THE EFFECT OF WHOLE RED RASPBERRY JUICE ON BODY COMPOSITION, PHYSICAL ACTIVITY, AND SERUM INFLAMMATION BIOMARKERS IN POSTMENOPAUSAL OSTEOPENIC WOMEN

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ABSTRACT

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The purpose of this study was to examine the effect of red raspberry juice on body composition, inflammatory biomarkers, and physical activity 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 70 = 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. Body composition was evaluated via DXA scans performed at baseline and final (180 days). Blood was obtained and self-reported physical activity questionnaires were completed at baseline, midpoint (90 days), and final visits. At the end of the 6-month study, there was a small reduction albeit not significant in visceral adipose tissue volume, visceral adipose tissue mass, android fat, gynoid fat, android to gynoid ratio, and total body fat observed in both raspberry and placebo groups. Serum leptin levels were higher in the placebo group compared to the raspberry group at the end of the study. There were no significant changes in recreational

activity patterns for walking, moderate, and vigorous physical activity for either the raspberry or the placebo group. The study findings suggest that inclusion of red raspberry in the diet of postmenopausal women may have a positive effect on body composition that may lead to reductions in inflammation and decrease disease risk.

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CHAPTER I

INTRODUCTION

Menopause is the natural cessation of menstruation usually occurring in women between the ages of 45 and 55. Women are considered postmenopausal 12 months after their last menstrual cycle. During menopause, women experience a decline in reproductive ovarian function resulting in a decrease in estrogen production (Moreno-Frias, & Malacara, 2015). Characteristics of menopause include low-grade chronic inflammation as well as vasomotor and emotional symptoms (Figureoa-Vega et al., 2015). In order to balance the physical and emotional states of a typical hormonal imbalance, women during menopause seek medicinal relief. Medical interventions include hormone therapy, selective serotonin reuptake inhibitors, serotonin norepinephrine reuptake inhibitors, gabapentin, and clonidine (Li et al., 2016). During this time, change in body composition, physical activity, and hormonal shifts may also impact disease risk (Figureoa-Vega et al., 2015; Sites et al., 2002). As a result of these hormonal shifts, there is higher risk for chronic disease, such as cardiovascular disease, osteoporosis, diabetes mellitus, and cancers (Figureoa-Vega et al., 2015; Sites et al., 2002).

In postmenopausal women, there is an increase in inflammatory cytokines that include tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) (Figureoa-Vega et al., 2015). This low-grade, chronic inflammation can lead to

dyslipidemia, insulin resistance, decreased high-density lipoprotein (HDL) concentrations, and weight gain (Figureoa-Vega et al., 2015; Nieto-Vazquez et al., 2008). When overexpressed, TNF-α is linked to insulin resistance by impairing insulin signaling at the level of the insulin receptor substrate (IRS) proteins (Nieto-Vazquez et al., 2008). Since sex hormones regulate TNF-α, menopausal women with decreased sex hormones have increased inflammation and risk of diabetes (Sites et al., 2002). TNF-α facilitates the production of IL-6 and up-regulates hepatocyte expression of C-reactive protein (CRP) (Foss de Oliveria, & Silva, 1993). During the release of IL-6, smooth muscle and endothelial cells also secrete chemokines to recruit more immune cells resulting in increased inflammation (Calabrese & Rose-John, 2014). In addition, IL-6 stimulates NF-κB ligand (RANKL) to promote osteoclast activity resulting in bone loss which may lead to osteoporosis and bone fractures (Tanaka & Kishimoto, 2014). IL-1β also promotes bone resorption as well as increases fever-inducing prostaglandin E_2 (PGE_2) and TNF-α (Gomes et al., 2016).

Estrogen deficiency in postmenopausal women may lead to health implications through changes in body composition, body fat distribution, bone health, and physical activity patterns (Sinaki & Offord, 1998). After menopause, there is generally an increase in fat mass and a decrease in lean body mass. The increase in fat mass is potentially due to a lack of estrogen and testosterone that normally participates in directing the distribution of accumulated body fat (Sinaki & Offord, 1998). Postmenopausal women also have a shift in body fat distribution from gynoid (fat in the lower abdomen and legs) to android (fat in the waist and upper body) (Gambacciani, Ciaponi, Cappagli, Simone,

Orlandi, & Genazzani, 2001). This excess of adipose tissue and change in body fat distribution leads to an increased risk of cardiovascular disease and diabetes (Czernichow et al 2002; Laffin, Majewski, Liao, & Bakris, 2016; Berk et al., 2016).

Postmenopausal women are more susceptible to obesity as aging is associated with increased leptin levels and leptin resistance, along with decreases in leptin receptors (Gulcelik, Halil, Ariogul, & Usman, 2013). Leptin is a hormone secreted by adipose tissue that interacts with neurotransmitters which act in various regions of the brain, including the hypothalamus, hindbrain, and mesolimbic neural circuits to control food intake and stimulate energy expenditure (Wierucka-Rybak, Wolak, Juszczack, Drobnick, & Bojanowska, 2016). It is known as the satiety hormone and communicates with the brain indicating that there is enough fat stored. Leptin resistance decreases the rate of leptin transporting across the blood-brain barrier into the brain, resulting in elevated serum leptin levels and obesity (Albala et al., 2016).

Dietary intake of fruits and vegetables are effective in reducing steady state low-grade inflammation present in postmenopausal women (Lau, Joseph, McDonald, & Kalt, 2009). Studies have shown that the consumption of polyphenolic rich diets are beneficial as they are inversely related to inflammation (Heinonen, Meyer, & Frankel, 1998; Mullen et al., 2002). In particular, in an antigen-induced arthritis rat model given an oral treatment of red raspberry extract, there was a significant reduction in inflammation. Ankles of rats in the model system were evaluated and showed inflammation was inhibited by 54% (Jean-Gilles et al., 2012). Fruit and vegetable intake have also been associated with reducing risk of major chronic diseases (Manach, Williamson, & Morand,

2005; Williamson, Barron, & Shimoi, 2005; Kroon, Clifford, & Crozier, 2004; Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Through high quantities of ellagitannins and anthocyanins, red raspberries promote antioxidant activity. They are also rich in potassium, manganese, iron, magnesium, copper, fiber, and vitamins A, B, C, E, and K. Research has shown that regular raspberry consumption may have a positive impact in postmenopausal body weight and chronic disease prevention (Heinonen et al., 1998; Stoner, Wang, & Casto, 2008).

Studies with red raspberry have been shown to decrease adipogenesis by inhibiting transcription factors while leading to reductions in weight gain by altering lipid metabolism (Burton-Freeman, Sandhu, & Edirisinghe, 2016; Jung et al., 2016; Tsai et al., 2017). Treatment with red raspberry reduced serum triglycerides and total cholesterol (Jung et al., 2016). Animal studies have found raspberry ketones resulted in prevention of increased body weight and visceral adipose tissue (Morimoto et al., 2005; Rios-Hoyo & Gutierrez-Salmean, 2016). However, there have been no studies with raspberries investigating its effect on body composition and inflammation in postmenopausal osteopenic women. This study examined the effect of whole red raspberries on body composition, fat distribution, physical activity, and inflammatory biomarkers in postmenopausal osteopenic women.

Hypothesis and Specific Aims

Hypothesis

The daily inclusion of red raspberry juice concentrate will have favorable effects on body composition, physical activity, and biomarkers of inflammation in

postmenopausal women who are mild to moderately osteopenic and not on hormone or other bone therapies.

Specific Aims

Aim 1. To evaluate whether daily intake of 2 ounces of whole red raspberry juice concentrate, in comparison to placebo juice, will have favorable effects on body composition (reduction in fat mass and improved body fat distribution) in postmenopausal women with osteopenia.

Aim 2. To determine whether daily intake of 2 ounces of whole red raspberry juice concentrate, in comparison to placebo juice, will improve physical activity and activity patterns associated with daily living in postmenopausal women with osteopenia.

Aim 3. To determine whether daily intake of 2 ounces of whole red raspberry juice concentrate, in comparison to placebo juice, will have favorable effects on biomarkers of inflammation in postmenopausal women with osteopenia.

CHAPTER II

REVIEW OF LITERATURE

Menopause

Menopause is the natural cessation of menstruation, typically occurring in women ages 45 to 55. During menopause, women experience a decline in reproductive ovarian function, resulting in decreased estrogen production. Characteristics of menopause include low-grade chronic inflammation as well as vasomotor and emotional symptoms (Figureoa-Vega et al., 2015). Women are considered postmenopausal 12 months after their last menstrual cycle. During this time, changes in body composition, physical activity, and hormonal shifts may negatively impact disease risk (Figureoa-Vega et al., 2015; Sites et al., 2002).

A estrogen decline in postmenopausal women contributes to possible health implications including changes in body composition, body fat distribution, and physical activity patterns (Figureoa-Vega et al., 2015). As a result of the hormonal shifts, there is a higher risk for chronic diseases, such as cardiovascular disease, osteoporosis, diabetes mellitus, and cancer (Figureoa-Vega et al., 2015). Overall, higher incidences of chronic disease contribute to an increased economic burden.

Postmenopausal Disease Risk

In the United States, cardiovascular disease is the leading cause of death in women (Roger et al., 2012). More than 42 million American women are currently living

with cardiovascular disease (Roger et al., 2012). In 2011, the annual direct and indirect cost of cardiovascular disease in the United States was an estimated $320.1 billion (Mozaffarian et al., 2014).

Postmenopausal women have a higher incidence of cardiovascular disease risk associated with atherosclerosis in comparison to premenopausal women (Cakir et al., 2016). Menopause is a risk factor for cardiovascular disease as the decline in estrogen can have a negative impact on cardiovascular function and metabolism (Rosano, Vitale, Marazzi, & Volterrani, 2007). Estrogen plays an important role in maintaining a healthy and functional endothelium (Figureoa-Vega et al., 2015). Postmenopausal women are found to have higher prevalences of endothelial dysfunction leading to cardiovascular disease risk (Figureoa-Vega et al., 2015). This occurs through inflammatory cell infiltration, which causes the thickening of arterial vessel walls and progression of stenosis, atherosclerosis, and occlusion. Women with five or more years since their last menses are at greater risk of endothelial dysfunction and inflammation due to a longer period of estrogen deprivation (Figureoa-Vega et al., 2015). This hormonal shift also increases central abdominal obesity, leading to other risk factors such as high blood pressure and type 2 diabetes in postmenopausal women (Laffin et al., 2016). The prevalence of hypertension doubles amongst women in their first 10 years following menopause (Rosano et al., 2007). Type 2 diabetes develops from insulin resistance and increases the risk of arterial thrombosis through platelet dysfunction (Samos et al., 2016). These risk factors are strongly associated with a decline in estrogen production in postmenopausal women.

Diabetes is a metabolic disease of insulin resistance or reduced insulin production creating elevated blood glucose levels. It affects 29 million Americans with an estimated total annual economic burden of $245 billion as reported in 2012 (ADA, 2013; CDC, 2015). Globally, the issue of obesity has also added to the incidence of type 2 diabetes, where an estimated 82-87% of type 2 diabetes patients are overweight or obese (Berk et al., 2016). Weight loss is ideal for obese patients to help improve glycemic control, lipid profiles, and blood pressure. Postmenopausal women may experience insulin resistance associated with aging, weight gain, and decreased physical activity, causing increases in blood pressure largely due to sodium and fluid retention (Rosano et al, 2007).

The second leading cause of death in women is cancer, particularly lung and breast cancer. One in eight women in the United States will be diagnosed with breast cancer in her lifetime (Matthews & Thompson, 2016). Breast cancer is diagnosed every two minutes and a woman dies from breast cancer every eleven minutes (Matthews, 2016). In 2012, 1.7 million new cases of breast cancer were diagnosed and responsible for nearly 700,000 deaths worldwide (Matthews & Thompson, 2016). In 2012, the Agency for Healthcare Research and Quality estimated that the direct medical costs associated with cancer to be $87.5 billion in the United States (National Cancer Institute, 2015).

Postmenopausal women are at risk for cancer due to advancing age, especially those who began menopause at a later age. According to the androgen excess theory, women exposed to more estrogen by beginning menopause at a later age have a higher risk of endometrial and breast cancer (Lobo et al., 2014; Secreto et al., 2016). There are

strong associations with high-circulating androgens promoting breast cancer development (Lobo et al., 2014; Secreto et al., 2016). The androgen excess theory reports that hormone-dependent breast cancer patients frequently have increased androgen levels. This helps to predict the clinical outcome of ovariectomized patients with high levels of pre-surgical urinary androgen excretion. These patients are more likely to have remission of metastases as androgen levels revert to pre-surgery elevated levels when compared with normal pre-surgical urinary androgen levels (Secreto & Zumoff, 2012).

Cancer development has also been linked to excess adiposity. Since menopause increases body weight and abdominal fat, postmenopausal women have a higher risk of cancer (Matthews & Thompson, 2016). Excess adipose tissue results in an over expression of adipokines with pro-angiogenic effects which facilitate tumors growth and spread (Matos et al., 2016). An imbalance within the immune system also results in an increase in inflammatory markers followed by a rise in activity and number of T and B lymphocytes, neutrophils, and mast cells, while insulin sensitizing eosinophils are decreased (Matos et al., 2016). The exact cause of cancer is unknown, but chronic inflammation is a major risk factor.

In 2014, the National Osteoporosis Foundation estimated osteoporosis and low bone mass affects 54 million United States adults age 50 and older, 80% of which are women (Bilek et al., 2016; NOF, 2014). Osteopenia, a mild-to-moderate degree of bone loss, may lead to osteoporosis which is a risk factor for fractures. Over 34 million postmenopausal American women have osteopenia (Bilek et al., 2016). The medical cost

for osteoporosis and fractures in 2008 was estimated at $22 billion (Blume & Curtis, 2010).

Osteoporosis causes bone to become more fragile and increases the risk for bone fracture. Osteoporotic fractures occur most commonly at the hip, lower forearm, and vertebrae (Crosignani, Policlinico, & Fanti, 2010). Overall, through their lifetime, women have a 40-50% risk for fractures, whereas men have a 13-22% risk (Crosignani et al., 2010). The risk for hip fractures increases significantly in women. Postmenopausal women are twice as likely to have hip fractures when compared to premenopausal women (Banks et al., 2009). Osteoporosis is defined as having a bone mineral density (BMD) that is 2.5 standard deviations below the mean of peak bone mass of healthy women, which is defined as a T-score of -2.5 or lower (Kwon, Park, Kim, Moon, & Kang, 2016). After menopause, increased activity of osteoclasts creates an imbalance in relation to osteoblasts, leading to a net decrease in bone mineral density (BMD) and increased risk of osteoporosis (Khatkhatay, Daswani, Gavali, Desai, M., & Patil, 2016). A reduction in estradiol results in lower skeletal health protection, as estrogen promotes osteoclast apoptosis (Kameda et al., 1997).

Metabolic Changes Associated with Menopause

Body Composition

As women approach menopause, a shift in body mass and body composition occurs during the transition period known as perimenopause (Harlow & Paramsothy, 2012). After menopause, there is generally an increase in fat mass and a decrease in lean body mass. Significant increases in body weight of 5 kg over 36 months have been

observed in early postmenopausal women (Gambacciani et al., 2001). An increase in fat mass is due possibly to a lack of estrogen and testosterone that normally helps promote the distribution of accumulated body fat and may also be connected to an increase in chronic low-grade inflammation (Sinaki & Offord, 1998). Postmenopausal women also have a shift in body fat distribution from gynoid (fat in the lower abdomen and legs) to android (fat in the waist and upper body) (Gambacciani et al., 2001). This excess of adipose tissue and change in body fat distribution lead to the conditions previously discussed, such as cardiovascular disease and diabetes (Czernichow et al., 2002; Laffin, Berk et al., 2016).

Postmenopausal women are more susceptible to obesity as aging is associated with increased leptin levels and leptin resistance, along with decreases in leptin receptors (Gulcelik et al., 2013). Leptin is a hormone secreted by adipose tissue that interacts with neurotransmitters which act in various regions of the brain, including the hypothalamus, hindbrain, and mesolimbic neural circuits to control food intake and stimulate energy expenditure (Wierucka-Rybak et al., 2016). Leptin resistance decreases the rate of leptin transport across the blood-brain barrier into the brain, resulting in elevated serum leptin levels and obesity (Albala et al., 2016).

Adipose tissue regulates metabolic homeostasis by secreting hormones, cytokines, growth factors, and enzymes. Visceral adipose tissue, also known as intra-abdominal fat, is found surrounding abdominal organs, whereas subcutaneous adipose tissue is directly underneath the skin. Insulin resistance and lipid dystrophy have been shown to increase in males and females with a higher ratio of visceral adipose tissue compared to

subcutaneous adipose tissue, even when age and gender were equally matched (Matsuzawa, Nakamura, Tokunaga, & Simomura, 1994). Visceral fat accumulation, induced by high sucrose intake and a deficiency of sex hormones, is also more active in synthesizing and releasing pro-inflammatory cytokines (Duran, Kosus, Kosus, & Turhan 2016; Gulcelik et al, 2013).

Inflammation

In postmenopausal women, there is an increase in inflammatory markers that TNF-α, IL-6, and IL-1β (Figureoa-Vega et al., 2015) This low-grade, chronic inflammation can lead to weight gain, dyslipidemia, insulin resistance, and decreased high-density lipoprotein (HDL) concentrations (Figureoa-Vega et al., 2015; Nieto-Vazquez et al., 2008). Inflammation is a natural response to obesity, causing obese postmenopausal women to have greater risk for various disease conditions (Matthews & Thompson, 2016).

Visceral and subcutaneous adipose tissues produce the pro-inflammatory cytokines IL-6 and TNF-α that stimulate lipolysis (Sites et al., 2002). This results in increased free fatty acids shuttle to the liver which induces hepatic triglyceride synthesis resulting in hypertriglyceridemia. TNF-α, produced mainly by macrophages, regulates cell differentiation, apoptosis, proliferation, lipid metabolism, and coagulation (Wajant, Pfizenmaier, & Scheurich 2003). When overexpressed, TNF-α is linked to insulin resistance by impairing insulin signaling at the level of the IRS proteins (Nieto-Vazquez et al., 2008). Aging and menopause are associated with increased inflammatory mediators and insulin resistance due in part to the shift in production of sex hormones (Sites et al.,

2002). Estradiol inhibits TNF-α activation and basal activity, while progesterone inhibits TNF-α mRNA and protein expression. Since sex hormones regulate TNF-α, menopausal women with decreased sex hormones have increased inflammation and risk of diabetes (Sites et al., 2002).

TNF-α also facilitates the production of IL-6 and up-regulates hepatocyte expression of C-reactive protein (CRP) (Foss et al., 1993). IL-6 indirectly stimulates hepatocytes to secrete acute-phase proteins, such as CRP, fibrinogen, and hepcidin, but decreases transferrin, albumin, and fibronectin (Calabrese & Rose-John, 2014; Tanaka & Kishimoto, 2014). IL-6 is produced by various cells, including adipocytes, monocytes, bone marrow stromal cells, T-cells, B-cells, smooth muscle cells, and endothelial cells (Mihara, Hashizume, Yoshida, Suzuki, & Shiina, 2012). During the release of IL-6, smooth muscle and endothelial cells also secrete chemokines to gather more immune cells resulting in increased inflammation (Calabrese & Rose-John, 2014). In addition, IL-6 stimulates NF-κB ligand (RANKL) to promote osteoclast activity and possibly lead to osteoporosis (Tanaka & Kishimoto, 2014).

Both pro-inflammatory cytokines, IL-1β and IL-6 stimulate B cell proliferation resulting in immunoglobulin production to neutralize pathogens (Schindler et al., 1990). Excess production of IL-1β can be found in people with fever, anemia, and hypotension (Church, Cook, & Mcdermott, 2008). Produced by macrophages and monocytes, IL-1β promotes bone resorption and increases fever-inducing PGE_2 and TNF-α (Gomes et al., 2016).

Oxidative Stress

Inflammatory markers stimulate neutrophils and macrophages that naturally produce free radicals, such as hydrogen peroxide, superoxide, and hydroxyl radical, to combat bacteria during oxidative bursts (Ozsurekci & Aykac, 2016). Free radicals are molecules with one or more unpaired electrons in their outer shell. Non-free radical oxygenated molecules and free radicals fall under the term reactive oxygen species (ROS). Exogenous sources of ROS include radiation, car exhaust, cigarette smoke, ozone, etc. (Irshad & Chaudhuri, 2002). Endogenous sources include products produced through beta-oxidation of fat in the peroxisome, auto-oxidation of amino acids, catecholamines, ischaemia reperfusion injury, reduction of oxygen to superoxide radical anions in the mitochondrial electron transport chain, and oxidative bursts by phagocytes (Irshad & Chaudhuri, 2002; Pisoschi & Pop, 2015). Oxidative stress occurs as a result of the production of ROS that cause cellular damage leading to ATP depletion, preventing controlled apoptotic death, or damage to DNA (Mullen et al., 2002). An imbalance favoring oxidative damage can lead to critical failure of biological systems, leading to diseases such as autoimmune disorders, malignancies, diabetes, renal diseases, atherosclerosis, and neurodegeneration (Ozsurekci & Aykac, 2016).

Postmenopausal women have increased oxidative stress and weakened antioxidant defense due to inflammation, advancing age, increased body mass index particularly due to increased body fat, and decreased vasoprotective nitric oxide associated with oxidative stress (Cakir et al., 2016). Antioxidant defense can be upregulated with regular exercise as a homeostatic adaptation. A study with athletes in a physical training program was

compared to sedentary controls and showed regular exercise increased antioxidant properties by elevating uric acid, ascorbic acid, and α-tocopherol (Brites et al., 1999).

Physical Activity and Postmenopausal Health

The 2008 Physical Activity Guidelines for Americans recommend aerobic exercise of moderate intensity for at least 150 minutes each week or 75 minutes each week of vigorous-intensity physical activity (U.S. Department of Health & Human Services [HSS], 2008). Resistance exercise for all of the major muscles groups, such as those of the legs, hips, abdomen, and back, in older adults are recommended for at least 2 days a week (HHS, 2008). Weight-bearing endurance activities and resistance exercises are especially important for postmenopausal women as they elicit a positive response on the skeletal system especially at the lumbar spine and femoral neck. Increased age leads to decreased physical performance, shown through a decline in tests for walking speed, functional reach, and grip strength (Aoyagi et al., 2000). Better physical performance correlated with higher bone mineral density. The mechanical forces from physical activity are a key contributor to better overall musculoskeletal health (Aoyagi et al., 2000; Morillas-Ruiz & Hernandez-Sanchez, 2015; Sternfeld & Dugan, 2011).

Postmenopausal women tend to become less active and sedentary as physical activity patterns tend to decline beginning in their early twenties (Sternfeld & Dugan, 2011; Stewart, 2005). Within a 3-year study period, middle-aged women gained an average of 4.5 to 4.9 pounds and increased waist circumference (Grindler & Santoro, 2015). Young women who were not physically active were also found to be less likely to be active later in life. Goal setting and self-monitoring contribute to habitual physical

activity, as well as confidence and enjoyment in the activity (Pate, 1995). Active individuals have been found to have less changes in body weight and body composition (Sternfeld & Dugan, 2011). Also, the level of physical activity is inversely associated with weight gain and intra-abdominal fat content (Sternfeld & Dugan, 2011). Increased abdominal fat leads to higher production of pro-inflammatory products, preventable with physical activity and dietary intervention (Pate, 1995).

Postmenopausal Health and Weight Gain

The World Health Organization released a report that indicated a doubling of obesity incidence since 1980 that reached epidemic proportions in the late 1990's (Vivarelli et al., 2016). Men and women of all ages, educational levels, ethnic groups, and from developed and developing countries have experienced an increase in obesity prevalence worldwide (Samper-Ternent & Snih, 2012). There is a variation in prevalence as the United States has a much higher obesity rates for both men and women in comparison to other countries. For example, in 2004 the USA had obesity rates of 37.9% for women and 30.7% for men, while Europe reported obesity rates of 24.2% for women and 17.6% for men (Michaud, Soest, & Andreyeva, 2007). Prevalence within the United States also varies across ethnicities where African Americans have the highest obesity rates with Hispanics being the second highest (Flegal, 2010). In order to improve overall health, dietary guidelines recommend increased consumption of fruits and vegetables as sources of dietary fiber, phytochemicals, and essential nutrients. Berries in particular are beneficial for their high content of antioxidant phytochemical, such as flavonoids (Vivarelli et al., 2016).

A slight loss of weight can be beneficial as a 11-22lbs loss may lower blood pressure and body mass index by 1-3kg/m^2, resulting in a 2-13% decrease in cardiovascular disease risk (Czernichow et al., 2002). A weight reduction of more than 4.4lbs has been implicated to increase systolic blood pressure the least and decrease diastolic blood pressure the most (Czernichow et al., 2002). While losing weight is important, preventing weight gain is also crucial as a 1kg/m^2 increase in BMI may increase risk of hypertension by 12% (Huang et al., 1998). A meta-analysis showed that gaining 10lbs or more between the ages of 40 and 60 was shown to increase diabetes risk by 40-70% and cancer risk by 24-59% (Bertoia et al., 2016). Diet in conjunction with physical activity is vital to reduce disease risk associated with menopause.

Diet and Postmenopausal Health

According to the USDA, Americans do not consume the recommended quantities and varieties of fruits and vegetables (Golmohamadi, Moller, Powers, & Nindo, 2013). The Dietary Guidelines for Americans 2015-2020 recommends women ages 31 to 50 consume 2.5 to 3 cups of vegetables and women ages 51 to 70 to consume 2 to 3 cups of vegetables daily. The intake of vegetables for American women 31 to 50 years of age is 1.6 cups and women 51 to 70 years of age is 1.8 cups daily. The recommended intake for fruits in women ages 31 to 70 is 1.5 to 2 cups, while the average actual intake for women 31 to 50 years of age is .9 cups and for the women ages 51 to 70 the average actual daily intake is 1.2 cups (U.S. Department of Health and Human Services & U.S. Department of Agriculture, 2015). The bioactive compounds within these foods are necessary, as they have shown to protect the body against various diseases (Golmohamadi, Moller, Powers,

& Nindo, 2013). An alternative to whole fruits is fruit juice made from whole fruits that provide a convenient source of nutrients. Americans are already consuming large quantities of juice in general, but most juices have high sugar content contributing to increased calorie consumption. Juice contains carbohydrates varying from 11g/100 mL (.44 kcal/mL) to more than 16g/100 mL (.64 kcal/mL) (Heyman & Abrams, 2017). In 2010, the United States juice retail market was valued at $16.2 billion in 2010 with Americans consuming 30.3L of juice per person (Golmohamadi et al., 2013).

Fruit and vegetable intake have been associated with reducing the risk of major chronic diseases, attributable to their anti-inflammatory and anti-oxidative actions (Kroon et al., 2004; Manach et al., 2004; Manach et al., 2005; Williamson et al., 2005). Fruits that have been most commonly researched include blueberries, blackcurrants, cranberries, blackberries, strawberries, cherries, and grapes. Berries in particular provide good sources of fiber, vitamin A, vitamin B9, vitamin C, vitamin E, vitamin K, carotenoids, calcium, phosphorus, iron, selenium, and polyphenols (Davicco, Wittrant, & Coxam, 2016). Fruits and vegetables that are associated with less weight gain are sources with high flavonoid content. Flavonoids have been shown to increase glucose uptake in muscle, decrease energy intake, and decrease glucose uptake in adipose tissue (Ashida et al., 2004). A study on anthocyanin found that this flavonoid subclass is associated with weight change through several possible mechanisms including inhibition of adipogenesis, decreased energy intake, decreasing fat absorption, increasing muscle glucose uptake, decreasing adipose tissue glucose uptake, and increasing energy expenditure (Bertoia et

al., 2016). The resulting weight loss was less than 1 pound per increased daily standard deviation, but each day had more than one standard deviation (Bertoia et al., 2016).

Berries also reduce obesity by decreasing lipogenesis, decreasing lipid absorption, inhibiting proinflammatory adipokine secretion, and reducing proliferation of preadipocytes (Kowalska & Olejnik, 2016). In addition, berry anthocyanins exhibit anti-inflammatory benefits through inhibition of the cyclooxygenase enzyme that produces inflammatory and vasoconstricting mediators, lipoxygenase which oxidizes polyunsaturated fatty acids, nitric oxide, and TNF-α production (Davicco et al., 2016; Figueira et al., 2016). Nitric oxide is a free radical increased during inflammation that activates nuclear factor-kB to induce osteoclast differentiation (Davicco et al., 2016).

Red Raspberry

As a healthy food choice, red raspberries are low in total calories with a 100g serving containing 52kcals, high in dietary fiber, and low in fructose (Golmohamadi et al., 2013). Raspberries have a Trolox Equivalent Antioxidant Capacity (TEAC) between 16-20 umol/g of fresh weight and between 200-300 mg/100g of dry weight for total anthocyanins. Trolox is a measure of antioxidant capacity similar to vitamin E used as a standard for comparison to the TEAC antioxidant capacity measurement (Beekwilder, Hall, & Vos, 2005; Kahkonen Hopia, & Heinonen, 2001).

Red raspberries are rich in potassium, manganese, iron, magnesium, copper, fiber, and vitamins A, B, C, E, and K, and differentiate themselves from other berries and fruits by having high amounts of ellagitannins and anthocyanins. Anthocyanins are a subclass of flavonoids, which is the largest subcategory of polyphenols (Pandey & Rizvi, 2009).

Polyphenols have antioxidant properties, increase HDL, improve endothelial function, and possess anti-platelet and anti-inflammatory effects (Manach et al., 2004; Pandey & Rizvi, 2009; Seeram, Momin, Bourquin, & Nair, 2001). Ellagitannins are a type of polyphenol under the tannin category that scavenge free radicals and protect against lipid and protein oxidation (Seeram et al., 2001). These tannins are precursors to ellagic acid, which have antiviral properties and protect against colon, lung, and esophageal cancers (Mullen et al., 2002). Ellagitannin and anthocyanin content vary among raspberry varieties depending on location and cultivating techniques. For example, greater pesticides use results in lower ellagitannin content as the plants have a reduced need to produce deterrents (Rao & Snyder, 2010).

Red Raspberry and Body Weight

Excess energy intake causes adipocyte hyperplasia, adipocyte hypertrophy, increased triglyceride levels in plasma and tissues, and tissue damage. Although the underlying mechanism is unclear, studies with red raspberry have shown to decrease adipogenesis by inhibiting CCAAT-enhancer-binding protein (C/EBPα) and peroxisome proliferator-activated receptor (PPARγ) (Jung et al., 2016; Tsai et al., 2017). C/EBPα and PPARγ are transcription factors in adipogenesis that accelerate lipogenesis and sustain lipid homeostasis. Raspberry has also shown to reduce weight gain by altering lipid metabolism (Burton-Freeman et al., 2016). Treatment with red raspberry led to reduced serum triglycerides, total cholesterol, body fat accumulation and weight gain (Jung et al., 2016).

In vitro studies using raspberry ketones resulted in increased fatty acid oxidation, increased secretion of adiponectin, and suppression of lipid accumulation in adipocytes (Park, 2010). Raspberry ketones are extracted from raspberries for use as flavoring by the food industry and are commonly used for research using *in vivo* and *in vitro* models. Studies have found that these ketones down regulate adipogenesis transcription factors and increase expression of genes that favor fatty acid oxidation (Morimoto et al., 2005; Park, 2010; Rios-Hoyo & Gutierrez-Salmean, 2016). In animal studies using rats on a high-fat diet fed raspberry ketones, there was a prevention of increased body weight and visceral adipose tissue (Morimoto , 2005; Rios-Hoyo, 2016). Although weight and hepatic triacylglycerol were initially increased by the high-fat diet, they eventually decreased with the addition of raspberry ketones. The ketones increased norepinephrine-induced lipolysis (Morimoto et al., 2005).

Red Raspberry and Inflammation and Oxidative Stress

Dietary intake of fruits and vegetables is effective in reducing the level of steady state low-grade inflammation present in postmenopausal women (Lau et al., 2009). In particular, studies have shown that the consumption of polyphenol rich diets is beneficial as they are inversely related to inflammation and oxidative stress (Heinonen et al., 1998; Mullen et al., 2002).

Flavonoids reduce the immune response and inflammation by inhibiting regulatory enzymes and pro-inflammatory mediators, such as angiotensin-converting enzyme, interleukin-1β, interleukin-6, TNF-α, and cyclooxygenase-2 (Mullen et al., 2002; Seeram et al., 2001). Antioxidant properties prevent inflammatory processes from

inducing oxidative stress and generating excess ROS (Mullen et al., 2002). In an *in vitro* study, antioxidant compounds associated with red raspberries were shown to inhibit the oxidation of low-density lipoproteins and liposomes (Heinonen et al., 1998). These compounds also neutralize the oxidative stress created by free radicals and help prevent disease by enhancing immune cell function (Ozsurekci & Aykac, 2016).

In an *in vivo* study, lyophilized raspberry incorporated into the diet at 2.5, 5, and 10% along with the colon carcinogen azoxymethane was fed to rats. In response to the diet, there was reduced oxidative DNA damage and tumor multiplicity (Stoner et al., 2008). Berry constituents influence signaling pathways through inhibition of cancer progression via decreases in cell proliferation and differentiation, increased apoptosis, and anti-angiogenesis (Stoner et al., 2008). Specifically, berries protect against oxidative DNA damage, inhibit DNA adduct formation, inhibit tumorigenesis, and enhance DNA repair (Stoner et al., 2008).

Role of Red Raspberry in Disease Conditions

Free radical damage and antioxidant defense biomarkers have been used for cardiovascular disease patients to decipher oxidative stress status, since both are closely related. Lipid peroxidation increases cardiovascular risk and low-density lipoprotein (LDL) oxidation in the oxidative modification hypothesis of atherosclerosis is said to initiate atherogenesis, which is the accumulation of cholesterol deposits in arterial macrophages (Cakir et al., 2016). The hindrance of blood flow induces thrombosis and low oxygen supply. Without oxygen, organs are not able to function properly, leading potentially to heart attack and stroke (Hardy & Cooper, 2009). The oxidative

modification hypothesis of atherosclerosis implicates oxidized LDL as having the highest potential pro-atherogenic activities with the potential to lead to atherosclerosis (Stocker & Keaney JF Jr., 2004). Oxidized LDL is chemotactic for T lymphocytes, monocytes, and macrophages, which stimulate arterial inflammation (Stocker & Keaney JF Jr., 2004). This increases to the importance of antioxidant protection of LDL and any associated oxidative events.

Red raspberry extract has shown to reduce inflammation and bone resorption. Since inflammation increases bone resorption and decreases bone formation, lessening inflammation would aid in reducing osteoporosis risk (Hardy & Cooper, 2009). In addition, chronic inflammatory diseases may reduce exercise frequency, leading to bone loss as a result of less mechanical stimulation of bone (Hardy & Cooper, 2009). Research has reported a significant reduction in inflammation and bone resorption occurrence in an antigen-induced arthritis rat model given an oral treatment of red raspberry extract of 120mg/Kg daily for 30 days after the injection of mycobacterium tuberculosis. Ankle evaluation of the rat model and showed inflammation was inhibited by 54% and bone resorption by 67% when compared with the non-treated diseased control. The rat models given the lower concentration oral treatment of 30mg/Kg red raspberry extract daily showed no sign of improvement (Jean-Gilles et al., 2012).

Another feature of red raspberries is their antiproliferative actions on cancer cells. Anthocyanins down-regulate the proinflammatory protein cyclooxygenase-II expression and activity while ellagic acid induces apoptosis (Rao et al., 2010). Anthocyanins also have been shown to inhibit malignant cell growth of colon and breast carcinoma cells by

inhibiting signaling cascades for cell proliferation. In general, plant phenols are anti-angiogenic, denying the vascular blood supply tumors require (Kassim et al., 2009).

The knowledge that flavonoids improve glucose control has been established, but the connection between diabetes and red raspberries is still a new area of research (Rao et al., 2010). Anthocyanins have been shown to induce insulin secretion, glucose uptake, and improve adipocyte function (Rao et al., 2010). Enzymes that are typically active during carbohydrate digestion are hindered by flavonoids, allowing for better blood sugar control (Zhao Iyer, Flores, Donhowe, & Kong, 2013). Anthocyanins inhibit α-amylases, while ellagitannins inhibit α-glucosidase (Rao et al., 2010.

Red raspberries are beneficial in preventing disease conditions by decreasing weight gain, serum trigylcerides, total cholesterol, and body fat. Red raspberries also increase HDLs and lead to anti-inflammatory effects through its rich elagitannin and anthocyanin content. These polyphenols could be advantageous for postmenopausal women as they experience changes in body composition, body fat, and physical activity. The purpose of this research study was to show the effect of whole red raspberries on body composition, fat distribution, physical activity, and inflammation in osteopenic postmenopausal women.

CHAPTER III

METHODOLOGY

Study Design and Subject Recruitment

A total of 57 postmenopausal (within 1 to 10 years of menopause) women, ranging in age from 45 to 70, with a mild to moderate degree of bone loss were recruited through private clinical practices and the local community of the Denton and Dallas Fort Worth Metroplex area. The double blind randomized placebo-controlled study placed subjects into one of two treatment groups ($n1 = 27$, $n2 = 30$) for a period of 6 months. Treatment Group A consumed 2 ounces of red raspberry juice concentrate daily. Treatment Group B served as the control group and consumed 2 ounces of a placebo juice concentrate equivalent to the red raspberry juice concentrate in appearance, energy, and fructose and dextrose content. The placebo and raspberry juice concentrates were reconstituted with 10 ounces of water immediately before consumption.

Inclusion/Exclusion Criteria

Participants were considered regardless of ethnicity or race. Participants were not on hormone or other bone therapy treatment for at least 6 months prior to the initiation of the study. Participants had lumbar spine or femoral neck bone density T-scores within -.5 to -2.5. Individuals excluded from participation included those on either endocrine drugs, neuroactive drugs, drugs influencing bone and calcium metabolism, or pharmacological doses of calcitonin, bisphosphonates, sodium fluoride, parathyroid hormone, or other

anabolic steroids. Subjects were excluded if they were heavy smokers, smoking more than 20 cigarettes daily.

Body Composition Assessment

Participants had body composition assessments performed by a certified technician using a dual-energy X-ray absorptiometry (DEXA)/quantitative digital radiography at baseline and at the end of the study. The DEXA was used to determined body mass index classification, android:gynoid fat ratio, total body fat, and lean body mass. Anthropometrics (height and weight) and blood pressure were also conducted at baseline, midpoint, and final visits. Blood pressure was assessed while participants were in a sitting position with a wrist blood pressure monitor held close to their heart.

Physical Activity/Treatment Compliance

Physical activity questionnaires were completed at baseline, 3 month, and the final study visit using a long form questionnaire known as the International Physical Activity Questionnaire (IPAC). The questionnaire assessed leisure, occupational, and home activities in the previous seven days. Activities were classified as moderate, hard, and very hard, and determined consistency, usual activity level, and deviations from baseline. Treatment compliance was tracked using calendars for daily consumption of raspberry or placebo juice. Calendars were given to participants at the baseline and 3 month visits and are collected during the next study visit. Participants were also randomly contacted to make sure they were complying with the treatment and to address any concerns regarding the study.

Blood Collection

Fasting venous blood was obtained by a trained phlebotimist at baseline, 3 months, and at the end of the study. Blood was centrifuged at 1500x g for ten minutes within two hours of collection. Plasma was separated and aliquoted for storage at -70°C until analysis of various inflammatory and adipokine markers.

Blood Biomarkers

A Human High Sensitivity T Cell Magnetic Bead Panel Multiplex kit from Millipore (Billerica, MA) was used to evaluate the following biomarkers: TNF-alpha, interleukin-1 beta, and interleukin-6. A Human Bone Magnetic Bead Panel Multiplex kit from Millipore (Billerica, MA) was used to evaluate leptin to assess body composition. Plasma samples from subjects obtained at each of the visits were analyzed in duplicate, with 25µL of plasma used for each well. The multiplex kits were developed as an immunoassay on the surface of fluorescent-coded magnetic beads (MagPlex™-C microspheres). The 96 well plate assays purchased from EMD Millipore included all the reagents as well as the appropriate plate required by the assay. Different coded bead groups, each of which is coated with a specific capture antibody, detect one of the biomarkers. A detection antibody is introduced and incubation with a streptavidin-phycoerythrin (PE) conjugate is performed to complete the reaction on the microspheres. The assay was analyzed using a Luminex 200 system. Within the device settings, 50 events per bead region were defined as minimum criterion. The beads coupled with the antibody bound to the specific analyte pass through two lasers, which excites the internal dyes and the signal of PE.

Statistical Analysis

A minimum sample size of 42 participants were needed in order to conduct analysis with the alpha = .05, power = .80, and a moderate effect size. Raw data for units of pg/mL: TNF-alpha, interleukin-1 beta, interleukin-6, and leptin were converted to pg/mL units for quantification. Descriptive statistics were calculated for all variables and included means, standard deviations, medians, minima, and maxima for continuous variables. The frequencies and percentages were calculated for all categorical demographic variables. For data not normally distributed, adjustments were made through either transformation data or non-parametric tests. Extreme outliers were evaluated to determine if they were due to technical or data entry error. The data was analyzed using SPSS v. 19.0. Independent sample t-tests were used to test for potential baseline differences in body composition, physical activity, and biomarkers of inflammation between raspberry and placebo groups. Baseline differences were controlled through covariate analysis, including ANOVA and regression.

CHAPTER IV

RESULTS

A total of 101 postmenopausal women were initially screened to participate in the study with 84 potential participants who met the inclusion criteria for a study visit. Of those 84 potential participants, 57 individuals qualified based on bone density assessment, meeting the criteria for bone loss. These individuals were randomly assigned to either the placebo (control) or raspberry (treatment) group. Of the 57 participants, 19 withdrew from the study for to various reasons (palatability of the treatment, lack of interest, conflicts in scheduling, or mild to moderate GI discomfort such as nausea and vomiting). The demographic data of the study participants is shown in Tables 1 and 2.

Body Composition and Plasma Leptin Concentrations

After 180 days of treatment, both the placebo and raspberry groups saw an insignificant increase in body mass index (BMI). The placebo group had an insignificantly lower systolic blood pressure and increased diastolic blood pressure at the final visit, whereas the raspberry treatment group showed a significant increase in both the systolic and diastolic blood pressures (see Table 3). Distribution of fat was evaluated by using DEXA and visceral adipose tissue, android fat, gynoid fat, android to gynoid ratio, and body fat was calculated using the software. After the 6-month treatment, there was an insignificant reduction in visceral adipose tissue volume, visceral adipose tissue mass, android fat, android to gynoid ratio, and total body fat in both raspberry and

placebo group. Gynoid fat was also decreased in the raspberry group, while it increased slightly in the placebo group (see Figures 1-4). Although insignificant, leptin had a higher trend at final values for the placebo group compared to the raspberry group (see Table 4).

Inflammation

Plasma levels of interleukin 1 beta (IL1 beta) were increased significantly from baseline to midpoint and remained high at final time point in the raspberry group. Plasma levels of IL-6 increased significantly from baseline to midpoint and decreased significantly at the end of treatment visit for both the raspberry and placebo groups. Plasma levels of TNF alpha increased from baseline to midpoint and decreased at final values for both intervention and placebo groups, although the placebo group showed significant differences between baseline to midpoint and midpoint to final values (see Table 5).

Physical Activity

Physical activity was measured using a physical activity questionnaire at baseline, midpoint, and final visits. There were no significant changes in recreational activity or home-related activity patterns for walking, moderate, and vigorous physical activity. There was a slight increase in walking minutes per week between baseline, midpoint, and final values for the raspberry group, whereas the placebo group had decreased from baseline to midpoint. Moderate physical activity for the raspberry group declined between baseline, midpoint, and final values. Moderate physical activity for the placebo

group increased between baseline to midpoint and decreased between midpoint and final visits. Vigorous physical activity decreased from baseline to midpoint in both treatment groups, but increased from midpoint to final only in the placebo group. The walking, moderate, and vigorous activities associated with home-related activity for the raspberry group decreased from baseline to midpoint and then increased at final visit. The walking, moderate, and vigorous home-related physical activity for the placebo group increased between baseline and midpoint but decreased at final visit (see Table 6).

CHAPTER V

DISCUSSION

The purpose of this thesis research was to investigate whether consumption of raspberry juice for a period of 6 months reduces inflammatory markers, positively impacting body weight and composition resulting in improvements in physical activity levels. Several studies investigating the effects of polyphenolic rich diets have demonstrated favorable effects related to body composition and inflammation (Heinonen et al., 1998; Rios-Hoyo & Gutierrez-Salmean, 2016; Mullen et al., 2002; Park, 2010). Increased inflammation and body fat accumulation are associated with increased risk for cardiovascular disease, diabetes and osteoporosis in postmenopausal women (Figureoa-Vega et al., 2015; Matthews & Thompson, 2016; Nieto-Vazquez et al., 2008). Treatment with red raspberries has been shown to reduce serum triglycerides, total cholesterol, body fat accumulation, and weight gain (Jung et al., 2016; Park, 2010; Morimoto et al., 2005; Rios-Hoyo & Gutierrez-Salmean, 2016). The benefits of antioxidant rich red raspberries have previously been studied *in vitro* and in animal models (Morimoto et al., 2005; Park, 2010; Rios-Hoyo & Gutierrez-Salmean, 2016). In this study, we randomized postmenopausal osteopenic women to receive raspberry juice or placebo juice to investigate changes in body composition, inflammation, and physical activity over a 6-month treatment period. Overall, the outcome of our study intervention incorporating raspberry juice did not result in any significant effect.

Body composition assessment and fat distribution was evaluated by measuring visceral adipose tissue, android fat, gynoid fat, android to gynoid ratio, and total body fat. Visceral adipose tissue surrounds abdominal organs and has been shown to increase insulin resistance and lipid dystrophy in people with a higher ratio of visceral adipose tissue compared to subcutaneous adipose tissue (Matsuzawa, Nakamura, Tokunaga, & Simomura, 1994). Visceral fat accumulation, induced by high sucrose intake and a deficiency of sex hormones results in more active synthesis and release of pro-inflammatory cytokines (Duran et al., 2016; Gulcelik et al., 2013). Excess adipose tissue and changes in body fat distribution also lead to morbidity and mortality risk associated with cardiovascular disease and diabetes (Czernichow et al., 2002; Laffin, 2016; Berk et al., 2016). *In vitro* studies using raspberry ketones have demonstrated increased secretion of adiponectin and suppression of lipid accumulation in adipocytes (Park, 2010). Raspberry ketones are extracted from raspberries for use as flavoring by the food industry and are commonly used for research using *in vivo* and *in vitro* models. Studies have found that these ketones down regulate adipogenesis transcription factors and increase fatty acid oxidation (Morimoto et al., 2005; Park, 2010; Rios-Hoyo & Gutierrez-Salmean, 2016).

An animal study using rats on a high-fat diet fed raspberry ketones resulted in prevention of increased body weight and visceral adipose tissue (Morimoto et al., 2005; Rios-Hoyo & Gutierrez-Salmean, 2016). Although weight and hepatic triacylglycerols were initially increased by the high-fat diet, they eventually decreased with the addition of raspberry ketones. The ketones increased norepinephrine-induced lipolysis (Morimoto

et al., 2005). This study showed a reduction in visceral adipose tissue volume, visceral adipose tissue mass, android fat, gynoid fat, android to gynoid ratio, and body fat total in the raspberry treatment group, although they were not significant reductions. BMI was not significantly increased while systolic blood pressure and diastolic blood pressure were significantly increased for the raspberry treatment group after the 6-month treatment duration. These results did not align with an *in vitro* study that reported that raspberry anthocyanins lower blood pressure. In the study, rabbit aortic vessels were cut into transverse ring segments and suspended in organ baths with raspberry concentrate. The aortic vessels were then assessed for vessel contraction and relaxation. The raspberry concentrate was shown to be a potent vasodilator, which would ultimately lower blood pressure (Mullen et al., 2002).

Leptin, a hormone secreted by adipose tissue that interacts with neurotransmitters acting in various regions of the brain, was slightly increased in the raspberry treatment group. The leptin increase in the raspberry group may be related to the raspberry group's BMI increase. Since adipose tissue produces leptin, leptin levels correlate with BMI (Albala et al., 2016). Subsequently, increased leptin leads to leptin resistance, decreasing the rate of leptin transport across the blood-brain barrier into the brain resulting in high serum leptin levels and obesity (Albala et al., 2016).

Plasma levels of inflammatory marker IL1 beta, which promotes fever-inducing PGE_2 and TNF-α, and IL6 were reported to increase significantly in a previous raspberry treatment group study. Plasma levels of inflammatory marker TNF alpha also showed an insignificant increase. These results of increased inflammatory markers do not align with

the results of previous studies that reported that consumption of polyphenolic rich diets were inversely related to inflammation (Heinonen et al., 1998; Mullen et al., 2002). Research has shown significant reduction in inflammation in an antigen-induced arthritis rat model given an oral treatment of red raspberry extract of 120mg/Kg daily for 30 days after the injection of mycobacterium tuberculosis. The rat model ankles were evaluated and showed inflammation was inhibited by 54% when compared with the non-treated diseased control (Jean-Gilles et al., 2012).

The hypothesis of this study was that the anticipated decrease in body mass, improvements in body composition, and reduction in inflammation would promote positive changes in physical activity patterns. After the 6-month study, there were no significant changes in recreational or home-related activity patterns for walking, moderate, and vigorous physical activity. Where recreational physical activity had a slight increase in walking minutes per week, moderate and vigorous recreational physical activity for the raspberry treatment group declined. Home-related physical activity for walking and moderate activity increased, whereas vigorous home-related physical activity decreased in the raspberry treatment group.

A limitation of the study was the high dropout rate, 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 dropout rate made the effect size smaller, shifted the

power analysis, and ultimately impacted our statistical results. The small sample size can make it difficult to generalize the findings of this research to the larger population of postmenopausal women. This study attempted to overcome this limitation by using the intent-to-treat (ITT) analysis. ITT analysis was used which is a more cautious analytical approach, but is more likely to prevent a type 1 error. In addition, self-reporting of the physical activity level allows for error and may have resulted in inaccurate measurements for baseline, midpoint, or final physical activity levels.

This study examined the effect of 2 ounces of daily raspberry consumption for 6 months on body weight and body composition, inflammation, and physical activity patterns. More research is warranted to determine the threshold of red raspberry intake for health improvements in postmenopausal women. Future studies may also focus on the anti-inflammatory markers and additional adipokines to determine the potential effect of long-term raspberry consumption by increasing the duration of the study, increasing sample size, and increasing dosage of the red raspberry.

Table 1

Participant Screening and Dropout Rate

Participants Screened	Bone Density Assessment	Qualified and Initiated Treatment	Completed Treatment	Participant Dropout	Dropout Rate
101	84	57	37	20	35%

Table 2

Study Participant Demographics

	Baseline (n)	Midpoint (n)	Final (n)	Age Range	Average Age	Drop Rate (midpoint)	Drop Rate (overall)
Placebo	27	21	20	46-68	57.11	22%	25%
Raspberry	30	19	17	46-71	59.27	37%	43%
Total	57	40	37	46-71	58.25	30%	35%

Table 3

Effect of Placebo VS Raspberry Treatment on Body Mass Index (BMI) (kg/m^2) and Blood Pressure (mmHg)

	Placebo		Raspberry	
	Mean	SEM	Mean	SEM
BMI				
Baseline	25.18	1.05	24.19	.87
Midpoint	25.33	1.14	24.35	.91
Final	25.41	1.17	24.29	.89
Blood Pressure Systolic				
Baseline	124.63	4.06	125.76	5.43
Midpoint	119.79	3.69	128.59*	4.04
Final	124.16	3.61	136.06*	4.63
Blood Pressure Diastolic				
Baseline	72.58	2.61	72.88	2.84
Midpoint	74.16	2.36	78.29*	2.78
Final	75.58	2.46	80.41*	2.73

N = 17 raspberry, n = 20 placebo. *Asterisk denotes significant difference ($p \leq .05$) from baseline.*

Table 4

Effect of Placebo VS Raspberry Treatment on Leptin (pg/mL)

	Placebo		Raspberry	
	Mean	SEM	Mean	SEM
Leptin				
Baseline	13138.30	2374.57	8909.55	1630.96
Midpoint	11592.95	1671.43	10357.74	1316.25
Final	13124.62	1977.11	9013.13	853.46

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

Table 5

Effect of Placebo VS Raspberry Treatment on Inflammatory Biomarkers (pg/mL)

	Placebo		Raspberry	
	Mean	SEM	Mean	SEM
TNF alpha				
Baseline	1.57	.17	1.53	.20
Midpoint	1.64*	.18	1.74	.13
Final	1.23*	.10	1.66	.16
IL1 beta				
Baseline	.55	.05	.64	.08
Midpoint	.62	.06	.75*	.08
FInal	.56	.05	.79*	.07
IL6				
Baseline	2.00	.27	2.19	.39
Midpoint	2.20*	.24	2.74*	.36
Final	1.83*	.21	2.50*	.39

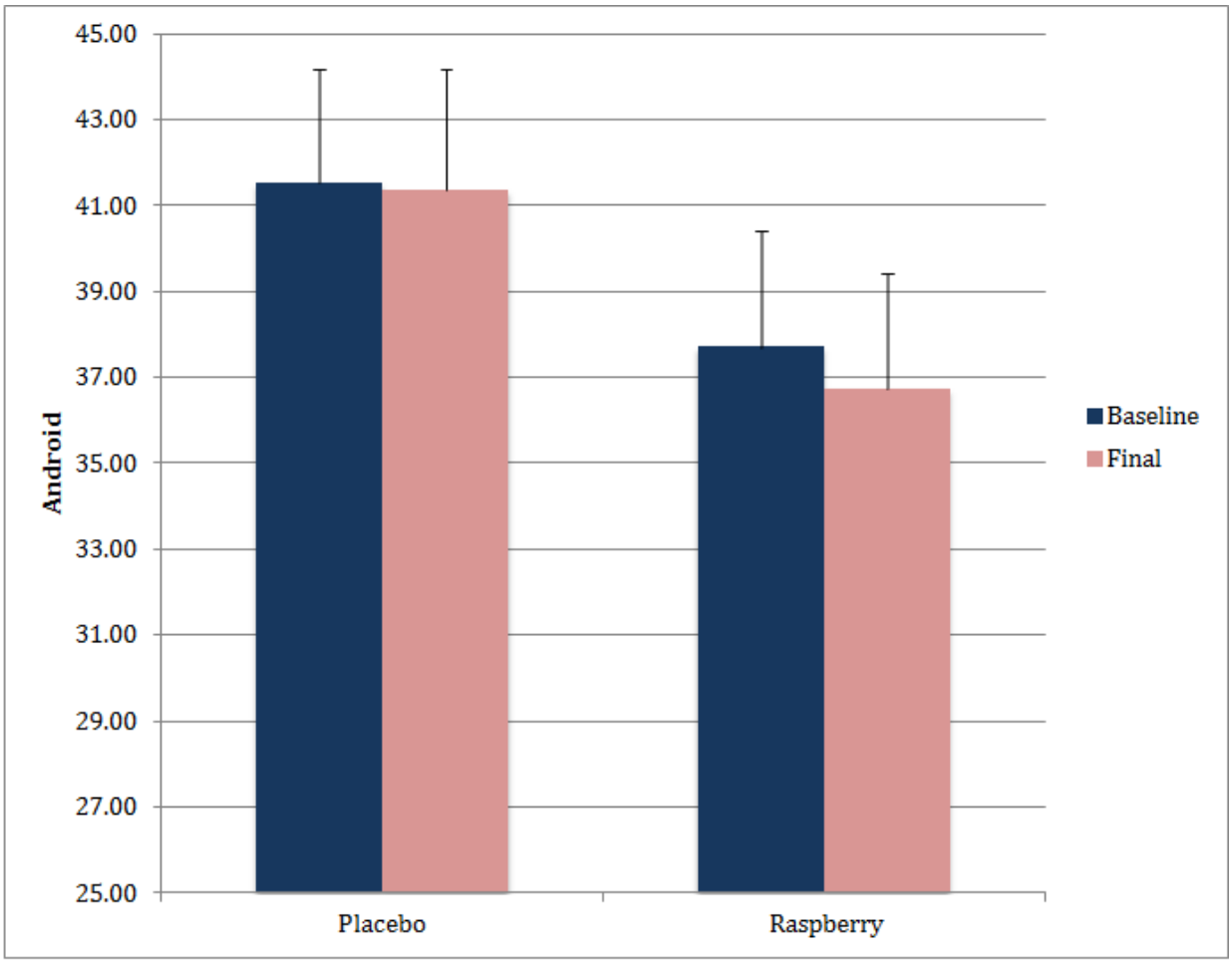
Mean±SEM. N = 17 raspberry, n = 20 placebo. Asterisk denotes significant difference ($p \leq .05$) from baseline.

Table 6

Effect of Placebo VS Raspberry Treatment on Physical Activity (min/week)

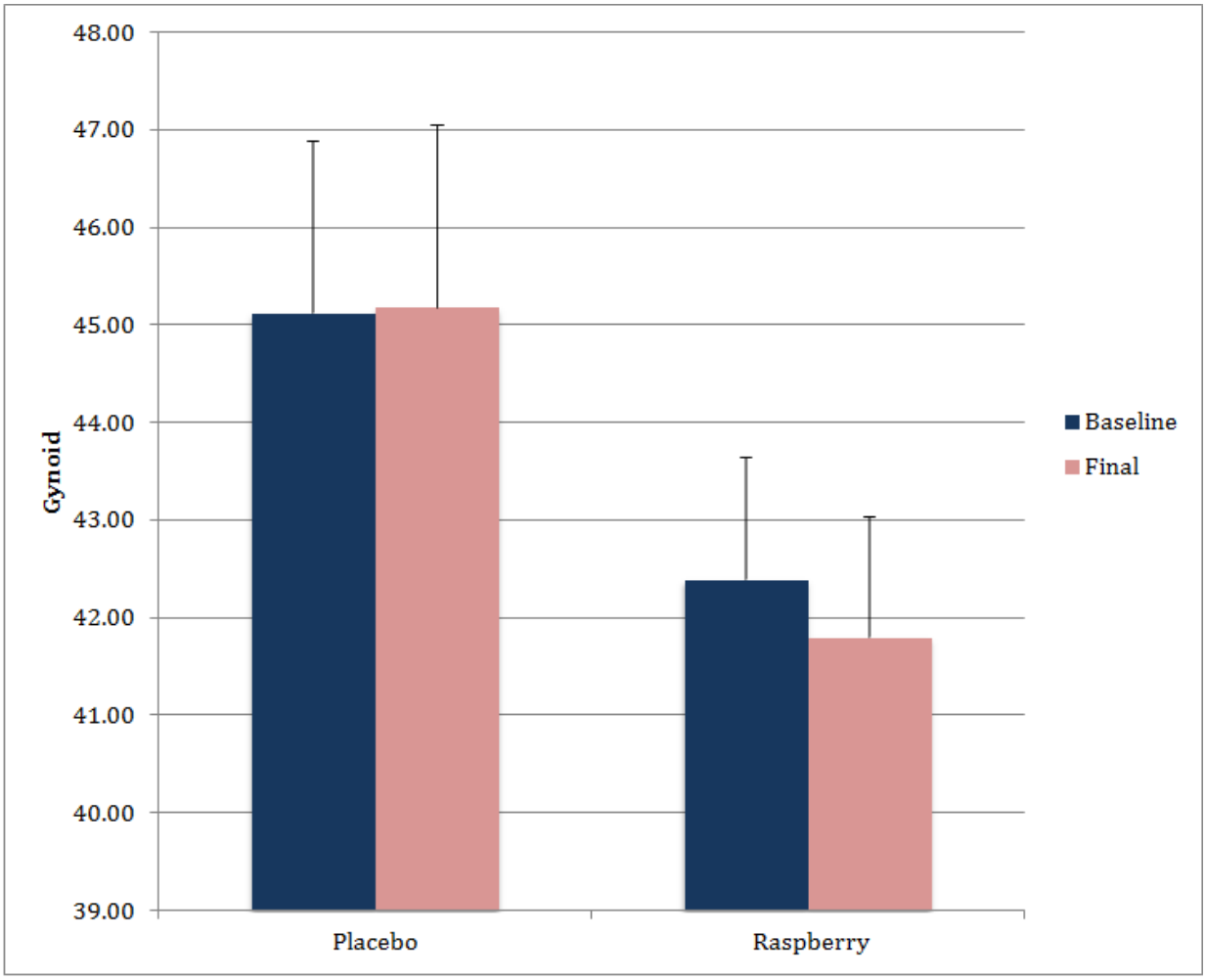
	Placebo		Raspberry	
	Mean	SEM	Mean	SEM
Walking Home Related Physical Activity				
Baseline	271.5	100.37	169.12	44.59
Midpoint	286.25	95.28	162.35	51.52
Final	150	32.2	222.35	63.02
Moderate Home Related Physical Activity				
Baseline	67.25	28.32	89.06	28.04
Midpoint	74.50	19.01	71.25	17.27
Final	61.75	26.32	101.25	39.39
Vigorous Home Related Physical Activity				
Baseline	52.5	26.06	75.88	24.15
Midpoint	56.25	22.93	45.59	18.38
Final	12.5	8.52	54.12	35.42
Walking Recreational Physical Activity				
Baseline	62.56	14.99	40.88	16.46
Midpoint	60.75	23.95	67.06	18.87
Final	72	23.32	70.88	23.22
Moderate Recreational Physical Activity				
Baseline	67.5	36.24	52.94	28.97
Midpoint	86.25	32.3	46.47	19.13
Final	73.75	34.65	30.29	13.94
Vigorous Recreational Physical Activity				
Baseline	81.38	34.05	35.29	21.86
Midpoint	70	21.69	24.12	13.91
Final	87.25	32.32	24.12	16.52

N = 17 raspberry, n = 20 placebo. *Asterisk denotes significant difference ($p \leq .05$) from baseline.*



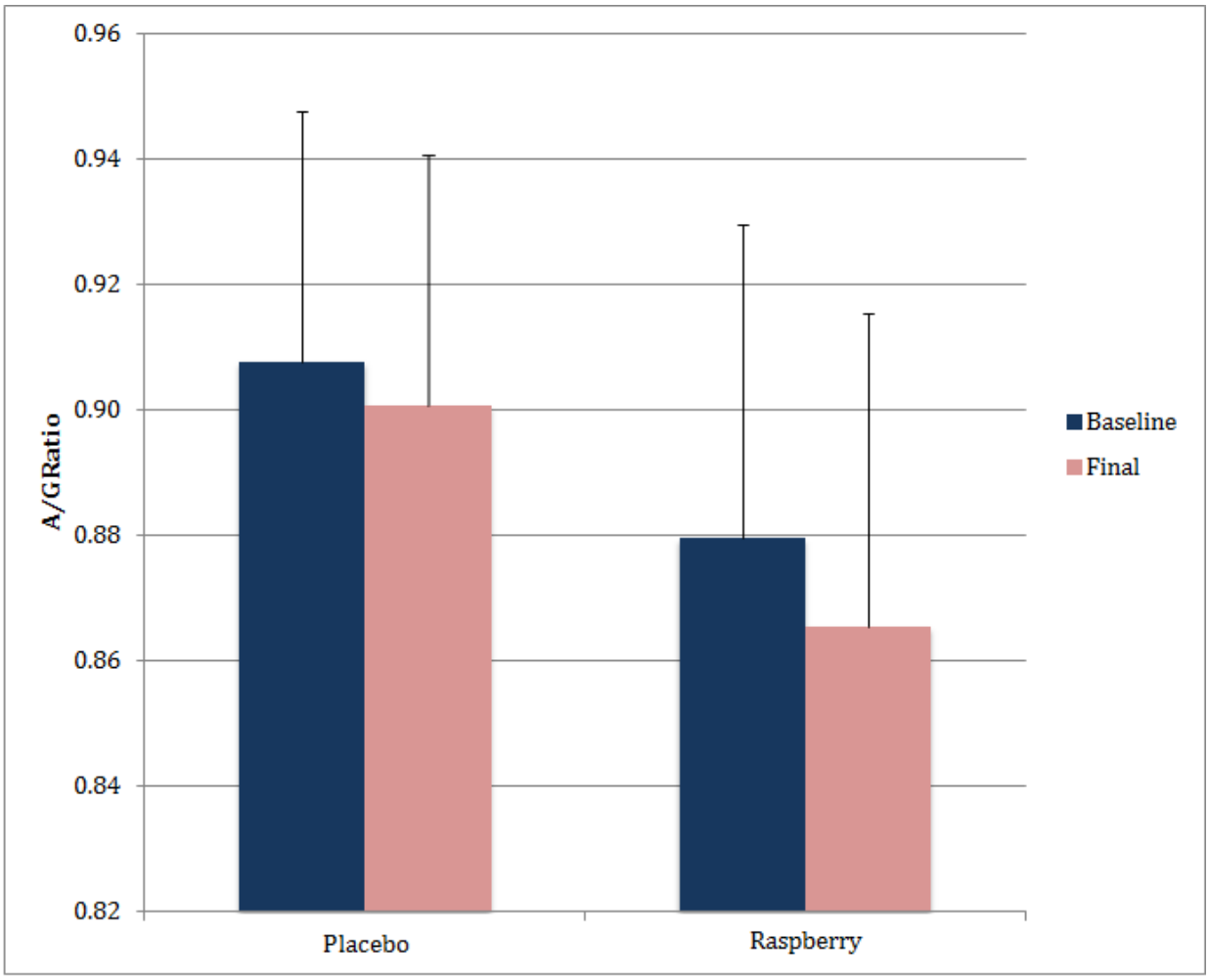
Mean±SEM. N=17 raspberry, n=20 placebo

Figure 1. Effect of Placebo VS Raspberry Treatment on Android (%) in Postmenopausal with Osteopenia



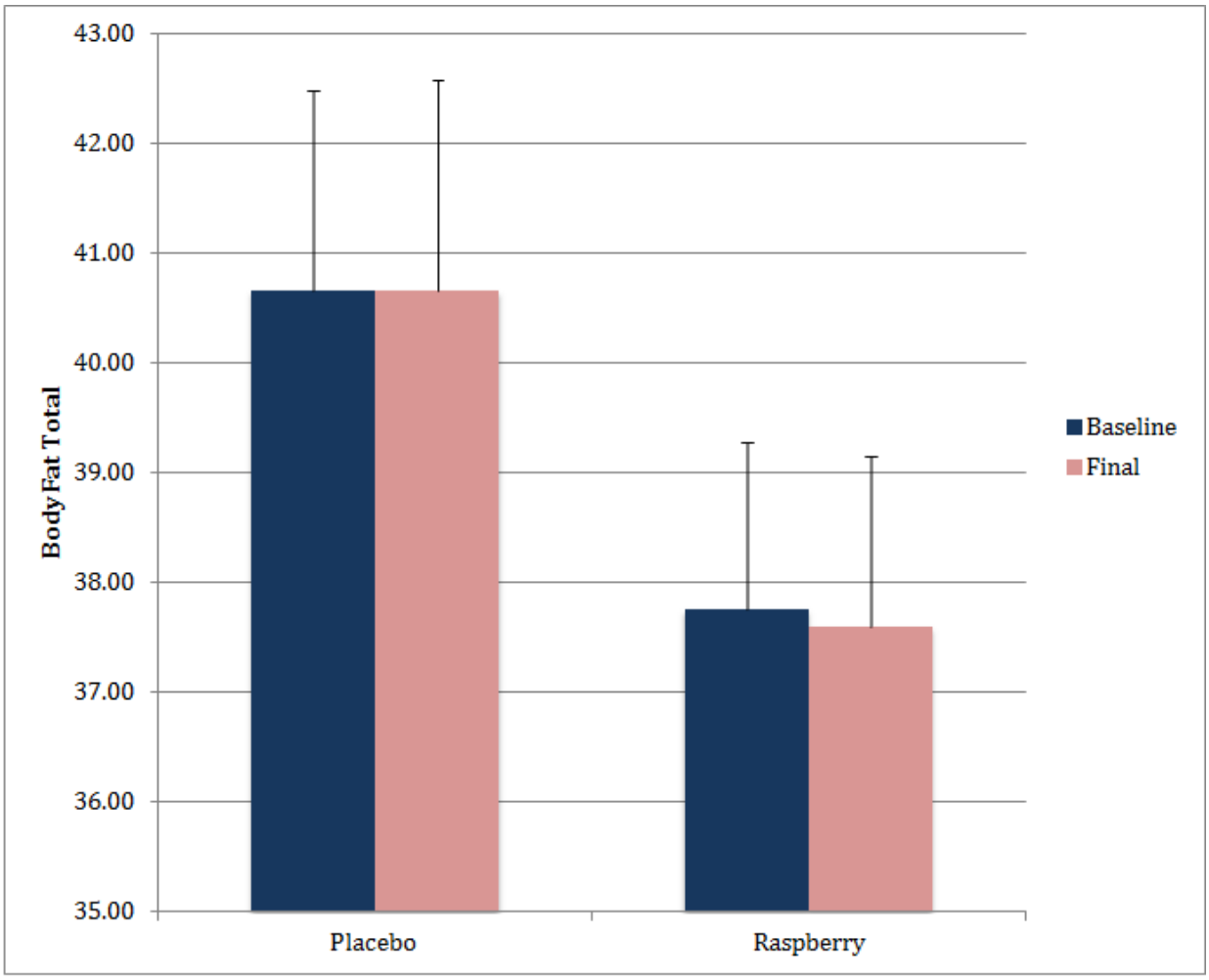
Mean±SEM. N=17 raspberry, n=20 placebo

Figure 2. Effect of Placebo VS Raspberry Treatment on Gynoid (%) in Postmenopausal with Osteopenia



Mean±SEM. N=17 raspberry, n=20 placebo

Figure 3. Effect of Placebo VS Raspberry Treatment on A/G Ratio in Postmenopausal with Osteopenia



Mean±SEM. N=17 raspberry, n=20 placebo

Figure 4. Effect of Placebo VS Raspberry Treatment on Body Fat Total (%) in Postmenopausal with Osteopenia

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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 3visits.

Benefits include: awareness of bone status and bone health, measurement of bone density and blood measures associated with bone status. Upon completion, you wi**11** 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. Shanii Juma, Department of Nutrition and Food Sciences s juma@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

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 QUESTIONAIRE

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			Size		
	Day	Week	Rarely/ Never	S	M	L
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	**How Often**			**Size**		
(Medium Serving)	**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	**How Often**			**Size**		
(Medium Serving)	**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 (Medium Serving)	**How Often**			**Size**		
	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/ Never**	**S**	**M**	**L**
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?
2.

☐ 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 yourwork**? 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.