<td>-0.308*</td>
<td>-0.013</td>
<td>-0.303*</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/cm²)</td>
<td>-0.186</td>
<td>0.018</td>
<td>0.079</td>
</tr>
<tr>
<td>L1-L4 BMD (g/cm²)</td>
<td>-0.135</td>
<td>-0.078</td>
<td>-0.044</td>
</tr>
<tr>
<td>Non-dominant arm total radius BMD (g/cm²)</td>
<td>-0.128</td>
<td>-0.131</td>
<td>0.197</td>
</tr>
<tr>
<td>Non-dominant arm 33% radius BMD (g/cm²)</td>
<td>-0.071</td>
<td>-0.153</td>
<td>0.135</td>
</tr>
<tr>
<td>Total T-score</td>
<td>-0.126</td>
<td>-0.072</td>
<td>0.046</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-0.184</td>
<td>0.049</td>
<td>0.213</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-0.095</td>
<td>-0.009</td>
<td>0.141</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-0.303*</td>
<td>-0.009</td>
<td>-0.301*</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-0.200</td>
<td>0.018</td>
<td>0.077</td>
</tr>
<tr>
<td>L1-L4 T-score</td>
<td>-0.138</td>
<td>-0.081</td>
<td>-0.043</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>-0.164</td>
<td>-0.037</td>
<td>0.098</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>-0.164</td>
<td>0.054</td>
<td>0.236</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>-0.093</td>
<td>-0.023</td>
<td>0.097</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>-0.336*</td>
<td>-0.083</td>
<td>0.274</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>-0.241</td>
<td>-0.073</td>
<td>0.188</td>
</tr>
<tr>
<td>L1-L4 BMC (g)</td>
<td>-0.168</td>
<td>-0.003</td>
<td>0.168</td>
</tr>
</tbody>
</table>

BMD=Bone mineral density; BMC= Bone mineral content

*p≤0.05
**Table 19.** Pearson’s correlation coefficients among blood biomarkers

<table>
<thead>
<tr>
<th></th>
<th>Sclerostin (ng/mL)</th>
<th>sTnT (pg/mL)</th>
<th>Leptin (ng/mL)</th>
<th>Adiponectin (µg/mL)</th>
<th>CRP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin (ng/mL)</td>
<td>-</td>
<td>0.3*</td>
<td>-0.106</td>
<td>0.164</td>
<td>-0.224</td>
</tr>
<tr>
<td>sTnT (pg/mL)</td>
<td>0.3*</td>
<td>-</td>
<td>0.16</td>
<td>-0.112</td>
<td>-0.116</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>-0.106</td>
<td>0.16</td>
<td>-</td>
<td>0.007</td>
<td>0.244</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>0.164</td>
<td>-0.112</td>
<td>0.007</td>
<td>-</td>
<td>-0.078</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>-0.224</td>
<td>-0.116</td>
<td>0.244</td>
<td>-0.078</td>
<td>-</td>
</tr>
</tbody>
</table>

CRP = C-reactive protein

* *p ≤ 0.05
**Table 20.** Pearson’s correlation coefficients among leptin, adiponectin, L:A and anthropometrics, body fat, dietary intake of calcium and vitamin D, energy intake total hours of physical activity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Leptin (ng/mL)</th>
<th>Adiponectin (µg/mL)</th>
<th>L/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.265*</td>
<td>0.084</td>
<td>-0.004</td>
</tr>
<tr>
<td>Age at menopause onset (years)</td>
<td>-0.110</td>
<td>-0.066</td>
<td>-0.162</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>0.257</td>
<td>0.116</td>
<td>0.091</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.318*</td>
<td>-0.136</td>
<td>0.175</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.1</td>
<td>-0.143</td>
<td>-0.042</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>0.385**</td>
<td>-0.064</td>
<td>0.191</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.415**</td>
<td>0.014</td>
<td>0.155</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.367**</td>
<td>0.032</td>
<td>0.094</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>0.483**</td>
<td>0.061</td>
<td>0.120</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.196</td>
<td>-0.038</td>
<td>0.134</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.032</td>
<td>0.185</td>
<td>-0.061</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.025</td>
<td>0.078</td>
<td>-0.012</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>0.429**</td>
<td>-0.293*</td>
<td>0.279*</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>0.397**</td>
<td>-0.19</td>
<td>0.208</td>
</tr>
<tr>
<td>Total lean (kg)</td>
<td>0.12</td>
<td>-0.003</td>
<td>0.035</td>
</tr>
<tr>
<td>Lean/fat ratio</td>
<td>-0.388**</td>
<td>0.314*</td>
<td>-0.260*</td>
</tr>
<tr>
<td>ALM</td>
<td>0.088</td>
<td>-0.068</td>
<td>0.070</td>
</tr>
<tr>
<td>ASMI</td>
<td>0.197</td>
<td>0.081</td>
<td>0.094</td>
</tr>
<tr>
<td>Energy intake (kcal/day)</td>
<td>-0.159</td>
<td>-0.029</td>
<td>-0.162</td>
</tr>
<tr>
<td>Total calcium intake (mg/day)</td>
<td>-0.192</td>
<td>-0.142</td>
<td>-0.194</td>
</tr>
<tr>
<td>Total vitamin D intake (IU/day)</td>
<td>-0.424**</td>
<td>-0.125</td>
<td>-0.037</td>
</tr>
<tr>
<td>Total hours of physical activity per week</td>
<td>-0.156</td>
<td>-0.109</td>
<td>-0.005</td>
</tr>
<tr>
<td>Number of steps climbed per day</td>
<td>-0.017</td>
<td>-0.107</td>
<td>0.074</td>
</tr>
</tbody>
</table>

ALM= Appendicular lean mass, ASMI= Appendicular skeletal muscle mass index; BMI= Body mass index

*\(p\leq0.05\)

**\(p\leq0.01\)
Table 21. Pearson’s correlation coefficients among leptin, adiponectin, L/A and variables of bone-mineral density, bone-mineral content and T-score

<table>
<thead>
<tr>
<th>Variables</th>
<th>Leptin (ng/mL)</th>
<th>Adiponectin (µg/mL)</th>
<th>L/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BMD (g/cm²)</td>
<td>-0.143</td>
<td>-0.094</td>
<td>0.179</td>
</tr>
<tr>
<td>Right femur BMD (g/cm²)</td>
<td>0.096</td>
<td>-0.360**</td>
<td>0.323*</td>
</tr>
<tr>
<td>Left femur BMD (g/cm²)</td>
<td>-0.123</td>
<td>-0.432**</td>
<td>0.320*</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/cm²)</td>
<td>-0.045</td>
<td>-0.248</td>
<td>0.105</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/cm²)</td>
<td>-0.196</td>
<td>-0.415**</td>
<td>0.207</td>
</tr>
<tr>
<td>L1-L4 BMD (g/cm²)</td>
<td>0.075</td>
<td>0.016</td>
<td>0.102</td>
</tr>
<tr>
<td>Non dominant arm total radius BMD (g/cm²)</td>
<td>-0.154</td>
<td>-0.185</td>
<td>0.153</td>
</tr>
<tr>
<td>Non dominant arm 33% radius BMD (g/cm²)</td>
<td>-0.218</td>
<td>-0.141</td>
<td>0.095</td>
</tr>
<tr>
<td>Total T-score</td>
<td>-0.140</td>
<td>-0.098</td>
<td>0.188</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-0.123</td>
<td>-0.379**</td>
<td>0.321*</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-0.120</td>
<td>-0.430**</td>
<td>0.325*</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-0.04</td>
<td>-0.246</td>
<td>0.107</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-0.194</td>
<td>-0.421**</td>
<td>0.204</td>
</tr>
<tr>
<td>Lumbar spine T-score (L₁-L₄)</td>
<td>0.078</td>
<td>0.015</td>
<td>0.1</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>-0.127</td>
<td>-0.126</td>
<td>0.078</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>-0.093</td>
<td>-0.294*</td>
<td>0.278*</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>-0.198</td>
<td>-0.329*</td>
<td>0.218</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>0.068</td>
<td>-0.189</td>
<td>0.028</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>0.0</td>
<td>-0.332*</td>
<td>0.066</td>
</tr>
<tr>
<td>L1-L4 BMC (g)</td>
<td>-0.029</td>
<td>-0.022</td>
<td>0.053</td>
</tr>
</tbody>
</table>

BMD=Bone mineral density; BMC= Bone mineral content
*p≤0.05
**p≤0.01
Table 22. Pearson’s correlation coefficients between leptin, adiponectin, L/A and variables of bone-mineral density, bone-mineral content and T-score after adjusted for weight and BMI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Leptin (ng/mL)</th>
<th>Leptin (ng/mL)</th>
<th>Adiponectin (µg/mL)</th>
<th>Adiponectin (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r&lt;sup&gt;a&lt;/sup&gt;</td>
<td>r&lt;sup&gt;b&lt;/sup&gt;</td>
<td>r&lt;sup&gt;a&lt;/sup&gt;</td>
<td>r&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.249</td>
<td>-0.253</td>
<td>-0.060</td>
<td>-0.082</td>
</tr>
<tr>
<td>Right femur BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.184</td>
<td>-0.217</td>
<td>-0.333*</td>
<td>-0.337*</td>
</tr>
<tr>
<td>Left femur BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.230</td>
<td>-0.268</td>
<td>-0.407**</td>
<td>-0.415**</td>
</tr>
<tr>
<td>Right femoral neck BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.232</td>
<td>-0.231</td>
<td>-0.214</td>
<td>-0.228</td>
</tr>
<tr>
<td>Left femoral neck BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.328*</td>
<td>-0.348*</td>
<td>-0.399**</td>
<td>-0.407**</td>
</tr>
<tr>
<td>L&lt;sub&gt;1&lt;/sub&gt;-L&lt;sub&gt;4&lt;/sub&gt; BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.071</td>
<td>-0.102</td>
<td>0.030</td>
<td>0.007</td>
</tr>
<tr>
<td>Non dominant arm total radius BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.208</td>
<td>-0.183</td>
<td>-0.171</td>
<td>-0.183</td>
</tr>
<tr>
<td>Non dominant arm 33% radius BMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>-0.244</td>
<td>-0.221</td>
<td>-0.136</td>
<td>-0.143</td>
</tr>
<tr>
<td>Total T-score</td>
<td>-0.250</td>
<td>-0.254</td>
<td>-0.063</td>
<td>-0.085</td>
</tr>
<tr>
<td>Right femur T-score</td>
<td>-0.210</td>
<td>-0.234</td>
<td>-0.353**</td>
<td>-0.358**</td>
</tr>
<tr>
<td>Left femur T-score</td>
<td>-0.225</td>
<td>-0.264</td>
<td>-0.405**</td>
<td>-0.413**</td>
</tr>
<tr>
<td>Right femoral neck T-score</td>
<td>-0.214</td>
<td>-0.215</td>
<td>-0.213</td>
<td>-0.226</td>
</tr>
<tr>
<td>Left femoral neck T-score</td>
<td>-0.330*</td>
<td>-0.349*</td>
<td>-0.405**</td>
<td>-0.413**</td>
</tr>
<tr>
<td>Lumbar spine T-score (L&lt;sub&gt;1&lt;/sub&gt;-L&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>-0.070</td>
<td>-0.102</td>
<td>0.029</td>
<td>0.005</td>
</tr>
<tr>
<td>Total BMC (g)</td>
<td>-0.325*</td>
<td>-0.248*</td>
<td>-0.073</td>
<td>-0.114</td>
</tr>
<tr>
<td>Right femur BMC (g)</td>
<td>-0.266</td>
<td>-0.271</td>
<td>-0.250*</td>
<td>-0.261*</td>
</tr>
<tr>
<td>Left femur BMC (g)</td>
<td>-0.401**</td>
<td>-0.412**</td>
<td>-0.281*</td>
<td>-0.295*</td>
</tr>
<tr>
<td>Right femoral neck BMC (g)</td>
<td>-0.175</td>
<td>-0.192</td>
<td>-0.140</td>
<td>-0.160</td>
</tr>
<tr>
<td>Left femoral neck BMC (g)</td>
<td>-0.244</td>
<td>-0.277</td>
<td>-0.310*</td>
<td>-0.325*</td>
</tr>
<tr>
<td>L1-L4 BMC (g)</td>
<td>-0.253</td>
<td>-0.221</td>
<td>0.002</td>
<td>-0.030</td>
</tr>
</tbody>
</table>

BMD=Bone mineral density; BMC= Bone mineral content; r<sup>a</sup>= Adjusted for weight; r<sup>b</sup>= Adjusted for BMI

*<i>p</i>≤0.05

**<i>p</i>≤0.01
**Table 23.** Descriptive statistic for lipid profile of all participants (N=59)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum/Maximum</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>195.0 ± 37.2</td>
<td>107.0/296.0</td>
<td>189.0</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>97.8 ± 41.7</td>
<td>37.0/200.0</td>
<td>163.0</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>110.8 ± 35.1</td>
<td>35.4/224.4</td>
<td>198.0</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>23.1 ± 26.5</td>
<td>7.4/199.8</td>
<td>192.4</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>64.6 ± 15.1</td>
<td>38.0/95.0</td>
<td>57.0</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dL)</td>
<td>130.0 ± 37.2</td>
<td>54.0/247.0</td>
<td>193.0</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio</td>
<td>3.1 ± 0.8</td>
<td>1.6/6.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

TG= Triglyceride; HDL= High density lipoprotein
<table>
<thead>
<tr>
<th>Variables</th>
<th>Osteopenic obese (N=35)</th>
<th>Obese-only (N=10)</th>
<th>OSO (N=10)</th>
<th>Osteopenic/ sarcopenic non-obese (N=4)</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>190.6 ± 34.5</td>
<td>191.7 ± 34.6</td>
<td>215.3 ± 49.6</td>
<td>187.3 ± 22.3</td>
<td>&lt;200</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>105.0 ± 42.9a</td>
<td>101.4 ± 47.0a</td>
<td>87.5 ± 27.6a</td>
<td>49.0 ± 10.8b</td>
<td>&lt;150</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>106.3 ± 30.5</td>
<td>112.5 ± 24.0</td>
<td>129.8 ± 55.0</td>
<td>93.2 ± 21.6</td>
<td>&lt;130</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>21.0 ± 8.6a</td>
<td>20.3 ± 9.4a</td>
<td>35.7 ± 57.9a</td>
<td>9.8 ± 2.2b</td>
<td>&lt;30</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>63.3 ± 15.6a</td>
<td>59.0 ± 11.5a</td>
<td>69.0 ± 13.9ab</td>
<td>84.3 ± 2.1b</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dL)</td>
<td>127.3 ± 31.2</td>
<td>132.7 ± 29.2</td>
<td>147.3 ± 31.2</td>
<td>103.0 ± 20.6</td>
<td>&lt;160</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio</td>
<td>3.1 ± 0.7</td>
<td>3.3 ± 0.6</td>
<td>3.3 ± 1.4</td>
<td>2.2 ± 0.2</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

OSO=Osteosarcopenic obesity; TG= Triglyceride; HDL= High density lipoprotein
Values within a row with different superscript sign indicate that they are significantly different from each other (p≤0.05).
There was no significant difference among groups using the ANOVA test. Kruskal-Wallis test was performed on the non-parametrical data which are presented in the table.
Figure 2. Flow-chart of participants from recruitment to analysis
Figure 3. Comparison of serum sclerostin concentrations in different groups. Bars with different script letters indicate that they are significantly different from each other ($p \leq 0.05$). Data are shown as mean ± standard error.
Figure 4. Comparison of serum sTnT concentrations in different groups. Bars with different script letters indicate that they are significantly different from each other ($p \leq 0.05$). Data are shown as mean ± standard error.
Figure 5. Comparison of serum leptin concentrations in different groups. Bars with different script letters indicate that they are significantly different from each other ($p \leq 0.05$). Data are shown as mean ± standard error.
Figure 6. Comparison of serum adiponectin concentrations in different groups. Bars with different script letters indicate that they are significantly different from each other ($p \leq 0.05$). Data are shown as mean ± standard error.
Figure 7. Comparison of CRP concentrations in different groups. Bars with different script letters indicate that they are significantly different from each other ($p \leq 0.05$). Data are shown as mean ± standard error.
CHAPTER 5

DISCUSSION

5.1 Overall Findings

The overall objective of this study was to investigate the metabolic profile of the OSO syndrome in the cohort of postmenopausal women, and evaluate it within the context of other parameters, including bone and body composition measurements, diet, and habitual physical activity. Based on our findings, the combination of high serum sclerostin, sTnT and leptin and low adiponectin can be used to better identify the characteristics of OSO syndrome and possibly apply as measurements for its diagnosis. Moreover, CRP could be used as a biomarker to better evaluate and measure the severity of OSO syndrome. In addition, OSO syndrome is associated with the most imbalanced lipid profile compared to other groups.

The current study revealed a statistically significant negative correlation between leptin and total vitamin D intake. These results may suggest the role of vitamin D in regulating body fat percentage. Other significant findings of our study are that protein intake has a positive correlation with right femoral neck BMD and T-score in the entire population, which may confirm the important role of protein intake on bone health. In addition, our results confirmed the important role of physical activity in maintaining the bone and muscle health.

The key findings of the present study demonstrate that there is a significant positive correlation between serum sclerostin and sTnT ($r=0.3; p\leq0.05$), indicating simultaneous mediation of these biomarkers in bone and muscle loss in postmenopausal women. The results showed that the serum sclerostin concentrations were significantly higher in the OSO and osteopenic obese population in comparison to the obese-only, and there was no significant difference among the osteopenic/sarcopenic non-obese group and the other groups. Therefore, the hypothesis was accepted as the sclerostin concentration was higher in the OSO group in comparison to the other groups. Serum sTnT concentration was also significantly higher in the OSO group in comparison to osteopenic obese and obese-only groups. Therefore, the hypothesis was accepted. In addition, OSO group had significantly greater leptin values than osteopenic/sarcopenic non-obese group, confirming our hypothesis.
These results confirm possible simultaneous effects of osteopenia/osteoporosis, sarcopenia, and adiposity on each other. Overall, women with the OSO syndrome have presented with the poorest outcomes for each variable including sclerostin, sTnT, leptin and CRP.

This is the first study to investigate the relationship between serum sclerostin and sTnT as predictors of bone and muscle health, respectively. Sclerostin concentration was previously used to evaluate the severity of bone loss as it inhibits the differentiation of osteoblasts and reduces bone formation (Wijenayaka et al. 2011). Moreover, a previous study showed that sclerostin increases the urinary excretion of calcium and phosphorus, thereby possibly reducing bone mineralization (Ryan et al. 2013). Serum sTnT concentration was associated with muscle injury and specifically sarcopenia (Chase et al. 2013). This study provides supporting evidence that sclerostin and sTnT are useful markers for bone and muscle turnover. Moreover, based on our results, the OSO group had significantly greater leptin concentrations than osteopenic/sarcopenic non-obese group. Therefore, the hypothesis was accepted as leptin concentration was higher in the OSO group in comparison to the other groups. Women in the osteopenic/sarcopenic non-obese group had a significantly greater adiponectin level than the women in other three groups. In fact, the obese-only group had the lowest leptin concentration of all. Therefore, the hypothesis was rejected as the lowest adiponectin concentration was not observed in the OSO group. In addition, although the OSO group had the highest CRP concentration, there was no significant difference among groups. Therefore our hypothesis is partially accepted. Based on the current study, osteopenic/sarcopenic non-obese group had the lowest concentrations of cholesterol, TG, LDL, VLDL and the highest HDL concentration. Since there were no significant differences between groups, our hypothesis is partially accepted. The overall findings have been discussed in more details in the below sections.

5.2 Serum Biomarkers

5.2.1 Association between Serum Sclerostin and Bone

Previous studies have reported the association of high serum sclerostin with low BMD and increased risk of osteoporotic fractures (Mirza et al. 2010; Amrein et al. 2012; Ardawi et al. 2012; Zou et al. 2016). This is the first study that assessed the relationship between sclerostin, and bone mineral density in OSO syndrome (where osteopenia, sarcopenia and increased adiposity are evaluated at the same time).
Our results showed that serum sclerostin had a significant negative correlation with right femoral neck BMD, T-score and BMC ($r=-0.308$, $r=-0.303$, $r=-0.336$, respectively; $p \leq 0.05$) in the entire population. This indicated that higher serum sclerostin concentrations were associated with lower bone density. Our results were consistent with the findings in prior studies (Amrein et al. 2012; Zou et al. 2016). In addition, we found that serum sclerostin concentrations were significantly higher in the OSO and osteopenic obese groups in comparison to the obese-only group. This indicates the simultaneous effect of osteopenia/osteoporosis and sarcopenia on bone mineral density and body composition (including muscle and adipose tissue). Overall sclerostin could be used as a reliable biomarker for the OSO diagnosis.

5.2.2 Association between Serum sTnT and Muscle

Serum concentration of sTnT has been shown to have strong association with sarcopenia (Chase et al. 2013). This is the first study that assessed the relationship between sTnT, and muscle loss in OSO syndrome and its correlation with sclerostin.

Serum sTnT has previously been used to evaluate skeletal muscle health (Abreu et al. 2014; Magnusson-Lind et al. 2014). Results of our study showed a negative association between serum sTnT concentrations and total lean, lean/fat ratio, ALM and ASMI and positive association with total body fat, total fat percentage, age and weight in the entire population. Serum sTnT concentration was significantly higher in the OSO group in comparison to the osteopenic obese and obese-only groups, which indicates the simultaneous effect of osteopenia/osteoporosis and sarcopenia on muscle mass and body composition. The results indicate that troponin could be used as a biomarker of muscle health for the older adults and as a reliable indicator for the OSO diagnosis.

5.2.3 Association between Serum Adipokines and Bone and Body Composition

Leptin and adiponectin, as adipocyte-secreted hormones, have been speculated to be possible links between fat and bone. Conflicting results of the relationship between leptin and bone were reported in both men and women (Iwamoto et al. 2000; Dennison et al. 2004; Weiss et al. 2006; Koroglu et al. 2011; Barbour et al. 2012). Moreover, several studies demonstrated that adiponectin is either inversely or not related to BMD (Richards et al. 2007; Kanazawa et al. 2009; Ozkurt et al. 2009; Miazgowski et al. 2012; Tenta et al. 2012). Our study focused on the relationship of adipokines and bone parameters and body composition in postmenopausal women. Increased fat mass has been shown to be a predictor of circulating leptin concentrations.
(Baskin et al. 1999; Thomas et al. 2000). Moreover, serum adiponectin is negatively correlated with adiposity (Bahceci et al. 2007; Goropashnaya et al. 2009; JafariNasabian et al. 2015, 2017b). Our results corroborated those findings. In our study, leptin concentration had a positive correlation with body fat percentage and total body fat ($p \leq 0.01$). Moreover, negative correlation of serum adiponectin with body fat percentage ($p \leq 0.05$) was found. The highest concentrations of leptin were observed in the OSO group (17.3 ± 4.5 ng/mL) and women in the OSO group had significantly greater leptin values than the osteopenic/sarcopenic non-obese group. It has been reported that leptin is positively correlated with BMI, body fat percentage, waist/hip ratio, waist and hip circumference (Smith et al. 2006). Our results were consistent with the findings in prior studies, showing a significant positive correlation among leptin and total body fat, age, BMI, waist, hip and abdominal circumference. A significant negative correlation was reported, after adjusting for weight/BMI, between leptin and ALM. A similar significant negative correlation were noticed between leptin and left femoral BMD, T-score and BMC after adjusting for weight/BMI. The OSO group had the highest concentrations of leptin in comparison to other groups; therefore, higher leptin may result in poorer outcome for bone and muscle mass.

Our results also showed that women in the osteopenic/sarcopenic non-obese group had significantly greater adiponectin values than the women in the other three groups ($p \leq 0.05$). Moreover, statistically significant negative correlation of serum adiponectin with body fat percentage was noted in the whole population. Our results corroborated findings of the previous literature (Bahceci et al. 2007; Goropashnaya et al. 2009; JafariNasabian et al. 2015).

A previous study conducted by Jurimae et al. (Jürimäe et al. 2005) revealed a positive correlation between serum leptin and BMD of total body and femoral neck in overweight healthy postmenopausal women. A significant negative association was reported between serum adiponectin and BMD at several skeletal sites including total body, lumbar spine, and femoral neck (Jürimäe et al. 2005). However, in a more recent study conducted on 104 Hispanic and Caucasian women, no significant correlation was observed between leptin and BMD. In addition, adiponectin was inversely correlated with BMD in Caucasian women only (King et al. 2010).

The results of our study revealed that leptin was not a significant predictor of BMD, T-score or BMC of different skeletal sites before adjusting for weight and/or BMI. However, after the adjustment, a significant negative correlation was observed between leptin and left femoral BMD, BMC and T-score. These results indicate that the effects of leptin on bone could be
mediated through combination of multiple pathways and based on site of action the effects could be different (Thomas et al. 1999; Ducy et al. 2000; Holloway et al. 2002).

As adiponectin receptors are expressed in bone-forming cells, it can provide a signaling pathway between adipose tissue and bone (Berner et al. 2004). Adiponectin appears to indirectly increase osteoclast formation by stimulating RANKL pathway (Luo et al. 2006). Several studies have revealed an inverse correlation between adiponectin and BMD (King et al. 2010; Cervellati et al. 2016). Similarly, in our study, a statistically significant negative correlation of serum adiponectin with BMD, BMC and T-scores of several skeletal sites were observed, before and after adjusting for weight and/or BMI suggesting that the effect of adiponectin on bone is not weight dependent.

The lowest ratio of L/A were reported in the osteopenic/sarcopenic non-obese group. The association between L/A ratio and BMD has been previously investigated (Chi et al. 2013). It has been reported that serum L/A ratio was positively correlated to both femoral neck and total femur BMD. Our results also confirmed significant positive correlations between L/A ratio and BMD, T-score and BMC of femur further suggesting that the influence may be site-specific. Moreover, L/A ratio could be considered as an efficient index for evaluating the effects of adipokines on bone and body composition.

5.2.4 Association between Serum CRP and Bone and Body Composition

An inverse association of CRP concentration and BMD has been reported in both men and women (Ganesan et al. 2005; de Pablo et al. 2012). Our results have revealed a significant negative correlation between CRP and right femoral BMD and T-score but not in other skeletal sites. The lack of association between BMD and CRP concentration at other skeletal sites may be related to regional bone characteristics, which may also explain why bone disorders may have tendency toward certain regions. CRP concentration was positively correlated with total body fat and fat percentage and negatively correlated with lean/fat ratio. Therefore, CRP could be used as a biomarker for evaluation of OSO syndrome.

5.2.5 Association between Serum Sclerostin, sTnT and Adipokines

This is the first study that investigated the correlation of serum sclerostin and sTnT as predictors of bone and muscle parameters, respectively. A significant positive association was observed between serum sclerostin and sTnT in postmenopausal women which will further confirm that bone and muscle are interrelated. Also, this is the first study investigating the
association between sTnT and adipokines. Our results revealed no significant correlation between sTnT and leptin, adiponectin or L/A ratio. Previous studies have investigated the correlation between serum sclerostin and leptin (Armamento-Villareal et al. 2012; Grethen et al. 2012). No significant association between leptin and sclerostin has been reported. Our results also confirmed no significant correlation between these biomarkers. Serum sclerostin has been reported to be associated with adiponectin (González-Reimers et al. 2014; Jürimäe et al. 2016). Gonzalez-Reimers et al reported a significant direct correlation between serum sclerostin and adiponectin. However, the target population was 20 years old that didn’t have osteoporosis. Jürimäe et al. also reported the same results in a similar population (9-10 years old). Therefore, to our knowledge this is the first study that investigated the relationship between sclerostin and adiponectin in the content of older individuals. Our results revealed no significant association between the mentioned biomarkers in older adult women.

5.3 Dietary Intake from Food & Supplements

The average vitamin D, calcium and protein intake was higher than the RDA, and the average protein intake did not meet the recommended 1.4-1.6 g/kg/day (Campbell et al. 1994; Breen & Phillips 2013; Churchward-Venne et al. 2014) with only 26.66% and 18.3% of participants consuming more than 1.4 and 1.6 protein g/kg/day, respectively. The lowest amount of protein intake was observed in the OSO group. A previous study reported that 10%–25% of older adults consume less protein than the RDA and 5%–9% consumed less than the EAR (Fulgoni 2008).

The current study revealed a statistically significant negative correlation between leptin and total vitamin D intake. These results may suggest the role of vitamin D in regulating body fat percentage. Previous studies have reported an association between obesity and vitamin D deficiency (Lira et al. 2011; Ding et al. 2012). It has been reported that obese people have lower vitamin D status (Ding et al. 2012). The combination of our results and previous studies may indicate that vitamin D plays an important role in preventing obesity.

Elderly women living in an assisted living facility tend to have a sedentary lifestyle. They don’t shop for their food and rely on someone else for food preparation. This lifestyle may result in reduced protein and total energy intake (Volpi et al. 2013; Kelly et al. 2016a). Low physical activity combined with inadequate protein intake may further aggravate the decline in muscle protein synthesis and contribute to muscle loss in older adults. Extensive high protein
diet is associated with urinary calcium loss (Heaney & Layman 2008); therefore, it could affect bone mineral density (Cuenca-Sánchez et al. 2015). The relationship between protein intake and bone health is largely focused on the balance between dietary protein and calcium consumption. It is important to balance high protein intake with calcium, vitamin D and other nutrients related to bone health (Heaney & Layman 2008). In addition, dietary protein may positively impact bone mineral density as a proxy from increased muscle mass. Our results showed significant positive correlation between total amount of protein intake and right femoral neck BMD and T-score in the entire population. These results may confirm the positive effect of protein intake on bone health as long as there is an appropriate balance between protein and calcium intake specifically for the OSO syndrome.

5.3.1 OSO Syndrome Management and Life-style Recommendations Based on Diet

Lifestyle choices including dietary pattern plays an important role in the etiology of OSO syndrome. Diet plays a role in metabolic homeostasis of bone and muscle mass and balancing an optimal body weight (Kelly et al. 2016a, b; JafariNasabian et al. 2017a). Older adults often consume fewer nutrients including protein, calcium, magnesium and vitamin D due to decreased appetite, side effects associated with their medications, dementia or a desire for weight loss (Inglis et al. 2016; JafariNasabian et al. 2017a). Moreover, healthy fruit and vegetable intake, often low in older people, is associated with improved muscle strength (Neville et al. 2013) and increase muscle mass (JafariNasabian et al. 2013) and reduced risk of sarcopenia (Kim et al. 2015). In addition, nutrients are needed to maintain and build bone and muscle mass (Price et al. 2012). Absorption of most vitamins and minerals is diminished with aging (Pray et al. 2010). Older adults in the U.S. are potentially at nutritional risk due to increased consumption of high-energy and low-nutrient-dense diet, inadequate dietary fiber, and decreased ability to absorb or utilize some essential nutrients (Pray et al. 2010; JafariNasabian et al. 2017a). Over time, this may lead to bone and muscle loss and increased adiposity, as well as increased insulin resistance and low-grade chronic inflammation (Lattimer & Haub 2010; Ilich et al. 2014b). Moreover, a high dietary ratio of omega-6 to omega-3 polyunsaturated fatty acids may contribute to low-grade chronic inflammation (Ilich et al. 2014b; Kelly et al. 2016a, b). The important role of calcium and vitamin D on bone health has been thoroughly discussed previously in chapter two. Moreover, it has been shown that energy and protein intake has an independent correlation to BMD in several skeletal sites (Ilich & Brownbill 2008). A diet insufficient in calcium could
increase circulating calcitriol (1, 25-dihydroxyvitamin D). Higher calcitriol concentrations will stimulate adipogenesis via a specific-membrane vitamin D receptor (Zemel et al. 2005). In addition, insufficient energy and protein intake can increase muscle wasting (Morley 2001; Bales & Ritchie 2002; JafariNasabian et al. 2017a). Inadequate protein intake in aging adults combined with sedentary lifestyle may further exacerbate the decline in muscle protein synthesis associated with aging. As discussed in section 5.1, the RDA for protein (0.8 g/kg/day) is insufficient to maintain muscle mass in older adults. Nitrogen balance studies suggest that older adults need to consume a higher protein level (1.0-1.6 g/kg/day) (Campbell et al. 1994; Breen & Phillips 2013; Churchward-Venne et al. 2014; Nowson & O’Connell 2015). A recent analysis of NHANES data showed that energy imbalance, lower protein intake, excess consumption of simple carbohydrates, calcium, magnesium and potassium deficiency, excess phosphorus, sodium and iron intake and low long-chain polyunsaturated fatty acid intake may contribute to the OSO syndrome (Kelly et al. 2016a, b). The overall the dietary composition of both macro and micro nutrients plays an important role in preventing the OSO syndrome (JafariNasabian et al. 2017a).

5.4 Physical Activity

In general, physical activity is needed for maintenance and improvement of all components of body composition (Booth et al. 2000). Specifically in OSO syndrome, physical activity, even in the form of low intensity or habitual activity, could be used to maintain and improve BMD (Hughes et al. 2001; Ilich & Brownbill 2008; Kelly & Gilman 2016). Previous studies have supported the role of exercise in maintaining muscle strength and muscle quality, improving balance, and reducing adiposity and inflammation, with aging (Deckx et al. 2016; Kelly & Gilman 2016). Our results showed that there was a significant negative correlation between total hours of physical activity per week and waist circumference. In addition, total hours of habitual physical activity and average number of steps climbed were negatively correlated with total body fat, total body fat percentage, weight, BMI, hip circumference, abdominal circumference and energy intake. In conclusion, even low intensity exercise or habitual activities such as gardening, house work, etc. may help maintain the bone mineral density and muscle mass and reduce the risk of obesity for OSO syndrome.
5.4.1 OSO Syndrome Management and Life-style Recommendations Based on Physical Activity

Physical activity, even in the form of low intensity or habitual activity, is needed to maintain or improve BMD, (Hughes et al. 2001; Ilich & Brownbill 2008; Kelly & Gilman 2016) maintain muscle mass and muscle quality, improve balance, and reduce adiposity and inflammation, with aging.(Deckx et al. 2016; Kelly & Gilman 2016) and overall reduce the risk of OSO syndrome (JafariNasabian et al. 2017a). Different studies have revealed that weight-bearing physical activity can attenuate the rate of BMD loss by aging (Lanyon 1984; Wolff et al. 1999; Garber et al. 2011). A comprehensive exercise program for patients with OSO syndrome should include aerobic, strength, flexibility, and balance training (JafariNasabian et al. 2017a). All four components are crucial for the maintenance of body composition and bone health. Recent findings showed that resistance exercise increases lean mass most effectively, however, it’s best for older adults to exercise within their limits (Kelly & Gilman 2016; JafariNasabian et al. 2017a). Reducing adiposity through aerobic exercise and strength training is substantial in reducing inflammation and as a result in reducing bone and muscle loss and inhibiting the progression of OSO syndrome (Meng & Yu 2010; Chung et al. 2011; JafariNasabian et al. 2017a).

5.5 Concluding Remarks

OSO syndrome is a geriatric disease with multifactorial changes including bone and muscle loss combined with increased or redistributed adiposity (into the bone and muscle). This complex syndrome is not caused by one single factor, but by a mixture of elements such as lack of physical activity, poor nutrition, and low-grade chronic inflammation. The lack of established diagnostic criteria for OSO syndrome in the past few years attracted our focus to this issue. The results of this study could be used to establish diagnostic criteria for OSO syndrome in postmenopausal women. This study was the first study to examine the relationship between serum sclerostin and sTnT as predictors of bone and muscle parameters, respectively. The positive correlation we found between bone and muscle biomarkers has further confirmed that bone and muscle mass are interrelated. In conclusion, the combination of high serum sclerostin, sTnT and leptin and low adiponectin can be used to better identify the metabolic profile of OSO syndrome and possibly apply as measurements for its diagnostic criteria. In addition, CRP could be used as a biomarker to better evaluate and measure the severity of OSO syndrome. A
comprehensive nutritional intervention, in combination with physical activity and exercise training, are essential for the prevention and management of OSO syndrome.
APPENDIX A

IRB APPROVAL LETTERS

Floridiana 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: 02/11/2016
To: [Redacted]
Address: [Redacted]
Dept: NUTRITION FOOD AND EXERCISE SCIENCES
From: Thomas L. Jacobson, Chair
Re: Use of Human Subjects in Research

Analyzing bone, muscle and adipose tissue biomarkers to identify osteoarthritis obesity syndrome in older 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 11/18/2015.

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 relating to potential risk and benefit. This approval does not replace any institutional 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 11/16/2016 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: Joannikia Fisch-Enzi - fisch-enz@fsu.edu, Advisor
HSC No: 2015.16581
RE-APPROVAL MEMORANDUM

Date: 10/13/2016

To: Pegah JafariNasabian

Address: [Redacted]

Dept.: NUTRITION FOOD AND EXERCISE SCIENCES

From: Thomas L. Jacobson, Chair

Re: Re-approval of Use of Human subjects in Research:
   Analyzing bone, muscle and adipose tissue biomarkers to identify osteosarcopenic obesity syndrome in older women

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 10/11/2017, you are must request renewed approval by the Committee.

If you submitted a proposed consent form with your renewal request, the approved stamped consent form is attached to this re-approval notice. Only the stamped version of the consent form may be used in recruiting of research subjects. You are reminded 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 are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols as often as necessary to ensure that the project is being conducted in compliance with our institution and with DHHS regulations.

Cc: HSC No. 2016.19216
APPENDIX B

INITIAL SCREENING FORM

Initial screening

Date: ______________________

Name: ____________________________________________

Date of Birth: ________________

Must be ≥70 (born in or before 1945)

Address: ____________________________________________

Telephone: ________________________ E-mail _________________________

Self-reported

Weight: ________ lbs  Height:______ feet ______ inch  BMI:____________________

(Lbs*703/inch²)

1) Can you move around on your own?

Yes  No

If NO, what kind of aid do you need for moving around?
Walk, cane OK; wheelchair is disqualifier

2) Do you have Parkinson’s disease or multiple sclerosis

Telephone: ________________________ E-mail _________________________

3) Do you have systemic lupus or thyroid disease?

4) Do you have severe rheumatoid arthritis or osteoarthritis?

5) Do you have chronic fatigue or vitiligo?
6) Have you ever had cancer or presently have cancer?
   If YES, what kind and how long ago?
   **OK, if cancer-free** for 3 years

7) Do you have high blood pressure?
   If YES, are you taking medications, what kind and for how long?

8) Do you have severe osteoporosis?
   If YES, are you taking medications, what kind and for how long?

9) Do you have diabetes?
   If YES, are you taking medications, what kind and for how long?

10) Have you taken corticosteroids in the last 3 months?

11) Are you taking hormone replacement therapy or other postmenopausal drugs
   **OK, if free** for 3 mos. And not planning to resume

12) Are you presently taking diuretics?

13) Are you taking any supplements?
   Please list any supplements you are taking and bring them with you at the appointment
   ________________________________

14) Are you taking any medications ________________________________
   Please list any medications you are taking and bring them with you at the appointment
APPENDIX C

TELEPHONE INTERVIEW

First Contact/Appointment

“Hello I’m Pegah J. Nasabian, calling from Florida State University. You’ve previously expressed interest in the frailty study. We are conducting a research study that will evaluate the impact of bone and muscle loss and body fat on bone, muscle and inflammatory biomarkers. The study will be completed within 2 appointments at the nursing home/residency and/or visits to the university campus. Based on our preliminary studies, women who are dealing with all three conditions of bone loss, muscle loss and obesity (osteosarcopenic obesity) have higher risk of falls and fractures and possible long-term disabilities, compared to age matched obese-only women. The purpose of our project is to identify the effect of this condition on muscle and bone markers in older women. We will measure your height, weight, waist and hip circumferences, resting heart rate and blood pressure. You will be asked to fill out few questionnaires regarding your food intake, physical activity and your health. Your visit could last between 2-3 hours. You will need to be fasted on the day of your appointment since we will collect a blood sample for certain biomarkers related to your bone and muscle health. You will provide you with copies of your test results when you have completed the study. We are very grateful for your time. Please sign/initial this form if you are willing to participate in this study.

Do you have any questions? If not, Can I schedule your appointment with us?

Night before Appointment

“Hello this is Pegah J. Nasabian calling from Florida State University. I wanted to remind you of your appointment tomorrow at (time).”

Remind her to:

- Drink as much water as possible to make blood draw easier
- Not to eat later than 9pm or at least 8-12 hours before your appointment
- Bring a snack or breakfast to eat after blood draw so you aren’t hungry for the visit
- Bring all medications that you are currently taking
- Bring your eyeglasses (if your wear them) for reading forms
Give directions to Sandels from Westminster Oaks…

Start: 4449 Meandering Way, Tallahassee, FL

1. Exit gate, Left on Dempsey Mayo
2. Right on Mahan Dr.; continue on Mahan Dr. /Tennessee St. for 5 miles
3. Left on N. Copeland St.
4. Right on W. Call St.
5. 1st Left on Convocation Way; Sandals Building on corner

End: 120 Convocation Way, Tallahassee, FL (You can park in the two spaces in the loading dock area by the dumpsters in front of the Sandels building or in the circular drive in beside the Sandels marked for participant parking only.

Thank you very much for your time and we are looking forward to working with you shortly.
APPENDIX D

INFORMED CONSENT DOCUMENT

THE FRAILTY STUDY
CONSENT FORM FOR PARTICIPATION IN A RESEARCH PROJECT
AT FLORIDA STATE UNIVERSITY

Principal Investigator Name: Pegah Jafar Nasabian PhD Candidate
Co-Investigators: Jasminka Ilich-Ernst, PhD, RD
Project Title: Analyzing bone, muscle and adipose tissue biomarkers to identify osteosarcopenic obesity syndrome in older women

Invitation to Participate
You are invited to participate in a research study that will evaluate the impact of bone and muscle loss and body fat on bone, muscle and inflammatory biomarkers. The study will last two years, but each participant's enrollment will be completed within 2 appointments within a 6 month period at the nursing home/residency and/or visits to the university campus (Florida State University).

Description of the Study
Our lab has recently identified a condition characterized by the coexistence of decreased bone mass, decreased muscle mass and strength, and increased adiposity and termed it osteosarcopenic obesity syndrome. Elderly women with osteosarcopenic obesity have higher risk of falls and fractures and possible long-term disabilities, compared to age matched obese-only women.

This project aims to identify osteosarcopenic obesity in older (65 years or older) women who might not be clinically obese (e.g. determined by body mass index), but yet have increased adiposity (fat) levels and fat infiltration both in the bone and muscle resulting in either osteopenic obesity or sarcopenic obesity, respectively, or combined osteosarcopenic obesity. We will identify the effect of these conditions on specific biomarkers collected from participants' blood samples and compare them among osteosarcopenic obese women, osteopenic obese, and obese-only women. These tests will enable us to develop the cut-off points for the official medical diagnosis of osteosarcopenic obesity. Subsequently, this identification will provide an opportunity for these women to improve their condition or at least maintain the status quo, using appropriate physical activity measures, along with dietary modification.

Timing and procedures:
Initial introduction of the study and distribution of screening forms and consent
First visit: You will come to the Florida State University Lab for a fasting blood draw. You need to be fasted for 8-12 hours ( abstain from foods or drinks). Please avoid drinking caffeine in the last 12 hours and also avoid moderate to heavy physical activity in the last 24 hours before your visit. We will measure your height, weight, waist and hip circumferences, heart rate and blood pressure and you will be asked to fill out few questionnaires regarding your physical activity and your health (all can be done in the first visit and it last between 2-3 hours). By the end of this visit you will receive free Calcium, vitamin D and Magnesium supplementation for the total duration of six months. You will be provided with an instruction for your supplement intake (650 mg of Calcium/ 400 IU vitamin D and 300 mg of Magnesium per day). Supplements have been donated by Bayer HealthCare Company. (36 Columbia Road, Morristown, NJ 07960). If you are already using similar supplements (calcium, magnesium, and vitamin D), it is requested that you switch to the supplements that we will provide for the sake of consistency in the study.
Second visit: As a follow up at the end of 6 months, you will come back to our lab for a fasting blood draw (You need to be fasted for 8-12 hours). Please avoid drinking caffeine in the last 12 hours and also avoid
moderate to heavy physical activity in the last 24 hours before your visit. We will re-measure your height, weight, waist and hip circumferences, heart rate and blood pressure and you will be asked to fill out few questionnaires regarding your physical activity and your health (if last between 2-3 hours).

You will need to be fasted for your visits (between 8-12 hours). Blood will be drawn in both first and the final visits (3 tubes with ~10 cc each, or 30 cc total) to measure bone and muscle biomarkers.

Disqualifications and Exclusion Criteria
If you have any of the following conditions, they will be considered disqualifiers and you will be excluded from the study: Parkinson’s disease, multiple sclerosis, systemic lupus, thyroid disease, severe rheumatoid arthritis, osteoarthritis, chronic fatigue, vitiligo, history of cancer, history of or current kidney stones, kidney disease or insufficiency, hyperparathyroidism, vitamin D toxicity, hypercalcemia, hypophosphatemia, history of or current gastrointestinal bleeding and diagnosed cognitive impairment disorder.

Risks and Inconveniences
There might be a discomfort caused by taking anthropometric measurements and collecting demographic, health, dietary and life-style information. The completion of the questionnaires may take around 45-60 minutes. To minimize this discomfort, a professional, caring, non-judgmental staff will be employed and confidentiality will be in place at all times.

There is a small risk with taking blood samples and there may be some bruising at the site where the blood is drawn. There is also the possibility of risk of infection due to breakage of the skin. To minimize this risk and discomfort, a trained individual will draw blood strictly following the universal precaution’s measures and sanitary rules.

Possible Side Effects of Supplements
Possible Side-effects from calcium: Hypercalcemia (high calcium level in the blood), hypercalciuria (high calcium level in urine), kidney stone, hypomagnesemia (low magnesium level in blood), constipation, nausea.

Possible Side-effects from magnesium: Abdominal pain, constipation, dehydration, diarrhea, nausea, vomiting.

Possible Side-effects from vitamin D: Hypercalcemia (high calcium level in the blood), hypercalciuria (high calcium level in urine).

If you present any of the above symptoms, please report the side effects to your healthcare provider and stop taking the supplements.

Benefits
Results from all measurements will be shared with each participant, enabling them a better insight and awareness of their health and diet.

Confidentiality
Information pertaining to each participant will be kept confidential to the extent by law, locked in a file cabinet for 10 years and not disclosed, after which the files will be destroyed. We will use a code number to refer to the participants and the names will not appear in any publication. The FSU Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records.

FSU Human Subjects Committee approved on 2/11/18. VOID 11/18/18. HSC # 2015.18661
In Case of Injury
The FSU does not provide insurance coverage to compensate you if you are injured during the research. However, you may still be eligible for compensation.

Voluntary Participation
The participation in this study is voluntary and there is no penalty for nonparticipation. You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time.

Questions
Take as long as you need before you make a decision about participation. We will be happy to answer any questions you have about this study. If you have further questions about this project or if you have a research-related problem, you may contact the principal investigator, Pegah JafariNasabian, [redacted] or Dr. Jasmina Lich-Ernst, [redacted]. You may also contact the FSU IRB Human Subjects Office at (850) 644-7900.

Authorization:
I, ____________________________, have read this consent form and will participate in the project as described above. The purpose, involvement, and possible hazards/inconveniences of the study have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.
Signature: ____________________________ Date: ____________________________

Signature of Principal Investigator or Person Obtaining Consent
APPENDIX E

ANTHROPOMETRICS MEASUREMENT FORM

ID______________
Date_____________
Visit#____________

Anthropometrics

Weight: ________________kg
Height: _______________cm
BMI: _________________kg/m^2
Waist Circumference: _________________cm
Hip Circumference: _________________cm
Waist-to-Hip Ratio: ________________
Blood Pressure: _________mmHg _________mmHg       Average _________mmHg
Heart Rate: ________bpm ________bpm  Average ________bpm

Research Staff (Print Name): ----------------------------------
APPENDIX F

PHYSICAL PERFORMANCE TEST DATA SHEET

ID______________
Date_____________
Visit#____________

Physical Performance Tests Data Sheet

Handgrip Strength:

   Right hand: _____________ (kg)

   Left hand: _____________ (kg)

Knee Extension (One-repetition maximum (1-RM))

   Leg Extension (max): ________ lbs

Dominant Arm 5 lbs Dumbbell Row: _______ reps. (30 seconds)

Timed Sit-to-Rise (30 sec): ________________ (sec)

One Leg Stance (30 seconds each):     R _________ (sec)     L _________ (sec)

Four-Meter Timed Normal Walk Test: ______________ (sec)

Four-Meter Timed Brisk Walk Test: ______________ (sec)

Two-Minute Walk Test (50 ft): ________________ (ft, in)

Research Staff (Print Name): ________________________________

*kilogram, kg; degrees/second, deg/sec; second, sec; feet, ft; inches, in
APPENDIX G

3-DAY DIETARY RECORD

Subject ID:_____________
Name:_____________
Visit No.:_____________
Date:__________ (weekday/weekend)

<table>
<thead>
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<th>Day/Time</th>
<th>Kind of food</th>
<th>How prepared or brand name</th>
<th>Comments</th>
</tr>
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APPENDIX I

MEDICATION AND SUPPLEMENTS FORM

ID______________

Date______________

Visit#______________

<table>
<thead>
<tr>
<th>Medications</th>
<th>Brand Name (Pharmaceutical name)</th>
<th>Dosage</th>
<th>Frequency Taken (daily, weekly, as needed, etc.)</th>
<th>Reason for Taking</th>
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</table>
## Supplements

<table>
<thead>
<tr>
<th>Supplement Name</th>
<th>Dosage</th>
<th>Frequency Taken (daily, weekly, as needed, etc.)</th>
<th>Reason for Taking</th>
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<tbody>
<tr>
<td>Calcium (What form?)</td>
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<tr>
<td>Vitamin D</td>
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<tr>
<td>Multivitamin (Include all the details)</td>
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<tr>
<td>Others</td>
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BIOGRAPHICAL SKETCH

Pegah JafariNasabian was born and raised in Tehran, Iran. After graduating from Azad University Medical School in 2009, Pegah joined the Tehran University of Medical Sciences as a General Practitioner. During her service, she also volunteered to serve Afghan refugees who were being treated for tuberculosis. She was promoted as the head of the Diabetes Division at Shahabadi Health Center in 2011. Pegah left Tehran University of Medical Sciences in 2012 to join Florida State University and pursue a Doctor of Philosophy degree in Nutrition Sciences. During her doctoral training, Pegah also served as an Instructor/Graduate Teaching Assistant on four different courses and was awarded several scholarships, awards and honors. She has also served as a mentor on several Undergraduate Research Opportunity (UROP) projects. For her PhD dissertation project, she recruited participants from retirement centers and nursing houses located in Tallahassee, which in turn has opened the doors for future collaborations between Florida State University and these centers. Her research focuses on geriatric syndromes, mainly a recently identified syndrome named osteosarcopenic obesity.