THE EFFECT OF WHOLE RED RASPBERRY JUICE ON BONE DENSITY AND BIOMARKERS OF BONE IN POSTMENOPAUSAL OSTEOPENIC WOMEN

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ABSTRACT

THE EFFECT OF WHOLE RED RASPBERRY JUICE ON BONE DENSITY AND BIOMARKERS OF BONE IN POSTMENOPAUSAL OSTEOPENIC WOMEN

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The purpose of this study was to examine the effect of red raspberry juice on bone mineral density and bone biomarkers in postmenopausal women with mild-to-moderate bone loss. A total of 57 women were recruited and randomized into two groups for a period of 6 months (180 days). The treatment group (n= 30) consumed 2 oz of red raspberry concentrate daily (reconstituted with 10 oz water). The placebo group (n = 27) served as the control and consumed 2 ounces of a placebo mixture equivalent to the red raspberry juice concentrate in appearance, energy, and sugar content (fructose and dextrose) devoid of red raspberries. DXA scans were performed at baseline and final (180 days) visits to evaluate bone mineral density (BMD). Serum and urinary samples were collected at baseline, midpoint (90 days) and final visits to assess changes in bone markers. At the end of the 6 month study, total BMD had decreased in the control group, but was maintained or slightly improved in the treatment group. Biomarkers of bone formation were maintained in the treatment group and decreased in the placebo group. Additionally, biomarkers of bone resorption were decreased in the treatment group and remained unchanged in the placebo. The findings of this

study indicate that regular consumption of red raspberry may have a positive impact on bone health by inhibiting or protecting bone against resorption, and therefore, positively influences osteoporosis disease risk.

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CHAPTER I

INTRODUCTION

Menopause refers to the permeant cessation of menstrual periods that occurs naturally, or is induced by surgery, chemotherapy, or radiation (Sassarini & Lumsden, 2015). It is often preceded by months or years of irregular cycles, and up to 75% of women will experience various adverse symptoms. Symptoms include vasomotor symptoms (hot flashes and night sweats), vaginal dryness and sexual dysfunction, urinary incontinence, trouble sleeping, depression, anxiety, labile mood, memory loss, fatigue, headaches, joint pain, and weight gain (Sassaarini & Lumsden, 2015). The hormonal changes that occur due to menopause can negatively affect disease risk in women as well (Keteepe-Arachi and Sharma, 2016; Sassarini & Lumsden, 2015). The risk for developing cardiovascular disease, diabetes, and hypertension increases during this time (Rosano, 2007; Karvonen-Gutierrez et al., 2016; Keteepe-Arachi & Sharma, 2016; Staessen et al. 1997).

A woman's bone health is also negatively affected during menopause due to the decrease in estrogen production. Osteoporosis, a systemic skeletal disease is characterized by an imbalance in bone remodeling that leads to decreased bone density and bone strength, increasing risk for fracture. The spine, hip, wrist, humerus, and pelvis are the most common fracture sites observed in individuals with osteoporosis. Fractures of the hip and spine are associated with an increased mortality rate of 10-20% because

they often result in mobility limitations, depression, loss of independence and chronic pain (Lewiecki, 2017). An estimated 10 million Americans have osteoporosis and an additional 44 million are at risk for fracture due to low bone mineral density (National Osteoporosis Foundation, 2017). Osteoporosis is often diagnosed late making it a major public health threat as well as a financial issue. In the United States osteoporosis is responsible for 2 million broken bones and incurs costs of $19 billion annually to patients, families, and the health care system. These numbers are expected to increase to 3 million fractures and $25.3 billion annually by the year 2025 (National Osteoporosis Foundation, 2017).

Recent research has revealed estrogen plays a role in regulating bone homeostasis through its regulatory effects on the immune system and on oxidative stress which acts on bone cells (Weitzmann & Pacifici, 2006). Bone undergoes continuous remodeling by the corresponding resorption and formation activities of osteoclasts and osteoblasts (Cervellati et al., 2014). Osteoblasts are specialized cells that produce extracellular matrix and regulate bone mineralization, while osteoclasts are responsible for bone break down during bone resorption (Trzeciakiewicz, Habauzit, and Horcajada, 2009). Estrogen has been shown to induce osteoclast apoptosis and inhibit osteoblast apoptosis, protecting bone from an imbalance in turnover (Weitzmann and Pacifici, 2006). The estrogen decline that occurs in women after menopause often leads to an imbalance in bone homeostasis, with an increased bone turnover rate where resorption exceeds formation (Cervellati et al., 2014).

Currently there are several treatment options to prevent or stall osteoporosis, however they are mostly pharmacological methods with possible adverse effects from long-term use. Phytochemicals found abundantly in plants, fruits, and vegetables may be beneficial in protecting the body against reactive oxygen species that increase disease risk, including bone loss (Alvarez et al., 2016). Studies using animal as well as short term clinical studies have found that small doses of phytochemicals can have a positive effect on bone health and bone mineral density by activating cell signaling pathways, enzyme production, and directing the differentiation of osteoblasts and osteoclasts (Gunn, Weber, McGill, & Kruger, 2015; Zimmerman & Hooshmand, 2017).

Raspberries are one of the most widely consumed berries worldwide, with the most commercially produced being the red raspberry (Rudrappa, 2017; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). Raspberries have been demonstrated to be rich in dietary antioxidants and have been used in traditional and alternative medicine for various ailments (Skrovankova et al., 2015). Several studies have shown raspberries to have antioxidant effects in cancer prevention using rat models (Stoner et al., 2007). However, there have been no studies with raspberries investigating its effects on bone health. This study will examine the effect of raspberry consumption on bone health using postmenopausal women with mild to moderate osteopenic bone.

Hypothesis and Specific Aims

Hypothesis

The daily consumption of whole red raspberry juice concentrate will maintain or increase lumbar spine bone mineral density (BMD) in postmenopausal women who are mild to moderately osteopenic and not on hormone or other bone therapies.

Specific Aims

Aim 1. To determine whether the inclusion of whole red raspberry juice concentrate into the diets of postmenopausal women with mild to moderate bone loss (osteopenia) will maintain or increase bone mineral density of the lumbar spine, femoral neck, and wrist.

Aim 2. To evaluate whether whole red raspberry juice concentrate can positively influence selective plasma and urinary markers of bone metabolism in postmenopausal women with mild to moderate bone loss.

CHAPTER II

REVIEW OF LITERATURE

Menopause

Menopause refers to the permanent cessation of menstrual periods that occurs naturally, or is induced by surgery, chemotherapy, or radiation (Sassarini & Lumsden, 2015). Natural menopause is acknowledged retrospectively after 12 months of amenorrhea, and is a natural part of aging for women. It occurs at an average age of 52, however that age can vary widely from 40-58 years old (NAMS, 2017). Menopause is often preceded by months or years of irregular cycles, and up to 75% of women will experience adverse symptoms. The Stages of Reproductive Aging Workshop (STRAW) established a nomenclature and staging system for the female reproductive aging continuum in 2001, and was then revised in 2011 (NAMS, 2017). According to STRAW, 'menopause transition' refers to the span of time before menopause is diagnosed, when menstrual cycle and endocrine changes occur. This transition begins with a variation in the length of menstrual cycles and ends with the final menstrual period, indicating loss of ovarian follicular function. Symptoms during this time period include vasomotor symptoms (hot flashes and night sweats), vaginal dryness and sexual dysfunction, urinary incontinence, trouble sleeping, depression, anxiety, labile mood, memory loss, fatigue, headaches, joint pain, and weight gain (Sassaarini & Lumsden 2015).

Post-Menopausal Disease Risk

The hormonal changes that occur due to menopause can negatively affect disease risk in women (Keteepe-Arachi & Sharma, 2016; Sassarini & Lumsden, 2015). Cardiovascular disease (CVD) is the leading cause of death in women worldwide, and the leading cause of mortality and morbidity in developed countries in women over age 50 (Rosano et al., 2007). Ischemic heart disease in women generally presents about a decade later than in men due to the decline in estrogen production seen in menopause (Keteepe-Arachi & Sharma 2016). The hormonal changes that occur during menopause can result in an accelerated increase in low-density lipoprotein cholesterol (LDL-C) in the year following menopause (NAMS: CVD Risk, 2017). Postmenopausal women will have an increase in total cholesterol due to this increase in LDL-C without a reciprocal increase in high-density lipoprotein cholesterol (HDL-C) (Keteepe-Arachi & Sharma, 2016). After menopause, women may also experience an increase in body weight, alteration in fat distribution, centroid obesity and visceral fat deposition, as well as an increase in other CVD risk factors, such as diabetes mellitus (DM) (Keteepe-Arachi & Sharma 2016; Rosano et al., 2007).

Diabetes is of particular concern in women. A meta-analysis of 37 studies showed a 50% greater relative risk of fatal CVD in women with DM compared to men with DM (Huxley, Barzi, &Woodward et al., 2006). This significant difference in mortality is due to a more adverse risk factor profile, smaller coronary vessel diameter, and inadequate treatment of diabetes in women (Keteepe-Arachi & Sharma 2016). However, there is still debate about whether an increased prevalence of diabetes during midlife is due to

menopause or chronological aging (Karvonen-Gutierrez, Park, & Kim et al., 2016). Studies have shown that mid-life is a vulnerable time to develop obesity, because body composition and body fat distribution changes can be related to both ageing and menopause (Karvonen-Gutierrez et al., 2016; Sowers et al., 2007; Toth et al., 2000). The changes in body composition and increase in inflammatory cytokines that occurs during perimenopause have been associated with decreased insulin sensitivity and glucose tolerance (Karvonen-Gutierrez et al., 2016).

Cross sectional studies have shown an increase in blood pressure (systolic and diastolic) following the onset of menopause (Weiss, 1972; Staessen, Bulpitt, Fagard, Lijnen, & Amery, 1989; Staessen. Ginnocio, Thijs,& Fagard, 1997). Additionally, compared with pre- and perimenopausal women, postmenopausal women have a higher systolic blood pressure (Staessen et al., 1997). Because menopause is associated with a decrease in estrogen production, it is likely the changes in blood pressure are due to that reduction. This conclusion is partially supported by the observation that during the menstrual cycle, blood pressure is lower during the luteal phase (peak of estrogen levels) than during the follicular phase (Dubey, 2002).

Bone health is also affected by menopause. Research over the last several years has revealed a connection between estrogen and bone homeostasis. Due to the decrease in estrogen production as a result of menopause, decreased bone mineral density and osteoporosis are of significant concern (Weitzmann & Pacifici, 2006).

Osteoporosis

Osteoporosis is a systemic skeletal disorder characterized by an imbalance in bone remodeling that leads to decreased bone mineral density and bone strength. This decrease in strength and density increases susceptibility to fractures (Sandhu & Hampson, 2011). Osteoporosis is often underdiagnosed and poorly understood (Cooper, 2010). It occurs because of a failure to reach peak bone mass and excessive bone resorption and/or decreased bone formation during bone remodeling (Sandhu & Hampson, 2011). Attainment of peak bone mass is important in preventing osteoporosis and consequent fractures in adulthood (Sandhu & Hampson, 2011). Genetic factors are major determinants of peak bone mass; however, environmental factors such as nutrition, exercise, and smoking play an important role in achieving peak bone mass during childhood and early adulthood (Sandhu & Hampson, 2011). The disease is characterized by low bone mineral density and deterioration of bone tissue, with an increase in bone fragility and increase risk for fracture (Cooper, 2010).

Osteoporosis is often diagnosed late and therefore, is a major public health threat, as well as a financial issue. According to the National Osteoporosis Foundation, an estimated 10 million Americans have osteoporosis and an additional 44 million are affected by low bone mineral density putting them at increased risk for fracture (National Osteoporosis Foundation, 2017). Decrease in bone mineral density and bone mineral content result in increased bone fragility and susceptibility to fractures (Cooper, 2010). This could result in increased rates of morbidity, loss of independence, as well as increased mortality at advanced stages, or if left untreated (Cooper, 2010). Fractures can

have devastating impact on quality of life and those who suffer from osteoporotic fracture impose an economic burden on health care systems. Approximately one in two women, and one in four men over age 50 will break a bone due to low bone mineral density. (National Osteoporosis Foundation, 2017).

Economic Burden

Osteoporosis is an international problem with an estimated 8.9 million fractures caused by osteoporosis annually (Johnell & Kanis, 2006). In the United States alone, osteoporosis is responsible for 2 million broken bones and costs patients, families, and the health care system $19 billion annually (National Osteoporosis Foundation, 2017). It is estimated that by the year 2025, those numbers will increase to 3 million fractures and $25.3 billion annually (National Osteoporosis Foundation, 2017). A 2002 study of 30.2 million elderly Medicare recipients showed that 1.6 million were treated for a fracture and an additional 7.2 million had osteoporosis without a fracture. The estimated mean cost of the medical costs for each person treated for a fracture was at $8,600, and a percentage of the non-fracture patients received drug treatment averaging $500 per person. The population is aging because people are living longer, as a result the cost of osteoporosis/low BMD is quickly becoming a significant public health concern (Blume & Curtis, 2011).

Risk Factors

Osteoporosis has been called a “silent disease” because bone loss occurs over time without any obvious signs or symptoms (Gerend, Erchull, Aiken, Maner, 2006). Low bone mineral density is currently the best predictor of fracture risk in asymptomatic

patients (Siris et al., 2001). The majority of people are unaware of their condition until a fracture occurs. Most bone growth occurs during childhood; however, preventative actions adopted during adulthood can prevent or delay the onset of osteoporosis (Gerend et al., 2006). Genetic and physiological factors associated with increased risk for development of the disease include family history, advanced age, oophorectomy, early menopause, race (Asian and Caucasians have a higher risk), being small-boned, and having a low body weight (Gerend et al., 2006). Behavioral aspects include diet and physical activity. Adequate calcium and Vitamin D intake is essential for the development and maintenance of healthy bone (Gerend et al., 2006). High-impact activities that involve jumping and sprinting, as well as weight-bearing, are recommended for osteoporosis prevention (Gerend et al., 2006; Shen et al., 2012). Lifestyle factors including excessive alcohol intake and smoking have a negative impact on bone health (Siris et al., 2001). Postmenopausal women make up a large portion of those with osteoporosis induced by decreased estrogen levels and the benefits their estrogen provides.

Bone Remodeling

Bone undergoes continuous remodeling by the coordinated resorption and formation activities of osteoclasts and osteoblasts (Cervellati et al., 2014). Osteoblasts are the specialized cells within bone that produce extracellular matrix and regulate bone mineralization. They are derived from stem cells originating in the bone marrow that can differentiate into several kinds of tissue-specific cells. Each separate pathway is regulated by transcription factors. Specific to osteoblasts, different stages are involved in

osteoblastgenesis including proliferation, extracellular matrix synthesis, maturation, and mineralization (Trzeciakiewicz et al., 2009). Transcription factors play a role in this pathway, and expression of these factors is regulated by several hormones, including estrogen. Once the osteoblasts mature, they are surrounded by bone matrix and become osteocytes. It is probable that these osteocytes function as mechanosensors to regulate a bone response to mechanical stimuli (Trzeciakiewicz et al., 2009).

Osteoblasts regulate osteoclastogenesis and play an essential role in the regulation of osteoclast function. Osteoclasts are responsible for bone break down during bone resorption. Macrophage colony-stimulated factor produced by osteoblasts is required for osteoclast formation and development. It has been shown that the osteoprotegerin (OPG)/receptor activator of NF-kB (RANK) ligand (RANKL)/RANK system plays a role in osteoclastogenesis. The presence of estrogen seems to decrease the sensitivity of maturing osteoclasts to the osteoclastogenic factor receptor activator of NF-kB (RANK) ligand (RANKL) (Trzeciakiewicz et al., 2009).

The activities of osteoclasts and osteoblasts are combined into defined anatomical spaces called basic multicellular units (BMUs) (Weitzmann & Pacifici, 2006). Osteoblasts are responsible for the synthesis and mineralization of bone, whereas the osteoclasts break down bone during resorption (Trzeciakiewicz et al., 2009). Once activated, a portion of bone is first resorbed by osteoclasts (Erben, 2015). Following resorption, osteoblasts fill the resorption cavity with new bone (Erben, 2015). However, bone formation and resorption occur independently of each other resulting in resorption or formation drifts which alter bone structure (Erben, 2015). It is currently believed that

remodeling is initiated by either stochastic, hormone-driven, or targeted, microdamage-driven mechanisms (Erben, 2015). Regardless of which mechanism is used, it is likely that the initial event for bone resorption is detachment of bone lining cells from the bone surface (Erben, 2015).

Menopausal Changes Affecting Bone Remodeling

Estrogen plays a vital role in skeletal growth and homeostasis within the body (Weitzmann & Pacifici, 2006). Research over the last decade indicates that estrogen regulates bone homeostasis through regulatory effects on the immune system and direct effects on bone cells. Osteoblasts, osteocytes, and osteoclasts express functional estrogen receptors (Weitzmann & Pacifici, 2006). Bone cells contain two receptors that respond to estrogen, ERa and ERb. While bone cells contain both receptors, the distribution of these receptors is not equal within bone. Ligand binding to these ERs produces a change that promotes receptor dimerization and binding to specific DNA sequences called estrogen response elements. The ligand-bound ER forms a complex with coactivator proteins, which activates the transcriptional process and increases expression of target genes. Estrogen has also been shown to repress the expression of genes responsible for osteoclastic factors such as IL-6, TNF-a, and M-CSF (Weitzmann & Pacifici, 2006). Furthermore, estrogen decreases the sensitivity of aging osteoclasts to the osteoclastogenic factor receptor activator of NF-kB (RANK) ligand (RANKL). Estrogen has also been shown to induce osteoclast apoptosis and inhibit osteoblast apoptosis, further protecting bone from an imbalance in turnover (Weitzmann & Pacifici, 2006).

Estrogen decline that occurs in women after menopause often leads to imbalance of homeostasis of bone, with an increased bone turnover rate where resorption exceeds formation (Weitzmann & Pacifici, 2006; Cervellati et al., 2014). Estrogen deficiency leads to an increase in the number of BMUs through increased activation frequency, meaning the number of new remodeling units activated in each unit of time (Weitzmann & Pacifici, 2006). Increased activation frequency expands the remodeling space, increases cortical porosity, and enlarges the resorption area on trabecular surfaces contributing to low bone mineral density. Low estrogen also amplifies erosion depth by prolonging the resorption phase of the remodeling cycle due to the reduced apoptosis of osteoclasts (Weitzmann & Pacifici, 2006). Bone loss is limited by a compensatory increase of bone formation within each BMU, however the net increase in bone formation is inadequate due to the increase in osteoblast apoptosis induced by the estrogen deficiency (Weitzmann & Pacifici, 2006). *In vitro* and *in vivo* experiments have shown that estrogen withdrawal increases potential for formation of reactive oxygen species and decreases the antioxidant defense capacity of the cell, leading to an accumulation of these oxidants that are able to stimulate osteoclast formation and increase resorption activity (Cervellati et al., 2014).

Bone Biomarkers

Bone remodeling is frequently evaluated using bone turnover markers (BTMs) assayed in serum and urine because high bone turnover is associated with accelerated bone loss and increased risk for fracture (Biver et al., 2012; Chopin et al., 2012). Due to their rapid response to therapy, they are useful measures of short-term effects on bone

(Frunck-Brentano et al., 2011). Bone formation can be assessed by measuring bone-specific alkaline phosphatase (BAP). BAP is an enzyme located on the outer surface of osteoblasts, and is a major regulator of bone matrix mineralization (Szulc, 2012). BAP cleaves phosphates and pyrophosphate (mineralization inhibitor), and in so doing, provides inorganic orthophosphate, a substrate for the synthesis of hydroxyapatite, a mineralized form of calcium essential for bone (Szulc, 2012). Serum levels of BAP are correlated positively with bone formation (Szulc, 2012). Osteoprotegerin (OPG) is a biomarker that can also be used when evaluating bone (Simonet et al., 1997). OPG is a glycoprotein member of the tumor necrosis factor (TNF) receptor superfamily. OPG acts as a decoy receptor for the RANK/RANKL signaling pathway which is responsible for stimulating osteoclastogenesis. By binding to RANKL, OPG prevents RANK activation and subsequent osteoclastogenesis, thereby inhibiting bone resorption (Tat et al., 2008). *In vivo*, hepatic expression of OPG in mice resulted in osteopetrosis, coincident with a decrease in later stages of osteoclast differentiation (Simonet et al., 1997). *In vitro*, using an osteoclast-forming assay, found that all active forms of OPG inhibited osteoclastogenesis in a dose-dependent manner (Simonet et al., 1997). These findings could indicate that OPG is a key determinant in regulating bone remodeling.

Bone resorption can be quantified using deoxypyridinoline (DPD) found in urine (Szulc, 2012). DPD is a product of catabolism of type I collagen and correlates positively with bone resorption (Szulc, 2012). Several studies have concluded that increased levels of BTM, especially bone resorption ones, in postmenopausal women are associated with an increased fracture risk and can be considered an independent risk factor for fractures

in the future (Chopin et al., 2012). Sclerostin is a small protein expressed on the SOST gene in osteocytes that appears to play a role in bone remodeling (Lewiecki, 2014). When sclerostin binds to its receptor on osteoblasts, a signaling cascade begins that ultimately inhibits bone formation (Lewiecki, 2014). Low levels of serum sclerostin have been found in rare genetic disorders, such as sclerosteosis and van Buchem disease, which are characterized by high bone mineral density and low risk of fracture (Lewiecki, 2014). It is therefore believed that a low sclerostin level would indicate reduced bone resorption and/or increase bone formation (Lewiecki, 2014). Animal studies investigating the role of sclerostin in Wnt/B-catenin signaling have led to consideration of sclerostin as a potential target for treatment of osteoporosis (Lewiecki, 2014).

Current Dietary Treatment Options

Currently there are several treatment options to prevent or stall osteoporosis. One method is supplementation of calcium and vitamin D. The role of calcium and vitamin D in reducing fractures and falls in the elderly is unclear, but it has been shown that a reduction in calcium intake or absorption and/or vitamin D deficiency/insufficiency leads to secondary hyperparathyroidism (Lips, 2001; Sandhu & Hampson, 2011). This can contribute to accelerated bone loss in the elderly (Sandhu & Hampson, 2011). This method of treatment, however, displays conflicting findings among numerous studies. A large, randomized study found that supplementation with either calcium, vitamin D, or both for secondary fracture prevention was ineffective (Curtis, Moon, Dennison, Harvey, & Cooper, 2016: Grant, Avenell, & Campbell, 2005). However, in high-risk settings such as nursing homes where deficiencies are present, supplementation may be beneficial

(Chapuy et al., 2002; Curtis et al., 2016). A 3-year factorial, cluster-randomized, pragmatic, intervention study of 9605 elderly people living in Northern Europe found that there was a reduced risk of fractures in residents offered vitamin D and calcium supplementation compared to the control group and those not receiving supplementation (Larsen, Mosekilde, & Foldspang, 2004). The treatment group received 1000 mg of calcium carbonate and 400 IU of vitamin D3 (Larsen et al., 2004). Furthermore, in a separate study, there appeared to be a modest benefit from calcium and vitamin D supplementation for hip fractures and total fractures, but there was no benefit from vitamin D alone (Abrahamsen et al., 2010).

Current Pharmacological Treatment Options

There are several pharmacological methods for treating osteoporosis. Bisphosphonates are synthetic analogues of a naturally occurring compound that inhibits resorption by inactivating osteoclasts. The most commonly prescribed is Alendronate. An intravenous bisphosphonate can be used as an alternative if an oral bisphosphonate is contraindicated (malabsorption or dysphagia) (Curtis et al., 2016).

Denosumab acts as an antibody to RANKL, which is secreted by osteoblasts and activates osteoclastic activity. Denosumab is administered as a subcutaneous injection every six months and its efficacy has been demonstrated in patients with renal impairment (Curtis et al., 2016). Three year fracture data showed a 68% reduction in vertebral fractures and a 40% reduction in hip fractures (Cummings et al., 2009).

Strontium ranelate is taken as a daily oral dose and is believed to increase bone strength by altering bone material properties, however, the mechanism of its action is still under

research. Strontium ranelate leads to an increase in BMD at the spine and hip, though part of this increase is artefactual due to the fact that strontium has a greater atomic weight than calcium. However, strontium ranelate has been shown to increase cardiovascular disease risk in addition to previously known risk of deep vein thrombosis (DVT). Its use is now limited to treatment of severe osteoporosis in postmenopausal women with high risk for fracture and in men at an increased risk for fracture, with no cardiovascular disease.

Raloxifene is a selective estrogen receptor modulator that has antiresorptive effects on the skeleton without affecting the breasts (Curtis et al., 2016). Its use has been associated with a significant decrease in the risk of breast cancer. While this drug does prove to be effective in preventing postmenopausal bone loss and preventing vertebral fractures, there is no evidence that it prevents hip or nonvertebral fractures (Ettinger et al., 2009).

Teriparatide, a recombinant human parathryroid hormone peptide, is the only agent in current widespread use that has truly anabolic effects on bone. It increases bone formation and produces large increases in BMD, leading to an estimated 70% reduction in incidence of new vertebral fracture over 18 months of treatment (Neer et al., 2001). Additional studies have shown an increased benefit in combination treatments such as teriparatide plus denosumab or zolendronate (bisphosphonate) (Tsai et al., 2013).

Hormone replacement therapy (HRT) has been recognized as an effective treatment for menopausal symptoms that does offer protection for bone. However, HRT has been linked with an increase in the risks of breast cancer, DVT, and stroke (Bowring & Francis, 2011). HRT can be considered for women younger than 60 when the benefits

outweigh the risks. HRT has been shown to decrease the number of fractures at the hip and spine (Cauley et al., 2003). The effect on BMD once the treatment has stopped is still controversial. Conflicting evidence states HRT can offer a protective effect for several years after treatment has stopped, while other studies show that HRT is only protective while it's being taken (Bowring & Francis, 2011). In any case, the lowest effective dose of HRT should be used for the shortest amount of time.

Polyphenols

Phytochemicals are found abundantly in plants, particularly tea leaves, fruits, and vegetables. Studies have shown that the content of antioxidants and polyphenols found in fruits and vegetables may be beneficial in protecting the body against reactive oxygen species (Alvarez et al., 2016). Polyphenols have been shown to inhibit enzymes involved in prostaglandin and leukotriene synthesis, prevent free radical formation, decrease proinflammatory cytokine production, and block the activity of proinflammatory signaling factors (Franz, 2014). To date, approximately 5000 polyphenols have bene identified in foods regularly consumed by the public. Chemically, polyphenols are classified according to the number of phenol rings they contain, and which structural elements are bound to these rings (Sacco, Horcajada, & Offord, 2012). Polyphenols can be classified as phenolic acids, flavonoids, stilbenes, tannins, coumarins, and lignans. Polyphenols are absorbed into the bloodstream in their aglycone forms and metabolized into conjugates of gluconorate or sulfate, and/or eventually eliminated. These circulating forms likely possess different properties within cells and tissues than the original polyphenol aglycones (Sacco et al., 2012).

Polyphenols and Bone Health-Animal Studies

Small doses of specific phytochemicals have been shown to inhibit the body's inflammatory response by activating cell signaling pathways, enzyme production, and directing the differentiation of osteoblasts and osteoclasts (Gunn et al., 2015). Several animal studies have been performed assessing the benefits of these chemicals. Dried plums have a high phenolic content and have been shown to protect against or even reverse bone loss (Zimmerman & Hooshmand, 2017). An animal study designed to identify the means by which dried plum prevents bone weakening found that the protective effects were due in part to the decrease in osteoclast creation. The gene expression for RANKL was downregulated while an increase in serum insulin-like growth factor (IGF-1) was noted, indicating an increase in bone formation (Franklin et al., 2006). Blueberries are another highly phenolic fruit that is often studied. A small study using 4-month-old rats and four different blueberry-enriched diets was performed in the hopes of determining an effective dose of blueberry to increase bone net calcium retention, and thus improve bone accrual. The study found that a 5% blueberry diet increased bone net calcium by 25%, however, higher doses (10% and 15%) did not have an effect on calcium retention (Rodriguez et al., 2017). A similar study researching blueberry's effect on bone formation in rats found that after 34 days of being fed 10% powdered whole blueberries the male and female rats had increased bone mass, including bone mineral density and bone mineral content. Furthermore, they found a significant increase in bone formation markers BAP and osteocalcin (OCN) in the rats fed the blueberry diet as compared to the control group. However, at 34 days there was no

difference in the bone resorption marker, procollagen cross-links. At 61 days of being fed the blueberry diet, there was a decrease in the bone resorption marker, while bone formation markers observed at 31 days remained stable (Chen et al., 2010). Additionally, a study investigating phloridzin, a flavonoid found in apples, showed an increase in mineralization of bone in ovariectomized rats (Puel et al., 2005).

Polyphenols and Bone Health- Clinical Trials

Several observational studies have shown a beneficial effect of fruit, vegetable, and potassium intake on bone health in young boys and girls, pre-,peri-, and postmenopausal women, and elderly men and women (New, 2003). Epidemiological studies have shown a reduced risk of hip fracture and higher BMD in habitual tea drinkers, a beverage high in polyphenols and flavanols (Sacco et al., 2012; Shen et al., 2008). A small clinical trial looking at green tea polyphenol supplementation in postmenopausal osteopenic women demonstrated an increase in serum bone alkaline phosphatase (BAP) levels after one month, which was not seen in the control group (Shen et al., 2012). BAP is a bone biomarker that has been positively correlated with increased BMD. Similar evidence of dietary intervention has been found in reference to dried plums, or prunes. In studies using human subjects, similar improvements have been seen when using dried plums. In 2002, a study found that dried plum supplementation for 3 months significantly increased serum levels of IGF-1 and BAP. Both of these serum biomarkers are associated with an increase rate of bone formation (Arjmandi et al., 2002). A study using postmenopausal women consuming either 0g dried plum/day, 50 g dried plum/day, or 100 g dried plum/day for 6 months found that both doses of dried plum

prevented BMD loss, indicating that a practical dose of dried plums could be adequate for treatment and prevention of weakening bones (Hooshmand et al., 2016). These findings are promising as many people are more interested in a nutritional strategy as opposed to long-term drug intervention for bone health.

Raspberry

Raspberries remain one of the most consumed berries worldwide. They can range in color from red and black varieties, to purple, yellow, or golden. With the different colors comes a different composition of vitamins, minerals, and antioxidants (Ware, 2016). Raspberry is a small shrub belonging to the *Rosaceae* family. It is native to Europe, but today is widely cultivated in temperate regions all over the world. The largest producers of raspberries are Poland, The United States, Germany, and Chile (Rudrappa, 2017). The most commonly commercially produced raspberry is the red raspberry. The whole berry is a collection of small drupe fruits arranged in a circular fashion around a hollow central cavity. Each tiny drupelet has a small juicy pulp with a single white seed. The taste of raspberries ranges from sweet to acidic depending on the cultivar, similar to strawberries (Rudrappa, 2017; Skrovankova et al., 2015). Raspberries are low in calories and fat, and rich in dietary fiber, providing 6.5 grams or 16% of the daily recommended intake for fiber per serving. They are considered an excellent source of vitamin C, a powerful antioxidant, containing around 47% of the DRI for vitamin C. Raspberries also contain a sufficient amount of minerals such as potassium, manganese, copper, iron, and magnesium (USDA Research Basic Report). Raspberries have been proven to be one of the richest sources of dietary antioxidants (Beekwilder, Hall, & Vic de Vos, 2005). They

contain the phytochemicals anthocyanins, ellagic acid, quercetin, gallic acid, cyanidins, pelargonidins, catechins, kaempferol, and salicyclic acid (Rudrappa, 2017). Raspberries have been used in traditional and alternative medicine to cure wounds, colic, diarrhea, and renal illnesses (Skrovankova et al., 2015). Raspberry extract has also been shown to have anti-proliferative effects to suppress the growth of human colon, prostate, breast, and oral tumor cells (Skrovankova et al., 2015).

Raspberries and Bone Health

Research focusing specifically on bone health has been directed mostly towards the flavonoids group which can be broken down into flavones, flavonols, flavanones, isoflavones, and anthocyanins (Sacco et al., 2012). Anthocyanins contribute to about 25% of the antioxidant activity of red raspberries, the most prominent being cyanidin and pelargonidin glycosides (Beekwilder et al., 2005). Anthocyanins are flavonoids and contribute to the pigmentation of flowers and fruits (Beekwilder et al., 2005). The largest contributor of antioxidants in raspberries is ellagitannins, making up more than 50% of the total antioxidant capacity (Beekwilder et al., 2005). An *in vitro* study researching the effect of raspberry ketones on stem cells found that the raspberry promoted osteoblast differentiation, which would in turn promote bone formation. Using 4-(4-hydroxyphenyl)butane-2-one (RK), one of the major aromatic compounds found in red raspberry, C3H10T1/2 stem cells were treated for 6 days with 10-100 *u*g/mL of RK in culture medium containing 10nM all-*trans*-retinoic acid (ATRA) or 300 ng/mL recombinant human bone morphogenetic protein (rhBMP)-2 as an osteoblast-differentiating agent (Takata & Morimoto, 2014). RK in the presence of ATRA and

rhBMP-2 increased BAP activity, indicating an increase in bone formation (Takata & Morimoto, 2014). Similar results were found in a study using arthritis-induced rat models treated with red raspberry extract (REE). The diseased rats were provided a 30 mg/kg or 120 mg/kg of REE, while a control group was given no treatment. The researchers examined the ankle joints of each treatment group and found that the group receiving 120 mg/kg of the red raspberry extract had mild inflammation and marked bone resorption, compared to the control and 30 mg/kg treatment group which exhibited severe inflammation and bone resorption. Clinical scoring showed that at the higher dose of 120 mg/kg, REE significantly inhibited inflammation (54%), pannus formation (74%), cartilage damage (67%), and bone resorption (67%), while there was no improvement in the 30 mg/kg treatment group (Jean-Giles et al., 2011).

The antioxidant activity of many fruits and vegetables has become a common topic of research. Raspberries have been labeled as one of the richest sources of antioxidants. Several studies have been performed to show its effects on cancer prevention using rat models (Stoner et al., 2007). However, there have been no studies using raspberries focusing on its effects on bone mineral density. Therefore, the role of raspberries and its effect on bone is being addressed in this thesis research using postmenopausal women with mild to moderate osteopenic bone.

CHAPTER III

METHODOLOGY

Study Design and Subject Recruitment

A total of 57 postmenopausal (within 2 to 10 years of menopause) women, ranging in age from 45-70, with mild to moderate degree of bone loss who were not on hormone or other bone therapies were recruited through private clinical settings and the local community of the Denton and Dallas/Fort Worth Metroplex. The method of recruitment included emails as well as flyers at various locations around the community. Using a double blind, randomized placebo controlled study design, subjects were placed in two treatment groups (n = 30 and 27 per treatment group) for 6 months. Group 1 consumed 2 ounces of red raspberry juice concentrate daily (Stoner et al., 2007). Group 2 served as the control and consumed 2 ounces of a placebo mixture equivalent to the red raspberry juice concentrate in appearance, energy, and sugar content (fructose and dextrose). The placebo and raspberry juice concentrates were reconstituted with 10 ounces of water before daily consumption.

Inclusion/Exclusion Criteria

Study participants were postmenopausal women who were not currently or had not been on hormone or other bone therapies for at least six months prior to the initiation of this study, and whose lumbar spine bone mineral density z-score was between -0.5 to -2.5 of the young adult reference group. Subjects who met the inclusion criteria were considered for the study regardless of ethnicity and race. Participants were excluded from

the study if they received endocrine or neuroactive drugs, or any drugs influencing bone and calcium metabolism, or other anabolic steroid up to 6 months prior to participating in this study. Subjects smoking more than 20 cigarettes daily were also excluded from participating in this study.

Screening and Phone Questionnaire

Interested participants were instructed to call or email the principal investigator for a short phone interview to assess if they qualified for the initial bone density assessment. During the phone interview, interested participants were asked about smoking habits, medications, special dietary requirements, food allergies, and menopausal status. Once it was determined that the subject qualified for the initial bone density assessment, an appointment was scheduled at the Texas Woman's University Institute of Women's Health located on the Denton campus. The participant was emailed a copy of a Consent to Participate in Research Study form to review. The form included information about the purpose of the research, as well as the research procedures, time commitment, potential risks, and participant benefits. The participant was instructed to arrive at the baseline appointment fasted (no food for at least 10 hours) overnight to ensure an accurate blood draw and urine sample.

Bone Density Assessment

The bone density measurements were performed at the Institute for Women's Health at Texas Woman's University. For each study participant, bone mineral density was measured in the lumbar spine (L1-L4), hip (femoral neck, femoral trochanter, and total), and wrist using dual-energy X-ray absorptiometry (DXA) (QDR-4500; Hologic,

Inc., Waltham, MA) instrument at baseline and at the end of the study. DXA scans have bene shown to have a number of advantages when compared to other bone density measures. One advantage is the BMD results can be interpreted using the World Health Organizations T-score definition of osteoporosis and osteopenia. These scans allow for proper diagnosis of osteoporosis, assessment of fracture risk, and monitoring response to treatment (Blake & Fogelman, 2007). Bone density assessments were conducted by a trained technician.

Treatment Compliance

Treatment compliance was tracked using a calendar for daily consumption of raspberry or placebo juice. Calendars were provided to participants at baseline and the 3-month visit and participants were instructed to bring the calendar back on the following visit. Participants were also called for follow up to ensure compliance and to address any concerns.

Blood and Urine Collection and Storage

Blood and urine samples were collected from study participants at baseline, 3 months, and at the end of the study (6 months). Plasma was separated from the blood (centrifuged at 1500x g for 10 minutes) within two hours of collection and stored at -70 degrees Celsius for analysis at a later date. Urine specimens were aliquoted into microcentrifuge tubes and stored in a freezer at -70 degrees Celsius for analysis at a later date.

Plasma and Urine Biomarkers

Four bone biomarkers were assessed to determine bone health. Bone specific alkaline phosphatase (B-ALP), osteoprotegerin (OPG), and sclerostin were used to assess bone formation. OPG, B-ALP, and sclerostin were assessed at baseline, 3 months, and at the end of the study. An ELISA test was used to assess B-ALP (Pyrilinks-D, Quidel Corp, Mountain View, CA). B-ALP was measured using an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a pNPP substrate. The samples were prepared according to the kit instructions, and read at an optical density of 405 nm using quadratic calibration curve fitting software. OPG was analyzed using a Human High Sensitivity T Cell Magnetic Bead Panel (Milliplex, Millipore Corp, Billerica, MA). This test utilizes Luminex technology which allows for several results to be obtained from each sample. Luminex uses techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500 5.6 um polystyrene microspheres or 80 6.45 um magnetic microspheres can be created, each coated with a specific capture antibody. After an analyte from a test sample is captured by the bead, a biotinylated detection body is introduced. The reaction mixture is incubated with Streptavidin-PE conjugate to complete the reaction on each microsphere. Luminex 200 software was used to analyze the data. Each microsphere was identified and the results of its bioassay were quantified based on fluorescent reporter signals. Sclerostin was also used as an indicator of increased bone resorption. Low levels of sclerostin have been associated with

conditions of increased bone mineral density. Sclerostin was analyzed using the same Human High Sensitivity T Cell Magnetic Bead Panel described above. Luminex 200 software was used to analyze the data.

The marker used to assess bone resorption was deoxypyridinoline (DPD). As a urinary marker of bone resorption, DPD was assessed at baseline, 3 months, and at the end of the study. DPD was measured by an ELISA test (Pyrilinks-D, Quidel Corp, Mountain View, CA). This test is a competitive enzyme immunoassay in a microtiter stripwell format utilizing a monoclonal anti-DPD antibody coated on the strip to capture DPD. DPD in the sample competes with conjugated DPD-alkaline phosphatase for the antibody. The reaction is detected with a pNPP substrate. These results were then corrected for urinary concentration by creatinine, and were expressed as nmol DPD/mmol Creatinine. The kits were run according to the instructions, and read at an optical density of 405 nm using 4-parameter calibration curve fitting software.

Statistical Analysis

A minimum sample size of 42 participants was needed in order to conduct analysis with the alpha = 0.5, power = .80, and a moderate effect size. Descriptive statistics were calculated for all variables, compromising means, standard deviations, medians, minima and maxima for continuous variables, while frequencies and percentages were calculated for all categorical demographic variables. Distributions of the continuous variables were examined to determine if normality assumptions are met and parametric testing is appropriate, or whether transformed data or non-parametric tests should be used. Extreme outliers were investigated for technical or clerical error.

Independent sample t-tests tested for potential baseline differences of site specific bone mineral densities and bone mineral content. Repeated Measures Analysis of Variance (ANOVA) was used for evaluation of changes in biochemical markers of bone formation and bone resorption between baseline, midpoint, and end of study. Covariate analysis, including ANOVA and regression, were conducted to control for baseline differences. The data was analyzed using SPSS V 19.0.

Intent to treat analysis (ITT) was deemed appropriate for this study due to the high attrition rate. Randomized control trials (RCT) are the ideal study design in assessing the efficacy of a treatment, however, it is hard to control every aspect and problem such as treatment compliance and missing outcomes due to drop-out are common (Gupta, 2011). Using ITT analysis allows for preservation of the sample size because it includes all randomized subjects regardless of treatment received, withdrawal from the study or deviation from the protocol (Gupta, 2011). Missing value analysis was used to determine the missing data points caused by drop-out. Missing value analysis describes the pattern of missing values, estimates means, standard deviations, covariances, and correlations for different missing value methods (listwise, pairwise, regression, or expectation-maximization). Multiple imputation was used by filling in the missing entries of the incomplete data sets *m* times. Each of the *m* completed sets are analyzed resulting in *m* analyses. This data is then pooled into a final result (Van Buuren, 2012).

CHAPTER IV

RESULTS

A total of 100 postmenopausal women were initially screened to participate in the study. Of those screened, 61 women met the criteria and were scheduled for the initial bone density scan. Of the 61 potential participants who met the inclusion criteria in the screening process, 57 qualified based on bone density assessment and bone loss and were randomized to initiate either the raspberry or placebo juice treatment for a period of 6 months. Over the course of the study, there were 19 individuals who withdrew from the study due issues such as taste and palatability of treatment, mild to moderate GI discomfort such as bloating, gas, and diarrhea, lack of interest, or conflicts in study visit scheduling. Demographic data associated with study participants is provided in Table 1.1 and Table 1.2.

Bone Mineral Density

Bone mineral density (BMD) was measured using dual energy x-ray absorptiometry (DXA). Sites of measurement included the lumbar spine, femur, and radius. Total BMD was also measured. The total body BMD in the raspberry group decreased, but was not considered significant. There was, however, a significant decrease in total body BMD seen in the placebo group. 4^{th} Lumbar BMD, a major site of spine fracture, was increased in the raspberry treatment group from baseline to final, and was decreased in the placebo group (Figure 1.1). These changes were not statistically

significant. Lumbar vertebrae 1-4 BMD showed an increase in the raspberry treatment group and decrease in the placebo group (Figure 1.2). Again, these changes did not reach a level of significance. BMD measures in the wrist (upper radius), the primary site of wrist fracture, increased from baseline to final in the raspberry intervention group, and remained stable in the placebo group. Total radius BMD decreased in the placebo group and remained stable in the raspberry treatment group. Femoral neck BMD, a site associated with hip fracture, remained unchanged in the raspberry group and decreased in the placebo group (Table 1.3). These changes did not reach a level of significance.

Bone Formation Biomarkers

Plasma levels of bone specific alkaline phosphatase (BAP), a marker of bone formation, in the raspberry treatment group did not change from baseline to final. However, there was a significant decrease in BAP levels in the placebo group from baseline to midpoint and from baseline to final (Figure 1.3).

Sclerostin (SOST) is a small protein that renders osteoblasts nonfunctional when expressed by the *SOST* gene. Elevated levels of sclerostin have been negatively correlated with bone formation. Sclerostin increased from baseline to midpoint in the raspberry treatment group, and subsequently decreased from midpoint to final. However, these changes were not considered significant. The levels of SOST in the placebo group stayed relatively unchanged from baseline to final (Figure 1.4).

In the raspberry treatment group, osteoprotegerin (OPG) increased from baseline to midpoint, and then remained stable from midpoint to final. In comparison, OPG levels

increased from baseline to midpoint, and then decreased from midpoint to final in the placebo group. However, neither the raspberry treatment nor placebo group saw changes that would be considered significant (Figure 1.5).

Bone Resorption Biomarkers

Deoxypyridinoline (DPD), a urinary marker of bone resorption which is corrected based on creatinine level in urine, showed significant changes ($p \leq 0.05$) in the raspberry treatment group. There was a significant decrease in the raspberry intervention group from baseline to midpoint, and baseline to final. In the placebo group, the level of DPD did decrease from baseline to final, but it was not statistically significant (Figure 1.6).

CHAPTER V

DISCUSSION

The findings of our study demonstrate that raspberry juice consumption for a period of 6 months resulted in improvements in bone mineral density and also had positive impact on biomarkers of bone remodeling. Recent research examining phytochemicals found in plants, such as fruits and vegetables, has revealed a protective effect against inflammation and oxidative stress. Increased inflammation and oxidative stress are associated with increased risk for cardiovascular disease, diabetes and osteoporosis in postmenopausal women (Alvarez et al., 2016). Several studies investigating the effects of phytochemicals from plum, green tea, and blueberries have been conducted in relation to skeletal health. All of these studies have shown some level of favorable effects regarding bone health (Zimmerman & Hooshmand, 2017; Rodriguez et al., 2017; New, 2003; Chen et al., 2010). Raspberries are one of the most widely consumed berries, and one of the richest sources of antioxidants; however, there have been no human studies examining the effect of raspberries on bone health (Beekwilder et al., 2005). This study was designed as a 6-month randomized study using raspberry juice in comparison to a placebo juice to assess if whole raspberry had an effect on bone health in postmenopausal osteopenic women by analyzing bone biomarkers as well as bone mineral density at crucial fracture sites such as the spine, femoral neck, and radius.

Bone mineral density (BMD) results showed that the 4^{th} lumbar BMD, lumbar vertebrae 1-4 BMD, and upper radius (wrist) BMD all increased in the raspberry

treatment group after 180 days. These results are encouraging as decreases in BMD are associated with elevated risk for fracture. These results of improved bone mineral density align with results from similar studies using dose-dependent study designs examining blueberry and plum polyphenols. Both fruits have produced favorable results for bone health. Using the rat model of bone loss, investigators at the University of Arkansas examined the dose-dependent effect blueberries have on bone. Their results showed BMD and bone mineral content (BMC) were dose-dependently increased in the blueberry groups in comparison to controls. They also found that the expression of receptor activator of NF-kB ligand (RANKL), a protein essential for osteoclastogenesis was dose-dependently decreased in the blueberry-fed rats (Zhang et al., 2013). In a clinical study by Hooshmand et al.. daily consumption of 50 g of dried plum for 6 months was found to be as effective as 100 g of dried plum in preventing bone loss in postmenopausal, osteopenic women (Hooshmand et al., 2016). They indicated that the effects may be attributed to polyphenols found in dried plums inhibiting bone resorption while maintaining current rates of bone formation. (Hooshmand et al.,2016). Their results somewhat support the findings of this study demonstrating that polyphenols from raspberries may have a positive effect on bone health by inhibiting bone resorption causing decreased osteoclast differentiation and activity. While the changes seen in our study were not statistically significant in the raspberry treatment group, it warrants further investigation using a larger sample size.

Deoxypyridinoline (DPD), a urinary bone marker for bone resorption, was found to significantly decrease in the raspberry treatment group from baseline to midpoint (90

days), and baseline to final. Furthermore, osteoprotegerin (OPG), a marker that acts as a decoy receptor for RANKL expression and an inhibitor of osteoclastogenesis, was found to have increased and remained stable in the raspberry treatment group from baseline to final. Although not significant, these findings combined with the significant change seen in DPD, could indicate that raspberry treatment decreased bone resorption. These findings also support the positive change in BMD in the lumbar spine and wrist. A 6-month study examining soy isoflavones and their effect on BMD in postmenopausal women support the results of our study as well. A study conducted by Yan-Bing Ye and colleagues found a significantly lower level of DPD as well as an increase in BMD in the high-dose isoflavone treatment group compared with the low-dose isoflavone treatment groups as well as placebo group (Ye, Tang, Verbruggen, & Su, 2006). Another study using dried plums examined the mechanism of the protective effect. Researchers measured serum RANKL, OPG and sclerostin (SOST) levels in postmenopausal women receiving dried plum treatment. Sclerostin is a small protein expressed by the SOST gene in osteocytes. When bound to its receptors on osteoblasts, sclerostin renders osteoblasts nonfunctional (Lewiecki, 2014). They found that plum treatment suppressed RANKL production, promoted OPG, and inhibited SOST, all of which positively affect BMD (Hooshmand e al., 2014). SOST was measured in our study; however no significant changes were noted. The suppression of RANKL has been seen in various studies investigating polyphenols in relation to bone health. Estrogen has been shown to have a protective role in suppressing RANKL expression and inhibiting bone resorption

(Weitzmann & Pacifici, 2006). These findings are encouraging as estrogen levels decrease during menopause.

Bone specific alkaline phosphatase (B-ALP) is a biomarker associated with bone formation. No changes in B-ALP were seen in the raspberry treatment group; however there was a significant decrease in B-ALP in the placebo juice group. A 6-month study performed by Laura Harkness and colleagues (Harkness, Fielder, Sehgal, Oravec, & Lerner, 2004) examining the effect of soy isoflavone supplementation on BMD and markers of bone turnover produced similar results to this study. The isoflavone treatment group saw a decrease in their selected bone marker for resorption (type 1 collagen a1-chain helical peptide) and saw no significant difference in bone formation markers (Harkness et al., 2004). The relatively short duration of our study may have contributed to the lack of significant changes seen in the treatment group. The bone remodeling process is slow and can take up to 18 months to reach a new equilibrium (Ye et al., 2006).

The outcomes of this study in relation to the effect of raspberry on bone health are promising and contribute to the body of research. When looking at our results as a whole, bone mineral density was maintained or improved in the raspberry-fed group with a concurrent decrease in rates of bone resorption as represented by lower DPD levels in urine. These results support several studies that claim bone resorption is inhibited or slowed when treated with polyphenols from various plant sources (Harkness et al., 2004; Hooshmand et al., 2016; Ye et al., 2006). Due to funding limitations and the scope of this thesis project, we were only able to measure a limited number of bone biomarkers. Future

studies would benefit from investigating a greater number of biomarkers to better understand the bone protective action of raspberries. Additional biomarkers to consider would be procollagen type IN-terminal peptide (PINP) (formation), collagen type-1 c-telopeptide (CTX) (released during resorption), and TRAP-5b/BAP ratio. Furthermore, it would be interesting to investigate the effect on bone activity at the molecular level. Bone morphogenic proteins (BMPs) are multi-functional growth factors belonging to the transforming growth factor beta (TGFbeta) superfamily. BMP signaling plays a critical role in heart, neural and cartilage development, and postnatal bone formation (Chen et al., 2004). Investigating the effects of raspberry on BMPs would allow us to look closer at BMP signal transduction and cellular function in relation to osteoblasts. More research is warranted to determine the threshold of raspberry intake for optimal effect on bone health in postmenopausal women as well as the mechanism of its action. Additionally, a study examining the dose-dependent effect of raspberry on bone health may be more desirable to see if lower doses are found to be beneficial.

We acknowledge that our study did have several limitations that may impact the outcomes. The drop-out rate was high for our study making significant changes harder to detect. This study was based on participant compliance with consumption of red raspberry or placebo concentrate. GI side effects or the palatability of the product led some of the participants to withdraw from the study. Reformulation of the raspberry concentrate may be needed for future studies to make it more desirable to use in a clinical trial of longer duration. The high drop-out rate made our effect size smaller and shifted the power analysis and ultimately impacted our statistical results. This can also make it

difficult to generalize the findings of our research to the larger population of postmenopausal-age women with bone loss. We attempted to overcome this limitation by using the intent-to-treat (ITT) analysis. ITT analysis was used which is a more cautious analysis approach, but is more likely to prevent a type 1 error. Lastly, due to the nature of randomization, we were not able to evenly distribute levels of bone loss among the two groups making it harder to achieve statistically significant results.

In summary, our study is the first of its kind to investigate raspberries in postmenopausal women as a treatment to prevent bone loss. Our findings suggest that raspberries may have a positive effect on bone health by inhibiting bone resorption. Futures study designs should include a larger sample size and potentially a longer treatment period to see more significant changes in BMD as the time required for a bone remodeling cycle to reach completion increases with age.

Table 1.1

Participant Screening and Drop Out Rate

Participants Screened	Bone Density Assessment	Qualified and Initiated Treatment	Completed Treatment	Participant Drop Out	Drop Out Rate
100	**61**	**57**	**37**	**20**	**35%**

Table 1.2

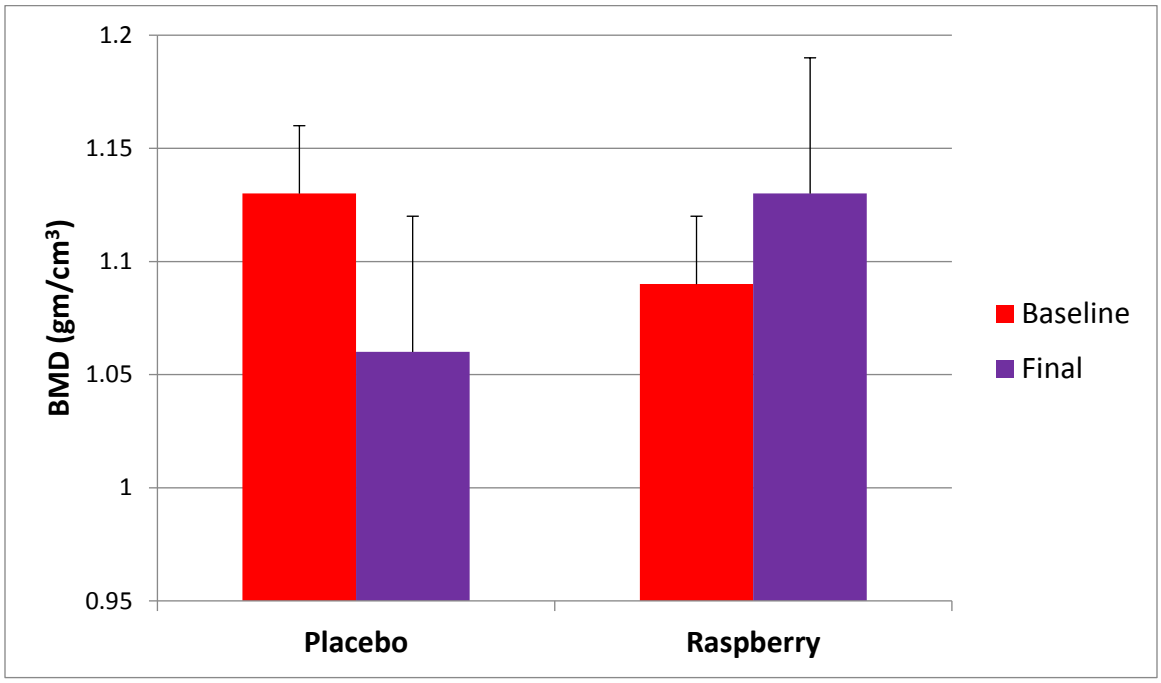
Demographics of Study Participants

	Baseline (n)	Midpoint (n)	Final (n)	Age Range	Average Age	Drop Rate Midpoint	Drop Rate Overall
Placebo		**21**	**20**	**46-68**	**57.11**	**22%**	**25%**
	30	**19**	**17**	**46-71**	**59.27**	**37%**	**43%**
		40	**37**	**46-71**	**58.25**	**30%**	**35%**

Table 1.3
Bone Mineral Density of Radius, Femoral Neck, and Total Body

	Placebo		Raspberry	
	Mean	**SEM**	**Mean**	**SEM**
Radius UD BMD				
Baseline	0.39	0.01	0.37	0.02
Final	0.39	0.01	0.38	0.02
Radius-Total BMD				
Baseline	0.61	0.02	0.57	0.02
Final	0.60	0.02	0.57	0.02
L-Femur Neck BMD				
Baseline	0.85	0.02	0.82	0.02
Final	0.84	0.02	0.82	0.02
Total BMD				
Baseline	1.07	0.01	1.05	0.02
Final	1.06*	0.02	1.04	0.02

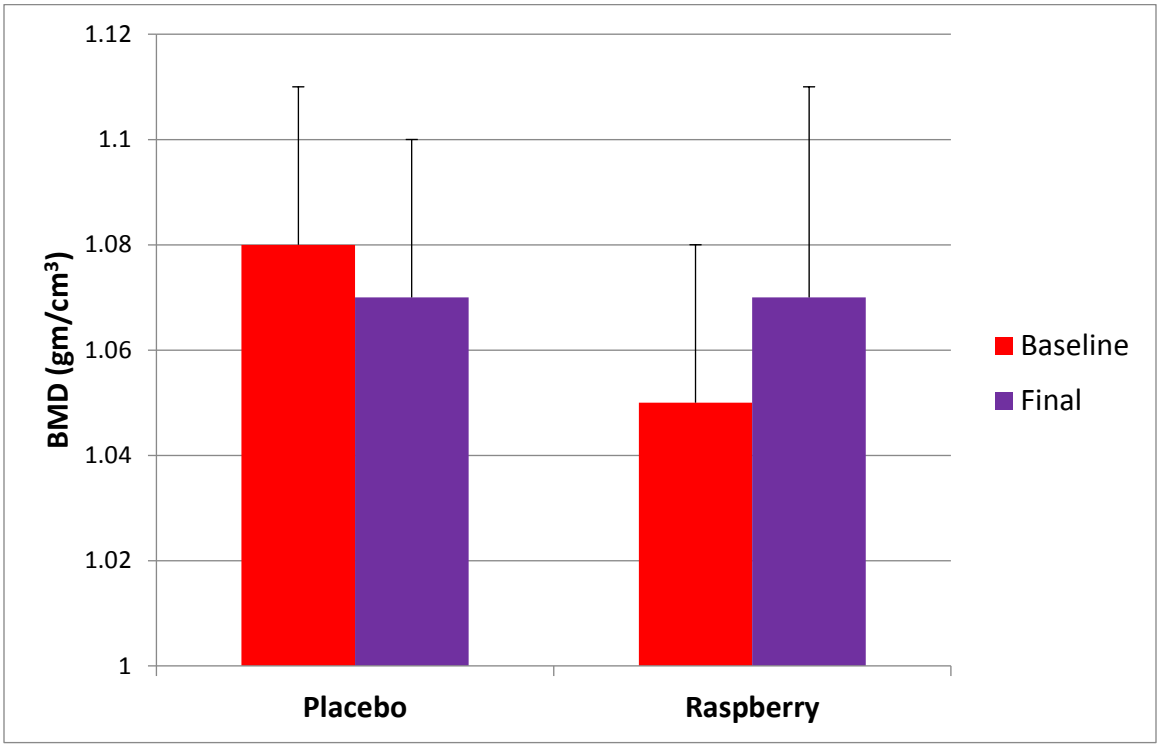
Mean ±SEM. N = 17 raspberry, n = 20 placebo. Asterisk denotes significant difference ($p < .05$) from baseline



Mean ±SEM. N= 17 for raspberry, n=20 placebo. Asterisk denotes significant difference (p≤.05) from baseline.

Figure 1.1

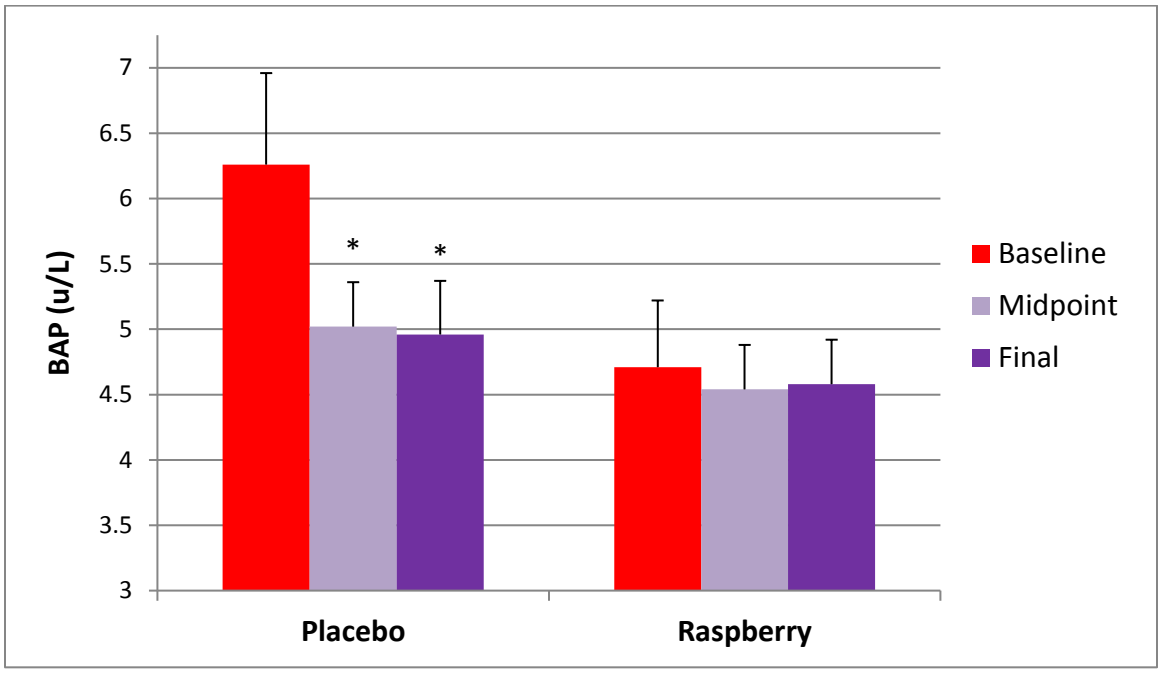
Effect of Placebo versus Raspberry on L4 Bone Mineral Density (BMD)



Mean ±SEM. N = 17 for raspberry, n = 20 placebo. Asterisk denotes significant difference (p<.05) from baseline

Figure 1.2

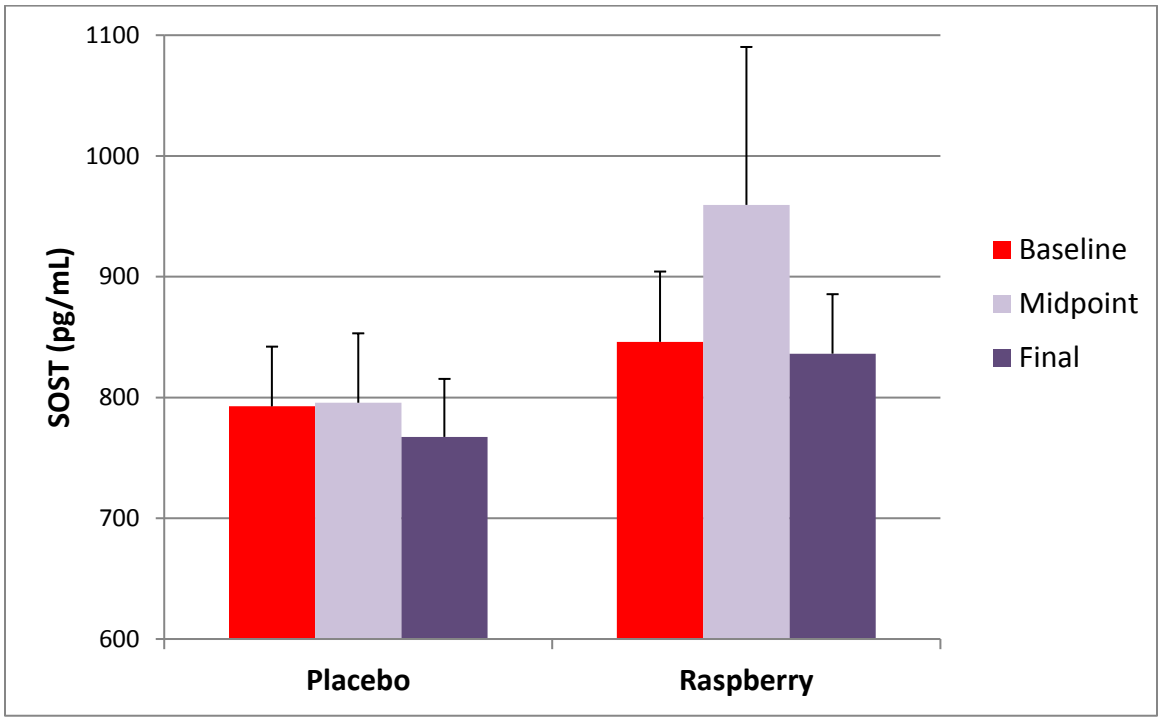
Effect of Placebo versus Raspberry on L1-L4 Bone Mineral Density (BMD)



Mean ±SEM. N = 30 for raspberry, n = 27 placebo. Asterisk denotes significant difference ($p<.05$) from baseline.

Figure 1.3

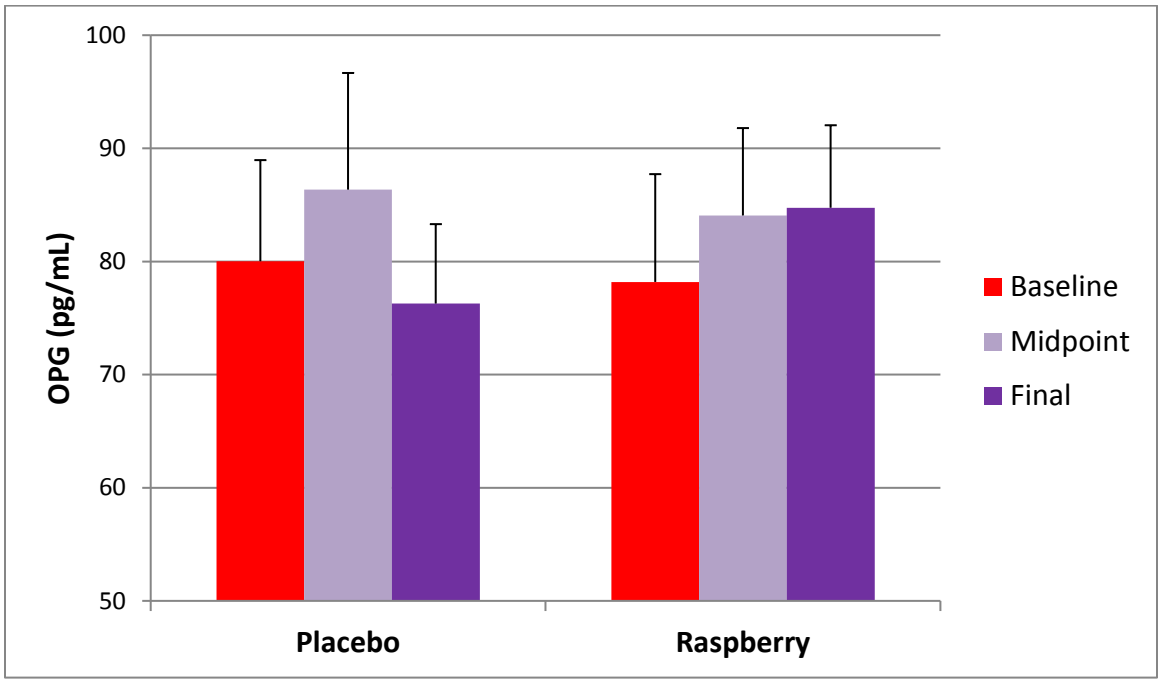
Effect of Placebo versus Raspberry on Plasma Bone Alkaline Phosphatase (BAP) Levels



Mean ±SEM. N = 30 for raspberry, n = 27 placebo. Asterisk denotes significant difference ($p<.05$) from baseline.

Figure 1.4

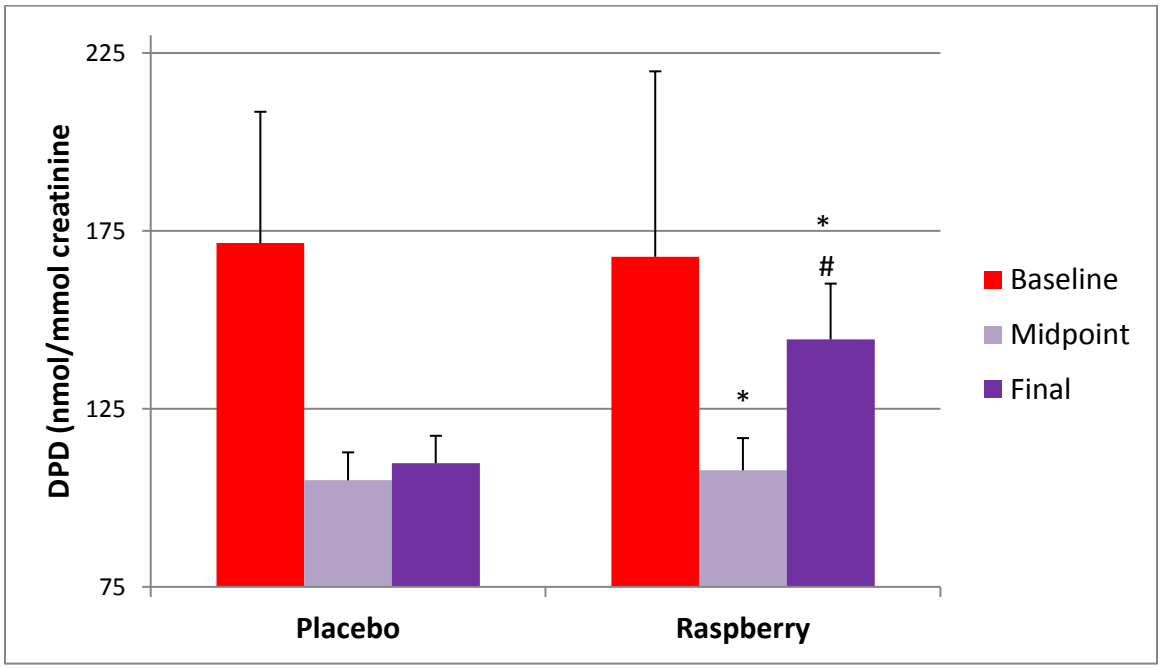
Effect of Placebo versus Raspberry on Plasma Sclerostin (SOST) Levels



Mean ±SEM. N = 30 for raspberry, n = 27 placebo. Asterisk denotes significant difference ($p<.05$) from baseline.

Figure 1.5

Effect of Placebo versus Raspberry on Plasma Osteoprotegerin (OPG) Levels



Mean ±SEM. N = 30 for raspberry, n = 27 placebo. Asterisk denotes significant difference (p<.05) from baseline, pound sign indicates significant difference (p < .05) from midpoint.

Figure 1.6
Effect of Placebo versus Raspberry on Plasma Deoxypyridinoline (DPD) Levels

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APPENDIX A

Participation Recruitment Flyer

Need Research Volunteers

Are you a Postmenopausal Woman

- Are you between 45 – 70 years old
- Do you feel that your bones are getting weak
- Do you experience pain in your back
- Are you otherwise healthy and mobile
- Would you be willing to participate in a study where you may be asked to consume red raspberry juice daily for 6 months

If you have answered **YES** to all of the above, then you may be eligible to participate in a 6 month research study to look at the beneficial effect of red raspberry on bone density and bone status.

Criteria include meeting the requirements listed above and willing to consume either red raspberry juice or a juice without red raspberry for a period of 6 months. There will be one blood draw at the start, midpoint, and at the end of the study. You will also provide urine specimen at the start, midpoint, and at the end of the study. Bone density of your spine and whole body will be measured at the start and the end of the study. The total time you need to spend for the study is 3 hours and 45 minutes over 3 months involving 3 visits.

Benefits include: awareness of bone status and bone health, measurement of bone density and blood measures associated with bone status. Upon completion, you will receive a compensation of $100 for your time in partial payments of $50 at the midpoint and $50 at the final follow-up visits.

If interested, please email or call for more information:
Dr. Shanil Juma, Department of Nutrition and Food Sciences sjuma@twu.edu; 940-898-2704
There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

APPENDIX B

Screening Questionnaire

Screening Questionnaire

ID:	Sex:	Age:
Telephone(s):	e-mail:	
Do you smoke?: ____ Yes _____ No	Cigarettes per day _____	
Medical condition you are taking medicine for:		
Hypertension ___ High cholesterol ___ Kidney disease ____ Lung disease ____ Diabetes ___ Heart disease ____ Liver disease ___ Thyroid condition ____ Bone Condition _____		
List any medications, drugs, prescription drugs, over the counter drugs, vitamins or food Supplements you are taking: List amount (mg) and times taken (daily, weekly etc.)		
Are you on a special diet? __No __weight loss __Medical condition __ Vegetarian		
___ Low salt ____ Low cholesterol ____ Weight gain		
Do you have any food allergies? ___ No ___Yes (list them)		
Here is the list of items (drugs/foods) you, as the participant, will be exposed to during the study: Raspberry Juice or Placebo Juice without Raspberry		

APPENDIX C

Informed Consent and IRB Approval

Appendix C

Texas Woman's University
Consent to Participate in Research

Study Title: Bone Protective Effect of Whole Red Raspberries in Postmenopausal Women with Osteopenia

Investigators:	Shanil Juma, PhD	940-898-2704	sjuma@twu.edu
	Nancy DiMarco	940-898-2785	ndimarco@twu.edu
	Parakat Vijayagopal, PhD	940-898-2709	pvijayagopal@twu.edu

Explanation and Purpose of Research

We are asking you to participate in a research study at Texas Woman's University. The purpose of the study is to find out if consumption of red raspberry juice (12 ounces daily) for 6 months will improve bone status in postmenopausal women who have mild-to-moderate bone loss. We will ask the following questions:

a) Will consuming red raspberry juice for 6 months improve bone health?
b) Will consuming red raspberry juice influence serum and urinary markers of bone status?

Research Procedures

For this study, the baseline visit will first involve obtaining consent for your participation in this study. At this visit, we will do a bone density measurement of your spine, hip (femoral neck), and whole body to determine if you have mild-to-moderate bone loss and qualify for the study. This will be done by a trained and certified technician. Based on the bone density assessment using the dual x-ray absorptiometry instrument known as DEXA, if you qualify and agree to participate we will proceed with additional data analysis and data collection associated with the study. As part of the consent, you agree that you will not initiate any new bone therapies during the duration of the treatment period. If you do decide to initiate a new bone therapy, please contact the principal investigator to determine if you still qualify to continue participating in this study.

During the baseline visit you will be asked to come fasted (not to eat any food overnight or at least 10 hours). A phlebotomist (person taking the blood) will draw 3 table spoons of your blood from one of the veins of your arms. We will then provide you with a snack and drink (cookies, crackers, and orange juice). This will be followed with a spot urine collection. A sterile specimen cup will be provided to collect a small urine specimen after the first morning void. A trained female personnel will take your height and weight measurements. Filtered water and a light snack will be available for you at the study site. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week. At the end of the baseline visit, you will be randomly assigned to a treatment based on chance, like a flip of a coin. Neither you nor the researcher chooses your assigned treatment group. You will have an equal chance of being in either group. You will be provided a 90 day supply of either the study treatment (red raspberry juice) or a control (comparative placebo juice without red raspberry). At the 90 day visit (midpoint), you will again be asked not to eat any food overnight (10 hours). A trained female personnel will take your height and weight

Participant Initials _____

measurements. A phlebotomist (person taking the blood) will draw 3 table spoons of your blood from one of the veins of your arms. We will then provide you with a snack and drink (cookies, crackers, and orange juice). A spot urine specimen will be obtained in a sterile specimen cup. Filtered water and a light snack will be available for you at the study site. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week. (You will be provided a 90 day supply of either the study treatment (red raspberry juice) or a control (comparative placebo juice without red raspberry). At the end of the study (6 months), you will be asked to come in for your last visit and not to eat any food overnight (10 hours) for a blood draw (3 tablespoons of blood will be obtained). You will be provided with snacks and filtered water. A spot urine specimen will be obtained. A trained and certified technician will measure bone density of your spine, hip (femoral neck), and whole body. A trained female personnel will measure height and weight. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week.

Time Commitment

The study treatment period is 6 months. The study volunteer time commitment includes initial screening questions (~15 minutes), consent form (15 minutes), bone density assessment (30 minutes each during baseline and three months), physical activity and food frequency questionnaire (30 minutes each during baseline, midpoint(90 days), and end of study(180 days)), anthropometrics-height and weight (5 minutes each during baseline, midpoint (90 days), and 180 days), and blood draw and urine collection (10 minutes each at baseline, midpoint (90 days) and final). Total time commitment for each participant is approximately 3 hours and 45 minutes.

Potential Risks

A potential risk to you as a participant in this study is release of confidential information. Confidentiality will be protected to the extent that is allowed by law. To protect confidentiality, you will be given a code number which will be used in all records. Only Dr. Juma will know your identity. All records will be stored in a locked filing cabinet in Dr. Juma's office. The records will be shredded within 5 years of completion of the study. Your name or any other identifying information will not be included in any publication that may result from the study. There is a potential risk of loss of confidentiality in email, downloading, and internet transactions.

A second possible risk is that you may not like the red raspberry juice or the comparative placebo juice without red raspberry. If you do not like the randomized treatment, there is no penalty for not consuming it. You are free to quit the study at any time. Red raspberry juice and the placebo juice without red raspberry is from a whole fruit source or equivalent to the sugar content of the whole fruit that has been custom prepared and packaged for our study. It has been previously used in other human clinical studies and is deemed safe for consumption and not harmful in any way.

Another possible risk to you as a participant in this study includes the discomfort of blood drawings. The phlebotomist will ask you about any concerns or previous issues with having

Participant Initials _____

a blood draw. If there are serious concerns or reactions to blood draw, we will ask you that you have the option to withdraw from participating in the study at any time. Blood draw may cause minor pain, bruising, discomfort, swelling, anxiety, infection or fainting. We will use a certified expert for blood draw. This will minimize the possibility of pain, bruising, discomfort, swelling, infection, and anxiety. A light snack and water will be made available at the draw site to avoid fainting.

Study volunteers will receive time to relax before and after blood draw. They will be offered the opportunity to watch television to reduce anxiety. If a participant faints during the blood draw, investigators will assist in laying him/her down and making him/her comfortable and providing any medical assistance if necessary. We will carefully watch the person until she regains consciousness and will not make another attempt to draw the person's blood again that day. We will also ask you to drink a lot of water before the blood draw.

You may be allergic to the latex gloves the phlebotomist wears for blood draw. In that case, the phlebotomist will use a different type of gloves. You will receive time to relax before and after blood draw. A light snack and water will be available to you. This will reduce the possibility of your fainting. If you faint during the blood draw, we will lay you down and make you comfortable. We will carefully watch you until you regain consciousness and will not make another attempt to draw your blood again that day.

Other possible risks to you are loss of time, fatigue, allergic reaction, and infection. You can watch videos or relax while you are waiting. Before we select you for the study, we will ask whether you are allergic to the food we use in the study. If you are allergic, we will not select you for the study. The phlebotomist will clean your arm with alcohol before taking blood and she will use a new needle. This will minimize the possibility of infection.

There is potential risk associated with assessment of bone density using the dual x-ray absorptiometry instrument known as DEXA. This scan includes exposure to small amount of radiation to get an image of the participant's body. All of us are exposed to small amounts of environmental radiation which is unavoidable. A single DEXA body scan is approximately 4 days worth of unavoidable radiation exposure. As a participant you are made aware of this exposure in the informed consent and verbally at the time before the scan. You must not be pregnant, lactating, or planning to become pregnant during the duration of this study. You may decide not to have the DEXA can and withdraw from the study at any time

In addition to the risks above, you may experience anxiety or embarrassment related to height, weight and body composition determination. In order to minimize this risk, you will be assured of complete confidentiality before taking these measurements. All measurements will be taken only by an experienced and trained female study personnel in a private room. The bone density measurement will be conducted by a trained and certified technician. Anthropometrics (height and body weight) measurements will be conducted bya trained female personnel. Blood draw will be done by a trained female phlebotomist.

Participant Initials _____

The study treatment consists of red raspberry juice contains whole red raspberries and the comparative placebo juice contains sugar equivalent to the red raspberry treatment without red raspberries. If participants are allergic to red raspberry or sugar she may consider not participating in the study. If any participant becomes allergic to either of the juices used in the study, she can withdraw from the study at any time.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation Benefits

Your participation in this research study is completely voluntary, and you may discontinue your participation in the study at any time without penalty. As a participant in the study, you will receive either the treatment or placebo juice for 6 months. You will also receive a cash incentive of $100, of which $50 will be paid at midpoint (90 days) and the remaining $50 after you complete the study. In addition, at completion of the study a summary of results as well as the results of your blood and urine analysis will be mailed to you upon request.

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you may ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

_____ _____

Signature of Participant Date

Participant Initials _____

This page will be detached and filled separately.

* If you would like to receive a summary of the results of this study, please provide an address to which this summary should be sent:

_____
_____
_____

Participant Initials _____

APPENDIX D

Protocol Approval Letter

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378 email: IRB@twu.edu
http://www.twu.edu/irb.html

DATE: February 29, 2016

TO: Dr. Shanil Juma
Nutrition & Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Extension for Bone Protective Effect of Whole Red Raspberries in Postmenopausal Women with Osteopenia (Protocol #: 17638)

The request for an extension of your IRB approval for the above referenced study has been reviewed by the TWU IRB (operating under FWA00000178) and appears to meet our requirements for the protection of individuals' rights.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. If subject recruitment is on-going, a copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

This extension is valid one year from March 7, 2016. Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any unanticipated incidents. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB. cc.

Dr. Shane Broughton, Nutrition & Food Sciences

APPENDIX E

Food Frequency Questionnaire

Date_____

Subject ID_____

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 1/2 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**
Grapefruit (1/2)		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			Si		
		W	Rar Nev			
DAIRY FOODS						
Whole milk (1 cup, 8 oz)						
2% milk (1cup, 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)						
FRUITS, FRUIT JUICES						
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)						
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)						
VEGETABLES, *VEGETABLE JUICE*						
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 (½cup)						
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**
EGGS, MEAT, ETC.						
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	**How Often**			**Size**		
(Medium Serving)	**Day**	**Week**	**Rarely/ Never**	**S**	**M**	**L**
BREADS, CEREALS, STARCHES						
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 (1cup)						
White rice (1cup)						
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	**How Often**			**Size**		

(Medium Serving)	Day	Week	Rarely/ Never	S	M	L
BEVERAGES						
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)						
SWEETS, BAKED GOODS, MISC.						
Chocolate (1 small bar or 1 oz)						
Candy bar (1 small bar)						
Candy without chocolate (1 oz)						
Cookies, home baked (2)						
Cookies, ready made (2)						
Brownies (2)						
Doughnuts (2)						
Cake, home baked (1 slice)						
Cake, ready made (1 slice)						
Sweet roll, coffee cake, or other pastry ready made (1 serving)						
Sweet roll, coffee cake, or other pastry home baked (1 serving)						
Pie, homemade (1 slice)						
Pie, ready made (1 slice)						
Jam, jelly, preserves, syrup, or Honey (1 Tbsp)						
Peanut butter (1 Tbsp)						
Popcorn (1 cup)						
Popcorn, air popped (1 cup)						

Type of Food	**How Often**			**Size**		
(Medium Serving)	**Day**	**Week**	**Rarely/**	**S**	**M**	**L**

			Never			
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

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.