

**COMPARING BODY MASS INDEX, FAT MASS, AND LEAN MASS IN  
PREDICTING CARDIOMETABOLIC RISK FACTORS AND BONE  
MINERAL DENSITY IN OSTEOPENIC POSTMENOPAUSAL WOMEN**

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition

Spring 2021

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## **ACKNOWLEDGMENTS**

I would like to express my utmost gratitude to my advisor, Dr. Sheau Ching Chai, for her continuous support, encouragement, and guidance through my graduate and research studies. Her passion for research is very inspiring. Under her guidance, I was able to learn so much about nutrition research. I would like to also thank the rest of my committee members, Dr. Richard Suminski and Dr. Bahram Arjmandi, for offering their time and expertise to support and guide me in preparation of my thesis.

Additionally, I would like to thank my colleagues, especially Safiyah Mansoori, Bharathi Ramesh, and Irene Li, who supported and assisted me emotionally and academically through the completion of my research and graduate studies. They were always there for me whenever I need an extra pair of hands or a second opinion about my research and other assignments.

I would like to immensely thank my family, especially my father Singar Ah Kin, my mother Rokiah Atong, and my sister Sheera Nabila Singar, for their unconditional love and support. Last but certainly not least, I would like to thank my best friends, Robert Girone and Roman Steiner. Thank you for making my college life bearable. I could not imagine getting this far without all of you.

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## ABSTRACT

Postmenopausal women have significantly lower estrogen levels than premenopausal women. Reduced estrogen levels affect body weight and adipose tissue distribution in postmenopausal women. Obesity is a risk factor for cardiovascular disease (CVD), though thought to have protective effects on bone health. This study aimed to investigate the associations between body mass index (BMI), fat mass (FM), and lean mass (LM) with CVD risk factors, bone turnover markers (BTM), and bone mineral density (BMD) in osteopenic postmenopausal women. Overnight fasting blood and urine were collected from 132 osteopenic postmenopausal (1 to 10 years) women not on hormone therapy and drugs known to affect bone metabolism. BMI, FM, and LM were assessed using dual-energy X-ray absorptiometry (DEXA). Multiple linear regression and Pearson correlation analysis were conducted to examine the associations between variables of interest and one-way ANOVA was conducted to compare the means of CVD risk factors between BMI categories. The present study found that BMI and FM, but not LM, were positively and significantly associated with CVD risk factors including fasting plasma glucose ( $p=0.002$  for both), c-reactive protein ( $p<0.001$  for both), apolipoprotein B ( $p=0.003$  &  $p=0.002$ , respectively), total cholesterol ( $p=0.008$  &  $p=0.004$ , respectively), low-density lipoprotein ( $p<0.001$  for both), and triglycerides ( $p=0.005$  &  $p=0.023$ , respectively) levels. BMI and LM, but not FM, were positively and significantly associated with BMD of multiple sites including L3 ( $p=0.024$  &  $p=0.004$ , respectively), radius ( $p<0.001$  &  $p=0.001$ , respectively), ulna ( $p=0.006$  for both), legs ( $p=0.014$  &  $p<0.001$ , respectively), ribs ( $p=0.002$  &  $p=0.043$ , respectively), spine ( $p=0.002$  for both), whole-body ( $p=0.019$  &

p=0.001, respectively), and trochanter bones (p=0.004 & p<0.001, respectively), though LM had bigger effect sizes than BMI for most sites. BMI, FM, and LM were not significantly associated with BTMs. These data suggest that BMI can be a simple, inexpensive, and reliable tool in predicting cardiometabolic risk factors whereas LM is a better predictor of BMD than BMI in osteopenic postmenopausal women.

## **Chapter 1**

### **INTRODUCTION**

Menopause is a normal part of aging in a woman's life, which marks the end of her menstrual cycles. Menopause is the time after the absence of a menstrual period for 12 consecutive months. Most women typically experience menopause in their 40s or 50s, but the average age of women experiencing menopause in the United States is 51. As menopause approaches, the ovaries produce less estrogen, causing the menstrual cycle to become irregular and eventually stop. This causes women to notice and experience some discomforting signs and symptoms as their bodies adapt to different levels of hormones.

The production of estrogen starts to reduce during perimenopause. Perimenopause can begin eight to ten years before menopause, although the average length of perimenopause is four years. Other than its widely known role in regulating reproductive cycle and sexual health, estrogen also plays other important roles relating to heart health<sup>1</sup>, bone health<sup>2</sup>, and energy metabolism<sup>3</sup> in a woman's body.

Menopause alone does not cause weight gain. However, reduced estrogen levels disrupt energy metabolism and adipose tissue distribution in postmenopausal women. Findings from animal studies<sup>4,5</sup> showed that estrogen depletion causes fat tissues to accumulate around the abdominal area, and this condition is improved when estrogen is administered. In human studies, waist circumference<sup>6</sup>, central abdominal fat<sup>7,8</sup>, total fat mass<sup>7</sup>, and visceral fat mass<sup>9</sup> increased significantly in postmenopausal women.

Obesity, especially among postmenopausal women should be addressed accordingly because it may have deleterious effects on their bone and heart health. Obesity is linked to heart disease. Although high body weight is thought to have a protective effect against osteoporosis, being female, menopause, and age over 50 are considered as risk factors of osteoporosis. The most recent data from the Centers for Disease Control and Prevention (CDC) indicate that over 40% of older women in this country are obese and severely obese.<sup>10</sup> CDC also reported that heart disease is still the leading cause of death in United States (US). In addition, a recent study found that more than 8 million and 27 million women over the age of 50 had osteoporosis and low bone mass.<sup>11</sup>

Both heart disease and osteoporosis are major public health issues as they contribute to increased morbidity and mortality rates, as well as higher economic burden. Although higher body weight is negatively associated with heart health but positively associated with bone health, these diseases share some common risk factors and pathophysiological mechanism. To date, there are limited studies that directly compare the associations between body mass index (BMI) and body composition (lean mass and fat mass) with heart health and bone health in postmenopausal women. Therefore, this study is proposed to compare BMI, fat mass (FM), and lean mass (LM) in predicting cardiometabolic risk factors and bone mineral density (BMD) in this population.

## **Chapter 2**

### **LITERATURE REVIEW**

#### **Menopausal Status and Estrogen Levels**

Menopause can occur either naturally or surgically (ovariectomy). In natural menopause, the stage before menopause is known as perimenopause whereas the stage after menopause is known as post-menopause. Estrogen levels in women start to decline during perimenopause. The decline in estrogen levels speeds up in the last one or two years before menopause occurs. During this period, women will experience some menopausal symptoms although they still have their menses. Perimenopause can start eight to ten years before menopause, but on average women enter their perimenopause stage four years before menopause occurs.

Postmenopausal women have significantly lower estrogen levels than premenopausal women. Normal premenopausal women have 17–200 pg/mL of estrone and 15–350 pg/ml of estradiol. However, the levels of these hormones sharply drop to 7–40 pg/mL and less than 10 pg/ml respectively in postmenopausal women.

The changes in estrogen levels occur because as women age, their ovarian follicles will deplete. The ovary is no longer able to respond to the signals both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). As a result, the production of estrogen and progesterone decreases and eventually stops. However, androgen is still being produced by the ovary regardless of the menopausal status. The peripheral aromatization of ovarian and adrenal androgens causes postmenopausal women to continue having low circulating estrogens levels in their body.<sup>12</sup>

## **Measurements of Adiposity and Body Composition**

Obesity is a major public health issue in the US. The most recent data from the CDC (2017–2018) indicate that more than 70% of American adults aged 20 and older are overweight and obese. There are various methods to measure adiposity and body mass index (BMI) is one of them. The BMI of an adult individual can be easily calculated by taking his weight in kilograms divided by the square of his height in meters. This inexpensive screening tool can help categorizing individuals into their weight class namely underweight (less than 18.5 kg/m<sup>2</sup>), normal weight (18.5 kg/m<sup>2</sup>–25 kg/m<sup>2</sup>), overweight (25 kg/m<sup>2</sup>–30 kg/m<sup>2</sup>), and obese (more than 30 kg/m<sup>2</sup>). BMI is not a direct translation of body fat level, although some research suggest that it is moderately correlated with direct body fat measurements obtained from methods such as skinfold thickness measurements, bioelectrical impedance (BIA), underwater weighing, and dual energy x-ray absorptiometry (DXA).<sup>13–15</sup> Multiple studies in the past also have demonstrated that BMI was strongly correlated with various poor health outcomes such as heart disease and other medical conditions. Those findings were consistent with the findings of studies that utilized direct body fatness measurements.<sup>16–21</sup>

One method that can be used to measure body composition is the DXA scan. It is considered as the gold standard for assessing body composition, especially in sports nutrition. The DXA scan has the ability to directly measure an individual's FM, LM, and BMD, among other things. In several studies, body fat percentage (BF%) was found to be correlated with the risk factors of cardiovascular disease and metabolic syndrome.<sup>22,23</sup> In addition, increased BF% was negatively associated with BMD in

certain populations.<sup>24,25</sup> While high BF% is associated with adverse health outcomes, lean body mass (LBM) is associated with positive health outcomes.<sup>26,27</sup>

### **Changes in Body Weight and Adipose Tissue Distribution in Postmenopausal Women**

As women age, weight gain becomes one of their major health concerns. Menopause does not cause weight gain, but the hormonal changes during menopause are associated with an increase in total body fat and AT accumulation in the abdominal region.<sup>28</sup> In a longitudinal epidemiologic study which included 16000 participants, self-reported BMI did not differ by menopausal status.<sup>29,30</sup> This result was confirmed by a sub-study in which the researchers conducted a small cross-sectional study to examine the participants' energy expenditure and body composition.<sup>31</sup> In several studies, postmenopausal women had higher amount of AT accumulation at the abdominal region compared to premenopausal women.<sup>32,33</sup>

In studies using animal models, hormonal changes at menopause have shown to have a serious impact on body composition and AT distribution.<sup>4,5</sup> In one study, oophorectomized mice had reduced energy expenditure although their energy intake was unaffected. They also had adipocyte hypertrophy, inflamed AT, and developed fatty liver. These conditions were reversed when the mice were supplemented with  $17\beta$ -estradiol.<sup>4,5</sup> In two other studies using aromatase gene knock-out (ArKO) mice and estrogen receptor- $\alpha$  knock-out (Er $\alpha$ KO) mice respectively, then mice became obese due to decreased energy expenditure.<sup>34-36</sup> These studies suggested that estrogen deficiency is associated with reduced energy expenditure and abdominal obesity.

## **The Influence of Body Weight and Body Composition on Heart Health**

A study examining the relationships between obesity and CVD in Colorado and four southern states using data from the CDC found that BMI was weakly associated with myocardial infarction (MI) rates, moderately associated with stroke rates, and strongly correlated with high blood pressure rates.<sup>37</sup> Besides, high BMI is also associated with hypertension, impaired fasting glucose, and dyslipidemia.<sup>38-40</sup> However, Qiang Zeng et al. in their studies argued that BF% is a better predictor of CVD risk factors compared to BMI.<sup>41</sup> In the study, <sup>41</sup> In the study, they measured the BMI and BF% of 3859 participants and found that BF%, rather than BMI, was independently associated with CVD risk factors.

In contrast, high LM is associated with lower major adverse cardiovascular events (MACE) in patients with coronary artery disease.<sup>27</sup> In the study, they found that patients in the fourth LM quartile had significantly lower risk of experiencing MACE compared to those in the first LM quartile ( $P=0.004$ ). BMI was not associated with MACE in the study. In addition, they also observed that those in the highest BF% quartile had almost double the risk of MACE when compared to those in the lowest BF% percentile ( $HR=1.89$ ,  $p=0.0008$ ). In a study investigating the relationship between muscle mass and fat mass to total and CVD mortality, Srikanthan et al. found that higher fat mass, muscle mass, and BMI were correlated with lower rates of CVD/total mortality.<sup>42</sup> In this study, they evaluated body composition data of 6451 CVD patients obtained from the National Health and Nutrition Examination Survey (NHANES) 1999 to 2004 and found that those in the high muscle and low fat mass group had the lowest mortality risk when compared to other body composition groups.

### **The Influence of Body Weight and Body Composition on Bone Health**

Obesity and osteoporosis are related as mesenchymal stromal cells from the bone marrow are required to produce both adipocytes and osteoblasts.<sup>43</sup> Traditionally, high body weight is thought to have a protective effect on the bones due to the positive mechanical loading effect. In several studies, body weight and BMI were positively correlated with BMD.<sup>44,45</sup> However, other studies using a larger number of participants did not find a similar correlation.<sup>46,47</sup>

A study in postmenopausal women found that both fat mass (FM) and LM were significantly associated with BMD at all sites, but LM was the strongest predictor of BMD at the femoral neck, hip, spine, and whole body.<sup>26</sup> Based on the available original findings, it is still unclear whether FM or LM is better at predicting bone health. Some studies<sup>48-50</sup> found that LM, and not FM is associated with bone mass, whereas other studies<sup>51,52</sup> suggested otherwise. An old study examining postmenopausal women suggested that both LM and FM are useful in predicting BMD.<sup>53</sup> The variation in the findings of these study may be due to the differences in the study design.

### **The Relationship between Heart Health and Bone Health**

Heart disease and osteoporosis are two age-related conditions that may share some common risk factors and pathophysiology mechanisms. The calcification of the artery during the development of atherosclerosis is similar to the mechanisms involved in bone mineralization.<sup>54,55</sup> Additionally, multiple epidemiologic studies reported that low bone mass in both men and women was a risk factor for cardiovascular mortality.<sup>56-61</sup> In other studies focusing on women, osteoporosis was significantly

associated with incident CVD events<sup>62</sup> and angiographically-determined coronary artery disease<sup>63</sup>, respectively. In another study consisting of Norwegian women, low femoral neck BMD was found to be significantly associated with an increased odds of stroke.<sup>64</sup> These findings suggest that in addition to old age, there may be other factors that link CVD and osteoporosis.

## Chapter 3

### AIMS OF THE STUDY

**Aim 1:** Compare BMI, FM, and LM in predicting CVD risk factors and inflammation status in osteopenic postmenopausal women.

**Aim 2:** Compare BMI, FM, and LM in predicting BTM and BMD in osteopenic postmenopausal women.

**Aim 3:** Investigate the associations between CVD risk factors and BMD in osteopenic postmenopausal women.

## **Chapter 4**

### **METHODS**

#### **Study Design and Study Participants**

This cross-sectional study was conducted at the Florida State University, Tallahassee, FL (Appendix A Figure 1). An approval from the Institutional Review Board (IRB) (Appendix B) was obtained prior the start of the study. A written informed consent was obtained from participants prior to data collection. A total of 132 postmenopausal women living in the area were recruited. Participants underwent phone and in-person screening (Appendix C and Appendix D). During the phone screening, participants' complete medical and nutrition history were obtained. When participants came for the in-person visit, their anthropometric measurements including weight and height were obtained. In addition, participants' BMD, FM, and LM were assessed using a dual-energy X-ray absorptiometry (DEXA). Moreover, overnight fasting blood and urine samples were also obtained from participants. Participants were eligible for the study if they were within 1-10 years postmenopausal, had a BMD t-score of -1 to -2.5 (osteopenic), and not on hormone replacement therapy (HRT). Participants were also excluded if they were treated with calcitonin, bisphosphonates, raloxifene and/or anabolic agents such as parathyroid hormone (PTH) and growth hormone (GH), or steroids. Other exclusion criteria include the presence of metabolic bone disease, renal disease, cancer, CVD, diabetes mellitus, respiratory disease, gastrointestinal disease, liver disease or other chronic diseases. Heavy smokers (more than twenty cigarettes/d) were also excluded from this study.

## **Anthropometrics, Bone Mineral Density, and Body Composition Assessments**

Participants' weight and height were measured using a digital scale and a stadiometer, respectively. For weight measurements, participants were requested to change into scrubs. Participants were then asked to take off their shoes, before standing straight with their chins up on a calibrated and leveled digital scale. For height measurements, with their shoes off and heels touching the base of the stadiometer, participants were requested to stand straight with their back and hip against the wall for proper measurement. Participants' BMI was calculated by taking their weight (in kg) divided by their height (in m) squared.

Participants' BMD, FM, and LM were assessed using a DEXA (GE Healthcare Lunar, Madison, WI, USA). The machine was equipped with a software for whole-body, lumbar spine, hip, and forearm BMD. In addition to whole-body BMD, BMD of specific sites including the lumbar spine, arms, legs, and hip were also assessed in participants.

## **Dietary Intake and Physical Activity**

Data on dietary intake were obtained by asking participants to complete a 7-day food frequency questionnaire (FFQ) (Appendix E) during the visit. A FFQ was used because it was a reliable tool in assessing long-term, habitual diet. A food analysis software (Food Processor version 7.50; ESHA Research, Salem, OR, USA) was utilized to perform the nutrient analysis. With an extensively researched food and nutrition database, this software was able to analyze more than 150 nutritional components, including mandatory and voluntary label nutrients.

Physical activity patterns of participants were also assessed during the visit. Participants' current physical activity was assessed using the validated Five-City Project Physical Activity Recall (Appendix F).<sup>65</sup> In addition, the physical activity recall was also used to obtain sleep and activity patterns of the participants including their leisure, occupational, and home activities.

### **Blood and Urine Analysis for Assessing Biomarkers**

Before coming for the in-person visit, participants were requested to not eat or drink anything (except water) for at least eight hours. Overnight fasting blood and urine samples were collected from participants at the very beginning of their visit. Upon collection, the blood samples were centrifuged at 4,000 rpm for 15 minutes at 4°C using an CL31R multispeed centrifuge (Thermo Electron Corporation) to separate the plasma and the serum. Samples were then aliquoted and stored in a freezer at -80 °C until further analysis. Serum was analyzed for TC, HDL-C, and TG. Friedewald equation was used to calculate LDL-C level. In addition, CRP, blood glucose, and alkaline phosphatase were analyzed using the SIRRUS Clinical Analyzer (Stanbio Laboratories, Boerne, TX). BTMs including bone-specific alkaline phosphatase (BALP), osteocalcin (OC), and tartrate-resistant acid phosphatase (TRAP) were analyzed using enzyme-linked immunosorbent assay (Quidel Corporation, San Diego, CA). Urine was analyzed for deoxypyridinoline (DPD).

### **Statistical Analysis**

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) version 27.0.1.0 software program. Descriptive statistics were

performed for all data. Multiple linear regression and bivariate correlation analysis were used to investigate the associations among the variables. For multiple linear regression, predicted values (PV) of dependent variables were calculated by multiplying standardized coefficient Beta (B) with standard deviation (SD) of the dependent variables. PV is used to describe the predicted value of a dependent variable caused by 1 SD increase or decrease of a predictor variable, when other predictor variables are being held constant. For bivariate correlation analysis, the strength of a correlation coefficient was considered as very small to none ( $r < 0.1$ ), small ( $0.1 < r < 0.3$ ), moderate ( $0.3 < r < 0.5$ ), large ( $0.5 < r < 0.7$ ), very large ( $0.7 < r < 0.9$ ), nearly perfect ( $0.9 < r < 1.0$ ), and perfect ( $r = 1.0$ ). In addition, one-way analysis of variance (ANOVA) was used to compare fasting plasma glucose, CRP, and lipid profiles based on participants' BMI categories. Then, a post-hoc analysis (Tukey's test) was conducted to investigate significance difference between groups. BMI of the participants were categorized as normal weight ( $18.5 \text{ kg/m}^2 - 24.9 \text{ kg/m}^2$ ), overweight ( $25.0 \text{ kg/m}^2 - 29.9 \text{ kg/m}^2$ ), or obese ( $> 30.0 \text{ kg/m}^2$ ). A p-value of  $< 0.05$  was considered significant for all tests. The statistical analysis performed to evaluate the aims of the study were described as follows:

Aim 1: Compare BMI, FM, and LM in predicting CVD risk factors, fasting blood glucose, and inflammation status in osteopenic postmenopausal women.

- A multiple linear regression analysis was used to investigate the associations between BMI, FM, and LM with CVD risk factors, fasting glucose level, and inflammation status (CRP) in osteopenic postmenopausal women. In addition to the covariates (age, years since menopause, physical activity, and total

energy intake), BMI was added to the model created. This process was repeated by substituting BMI with FM and then LM. The standardized coefficient Beta (B) was used to compare the strength of the effect of BMI, FM, and LM to the dependent variables.

Aim 2: Compare BMI and body composition in predicting BTM and BMD in osteopenic postmenopausal women.

- A multiple regression analysis was used to investigate the associations between BMI, FM, and LM with BTMs and BMD in osteopenic postmenopausal women. In addition to the covariates (age, years since menopause, physical activity, height, and total energy intake), BMI was added to the model created. This process was repeated by substituting BMI with FM and then LM. The standardized coefficient Beta (B) was used to compare the strength of the effect of BMI, FM, and LM to the dependent variables.

Aim 3: Investigate the correlations between CVD risk factors and BMD in osteopenic postmenopausal women.

- Bivariate correlation analysis was used to explore the correlations between CVD risk factors and BMD in osteopenic postmenopausal women.

## **Chapter 5**

### **RESULTS**

#### **Participants Characteristics, Anthropometric Measurements, Physical Activity, Bone Mineral Density, Body Composition, and Dietary Intake Assessments**

A flow chart of the study is presented in Appendix A (Figure 1). Of the 236 participants recruited and phone-screened, 132 participants were eligible and attended the in-person visit. A summary of participants' characteristics including age, years since menopause, anthropometric measurements, physical activity, whole body BMD, total FM, total LM, energy intake, and macronutrients intake are displayed within Table 1 (Appendix A). The mean age and years since menopause of the participants were 56.7 years old and 4.9 years, respectively. For anthropometry measurements, the mean weight, height, and BMI of the participants were 67.3kg, 164.5cm, and 24.9 kg/m<sup>2</sup>. Results from DEXA revealed that the mean whole-body BMD, fat mass, and lean of the participants were 1.1 g/cm<sup>3</sup>, 26425.6g, and 38064.5g, respectively. In addition, the mean physical activity was 856.8 kJ/day, so that is about 205kcal/day. For energy intake, the mean total energy intake was 1664 kcal, which came from 209.6g of carbohydrate, 70.2g of protein, and 60.3g of fat.

#### **The Associations between Body Mass Index and Body Composition with Cardiovascular Disease Risk Factors, Bone Turnover Markers, and Bone Mineral Density**

After adjusting for covariates, BMI, fat mass, and lean mass were significantly and positively associated with fasting plasma glucose (p=0.002, p=0.002, and p=0.014, respectively) and triglyceride (p=0.005, p=0.023, and p=0.047, respectively)

levels (Table 2). BMI and fat mass, but not lean mass, were significantly and positively associated with CRP ( $p < 0.001$  for both), ApoB ( $p = 0.003$  and  $p = 0.002$ , respectively), TC ( $p = 0.008$  and  $p = 0.004$ , respectively), and LDL-C ( $p < 0.001$  for both) levels. One standard deviation increase in BMI ( $4.4 \text{ kg/m}^2$ ) was predicted to increase the levels of glucose, triglyceride, CRP, ApoB, TC, and LDL-C by  $4.2 \text{ mg/dl}$ ,  $14.9 \text{ mg/dl}$ ,  $0.5 \text{ mg/dl}$ ,  $7.1 \text{ mg/dl}$ ,  $12.3 \text{ mg/dl}$ , and  $14.4 \text{ mg/dl}$ , respectively. When participants were divided based on the BMI categories, One-way ANOVA revealed that there were significant differences in the levels of fasting plasma glucose ( $p = 0.007$ ), CRP ( $p < 0.001$ ), triglyceride ( $p < 0.001$ ), HDL-C ( $p = 0.001$ ), LDL-C ( $p = 0.002$ ), and ApoB ( $p = 0.003$ ). Post-hoc analysis (Tukey's test) as presented in Figure 2 showed that the obese group had significantly higher CRP level than the overweight ( $p = 0.03$ ) and normal weight ( $p < 0.001$ ) groups. In addition, both overweight ( $p = 0.05$ ) and obese ( $p = 0.02$ ) groups had significantly higher plasma glucose levels than the normal weight group (Figure 3). For lipid profile (Figure 4), both overweight ( $p = 0.02$ ) and obese ( $p < 0.001$ ) groups had significantly higher serum triglyceride levels than the normal weight group. Overweight ( $p = 0.03$ ) and obese ( $p < 0.001$ ) groups also had significantly lower HDL-C levels than the normal weight group. Moreover, the overweight group had significantly higher levels of serum LDL-C ( $p < 0.001$ ) and ApoB ( $p < 0.001$ ) than the normal weight group.

For BTMs (Table 2), ALP was significantly and positively associated with fat mass ( $P = 0.019$ ). Other BTMs were not associated with BMI, fat mass, nor lean mass. Table 2 also showed the associations between BMI, fat mass, and lean mass with BMD of multiple sites. BMI and lean mass were significantly and positively associated with L3 ( $p = 0.024$  and  $p = 0.004$ , respectively) BMD, but only lean mass was

significantly and positively associated with L4 ( $p=0.019$ ) BMD. At the forearm, BMI, fat mass, and lean mass were significantly and positively associated with radius ( $p<0.001$ ,  $p<0.001$ , and  $p=0.001$ , respectively) BMD and ulna ( $p<0.001$ ,  $p=0.002$ , and  $p=0.001$ , respectively) BMD. BMI, fat mass, and lean mass were also significantly and positively associated with ribs ( $p=0.002$ ,  $p=0.004$ , and  $p=0.043$ , respectively) BMD and spine ( $p=0.002$ ,  $p=0.013$ , and  $p=0.002$ , respectively) BMD. BMI and lean mass were significantly and positively associated with arms ( $p=0.017$  and  $p<0.001$  respectively) BMD, legs ( $p=0.014$  and  $p<0.001$  respectively) BMD, and whole-body ( $p=0.019$  and  $p=0.001$  respectively) BMD. Only lean mass was significantly and positively associated with pelvis ( $p=0.003$ ) BMD. For hip BMD, both BMI and lean mass were significantly and positively associated with trochanter ( $p=0.004$  and  $p<0.001$  respectively) BMD. In addition, lean mass was significantly and positively associated with ward's triangle ( $p=0.017$ ) BMD whereas BMI was significantly and positively associated with shaft ( $p=0.050$ ) BMD. Femoral neck was not significantly associated with BMI, fat mass, or lean mass.

### **The Correlations between Cardiovascular Disease Risk Factors, Bone Turnover Markers, and Bone Mineral Density**

When exploring the relationships between CVD risk factors and BMD (Table 3), small to moderate correlations were found between fasting plasma glucose and BMD of multiple sites. There were positive and small correlations between fasting plasma glucose and pelvis ( $r=0.229$ ) BMD, whole-body BMD ( $r=0.251$ ), lower femoral neck ( $r=0.267$ ) BMD, trochanter ( $r=0.264$ ) BMD, and right femoral neck ( $r=0.251$ ) BMD. In addition, positive and moderate correlations were found between

fasting plasma glucose and L2 – L4 ( $r=0.334$ ,  $r=0.317$ , and  $r=0.349$ , respectively) BMDs and spine ( $r=0.335$ ) BMD. Other CVD risk factors were not significantly correlated with BMD in this population.

## Chapter 6

### DISCUSSION

In the present study, BMI, fat mass, and lean mass were significantly and positively associated with fasting plasma glucose and serum triglyceride levels. However, BMI and fat mass, not lean mass, appeared to have significant associations with more CVD risk factors including serum CRP, ApoB, TC, and LDL-C. A review study<sup>66</sup> by Lobo RA supported the findings of the present study. In the study, Lobo RA suggested that in postmenopausal women, the increased prevalence of cardiometabolic syndrome was primarily due to weight gain and obesity. In the review study, the author also reported that fat mass deposition when women become menopause is responsible for increased circulating adipocytokines, which affects insulin resistance and CVD.<sup>66</sup> In addition, the results of the present study are similar to the findings of a cross-sectional study<sup>67</sup> conducted in 100 postmenopausal Iranian women with metabolic syndromes. In the study, the authors observed that high BMI was positively correlated with high triglyceride and blood glucose levels. Multiple studies investigating postmenopausal or middle-aged women also observed elevated triglyceride<sup>68-70</sup> and glucose<sup>71</sup> levels in this population. The present study also observed a positive association between BMI and fat mass with TC and LDL-C in osteopenic postmenopausal women. It has been well established that increased cholesterol level is a significant predictor of CVD. In postmenopausal women, studies<sup>72-74</sup> have shown that menopause is positively associated with increased TC and LDL-C. The present study also showed that increased BMI and fat mass was positively associated with serum CRP level. Elevated CRP level has been associated with increased BMI and with CVD risk factors in many cross-sectional studies<sup>75-78</sup>

previously. A cross-sectional study<sup>79</sup> assessing 61 obese postmenopausal women also demonstrated that CRP level was positively associated with BMI and fat mass. The mechanism that linked CRP to obesity is still not well understood, but some studies<sup>76-78</sup> have proposed that in obese individuals, interleukin-6 (IL-6) secreted by adipose tissue may trigger CRP level to increase as well.

Previous studies<sup>80,81</sup> have consistently reported that postmenopausal women tend to have elevated serum ALP. In the present study, serum ALP was positively associated with fat mass in osteopenic postmenopausal women. An animal study<sup>82</sup> demonstrated that obese subjects had significantly higher ALP activity than non-obese subjects. A study<sup>83</sup> assessing 14224 middle-aged Koreans also observed a positive correlation between serum ALP and fat mass. The positive association between fat mass and serum ALP is potentially due to the presence of ALP activity in adipose tissue, and its enzyme activity is enhanced during adipogenesis<sup>84</sup>. The presence of ALP in adipose tissue suggests that an increase in fat mass may also increase serum ALP level.

In the present study, BMI and lean mass were positively associated with BMD, though lean mass was significantly associated with more sites of BMD than BMI. Other cross-sectional studies<sup>26,48</sup> assessing postmenopausal women also reported that lean mass had a stronger association with BMD in multiple sites. In addition, a longitudinal study<sup>85</sup> following 282 postmenopausal women for two years observed that lean mass was the best predictor of BMD at all sites. This suggests that increased body weight, and particularly lean mass, may have a protective role for bone in postmenopausal women. This can be explained by the greater mechanical loading on

the skeleton caused by the greater body mass, which will stimulate the osteocytes to increase osteoblasts activity<sup>86</sup>.

Fasting plasma glucose of osteopenic postmenopausal women in the present study was positively correlated with BMD. A study<sup>87</sup> assessing postmenopausal women with hyperparathyroidism also reported similar findings. In other studies<sup>88,89</sup> assessing diabetic patients, participants in those studies had significantly higher BMD, though glucose levels were not significantly associated with BMD in those patients. One potential reason may be due the anabolic effect of insulin on bone formation. Insulin interacts with insulin-like growth factor 1 (IGF-1) that present on osteoblasts and results in higher bone mass<sup>90,91</sup>.

The strength of this study was the utilization of DXA in assessing participants' BMD, fat mass, and lean mass. DXA is considered the gold standard in assessing body composition. In addition, various sites of BMD were assessed in this study. Moreover, only osteopenic 1-10 years postmenopausal women were recruited for this study. This population makes this study unique because no other studies have specifically looked at this subpopulation of postmenopausal women. One limitation of the present study was the small sample size. Besides, a big percentage of the postmenopausal women recruited in the study was Caucasian. So, the results of the present study might not be generalizable to postmenopausal women of other races and ethnicities. Future research should focus on further exploring the relationship between fasting plasma glucose and BMD in this population.

## **Chapter 7**

### **CONCLUSION**

This study concludes that BMI and fat mass are positively associated with CVD risk factors in osteopenic postmenopausal women. In a healthcare setting, BMI may be a simple, inexpensive, and reliable tool in predicting CVD risk factors in this population. In addition, BMI and lean mass are positively associated with BMD in osteopenic postmenopausal women, though lean mass is a better predictor than BMI. Future studies should have bigger sample size and include postmenopausal women of other races and ethnicities.

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## Appendix A

### TABLES AND FIGURES

Table 1 Participants' characteristics, anthropometric measurements, bone mineral density, body composition, dietary intake, and physical activity level.

	Mean	Minimum	Maximum	Std. Deviation
Age (years)	56.7	44.6	73	4.6
Weight (kg)	67.3	44.7	103	12.1
Height (cm)	164.5	151	177.5	5.5
Body Mass Index (kg/m <sup>2</sup> )	24.9	18.2	38.2	4.4
Fat Mass (g)	26425.6	11416	49777	8979.7
Lean Mass (g)	38064.5	26282	51883	4297.3
Whole-Body BMD (g/cm <sup>3</sup> )	1.1	0.9	1.4	0.1
Physical Activity (kJ/day)	856.8	227.9	2203.7	356.1
Years Since Menopause (years)	4.9	1.2	10	2.2
Energy (kcal)	1664	587.6	3939.1	535.2
Total Carbohydrate (g)	209.6	75	528.1	81.1
Protein (g)	70.2	25.7	175.4	25
Total Fat (g)	60.3	13.8	149.2	23.3

Table 2 Associations between BMI, fat mass, and lean mass with cardiovascular disease risk factors, bone turnover markers, and bone mineral density.

			B	t	Sig.	95% CI for B	
						Lower Bound	Upper Bound
Glucose, Inflammation Marker, & Lipid Profile	Glucose	BMI	0.374	3.142	0.002*	0.352	1.565
		FM	0.392	3.136	0.002*	0	0.001
		LM	0.288	2.517	0.014*	0	0.001
	CRP	BMI	0.455	3.695	0.000*	0.051	0.171
		FM	0.488	3.785	0.000*	0	0
		LM	0.227	1.852	0.067	0	0
	ApoA	BMI	-0.004	-0.029	0.977	-1.544	1.5
		FM	0.057	0.426	0.671	-0.001	0.001
		LM	-0.196	-1.664	0.1	-0.003	0
	ApoB	BMI	0.367	3.074	0.003*	0.561	2.613
		FM	0.393	3.136	0.002*	0	0.001
		LM	0.169	1.452	0.15	0	0.002
	TC	BMI	0.339	2.722	0.008*	0.744	4.768
		FM	0.381	2.928	0.004*	0	0.002
		LM	0.043	0.356	0.723	-0.002	0.002
	HDL-C	BMI	-0.232	-1.893	0.062	-2.047	0.05
		FM	-0.177	-1.362	0.177	-0.001	0
		LM	-0.303	-2.687	0.009*	-0.002	0
	LDL-C	BMI	0.433	3.618	0.000*	1.453	4.994
		FM	0.454	3.619	0.000*	0.001	0.003
		LM	0.181	1.493	0.139	0	0.003
Lp(a)	BMI	-0.206	-1.583	0.117	-2.563	0.291	
	FM	-0.164	-1.192	0.237	-0.001	0	
	LM	-0.095	-0.753	0.454	-0.002	0.001	
TG	BMI	0.352	2.893	0.005*	1.038	5.601	
	FM	0.301	2.315	0.023*	0	0.003	
	LM	0.237	2.017	0.047*	0	0.005	
BTMs	OC	BMI	-0.097	-0.715	0.477	-0.367	0.173
		FM	-0.05	-0.369	0.713	0	0
		LM	-0.144	-1.08	0.283	0	0
	TRAP	BMI	-0.188	-1.333	0.186	-0.132	0.026
		FM	-0.182	-1.294	0.199	0	0
		LM	-0.137	-0.973	0.333	0	0
	DPD	BMI	0.017	0.123	0.903	-0.341	0.386

		FM	-0.048	-0.347	0.729	0	0
		LM	0.103	0.745	0.459	0	0.001
		BMI	0.12	0.915	0.363	-0.164	0.445
	B-ALP	FM	0.16	1.223	0.225	0	0
		LM	-0.017	-0.133	0.894	0	0
	ALP	BMI	0.208	1.546	0.126	-0.266	2.128
		FM	0.315	2.38	0.019*	0	0.001
		LM	-0.081	-0.597	0.552	-0.002	0.001
	BMD - Lumbar Spine	L2	BMI	0.23	1.75	0.08	0
FM			0.21	1.55	0.12	0	0
LM			0.24	1.8	0.08	0	0
L3		BMI	0.3	2.3	0.024*	0	0.02
		FM	0.2	1.53	0.13	0	0
		LM	0.38	2.93	0.004*	0	0
L4		BMI	0.18	1.31	0.19	0	0.01
		FM	0.07	0.5	0.62	0	0
		LM	0.32	2.4	0.019*	0	0
BMD - Forearm	Radius	BMI	0.54	4.54	0.000*	0	0.01
		FM	0.46	3.72	0.000*	0	0
		LM	0.42	3.37	0.001*	0	0
	Ulna	BMI	0.36	2.79	0.006*	0	0.01
		FM	0.26	2.01	0.047*	0	0
		LM	0.36	2.84	0.006*	0	0
	Radius & Ulna	BMI	0.49	4.07	0.000*	0	0.01
		FM	0.4	3.2	0.002*	0	0
		LM	0.42	3.41	0.001*	0	0
BMD - Whole Body & Body Parts	Head	BMI	0.01	0.1	0.92	-0.01	0.02
		FM	0.06	0.45	0.65	0	0
		LM	-0.13	-0.98	0.33	0	0
	Arms	BMI	0.33	2.42	0.017*	0	0.01
		FM	0.19	1.36	0.18	0	0
		LM	0.48	3.75	0.000*	0	0
	Legs	BMI	0.33	2.52	0.014*	0	0.01
		FM	0.14	1.06	0.29	0	0
		LM	0.53	4.28	0.000*	0	0
	Ribs	BMI	0.43	3.26	0.002*	0	0.02
		FM	0.39	2.94	0.004*	0	0
		LM	0.28	2.05	0.043*	0	0
	Pelvis	BMI	0.19	1.46	0.15	0	0.01

	Spine	FM	0.05	0.35	0.73	0	0	
		LM	0.38	3.04	0.003*	0	0	
		BMI	0.43	3.27	0.002*	0	0.02	
	Whole Body	FM	0.34	2.52	0.013*	0	0	
		LM	0.41	3.18	0.002*	0	0	
		BMI	0.32	2.39	0.019*	0	0.01	
	BMD - Hip	Lower Neck	FM	0.18	1.3	0.2	0	0
			LM	0.44	3.33	0.001*	0	0
			BMI	0.2	1.51	0.14	0	0.01
Ward's Triangle		FM	0.12	0.88	0.38	0	0	
		LM	0.23	1.8	0.08	0	0	
		BMI	0.22	1.71	0.09	0	0.01	
Trochanter		FM	0.11	0.85	0.4	0	0	
		LM	0.31	2.44	0.017*	0	0	
		BMI	0.39	3	0.004*	0	0.01	
Shaft		FM	0.24	1.76	0.08	0	0	
		LM	0.48	3.85	0.000*	0	0	
		BMI	0.27	1.98	0.050*	0	0.02	
FN – Left		FM	0.21	1.53	0.13	0	0	
		LM	0.26	1.91	0.06	0	0	
		BMI	0.14	1.01	0.31	0	0.01	
FN – Right		FM	0.04	0.29	0.78	0	0	
		LM	0.24	1.82	0.07	0	0	
		BMI	0.1	0.77	0.44	0	0.01	
		FM	0.03	0.19	0.85	0	0	
		LM	0.21	1.59	0.12	0	0	
		BMI	0.1	0.77	0.44	0	0.01	
<p>BMI, body mass index; FM, fat mass; LM, lean mass; CRP, c-reactive protein; ApoA, apolipoprotein A; ApoB, Apolipoprotein B; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein; HDL-C, high-density lipoprotein; Lp(a), lipoprotein a; OC, osteocalcin; TRAP, tartrate-resistant acid phosphatase; DPD, deoxypyridinoline; B-ALP, bone-specific alkaline phosphatase; ALP, total serum alkaline phosphatase; FN, femoral neck</p> <p>* Values were significantly (p&lt;0.05) associated with BMI, FM, or LM, after adjusting for potential covariates (age, years since menopause, physical activity, height, and total energy intake).</p>								

Table 3 Correlations between cardiovascular disease risk factors, bone turnover markers, and bone mineral density.

BMD		Glucose	CRP	ApoA	ApoB	TC	HDL-C	LDL-C	Lp(a)	TG
L2	r	.334**	0.148	-0.098	0.039	-0.043	-0.071	-0.011	-0.028	0.075
L3	r	.317**	0.138	-0.033	0.065	0.004	-0.083	0.049	0.016	0.078
L4	r	.349**	0.017	0.051	0.049	0.037	0.018	0.044	0.111	-0.006
Radius & Ulna	r	0.004	0.058	-.252**	0.149	0.093	-.269**	.219*	-0.022	0.086
Pelvis	r	.229*	-0.07	-0.026	0.101	0.06	-0.01	0.083	0.022	-0.003
Spine	r	.335**	0.069	0.028	0.162	0.112	-0.008	0.129	-0.063	0.034
Whole Body	r	.251**	0.003	-0.069	0.136	0.091	-0.033	0.138	0.093	-0.064
Upper Neck	r	0.139	0.007	-0.098	0.048	0.075	0.009	0.077	0.021	0.036
Lower Neck	r	.267**	0.058	-0.103	0.14	0.08	-0.117	0.12	0.031	0.172
Ward's Triangle	r	0.172	0.08	-0.09	0.108	0.105	-0.051	0.134	-0.018	0.103
Trochanter	r	.264**	0.14	-0.089	0.116	0.129	-0.116	0.186	-0.048	0.131
Shaft	r	0.156	0.114	-0.158	0.11	0.093	-0.102	0.153	0.001	0.044
FN - Left	r	0.176	0.024	-0.139	0.071	0.05	-0.081	0.086	0.043	0.078
FN - Right	r	.251**	0.048	-0.076	0.127	0.113	-0.044	0.121	0.006	0.168

r, Pearson correlation coefficient; CRP, c-reactive protein; ApoA, apolipoprotein A; ApoB, apolipoprotein B; TC, total cholesterol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; Lp(a), lipoprotein a; TG, triglyceride

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

Figure 1 Flow of study

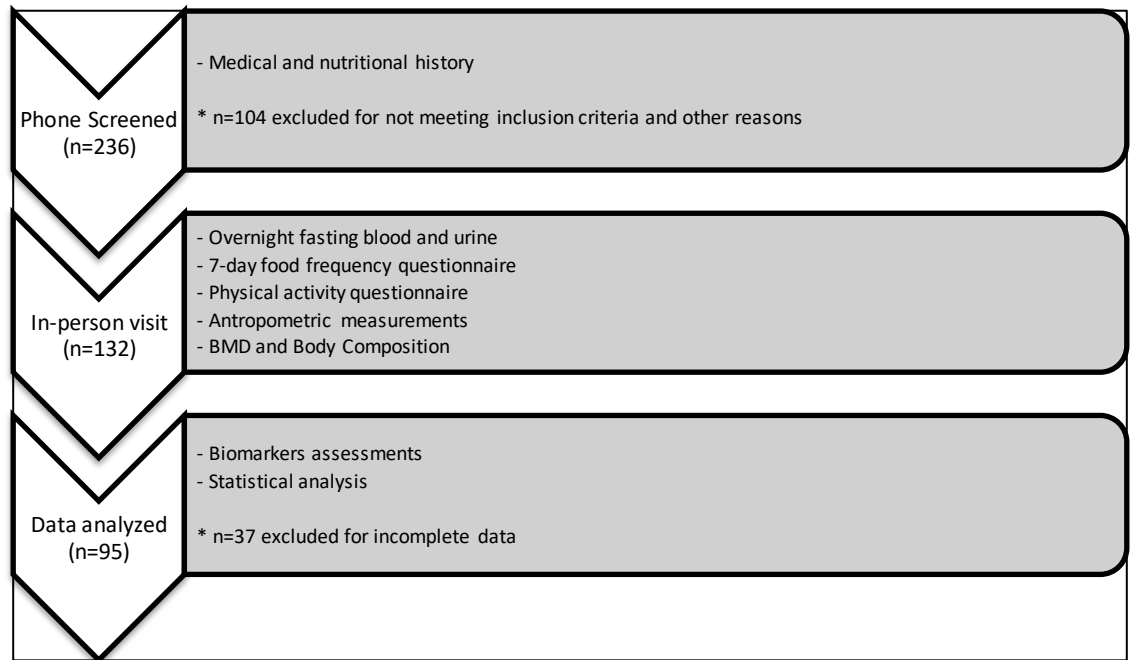


Figure 1 Flow of study including a summary of data collection and analysis.

Figure 2 CRP level based on BMI categories

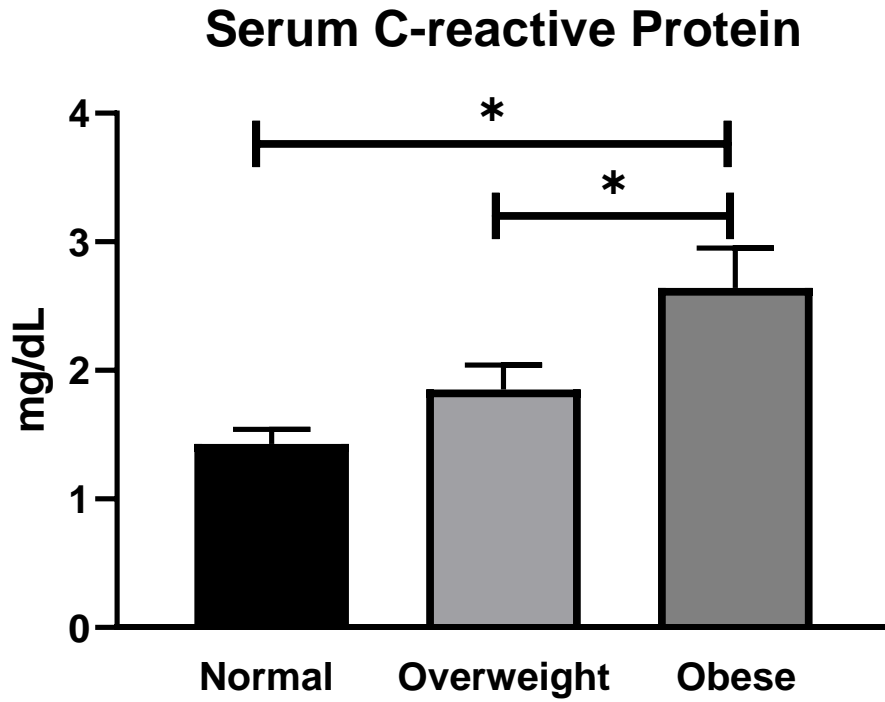


Figure 2 Values are reported as means  $\pm$  SEM. \*Asterisk indicates a statistically significant difference between the groups,  $P < 0.05$ .

Figure 3 Fasting plasma glucose level based on BMI categories

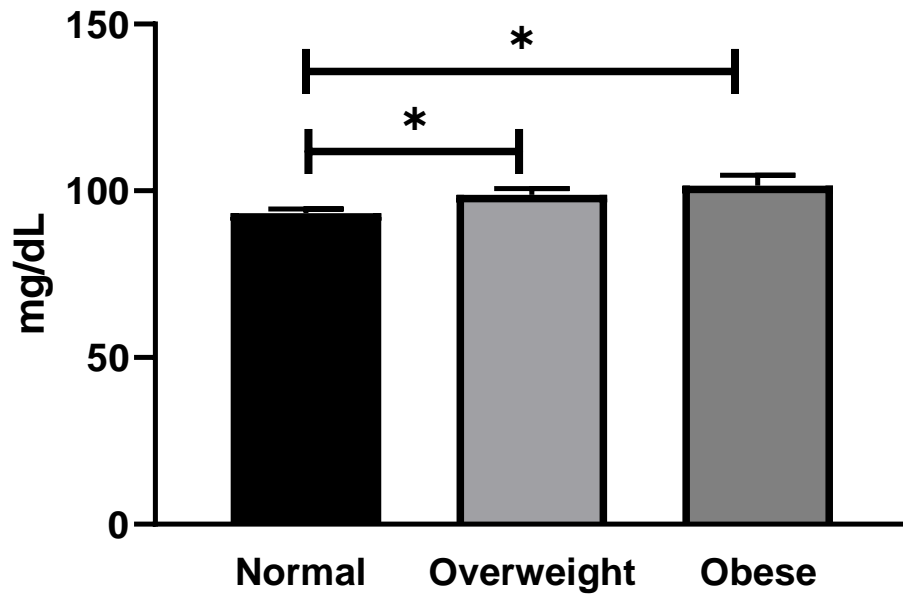


Figure 3 Values are reported as means  $\pm$  SEM. \*Asterisk indicates a statistically significant difference between the groups,  $P < 0.05$ .

Figure 4 Lipid profile based on BMI categories

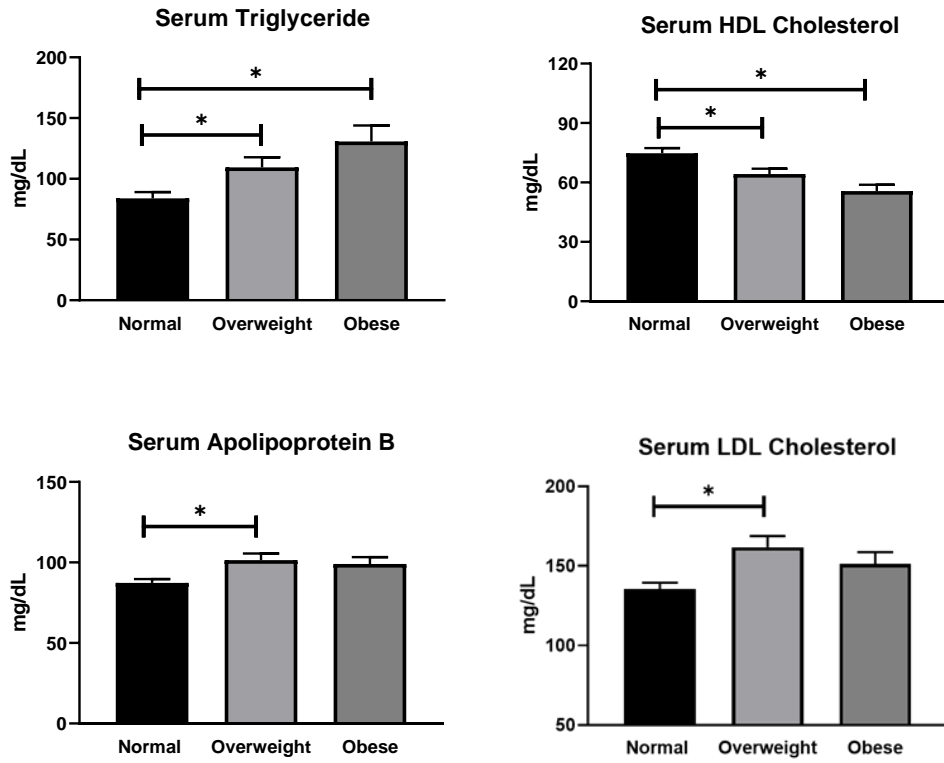


Figure 4 Values are reported as means  $\pm$  SEM. \*Asterisk indicates a statistically significant difference between the groups, P < 0.05.

**Appendix B**  
**IRB Approval Letter**

**IRB APPROVAL LETTRES & INFORMED CONSENT FORM**



Office of the Vice President For Research  
Human Subjects Committee  
Tallahassee, Florida 32306-2742  
(850) 644-8633· FAX (850) 644-4392

**REAPPROVAL MEMORANDUM**

Date: 6/20/2008

To:  
**Bahram Arjmandi**  
**436 Sandels Building, FSU**  
**Tallahassee, Florida 32306**

Dept.: **NUTRITION FOOD AND MOVEMENT SCIENCES**

From: **Thomas L. Jacobson, Chair**

A handwritten signature in black ink, appearing to read "Thomas Jacobson".

Re: **Reapproval of Use of Human subjects in Research:**  
**Dried Plum Reverses Bone Loss in Postmenopausal 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 4/9/2009 please request renewed approval.

You are reminded that a change in protocol in this project must be approved by resubmission of the project to the Committee for approval. Also, the principal investigator must report to the Chair promptly, and in writing, any unanticipated problems involving risks to 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 of such investigations as often as necessary to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

Cc:  
HSC No. 2008.0311-R

## Appendix C

### MEDICAL HISTORY QUESTIONNAIRE

#### MEDICAL HISTORY QUESTIONNAIRE

Subject ID: \_\_\_\_\_  
Interviewer: \_\_\_\_\_ Date \_\_\_\_\_

#### HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

Age \_\_\_\_\_ Height \_\_\_\_\_ Weight \_\_\_\_\_ BMI \_\_\_\_\_

#### **I. Medical History**

##### **A. Skeletal Health**

##### **Personal history of skeletal disorders:**

1. Not known \_\_\_\_\_
2. Yes: uncontrolled \_\_\_\_\_
3. Yes: Medications \_\_\_\_\_
4. Yes: Exercise program \_\_\_\_\_
5. Yes: Modified diet \_\_\_\_\_
6. Yes: Surgery \_\_\_\_\_
7. Yes: Combined program \_\_\_\_\_

Give details \_\_\_\_\_

Type of Medication(s) \_\_\_\_\_

Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_

How does this condition affect your activity?

##### **Family history of skeletal disorders:**

1. None \_\_\_\_\_
2. One parent \_\_\_\_\_
3. Both parents \_\_\_\_\_
4. One close relative \_\_\_\_\_
5. More than one close relative \_\_\_\_\_

Relative(s) \_\_\_\_\_

Comments: \_\_\_\_\_

##### **B. Cardiovascular Function**

##### **Personal history of cardiovascular disease:**

1. Not known \_\_\_\_\_
2. Yes: uncontrolled \_\_\_\_\_
3. Yes: Medications \_\_\_\_\_
4. Yes: Exercise program \_\_\_\_\_
5. Yes: Modified diet \_\_\_\_\_

- 6. Yes: Surgery \_\_\_\_\_
- 7. Yes: Combined program \_\_\_\_\_
- Give details \_\_\_\_\_
- Type of Medication(s) \_\_\_\_\_
- Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_
- How does this condition affect your activity? \_\_\_\_\_

**Family history of cardiovascular disease:**

- 1. None \_\_\_\_\_
- 2. One parent \_\_\_\_\_
- 3. Both parents \_\_\_\_\_
- 4. One close relative \_\_\_\_\_
- 5. More than one close relative \_\_\_\_\_

Relative(s) \_\_\_\_\_  
 Comments: \_\_\_\_\_

**C. Hypertension**

- 1. None known \_\_\_\_\_
- 2. Yes: uncontrolled \_\_\_\_\_
- 3. Yes: Medications \_\_\_\_\_
- 4. Yes: Exercise program \_\_\_\_\_
- 5. Yes: Modified diet \_\_\_\_\_
- 6. Yes: Yes: combined program \_\_\_\_\_
- 7. Most recent blood pressure \_\_\_\_\_
- Explain \_\_\_\_\_
- Type of Medication(s) \_\_\_\_\_
- Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_
- Ever taken thiazide diuretics? \_\_\_\_\_

**D. Diabetes**

- 1. No record or indication \_\_\_\_\_
- 2. In past, but not now \_\_\_\_\_
- 3. Yes, well controlled \_\_\_\_\_
- 4. Yes, not controlled \_\_\_\_\_
- Explain \_\_\_\_\_
- Type of Medication(s) \_\_\_\_\_
- Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_
- How does this condition affect your activity? \_\_\_\_\_

**E. Gastrointestinal/Digestive Problems**

- 1. No record or indication \_\_\_\_\_
- 2. In past, but not now \_\_\_\_\_
- 3. Yes, well controlled \_\_\_\_\_
- 4. Yes, not controlled \_\_\_\_\_
- Explain \_\_\_\_\_

Type of Medication(s) \_\_\_\_\_  
Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_  
Ever taken steroids (i.e., prednisone)? \_\_\_\_\_  
Currently taking antacids? \_\_\_\_\_  
How does this condition affect your activity? \_\_\_\_\_

**F. Liver Disease/ Problems**

1. No record or indication \_\_\_\_\_  
2. In past, but not now \_\_\_\_\_  
3. Yes, well controlled \_\_\_\_\_  
4. Yes, not controlled \_\_\_\_\_  
Explain \_\_\_\_\_  
Type of Medication(s) \_\_\_\_\_  
Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_  
How does this condition affect your activity? \_\_\_\_\_

**G. Respiratory Problems**

1. No record or indication \_\_\_\_\_  
2. In past, but not now \_\_\_\_\_  
3. Yes, well controlled \_\_\_\_\_  
4. Yes, not controlled \_\_\_\_\_  
Explain \_\_\_\_\_  
Type of Medication(s) \_\_\_\_\_  
Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_  
How does this condition affect your activity? \_\_\_\_\_

**H. Thyroid Disorder**

1. No record or indication \_\_\_\_\_  
2. In past, but not now \_\_\_\_\_  
Hyper? \_\_\_\_\_ Hypo? \_\_\_\_\_  
Explain \_\_\_\_\_  
Type of Medication(s) \_\_\_\_\_  
Ever taken thyroid hormones (i.e., Synthroid)? \_\_\_\_\_

**I. Reproductive history:** LMP \_\_\_\_\_, No. of children \_\_\_\_\_

**J. Kidney Diseases**

1. No record or indication \_\_\_\_\_  
2. In past, but not now \_\_\_\_\_  
3. Yes, well controlled \_\_\_\_\_  
4. Yes, not controlled \_\_\_\_\_  
Explain \_\_\_\_\_  
Type of Medication(s) \_\_\_\_\_  
Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_  
How does this condition affect your activity? \_\_\_\_\_

**K. Cancer**

1. No record or indication \_\_\_\_\_
  2. In past, but not now \_\_\_\_\_
  3. Yes, well controlled \_\_\_\_\_
  4. Yes, not controlled \_\_\_\_\_
- Explain \_\_\_\_\_  
Type of Medication(s) \_\_\_\_\_  
Current dosage \_\_\_\_\_ Years taken \_\_\_\_\_  
How does this condition affect your activity? \_\_\_\_\_

**II. Medication or Drug Use**

A. *Previous or Present Use of Any of the Following (Specify):*

1. Anabolic steroids \_\_\_\_\_
2. Corticosteroids or glucocorticoids \_\_\_\_\_ prednisone \_\_\_\_\_
3. Thiazide diuretics \_\_\_\_\_ Other diuretics \_\_\_\_\_
4. Vitamin D \_\_\_\_\_
5. Medications for bone:  
Fosamax: \_\_\_\_\_  
Evista (Raloxifene): \_\_\_\_\_  
Miacalcin (Calcitonin): \_\_\_\_\_  
Teriparatide (Forteo): \_\_\_\_\_
6. Others:  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

2. *Previous or Present Use of Alcoholic Beverages (Beer, wine, hard liquor).*

- Please indicate:  
Frequency of intake (Times/week or times/month): \_\_\_\_\_  
Number of servings at a sitting: \_\_\_\_\_  
Number of years of use: \_\_\_\_\_

C. *Estrogen or Hormone Replacement (ERT or HRT)*

1. Never \_\_\_ move along to section D.
2. Yes, in past \_\_\_ at what age? \_\_\_ & How long \_\_\_\_\_

D. *Currently /previously a smoker? \_\_\_\_\_ If yes, number of cigarettes per day \_\_\_\_\_*

**III. Physical Activity**

A. *Occupational Intensity (respond to 1, 2, 3, or 4):*

1. Majority of time: Sitting \_\_\_\_\_ Standing \_\_\_\_\_ Walking \_\_\_\_\_
2. Equal amount of time: Sitting and Standing \_\_\_\_\_  
Walking and Sitting \_\_\_\_\_  
Standing and Walking \_\_\_\_\_
3. Combination: Sitting, Standing, and Walking \_\_\_\_\_
4. Much of time: Lifting & Carrying \_\_\_\_\_

## Appendix D

### NUTRITION HISTORY QUESTIONNAIRE

#### NUTRITION HISTORY QUESTIONNAIRE

Subject ID: \_\_\_\_\_  
Interviewer: \_\_\_\_\_  
Date: \_\_\_\_\_

#### NUTRITION HISTORY QUESTIONNAIRE

##### NUTRITION HISTORY

**Modified/Specialized Diet(s) Followed:** \_\_\_\_\_

Recommended By \_\_\_\_\_ Length of time Followed \_\_\_\_\_

Recent Changes in Appetite? \_\_\_\_\_ Due to \_\_\_\_\_

Foods Avoided? \_\_\_\_\_ Due to \_\_\_\_\_

History of Problems Digesting Milk? \_\_\_\_\_ When? \_\_\_\_\_ How Long? \_\_\_\_\_

Diagnosis of Lactose Intolerance? \_\_\_\_\_ When? \_\_\_\_\_ By whom? \_\_\_\_\_

**Intake of Milk/Dairy Products & Calcium-Containing Foods** (No Times/Day, Wk or Month, Portion Size):

Milk \_\_\_\_\_ Yogurt \_\_\_\_\_ Frozen Yogurt \_\_\_\_\_

Ice Cream/Milk \_\_\_\_\_ Pudding/Custard \_\_\_\_\_ Hard Cheese \_\_\_\_\_

Mixed Dishes with Cheese \_\_\_\_\_ Soft Cheese \_\_\_\_\_ Grated Cheese \_\_\_\_\_

Donuts/Cakes/Cookies \_\_\_\_\_ Eggs \_\_\_\_\_ Dark Breads \_\_\_\_\_

How has this intake changed during that past three years?

**Intake of High Fiber Foods** (Specify Type, No Times/Day, Week or Month, Portion Size):

Whole Grain Products: Breads \_\_\_\_\_ Bran (wheat, oat) \_\_\_\_\_

Cereals \_\_\_\_\_ Crackers \_\_\_\_\_ Grains (i.e., popcorn) \_\_\_\_\_  
 Nuts \_\_\_\_\_ Seeds \_\_\_\_\_ Fruits \_\_\_\_\_ Dried  
 Fruits (prunes/dried apples) \_\_\_\_\_ Vegetables \_\_\_\_\_ Beans/Legumes  
 (i.e., chili) \_\_\_\_\_

How has this intake changed during the past three years?

---

**Nutritional Supplements** (Type, Dose, Times/Week, No Years):

Vitamin Supplements \_\_\_\_\_

Mineral Supplements \_\_\_\_\_

Vitamin/Mineral Supplements \_\_\_\_\_

Other Supplements (e.g., cod liver oil, protein powder) \_\_\_\_\_

**DAILY INTAKE** (*Typical weekday*)

<i>Time</i>	<i>Food/Description</i>	<i>Serving Size</i>	<i>Food Code</i>
-------------	-------------------------	---------------------	------------------

**Sodium Intake** (amount of salt used (tsp)/day): in cooking \_\_\_\_\_ at the table \_\_\_\_\_ Estimate  
 of Na<sup>+</sup> Intake/Day: Salt Shaker \_\_\_\_\_ High Na<sup>+</sup> foods: \_\_\_\_\_

**Beverages Consumed** (servings per day):

*Coffee: reg* \_\_\_\_\_ *decaf* \_\_\_\_\_ *Cocoa:* \_\_\_\_\_ *Tea: reg* \_\_\_\_\_ *decaf* \_\_\_\_\_

*Soda: reg (+caf)* \_\_\_\_\_ *diet (+caf)* \_\_\_\_\_ *reg (caf free)* \_\_\_\_\_ *diet (caf free)* \_\_\_\_\_

*Milk (oz/day): reg (3.5%)* \_\_\_\_\_ *low fat (2%)* \_\_\_\_\_ *skim(1% or <1%)* \_\_\_\_\_

During what period(s) of life have you been a milk drinker? As a(n):

\_\_\_1. Child \_\_\_2. Teenager \_\_\_3. Young adult (20-29) \_\_\_4. Adult (30-39)

\_\_\_5. mid-life adult (40-59) \_\_\_6. Older adult (60+) \_\_\_7. Never

If you excluded milk during any part of your life, was (is) this due to digestive problems?

(Explain): \_\_\_\_\_

Milk consumed/day: child \_\_\_\_\_ teenager \_\_\_\_\_ young adult (20-29) \_\_\_\_\_ adult (30-39) \_\_\_\_\_ mid-  
life adult (40-59) \_\_\_\_\_ older adult (60+) \_\_\_\_\_ Juice (type): \_\_\_\_\_ Calcium fortified Juice:  
\_\_\_\_\_ Other: \_\_\_\_\_

Alcoholic Beverages: \_\_\_\_\_ If yes, please specify:

Type Times/week or Mo Serving Size No Servings Total/Week or Mo

Beer: Reg  
Lite  
Dark

Wine: White  
Red  
Rose  
(Blush)

Mixed Drink (specify):

Other (specify):

**How did your food or beverage intake differ from the above during your younger years (teenager and young adulthood)?**

## Appendix E

### FOOD FREQUENCY QUESTIONNAIRE

#### FOOD FREQUENCY QUESTIONNAIRE

Subject Number \_\_\_\_\_

Date \_\_\_\_\_

#### Vitamin and Mineral Supplement

1. Do you take any vitamin or mineral supplement(s)?    Yes \_\_\_ No \_\_\_\_
2. If **Yes**, please, list all **names** of vitamin or mineral supplements, and **how often** do you take the supplement(s)?

Name \_\_\_\_\_ How often \_\_\_\_ per day Or \_\_\_\_ per week  
 Name \_\_\_\_\_ How often \_\_\_\_ per day Or \_\_\_\_ per week  
 Name \_\_\_\_\_ How often \_\_\_\_ per day Or \_\_\_\_ per week  
 Name \_\_\_\_\_ How often \_\_\_\_ per day Or \_\_\_\_ per week  
 Name \_\_\_\_\_ How often \_\_\_\_ per day Or \_\_\_\_ per week

#### Seven Day Food Frequency Questionnaire

This questionnaire asks you about your consumption of foods and beverages over the past week, which includes the time from exactly one week ago until the last meal you had before you fill out this questionnaire. The “How Often” columns are for day, week, or rarely/never. We want you to think back over the past week and tell us how many times (per day, if you consume the item every day, or per week) you consumed each item. A medium serving is in parentheses.

EXAMPLES:

- Ate ½ grapefruit about twice last week.  
 Ate 1 large hamburger four times last week.  
 Drank 2 cups of whole milk each day.

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
<u>Grapefruit (1/2)</u>		2			X	
Hamburger, regular (1 patty, 3 oz)		4				X
Whole milk (1 cup, 8 oz)	2				X	

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
<b>DAIRY FOODS</b>						
Whole milk (1 cup, 8 oz)						
2% milk (1 cup, 8 oz)						
Skim milk (1 cup, 8 oz)						
Cream, whipped (1 Tbsp)						
Sour cream (1 Tbsp)						
Coffee cream (1 Tbsp)						
Ice cream (½ cup)						
Low fat ice cream (½ cup)						
Frozen yogurt (½ cup)						
Yogurt (1 cup)						
Low fat yogurt (1 cup)						
Cottage cheese (½ cup)						
Cream cheese (1 oz)						
Low fat cream cheese (1 oz)						
Other cheese (1 slice or 1 oz)						
Low fat cheese (1 slice or 1 oz)						
Margarine (1 tsp)						
Butter (1 tsp)						
Reduced fat margarine (1 tsp)						
<b>FRUITS, FRUIT JUICES</b>						
Raisins (1 oz or 1 sm box)						
Grapes (20)						
Prunes (½ cup)						
Bananas						
Cantaloupe (¼ melon)						
Watermelon (1 slice)						
Apples, applesauce or pears (1 fresh, ½ cup)						
Dried Apples (1 oz)						
Apple juice (½ cup)						
Oranges						
Orange juice (½ cup)						
Grapefruit (½ cup)						
Grapefruit juice (½ cup)						
Other fruit juices (½ cup)						
Strawberries—fresh, frozen, or canned (½ cup)						
Blueberries—fresh, frozen, or canned (½ cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
Peaches (1 fresh, ½ cup canned)						
Apricots (1 fresh, ½ cup canned)						
Plums (1 fresh, ½ cup canned)						
Honeydew melon (¼ melon)						
<b><u>VEGETABLES, VEGETABLE JUICE</u></b>						
Tomatoes (1)						
Tomato juice (½ cup)						
Tomato sauce (½ cup)						
Spaghetti sauce (½ cup)						
Red chili sauce, taco sauce, or salsa (1 Tbsp)						
Tofu or soybeans (3-4 oz)						
String beans, green beans (½ cup)						
Broccoli (½ cup)						
Cabbage (½ cup)						
Cole slaw (½ cup)						
Cauliflower (½ cup)						
Brussels sprouts (½ cup)						
Carrots, raw (½ carrot or 2-4 sticks)						
Carrots, cooked (½ cup)						
Corn (1 ear or ½ cup frozen or canned)						
Peas (½ cup fresh, frozen or canned)						
Lima beans (½ cup frozen, or canned)						
Mixed vegetables (½ cup)						
Beans or lentils, baked or dried (½ cup)						
Summer or yellow squash (½ cup)						
Winter squash (½ cup)						
Zucchini (½ cup)						
Yam or sweet potato (½ cup)						
Spinach, (cooked ½ cup, raw 1 cup)						
Iceberg lettuce, romaine or leaf (1 cup)						
Celery (4" stick)						
Beets (½ cup)						
Alfalfa sprouts (½ cup)						
Kale, mustard, or chard greens (½ cup)						
Vegetable, vegetable beef, minestrone or tomato soup (1 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
<b><u>EGGS, MEAT, ETC.</u></b>						
Eggs (2)						
Chicken or turkey, roasted or broiled with skin (3-4 oz)						
Chicken or turkey, roasted or broiled skinless (3-4 oz)						
Chicken, fried with skin (3-4 oz)						
Bacon (2 slices)						
Hot dogs (2)						
Low fat hot dogs (2)						
Sausage (2 patties or 2 links)						
Bologna (1 slice)						
Other processed luncheon meat (1 slice)						
Liver, chicken or beef (3-4 oz)						
Hamburger, regular (1 patty, 3-4 oz)						
Hamburger, lean (1 patty, 3-4 oz)						
Meat loaf (3-4 oz)						
Pork, chops, roasts (3-4 oz)						
Lamb (3-4 oz)						
Beef, roast, steak (3-4 oz)						
Beef stew with vegetables (1 cup)						
Ham (3-4 oz)						
Tuna fish (3-4 oz)						
Tuna salad (½ cup)						
Fish, baked or broiled (3-4 oz)						
Fish, fried or fish sandwich (3-4 oz)						
Shrimp, Lobster, Scallops						
Pizza (2 slices)						
Mixed dishes with cheese (1 cup)						
Lasagna or meat pasta dishes (1 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
<b><u>BREADS, CEREALS, STARCHES</u></b>						
Cold breakfast cereal (1 cup)						
Cold breakfast cereal—fortified (1 cup)						
Cooked oatmeal (1 cup)						
Other cooked breakfast cereal (1 cup)						
White bread (1 slice)						

Pita bread (1 piece)						
Dark bread (1 slice)						
English muffin (1)						
Bagel (1)						
Dinner roll (1)						
Hamburger or hotdog bun (1)						
Muffin (1)						
Biscuit (1)						
Corn bread, corn muffin (1)						
Brown rice (1 cup)						
White rice (1 cup)						
Spaghetti noodles (1 cup)						
Macaroni noodles (1 cup)						
Other pasta noodles (1 cup)						
Bulgar, kasha, couscous (1 cup)						
Pancakes or waffles (2)						
Potatoes, french fries or fried (½ cup)						
Potatoes, baked or boiled (1)						
Mashed potatoes (1 cup)						
Potato chips or corn chips (small bag or 1 oz)						
Saltine crackers (5)						
Saltine crackers, low sodium (5)						
Saltine crackers, fat free (5)						
Other crackers (5)						
Other crackers, low fat (5)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
<b>BEVERAGES</b>						
Regular soft drink (1)						
Diet soft drink (1)						
Caffeine free soft drink (1)						
Caffeine free, Diet soft drink (1)						
Lemonade or other non-carbonated drink (1 glass, bottle, or can)						
Water (1 cup)						
Coffee (1 cup)						
Decaffeinated coffee (1 cup)						
Tea (1 cup)						
Herbal tea (1 cup)						
Beer (1 glass, bottle, or can)						
Red wine (4 oz glass)						

White wine (4 oz glass)						
Whiskey, gin, or other liquor (1 drink or shot)						
<b>SWEETS, BAKED GOODS, MISC.</b>						
Chocolate (1 small bar or 1 oz)						
Candy bar (1 small bar)						
Candy without chocolate (1 oz)						
Cookies, home baked (2)						
Cookies, readymade (2)						
Brownies (2)						
Doughnuts (2)						
Cake, home baked (1 slice)						
Cake, readymade (1 slice)						
Sweet roll, coffee cake, or other pastry readymade (1 serving)						
Sweet roll, coffee cake, or other pastry home baked (1 serving)						
Pie, homemade (1 slice)						
Pie, readymade (1 slice)						
Jam, jelly, preserves, syrup, or Honey (1 Tbsp)						
Peanut butter (1 Tbsp)						
Popcorn (1 cup)						
Popcorn, air popped (1 cup)						
<b>Type of Food</b> <b>(Medium Serving)</b>	<b>How Often</b>			<b>Size</b>		
	<b>Day</b>	<b>Week</b>	<b>Rarely/ Never</b>	<b>S</b>	<b>M</b>	<b>L</b>
Nuts (small packet or 1 oz)						
Bran, added to food (1 Tbsp)						
Wheat germ (1 Tbsp)						
Chowder or cream soup (1 cup)						
Oil and vinegar dressing (1 Tbsp)						
Mayonnaise or other creamy salad dressing, Regular (1 Tbsp)						
Mayonnaise or other creamy salad dressing, Low Fat or Reduced Calorie, Lite (1 Tbsp)						
Mayonnaise or other creamy salad dressing, Fat Free (1 Tbsp)						
Mustard, dry or prepared (1 tsp)						
Salt (1 shake)						
Pepper (1 shake)						

Can you think of any other food or drink that you had in the past week that was not on this form? If so, what was it? What was the amount? How many times did you have it this past week?

**Food** \_\_\_\_\_

**Amount** \_\_\_\_\_, **How often per day** \_\_\_\_\_, **per week** \_\_\_\_\_

**Food** \_\_\_\_\_

**Amount** \_\_\_\_\_, **How often per day** \_\_\_\_\_, **per week** \_\_\_\_\_

**Food** \_\_\_\_\_

**Amount** \_\_\_\_\_, **How often per day** \_\_\_\_\_, **per week** \_\_\_\_\_

**Food** \_\_\_\_\_

**Amount** \_\_\_\_\_, **How often per day** \_\_\_\_\_, **per week** \_\_\_\_\_

## Appendix F

### PHYSICAL ACTIVITY QUESTIONNAIRE

#### PHYSICAL ACTIVITY QUESTIONNAIRE

Subject ID: \_\_\_\_\_  
Date: \_\_\_\_\_

Interviewer: \_\_\_\_\_

#### Physical Activity Recall Items

First we would like to know about your physical activity during the past 7 days. But first, let me ask you about your sleep habits.

1. On the average, how many hours did you sleep each night during the last five weekday nights (Sunday-Thursday)? \_\_\_\_\_hours
3. On the average, how many hours did you sleep each night last Friday and Saturday nights? \_\_\_\_\_hours

Now I am going to ask you about your physical activity during the past 7 days, that is, the last 5 weekdays and last weekend, Saturday and Sunday.

We are not going to talk about light activities such as slow walking, light housework, or non-strenuous sports such as bowling, archery, or softball.

Please look at this list which shows some examples of what we consider moderate, hard, and very hard activities. (interviewer: hand subject the following card and allow time for the subject to read it over.)

People engage in many other types of activities, and if you are not sure where one of your activities fits, please ask me about it.

3. First, let's consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them?

Please tell me to the nearest half-hour. \_\_\_\_\_hours

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do?

(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

\_\_\_\_\_hours

5. Now, let's look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them?

Please tell me to the nearest half-hour. \_\_\_\_\_hours

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

\_\_\_\_\_ hours

7. Now, let's look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

\_\_\_\_\_ hours

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do?

(Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

\_\_\_\_\_ hours

9. Compared with your physical activity over the past 3 months, was last week's physical activity more, or less, or about the same?

- \_\_\_\_\_ 1. More
- \_\_\_\_\_ 2. Less
- \_\_\_\_\_ 3. About the same

Interviewer: Please list below any activities reported by the subject, which you don't know how to classify. Flag this record for review and completion.

Activity(brief description)	Hours: workday	Hours: weekend day
_____	_____	_____
_____	_____	_____
_____	_____	_____

## EXAMPLES OF ACTIVITIES IN EACH CATEGORY

### *Moderate activity (3-5 mets)*

- Occupational Tasks:**
- 1) Delivering mail or patrolling on foot
  - 2) House painting
  - 3) Truck driving (making deliveries, lifting/carrying light objects)
- Household Activities:**
- 1) Sweeping, mopping, cleaning windows
  - 2) Mowing the lawn with a power mower
  - 3) Raking the lawn and yardwork
  - 4) Light carpentry
- Sports:**  
*(actual playing time)*
- 1) Table tennis or Ping-Pong
  - 2) Softball, baseball
  - 3) Volleyball
  - 4) Dancing: folk, square, aerobics (low impact & intensity)
  - 5) Brisk walking (3 to 4 mile/hr; 15-20 min/mile)
  - 6) Bicycling on level ground (10-15 mile/hr)
  - 7) Golfing (walking and pulling/carrying own clubs)
- Calisthenics exercise and weight lifting

### *HARD ACTIVITY (5.1 – 6.9 METS)*

- Occupational Tasks:**
- 1) Heavy carpentry
  - 2) Construction work
- Household Tasks:**
- 1) Scrubbing floors
  - 2) Shoveling snow
  - 3) Moving (lifting furniture and boxes)
- Sports:**  
*(actual playing time)*
- 1) Racket Sports: badminton, paddleball, tennis (double)
  - 2) Basketball
  - 3) Rowing or canoeing leisurely
  - 4) Dancing: disco, jazz, aerobics (medium impact & intensity)
  - 5) Power walking (>mile/hr; <15 min/mile) or hiking
  - 6) Vigorous bicycling (16 – 20 mile/hr)
  - 7) Jogging ( $\geq 5$  mile/hr)
  - 8) Swimming
  - 9) Roller or ice skating
  - 10) Stationary bicycling

### *VERY HARD ACTIVITY ( $\geq 7.0$ METS)*

- Occupational Tasks:**
- 1) Digging or chopping with heavy tools
  - 2) Carrying heavy loads, such as bricks or lumber
- Sports:**  
*(actual playing time)*
- 1) Racket Sports: handball, racketball, squash, tennis (singles)
  - 2) Soccer
  - 3) Snow skiing (down hill and cross country)
  - 4) Dancing: aerobics (high impact & intensity)
  - 5) Jumping rope

- 6) Vigorous bicycling on hills
- 7) Jogging or running ( $\geq 8$  mile/hr)

**PHYSICAL ACTIVITY RECALL**

1. \_\_\_\_\_ HOURS week-day sleep   Total (sum of #1 and #2) \_\_\_\_\_
5. \_\_\_\_\_ HOURS week-end sleep
6. \_\_\_\_\_ HOURS week-day moderate/5 = \_\_\_\_\_   Average
7. \_\_\_\_\_ HOURS week-end moderate/2 = \_\_\_\_\_   Average
8. \_\_\_\_\_ HOURS week-day hard/5 = \_\_\_\_\_
9. \_\_\_\_\_ HOURS week-end hard/2 = \_\_\_\_\_   Average
10. \_\_\_\_\_ HOURS week-day very hard/5 = \_\_\_\_\_   Average
11. \_\_\_\_\_ HOURS week-end very hard/2 = \_\_\_\_\_
12. 1 \_\_\_\_\_ 2 \_\_\_\_\_ 3 \_\_\_\_\_
13. Total sleep hours \_\_\_\_\_ x 1 = \_\_\_\_\_
2. Average = hours moderate act. \_\_\_\_\_ x 4 = \_\_\_\_\_
3. Average = hours hard act. \_\_\_\_\_ x 6 = \_\_\_\_\_
4. Average = hours very hard act. \_\_\_\_\_ x 10 = \_\_\_\_\_
5. Sum of hours \_\_\_\_\_ - 24 \_\_\_\_\_ x 1.5 = \_\_\_\_\_
14. Sum of 1,2,3,4, & 5