# THE EFFECT OF FREEZE-DRIED GRAPE POWDER ON INFLAMMATORY MARKERS AND PHYSICAL ACTIVITY IN ADULTS WITH SELF-REPORTED KNEE OSTEOARTHRITIS

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REBECCA M. SMALL B.A., RDN, LD

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ABSTRACT

REBECCA M. SMALL BA, RDN, LD

AGE-ASSOCIATED EFFECT OF FREEZE-DRIED GRAPE POWDER ON INFLAMMATORY MARKERS AND PHYSICAL ACTIVITY IN ADULTS WITH KNEE OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common joint disease among US adults, leading to pain and disability. Treatment of OA is focused on symptom management since no cure has been found, and may involve expensive procedures including joint replacement and may lead many to seek natural dietary approaches. Grapes, rich in anti-inflammatory polyphenols may aid in management of OA symptoms. The purpose of this study was to assess the effect of daily consumption of freeze-dried grape powder on physical activity, and biochemical markers of inflammation and cartilage metabolism in individuals with self-reported knee osteoarthritis. A total of 72 men and women with knee OA were recruited and randomized into 2 groups. Group 1 (n=28, 21 female and 7 males) consumed 47 grams of freeze-dried grape powder (FDGP) daily for four months. Group 2 (n=28, 21 female and 7 males) consumed 47 grams of a comparable placebo. Serum specimens and self-reported physical activity were obtained at baseline and at four months. There was a significant decrease in very hard activity in the placebo group. However, an increase in moderate activity was seen for those $\leq$64 yr. in the placebo group. Participants $\geq$65 yr. reported a significant decline in moderate and hard activities

(P<.05) in both groups from baseline to final. A statistically significant increase in interleukin 1-beta (IL-1β) was observed in both groups, males greater than females. A greater increase occurred in the placebo group (637.33%) vs. FDGP (194.64%). Levels of IL-1β and cartilage oligomeric protein (COMP) increased more in those 65 years and older across both genders. In regards to cartilage turnover, both groups had a statistically significant increase in COMP (154.2% FDGP vs. 172.27% placebo). We showed that age has a significant impact on physical activity levels, inflammation, and cartilage metabolism in people with knee OA. FDGP supplementation may decrease age-related inflammation, decline in physical activity and cartilage matrix breakdown in those with OA.

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# CHAPTER I

## The Effect of Freeze-Dried Grape Powder on Inflammatory Markers and Physical Activity in Adults with Self-Reported Knee Osteoarthritis

Osteoarthritis (OA) is the most common joint disease occurring among one-in-five US adults and can be debilitating as a result of joint disfiguration and pain (CDC, 2010). One-third of US adults do not meet the 2008 Physical Activity Guidelines for American's recommendations for aerobic physical activity, which may partially be a result of increasing prevalence of OA (CDC, 2010; Dunlop et al., 2011; White et al., 2013). OA is among the leading causes of disability among US adults and leads to decreased health-related quality of life as well as increased mortality rates (CDC, 2010; Hochberg, 2008; Hoogeboom, 2013; Nguyen, 2011; Murphy, 2012).

Current treatment is aimed at symptom management, as a cure for OA is still to be discovered, leading many to resort to surgery to replace the affected joint(s) (Jordan et al., 2003; Ringdahl, 2011; Sinusas, 2012). Treatment of arthritis costs about 128 billion dollars annually, 4,741 dollars per patient, with 5 percent of healthcare costs incurred by joint-replacements (CDC, 2010; Helmick et al, 2008, London et al, 2010). Non-pharmacologic treatment options may include: physical therapy, weight loss, walking aids, and insoles (Conaghan et al. 2008; Jordan et al, 2003; Sinusas, 2012). Medications used to reduce inflammation and pain includes: Glucosamine and Chondroitin supplementation, non-steroidal anti-inflammatory (NSAIDs) medications (including COX-2 inhibitors) hyaluronic acid injections, and analgesics such as capsaicin cream and

opiates (Jordan et al, 2003; Sinusas, 2012). These medications, however, have negative side effects on the kidneys, gastrointestinal tract, and platelet aggregation, and have been shown to increase inflammatory cytokines in those with OA (Conaghan et al., 2008; Renda et al., 2006; Schumacher et al., 1996).

Inflammation is thought to play an integral a role in OA progression. The cyclooxygenase-2 (COX-2) pathway, part of the OA inflammatory process, has become a target of many drug therapies (Conaghan et al., 2008; Ou et al., 2012). Researchers have observed elevated serum levels of inflammatory cytokines and proteins including: interleukin 1- beta (IL1-β) and cartilage oligomeric matrix protein (COMP) among those with OA and are also involved in activation of the COX-2 pathway (Atkas et al., 2012; Blom et al., 2007; Denoble et al., 2010; Li et al., 2011; Marcu et al., 2010; Sun et al., 2011; Shahi et al., 2012; Verma & Dalal 2013; Zivanovic et al., 2011). Andriacchi et al. describe OA pathology in two phases, an initiation phase, stimulated by mechanical changes in the joint causing a shift in loading to an area of the joint not fit to bear that load, followed by the progression phase, in which metabolic pathways induce an inflammatory response, favoring catabolism of the extracellular matrix and adaptation of the subchondral bone (osteophyte formation) (Andriacchi et al. 2004; Goldring et al., 2010; Heijink et al., 2012).

Red grapes contain plant compounds known as polyphenols shown to reduce inflammation in OA and in other chronic diseases (Kovacic and Somanathan, 2010; Wu et al., 2006; Zhou and Raffoul, 2012). Resveratrol, one compound found in red grapes,

has been shown to reduce reactive oxygen species (ROS) during inflammation and can inhibit the activity of COX-1 and COX-2 enzymes, cytochrome P450 complex, and quinone reductase protecting cells from destruction by stress, infection, and environmental toxins (Gusman et al., 2001, Korolkiewicz RP et al., 2003, Liu et al., 2010; Mobasheri et al., 2012). In several animal studies, resveratrol was shown to protect knee cartilage from inflammatory cytokines known to promote OA progression including IL-1β induced pathways (Liu et al., 2010; Shakibaei et al., 2007; Shakibaei et al., 2008; Shen et al., 2012; Wang, et al., 2012). Delphinidin reduced PGE production in human chondrocytes by inhibiting IL-1β induced COX-2 expression (Hasseb et al., 2013). Studies using antioxidants extracted from grape skin and pomace have shown no reduction in oxidative stress in obese mice (Zhou and Raffoul, 2012), suggesting a role for the use of whole grapes in anti-inflammatory treatment. Few studies to date have examined the effect of whole grape on osteoarthritis in humans *in vivo*.

Seventy male and female adults between the age of 45 and 75 with self-reported knee osteoarthritis were recruited for this randomized controlled double blinded placebo study. Participants were randomized into two groups. The treatment group received 45 grams freeze-dried grape powder and the second group to receive 45 grams placebo daily for four months. Self-reported physical activity and blood serum samples were measured at baseline and at four months to determine changes in physical activity as well as in serum IL1-β and COMP to determine if red grapes have an effect on inflammation among those with knee pain.

## Central Hypothesis

Inclusion of whole grapes, which contain polyphenols, in the diet will reduce inflammatory markers associated with knee OA resulting in reduced pain, improved mobility, and a higher level of physical activity.

## Specific Aims

**Aim 1.** To examine the effects of freeze-dried grape powder in comparison to a placebo treatment on mobility in adults with self-reported knee OA. Primary measures will include assessing changes in subjective physical activity levels and mobility before and after four months of treatment.

**Aim 2.** To evaluate the effects of freeze-dried grape powder in comparison with a placebo treatment on selective serum biomarkers of inflammation in adults with self-reported knee OA before and after treatment. Primary measures will include assessing changes in levels of serum COMP and IL-1β in blood before and four months after treatment.

CHAPTER II

# Review of Literature

## Significance of the Problem

Among U.S. Adults, 7 in 10 die as a result of chronic disease every year (CDC, 2010). One modifiable behavior to reduce risk of developing chronic disease is physical activity (CDC, 2010). However, one-third of US adults do not meet the 2008 Physical Activity Guidelines for American's recommendations for aerobic physical activity, which may partially be a result of increasing prevalence of Osteoarthritis (OA) (CDC, 2010). As the most common joint disorder, OA is among the leading causes of disability among adults in the United States affecting individuals as early as 25 years of age most often after the age of 40 and leads to decreased health-related quality of life and increased mortality rates (CDC, 2010; Hochberg, 2008; Hoogeboom, 2013; Nguyen, 2011; Murphy, 2012). White et al. conducted a study to determine if adults with knee OA were able to meet the 2008 Physical Activity guidelines and determined that only 6 percent of men and 5 percent of the women met these guidelines, not far from the 12.9 percent of men and 7.7 percent of women with OA found to meet these same guidelines by the Osteoarthritis Initiative (Dunlop et al., 2011; White et al., 2013).

Osteoarthritis is a degenerative disease of the joints involving a progressive wearing away of articular cartilage with increased friction between joint forming bones resulting in pain, stiffness, asymmetric inflammation and osteophytes or bone spurs and reduced range of motion (Escott-Stump, 2008). This usually occurs in weight-bearing

joints (knees, hip, spine, and distal phalanges) and can lead to a reduced range of motion in the affected joints and overall mobility in those affected (CDC, 2010; Murphy, 2012; Escott-Stump, 2008).

Osteoarthritis quite prevalent as it is diagnosed in 1 in 5 U.S. adults; in 2010 this included 49.9 million people (CDC, 2010). Although the etiology of OA is unknown several factors have been found to increase risk of OA including: obesity, aging, increased repetitive load on the joints, estrogen deficiency, osteoporosis, inflammation, elevated C-reactive protein (CRP), genetic factors, gender (women have higher risk than men), or trauma/injury to the joints (CDC, 2010; Elbaz et. al, 2011; Kulcu, DG, et al, 2010; Muthuri et al., 2011; Yusuf et al, 2011).

**Economic Burden**

As a leading cause of disability among U.S. adults, costs include symptomatic treatment, lost workdays due to disability, and surgical intervention as well as additional emergency room visits. Treatment of arthritis costs about 128 billion dollars annually, 4,741 dollars per patient, with 5 percent of healthcare costs incurred by joint-replacements (CDC, 2010; Helmick et al, 2008, London et al, 2010). Hospital stays associated with a primary diagnosis of OA in 2009 cost 45,443 dollars per stay with an average out-of-pocket expense per person of 2,600 dollars every year (CDC, 2011; Murphy, 2011). Musculoskeletal conditions greatly impacted the economy in 1992 with expenses reaching 2.5 percent of the gross national product in the U.S. (March, 1997; Brown et al., 2006).

**Surgical and Non-Surgical Therapies**

As no cure has been found at this time for OA, treatment is focused on pain and symptom management (CDC, 2010). Treatment of OA is based off of severity of the disease with degrees ranging from mild to severe OA (Sinusas, 2012). Treatment is recommended to begin with non-pharmacologic treatments including: physical therapy, regular exercise, patient education, insoles, weight loss, or mobility aids such as walkers or canes (Conaghan et al. 2008; Jordan et al, 2003; Sinusas, 2012). If pain persists pharmacologic therapies are then considered beginning with analgesics (i.e. acetaminophen) (Sinusas, 2012).

Non-steroidal anti-inflammatory medication is used to reduce inflammation – these include: ibuprofen and naproxen (Jordan et al, 2003; Sinusas, 2012). Selective cyclooxygenase-2 (COX-2) inhibitors, celecoxib and rofecoxib, are the most common anti-inflammatory medications used in the treatment of OA. COX-2 inhibitors have been shown to effectively decrease chondrocyte apoptosis in the articular cartilage as well as reduce prostaglandin production and associated pain and inflammation (Ehrich et al., 1999; Hawkey et al., 1999; Conaghan et al., 2008; Ou et al, 2012; Simon et al., 1998; Renda et al, 2006). Glucosamine sulfate and Chondroitin supplementation have been found to modify the structure of affected joints (Sinusas, 2012). Topical creams for pain relief may include capsaicin or Lidocaine creams (Jordan et al, 2003).

If the previously mentioned medications are ineffective, opiods are considered under careful surveillance and Tramadol is discouraged if possible due to its negative side effects (Ringdahl, 2011; Sinusas, 2012). When immediate pain relief is needed, corticosteroid injections are often used as a short-term analgesic, one to two weeks (Reid et al, 2012). Hyaluronic acid, a glycosaminoglycan that makes up part of healthy synovial fluid, can be injected into the joint (i.e. Orthovisc, Symvisc) acting as a replacement for lost synovial fluid (Jordan et al., 2003; Ringdahl, 2011; Sinusas, 2012). Hyaluronic acid injections offer pain relief for an average of 6 months, however may cost up to 880 dollars per injection without insurance as compared to 17 dollars for the steroid injection and are used as a last resort before surgical intervention is considered (Ringdahl, 2011; Sinusas, 2012).

Prolonged use of OA medications may result in: nausea, diarrhea, constipation, abdominal pain, vomiting, heartburn, myocardial infarction, liver and renal failure (Escott-Stump, 2008; Jordan et al, 2003; Reid et al, 2012; Sinusas, 2012). Use of NSAIDs for only two weeks were shown to increase tumor necrosis factor (TNF) levels in synovial fluid of osteoarthritic patients (Schumacher et al., 1996). In the case of opiods, close monitoring by a physician is required as addiction or abuse may occur with extended use (Sinusas, 2012). Although COX-2 inhibitors have no known gastrointestinal side- effects or negative effects on platelet aggregation, adverse effects on the renal system have not been excluded (Simon et al., 1998; Ehrich et al., 1999; Hawkey et al., Laine et al. 1999; Simon et al., 1999; Renda et al., 2006). A meta-analysis conducted in

the UK found that prolonged use of COX-2 inhibitors and NSAIDS increased risk of gastrointestinal ulcers, which were reduced when patients received proton-pump inhibitors in addition to these medications (Conaghan et al., 2008). Increase in myocardial infarctions among those taking COX-2 inhibitors has been found as a result of changes in pro-/anti- thrombotic mediators (Conaghan et al., 2008).

If the patient does not respond well to pharmacologic or non-pharmacologic therapies, surgery is recommended (Jordan et al., 2003; Ringdahl, 2011; Sinusas, 2012). Types of surgical interventions for OA include: lavage, arthroplasty, arthroscopic debridement, or joint replacement with prosthetic devices (Altman, 2010; Jordan et al, 2003; Sinusas, 2012). Surgical intervention, though effective in reducing joint pain and improving quality of life involves risks associated with surgery, may reduce life expectancy, is costly, and often needs to be repeated as prosthetics are expected to function for 15 to 20 years on average before needing to be replaced (Ringdahl et al., 2011).

Unfortunately, a study by London et al, determined that 3.6 million U.S. adults live with pain from OA, including those who do not wish to have surgery but have tried other conventional treatments with no long-term relief (London et al., 2010). Those within this gap may live with untreated pain for an average of 20 years (London et al., 2010). Other cost-effective therapies are needed to aid patients that fall within this gap.

In response to this increasing gap, investigation of alternative and complementary therapies is growing among researchers. Therapies may include acupuncture to relieve pain and Yoga or Tai Chi to balance and protect bones. Dietary interventions such as omega-3 fatty acid supplementation, S-adenosylmethionine (SAM-e) supplementation, and the intake of various fruits, vegetables, herbs, and spices (i.e. ginger, turmeric, acai berry, grapes, capsaicin) have become an interest for numerous researchers and patients suffering from OA for their phytochemical properties, shown to reduce inflammation particularly in arthritis (Fouladbakhsh 2012; Jordan et al, 2003; Mendez C, VJ et al, 2005; Ringdahl et al., 2011; Sinusas, 2012).

**Osteoarthritis: Pathology and Inflammation**

Osteoarthritis is a multi-factoral disease involving biomechanical and metabolic pathways, including epigenetic modifications. Although a specific cause has still to be isolated, inflammation is thought to play an important role in OA pathology (Blom et al., 2007; Goldring et al., 2010; Sun et al., 2011). Andriacchi et al. describe OA pathology in two phases, an initiation phase, stimulated by mechanical changes in the joint causing a shift in loading to an area of the joint not fit to bear that load, followed by the progression phase, in which metabolic pathways induce an inflammatory response which favors catabolism of the extracellular matrix and adaptation of the subchondral bone (osteophyte formation) (Andriacchi et al., 2004; Goldring et al., 2010; Heijink et al., 2012).

In normal healthy joints, articular bones are separated by cartilage made up of an elastic extracellular matrix containing synovial fluid, macromolecules (proteoglycans, glycoproteins, matrix proteases, aggrecan, type II, XI, IX, and IV collagen fibers, and non-collagenous proteins), and chondrocytes responsible for maintaining balance of breakdown and repair of tissue within the joint such as release of lubricin (Andriacchi et al. 2004; Goldring et al., 2010; Heijink et al., 2012; Ou et al., 2012). When in balance, chondrocytes secrete matrix proteases as well as growth factors to breakdown and repair cartilage tissues as well as stimulate synthesis of lubricating factors for synovial fluid maintenance (i.e. aggrecan which holds fluid within the cartilage tissue) (Calich et al, 2010; Loeuille, 2005; Ou et al., 2012; Pelletier et al 2001).

This network is elastic, maintains high tensile strength, has low metabolic activity and vascularity to absorb compression and provide lubrication between bones during times of high mechanical loading (Ou et al., 2012; Heijink et al., 2012). In the knee, the central pressure is placed on the medial side of the tibiofemoral portion of the joint, and is able to bear up to three times a person's body weight while walking and six times body weight during stair climbing (Heijink et al., 2012). In OA an increase in shear stress, friction, weakened fibril network and breakage, as well as loss of proteoglycans reducing protection between subchondral bone and can cause articular surface damage (Andriacchi et al., 2004; Heijink et al., 2012). Reduced motion of the joint, malalignment from injury or trauma to the joint (i.e. meniscus or ACL tear), chronic stress from overuse of the joint as in exercise, aging, metabolic syndrome, and hormone imbalance (i.e. chronic

inflammation and oxidative stress) may be compounding factors during this first phase of OA (Andriacchi et al., 2004; Gkretsi et al., 2010; Goldring et al., 2010; Heijink et al., 2012; Helmark et al., 2012). Altered lipid profiles have been suggested to be correlated with OA progression and severity as elevated leptin levels and central adipose tissue have been correlated with risk of OA and cartilage loss (Gkretsi et al., 2011).

During the progression phase of OA mechanical stress and friction increases between articular bone surfaces as shear stress shifts to an area not normally equipped to bear additional force. Articular chondrocytes initiate an immune response in order to adapt to changes within the joint leading to disruption in the equilibrium normally kept by these cells. Research has found increased production of cytokines during OA leading to chondrocyte apoptosis, decreased synthesis of type II collagen, decrease in collagen thickness, and increase in secretion of growth factors involved in osteophyte formation (Andriacchi et al., 2004; Goldring et al., 2010; Heijink et al., 2012; Ou et al. 2012; Sun et al., 2011).

Several pathways have been proposed to be involved in the stress response of chondrocytes to OA progression. Attur et al. showed that under stress, as in the progression phase of OA, chondrocytes express elevated levels of prostaglandin $E_2$ ($PGE_2$) and LTB4, end products of the COX-2 and LOX pathways, as compared to normal human cartilage (Attur et al., 2012; Goldring et al., 2012; Sun et al., 2011). Phospholipase $A_2$ ($PLA_2$) is the enzyme responsible for the release of arachidonic acid (AA) from synovial cell membranes, a free fatty acid oxidized by COX-2 to synthesize

$PGE_2$ (Berenbown et al., 1996). In a study of microsomal prostaglandin E synthase-1 (mPGEs-1) knockout mice, Kamei et al. concluded that, $PGE_2$ increases neural sensitivity in those suffering from OA and is associated with the pain experienced by this population (Kamei et al., 2004). As a result of these findings, inhibiting the COX-2 pathway has become a target for drug and nutraceutical therapy to reduce the pain and inflammation associated with OA related to $PGE_2$ synthesis (Conaghan et al., 2008; Ou et al., 2012).

Interleukin-1 (IL-1) an inflammatory cytokine expressed primarily by macrophages activated by an inflammatory response and induce the expression of various catabolic and anabolic enzymes and proteins (Sun et al., 2011). IL-1 is not normally detected in synovial fluid, however, has been found to be mildly elevated in OA. Specifically, IL-1β has been seen to promote synthesis of COX-2 and proteases, which degrade extracellular matrix of the joint, including: MMPs (MMP-1, MMP-3, MMP-9, MMP-13, MMP-14) and aggrecanases (ADAMTS5, ADAMTS4, ADAMTS9). Inducible nitric oxide synthase (iNOS) and mPGEs-1 required for $PGE_2$ synthesis by COX-2 and nitric oxide (NO) release are also up regulated (Alvarez-Soria et al., 2008; Atkas et al., 2012; Blom et al., 2007; Denoble et al., 2010; Goldring et al., 2010; Marcu et al., 2010; Sun et al., 2011; Tanimoto et al., 2011).

Chowdhury et al., determined that the increase of NO and $PGE_2$ among chondrocytes induced by IL-1β is due to stimulation of the genetic transcription pathways of: mitogen-activated protein kinase (MAPK), activator protein-1 (AP-1), and nuclear factor kappa B (NF-kB) (Chowdhury et al., 2008; Marcu et al., 2010). In initiating these

pathways, IL-1β is involved in decreasing proteoglycan synthesis, elevating the release of $PGE_2$ and nitric oxide (NO) (Chowdhury et al., 2008). NO, though protective in some manner within the joint, also activates MMPs, increasing apoptosis of chondrocytes, and reduces aggrecan release thereby inhibiting synthesis of new collagen (Sun et al., 2011). Other transcription factors induced by localized inflammation of the joint include: Sox9, Runx2, ELF3 and the Wnt-signaling pathway (Attur et al., 2012; Loeser et al., 2013; Goldring et al., 2010).

Tumor necrosis factor (TNF-α), another cytokine upregulated in OA, works synergistically with IL-1β elevating its effects within cartilage and together are correlated with joint space narrowing and OA severity (Alvarez-Soria et al., 2008; Berenbrown et al., 1996; Denoble et al., 2010). Berenbrown et al, suggested that the mechanism of this synergistic action may involve increased release of AA from cell membranes by $PLA_2$ induced by of IL-1β and TNF-α. However, though Berenbrown et al. found and increase in $PGE_2$ synthesis in rabbit articular chondrocytes in the presence of IL-1β and TNF-α, no increase in AA release from cells was seen (Berenbrown et al., 1996).

Increased expression of catabolic proteins during OA progression in addition to IL-1 and TNF-α include: IL-17, IL-18, and oncostatin M (OSM) and have been linked to increased protease synthesis and expression as well as continuing to stimulate inflammatory response (Goldring et al., 2010; Blom 2007). Regulators of inflammation, which work to reverse or reduce the effects of catabolic cytokines by increasing growth factors and inhibiting inflammatory responses, include: IL-4, IL-6, IL-10, and IL-13

(Blom et al., 2007; Sun et al., 2011). Matrix proteins elevated during OA include: cartilage oligomeric matrix protein (COMP), collagens type II and X, aggrecan, link protein, osteopontin, and tenascin and may be used as markers of inflammation within the joint (Andriacchi et al., 2004; Denoble et al., 2010; Goldring et al., 2010).

Cartilage oligomeric matrix protein (COMP) or thrombospondin (TSP-5) is a non-collagen protein normally found in articular cartilage. COMP functions to maintain ECM stability by promoting connections between protein and collagen by binding to transforming growth factor (TGF-β) and bone morphogenetic protein-2 (BMP-2) promoting osteogenesis within cartilage (Haudenschild et al., 2011; Ishida et al., 2013; Li et al., 2011; Zivanovic et al., 2011). TGF-β also stimulates COMP synthesis by upregulating COMP mRNA to counter the effects of IL-1 (Li et al., 2011; Blom et al., 2007). As a mechano-stimulated gene, COMP is released from the ECM during inflammatory conditions as well as tissue damage. Elevated COMP levels in serum are positively correlated with IL-1β and under comparison with radiographical studies is elevated proportionate to severity of progression in knee OA (Li et al., 2011; Shahi et al., 2012; Verma & Dalal 2013; Zivanovic et al., 2011).

Anabolic growth factors such as TGFβ, BMP-2, -4, -7/OP-1, CTGF, and BFGF, have been shown to reduce inflammation by promoting synovial proliferation (Blom et al., 2007; Haudenschild et al., Ishida et al., 2013). However, these factors also increase the deposition of bone in the new areas of mechanical force within the joint leading to osteophyte formation commonly seen in weight-bearing OA joints (Blom et al., 2007).

Since elevated levels of COMP and IL-1β may be indicative of cartilage degradation as seen in OA pathogenesis, they were chosen for this study to measure inflammation of the knee before and after treatment versus placebo (Calich et al, 2010; Spector et al, 1997; Hunter et al, 2007; Daheshia, 2008). Inhibiting the induction of an inflammatory response by chondrocytes is a goal of drug and nutraceutical therapies for OA. Targeting these metabolic pathways is the beginning to decreasing pain and slowing progression of OA and eventually may prevent or reverse its occurrence at all.

**Influence of Diet and Body Weight**

Symptoms and occurrence of OA increase significantly with BMI and metabolic syndrome (CDC, 2010; Dunlop, 2010; Elbaz, 2011; Engstrom et al., 2009). The overall lifetime risk of symptomatic knee OA increases to two in three in among adults who are obese compared to those who are of normal weight (Murphy, 2012; Yusuf, 2011). A meta-analysis by Lee and Kean found a 7-fold increase in risk for knee OA if a person's BMI was greater than 30 $kg/m^2$ (classified as obese) (Lee and Kean, 2012). Among adults diagnosed with OA, 46.7 percent were obese and 52.9 percent were morbidly obese between 2007 and 2009 (CDC, 2010). Weight loss of 10 percent or more has been shown to improve symptoms among those with OA (Gudbergsen et al., 2012). Overweight and obesity are thought to increase cartilage damage and misalignment of the knee joint as a result of increasing mechanical load on the knees as well as inflammation caused by adipose tissue (Gkretsi et al., 2011; Pottie, 2006; deBoer et al., 2012).

Increased adipose tissue, especially in the central region of the body, has been associated with knee and hip OA as well as increased loss of cartilage (Gkretsi et al., 2011). Adipose, a metabolic tissue that also stores fat, secretes adipokines such as adiponectin, resistin, leptin and may be responsible for the elevated expression of genes for IL-1β, and TNF-α, which have been observed in those with OA and those with an increased BMI (deBoer et al., 2012; Gkretsi et al., 2011; Hotamisligil et al., 2006; Iwata et al., 2013). Elevated adipokines have been correlated with damage of cartilage matrix as well as inflammation of the synovium (Gkretsi et al., 2011; deBoer et al., 2012).

A high fat diet (60 percent of total calories) was shown to increase occurrence of OA when compared to a low fat (10 percent of total calories) diet in type 2 diabetic mice without contribution of weight gain (Mooney et al, 2011). Further, Iwata et al., reported elevated levels of TNF-α, VEGF and TGF-β levels in the articular tissues of OA induced mice fed a high fat diet (32 percent fat versus 4.8 percent fat in the control diet) for 8 to 12 weeks (Iwata et al., 2013). Increased osteophyte size, adipocytes (ie. leptin), synovial cells, and vascularization were also seen in the tissue (Iwata et al., 2013).

Concern of a fat-based diet leading to OA and prostaglandin synthesis is derived from the knowledge that stimulation of the COX-2 pathway increases $PGE_2$ via oxidation of fatty acids, namely eicosanoids into arachadonic acid (Knott et al., 2011). Also elevated oxidized low-density lipoproteins (LDL) were found in OA patients, suggesting increased risk of LDL oxidation for stimulation of COX-2 pathway (Gkretsi et al., 2011). Triantaphyllidou et al., used apolipoprotein A-I and high-density lipoprotein (HDL)

knockout mice to determine if HDL was important in the prevention or development of OA. The researchers found that in those mice that did not have HDL and were on a western type diet of 42 percent energy from fat were more prone to obesity and development of OA than the control group suggesting a pro-inflammatory role of the western type diet as well as a protective role of HDL (Triantaphyllidou et al., 2013).

However, these studies on a high fat diet did not take into account the composition of the fat within the diet. The high fat diet pellets used by Mooney et al., consisted mainly of lard (90 percent), primarily composed of saturated fat, and a smaller proportion of soybean oil (10 percent), primarily composed of mono- and poly-unsaturated fat (Mooney et al., 2011; Ulman, 2011). The low fat diet pellets were primarily soybean oil (55 percent) with 45 percent of the fat content from lard (Mooney et al., 2011; SoyConnection, 2013; Ulman, 2011). Dean and Hansen, suggest after a review of the literature on diet and OA, that those who follow a western style diet are more prone to chronic inflammation and development of OA versus those that follow a plant-based diet. An example of the plant-based diet found to be ant-inflammatory is the Mediterranean diet is higher in polyunsaturated fats including foods such as: olives, fish, vegetables, nuts, and fruits (Dean and Hansen, 2012).

Polyunsaturated fats, specifically, omega-3 fatty acids (found in fish, olives, flax, algae and chia) have shown beneficial effects for those with OA especially when taken in combination with glucosamine and chondroitin supplementation (Knott et al., 2011; Hurst et al., 2010; Kantor et al., 2012). Kantor et al., found that this combination reduced

high sensitivity serum C-RP levels based on data from the National Health and Nutrition Examination Survey between 1999 and 2000 (Kantor et al., 2012). Supplementation of omega-3 fatty acids was found to decrease joint pain in a Cochrane Review meta-analysis, especially eicosapentaenoic acid (EPA) (Goldberg and Katz, 2007; Hurst et al., 2010).

In addition the fat soluble-vitamin, 25-hydroxyvitamin D (25-(OH) D), may also be involved in the structural health of cartilage as decreased serum levels are associated with increased OA progression especially with loss of cartilage volume (Cao et al., 2013). Salmon calcitonin also prevents type II collagen loss (Nielsen et al., 2011). Lactobacillus casei (L. casei), a probiotic, was given orally with type II collagen and glucosamine and was found to reduce pain, cartilage loss, and lymphocyte levels as well as several inflammatory cytokines including: IL-1β, IL-2, -6, -12, -17, and TNF-α, MMP-1, 3, -and -13, and IFN-y as well as movement of NF-kB into the nucleus of chondrocytes (So et al., 2011). L. casei also increased IL-4 and IL-10, promoting collagen deposition (So et al., 2011).

Foods such as ginger, curcumin, olives, acai, and whole grapes, which contain compounds known as phenol compounds (phenolic antioxidants and flavonoids) are shown to have anti-inflammatory properties (Bhatt K.P and Pezzuto J.M., 2002; Dean and Hansen, 2012; Zhou and Raffoul, 2012; Jensen et al., 2011) and may benefit cartilage health (Dean and Hansen, 2012). Vitamin C, also in ginger, acai, whole grapes and other citrus containing foods, is associated with prevention of knee OA (Peregoy et al., 2011).

However, it is the polyphenols, which are of interest in reducing inflammation and cartilage destruction in OA. Resveratrol, for example, is a natural bioflavonoid in the skin of most grape varieties has been studied for its many pharmacological properties in cardio-protection and chemoprevention in cancer (Gusman J et al., 2001; Zern et al., 2005; Bhatt, K.P and Pezzuto JM, 2002; Demas et al., 2005, Stocco et al., 2012).

**Grapes and Inflammation**

As mentioned previously, red grapes are one of the many whole foods, which contain a variety of anti-inflammatory compounds, polyphenols and flavonoids, primarily located in the grape seed and skin. In addition to glucose and fructose, red grapes have been found to contain the anthocyanins (delphinidin, cyanidin, petunidin, peonidin, and malvidin), which are responsible for the pigment of the fruit (Wu et al., 2006). Other components of grapes include catechin, epicatechin, quercetin, myricetin, cinnamates, tyrosol, tannins, resveratrol, gallic acid, caffeic acid, syringic acid, p-coumaric acid, and gerulic acid (Zhou and Raffoul, 2012).

Resveratrol (3,4', 5-trihydoxystilbene), found in grape skin and peanuts, has been demonstrated to be protective against cartilage damage, cancer, cardiovascular disease, and other inflammatory diseases (Kovacic and Somanathan, 2010; Zhou and Raffoul, 2012). Cartilage is an estrogen receptor (ER) positive tissue allowing resveratrol, which is similar in structure to estrogen, to target cartilage by binding to and activating ERα and ERβ (Delmas et al., 2005; Ghem et al, 1997; Gusman J et al., 2001; Zhou and Raffoul, 2012). Resveratrol, touted as an anti-oxidant and anti-inflammatory has been shown to

reduce reactive oxygen species (ROS) during inflammation and can inhibit the activity of COX-1 and COX-2 enzymes, cytochrome P450 complex, and quinone reductase protecting cells from destruction by stress, infection, and environmental toxins (Gusman et al., 2001, Korolkiewicz RP et al., 2003, Liu et al., 2010; Mobasheri et al., 2012).

In addition to inhibiting the expression of COX-2, resveratrol also inhibited the expression of iNOS in various animal studies and subsequent synthesis of NO and $PGE_2$ in articular chondrocytes via the inhibition of NF-kB by activating the silent information regulator, SIRT1 even in the presence of IL-1β (Liu et al., 2010; Ming et al., 2013; Wang et al., 2012). In a study of rabbits induced with OA, resveratrol protected knee cartilage from destruction by reducing apoptosis rates and synthesis of NO and proteoglycan in the cartilage in a dose dependent manner (Wang, et al., 2012). Liu et al. showed additional reduction in MMP-13 activity and protection of type II collagen by resveratrol from advance glyocogen end products (AGEs) (Liu et al., 2010).

Various *in vitro* studies of the response of human articular chondrocytes to resveratrol (dosages ranging between 10 to100 μM) resulted in decreased IL-1β induced pathways including: caspase-3 activation, COX-2 expression, apoptosis, MMP-3, MMP-9, MMP-13 expression, ROS production, p53, and decrease in type II collagen and aggrecan synthesis (Shakibaei et al., 2007; Shakibaei et al., 2008; Shen et al., 2012). *In vivo*, resveratrol appears to inhibit inflammation in human articular chondrocytes by inhibiting the NF-kB activation and translocation of NF-kB to the nucleus, both of which

are stimulated by IL-1β and TNF-α (Kovacic and Somanathan, 2010; Shakibaei et al., 2008; Shen et al., 2012).

Grapes also contain tannins, which may aid in free radical scavenging via linkages between (+) catechin and (-) epicatachin monomers, which are found primarily in grape seeds (Basly et al., 2000; Castillo et al., 2000; Zhou and Raffoul, 2012). Grape seed proanthocyanidin extract given to rats induced with knee OA reduced MMP13 and IL-1β as well as destruction to the bone and osteophyte formation (Woo et al., 2011). Delphinidin is also found in bilberry, blueberries, black current, eggplant, and black beans among other foods (Wu et al., 2006). Haseeb et al. showed that delphinidin reduced PGE production in human chondrocytes by inhibiting IL-1β induced COX-2 expression, and phosphorylation of IL-1 receptor associated kinase-1, and inhibited translocation of NF-kB/p65 into the nucleus (Hasseb et al., 2013). Malvidin was administered in the form of Malvidin-3-O-β glucoside to human peripheral blood mononuclear cells (PBMC) and rats with chronic adjuvant induced arthritis and was found to inhibit synthesis of TNFα, IL1, IL-6, and NO in human cells and reduced NO in the rat cells (Decendit et al., 2013).

Studies using antioxidants extracted from grape skin and pomace did not reduce oxidative stress in obese mice (Zhou and Raffoul, 2012), suggesting a role for the use of whole grapes in anti-inflammatory treatment. Few studies to date have examined the effect of whole grape on osteoarthritis in humans *in vivo*.

**Joint Mobility and Impact of Physical Activity**

Osteoarthritis is found to limit activity such as: bending, standing, and walking in 42.4 percent of those diagnosed with arthritis and is accelerated by chronic disease, such as obesity and heart disease (CDC, 2010; Ettinger et al., 1994). OA is not caused or accelerated by regular moderate physical activity, determined by numerous animal and human studies (Buckwalter and Lane, 1996; Helmark et al., 2012). Rather, moderate exercise has been shown to be an effective preventive and therapeutic strategy for increasing flexibility and reducing pain in those suffering from OA (Deyle et al, 2000; Dunlop et al, 2010; Helmark et al., 2012; Jordan et al, 2003). Strenuous activity may promote joint inflammation, however, as reported by Neidhardt et al., in a study of marathon runners showing an elevation of serum COMP levels following the marathon (Neidhardt et al., 2000).

Cartilage matrix synthesis is stimulated by regular use of or loading of a joint but a sedentary lifestyle or static joint loading may lead to degradation of the matrix and joint and increased symptoms (Helmark et al., 2012; Holla et al., 2012; Goldring, 2006; Lane, 1995; Verwij et al., 2009). In adults with knee OA, only thirty minutes of single loading of the knee decreased synovial fluid COMP levels, but not serum levels (Helmark et al., 2012). It should be noted that this study looked at acute changes in serum levels directly after exercise (Helmark et al., 2012). After eleven weeks of structured exercise intervention, however, reduced serum COMP levels were observed in knee OA

participants as compared to a control group, who did not exercise and experienced an increase in serum COMP levels (Hunt et al., 2013).

A review of various exercise regimens by Brakke et al. found that strength training, aquatics, balance and perturbation therapies under close supervision of a personal trainer/therapist are most beneficial to those with OA (Brakke et al, 2012). Other studies have concluded that both structured regimens, including aerobic and resistance training, as well as lifestyle activities such as walking, bending up and down, etc. are beneficial to improved outcomes in those with knee OA. (Deyle et al., 2000; Dunlop et al, 2010; Rutjes et al, 2010, Ritjes et al, 2009; van Baar et al, 2001). However, lack of mobility as a result of pain from OA or lack of muscle strength associated with aging can make this a daunting task and may increase OA progression (Andriacchi et al., 2004; Holla et al., 2012).

Although exercise is encouraged in the management of OA symptoms, several Cochrane Reviews have found the effects of exercise to be short-lived (van Baar et al, 2001; Rutjes et al, 2010; Ritjes et al, 2009). In women with knee OA, acute isokintetic exercise produced mild inflammation (Germanou et al., 2012) and after a six month trial of high-intensity resistance training symptoms of OA were reduced in women, but not first peak knee or hip adduction compared to placebo (Foroughi et al., 2011). Though exercise is still considered beneficial and recommended as a preventive and first-line treatment for OA, additional strategies, such as dietary supplementation, which may manage inflammation in OA may be indicated.

CHAPTER III

# Methodology

## Study Design

A randomized double-blinded placebo controlled clinical trial was conducted over a four month time period. A total of 72 mobile adult volunteers, between the ages of 45 and 79, with self-reported mild to moderate degree of knee OA who met the inclusion criteria and provided consent to participate were included. Participants were randomly divided into two treatment groups. Group 1 (n=35) was given 47 g freeze-dried grape powder (FDGP) made from whole grapes and Group A (n=37) was given 47 g of a placebo treatment, as a control. The placebo powder consisted of equal parts fructose and dextrose, equivalent to FDGP in appearance and calorie content. Each group was informed to take 1 package of the treatment or placebo powder orally mixed with water or other liquid daily for 4 months.

Participants were asked to report physical activity levels at baseline and final visits (4 months). Serum samples were taken and bilateral knee range of motion was measured at baseline and final visits (4 months) as well. Participants were given a calendar on which to report missed days of treatment as well as days during which a pain reliever was taken. Serum samples were then analyzed for levels of IL-1β and COMP.

**Participant Recruitment for Study**

Participants were recruited through Texas Woman's University and orthopedic practices and clinics in Denton and the Dallas-Fort Worth Metroplex and community at large (Appendix B). To be included in the study participant were between the ages of 45 and 79 with self-reported mild to moderate knee OA. Exclusion criteria included: past medical history of severe liver, kidney, or other chronic or acute disease that affected their conditions and ability participate in this study. Ethnicity, gender, and race were not included in exclusion criteria.

The participants agreed to abstain from initiation of new medications known to influence OA and were not on prescribed medications classified as COX-2 inhibitors. Participants also agreed to avoid taking supplements including: chondroitin sulfate, glucosamine sulfate, glucosamine hydrochloride powder, grape powder or grape seed extracts. Subjects who wished to participate and reported taking these supplements were required to discontinue the supplements and undergo a one-month washout period prior to beginning this study. The Institutional Review Board at Texas Woman's University approved this study (Appendix A).

**Treatment**

The treatment group received 47 grams of freeze-dried grape powder (FDGP) (equal to 2 cups of whole grapes) to be consumed by mouth daily for 4 months (provided and by the California Table Grape Commission). Each package of FDGP contained approximately 70 μg of resveratrol, also determined to be in 2 cups of whole grapes. The

control group received a 47 g of a placebo containing an equal ratio of fructose and dextrose, which has a similar weight, appearance and energy content as FDGP (provided and by the California Table Grape Commission). The placebo was also to be consumed by mouth daily four 4 months. The phenolic and nutrient contents of the FDGP and placebo were standardized by the California Table Grape Commission. Each group was given enough treatment or placebo to consume 1 package orally, mixed with water or other liquid, daily for up to 4 months. Participants were instructed to store treatment and placebo powders in the freezer until use to retain patency of the powders.

**Physical Activity Assessment**

Physical activity levels were assessed for both groups by comparing participant responses to a physical activity questionnaire at baseline to responses at the final visit (4 months). The physical activity questionnaire (Appendix C) used in our study was modified from the Five City Project (Sallis, et al. 1985). This tool has been validated for assessment of participant self-reported level of physical activity (Blair, et al, 1985) and was used to evaluate whether the level of physical activity was positively impacted by the FDGP in comparison to placebo.

**Blood Collection and Analysis of Inflammatory Markers**

Fasting venous blood was taken from each participant once at baseline and once at the end of the treatment period (4 months) by a certified phlebotomist. Each participant was asked to fast for at least 8 hours prior to the blood draw. Blood specimens were centrifuged at 1500x g for 15 minutes, serum was aliquoted within 2 hours of collection and stored at -70°C until analysis.

Serum levels for each of the inflammatory and specific cartilage biomarkers (IL-1β and COMP) were compared between baseline and final visits to evaluate change for each participant. Degree of change in the levels of IL-1β and COMP for participants was also compared between placebo and treatment group. Enzyme-linked immunosorbant assay (ELISA) kits were used to measure levels of inflammatory markers in collected serum samples from participants at baseline and final visits. Human recombinant IL-1β ELISA kits were sourced from RayBio Tech (Norcross, GA) and the human COMP ELISA Kit from BioVendor (Chandler, NC). The rationale for using IL-1β as a marker of inflammation is in its perceived involvement in OA pathology, increased serum levels suggest increased inflammation in the body. In order to locate the source of inflammation, serum COMP was measured as this cartilage protein is released into the blood after mechanical or chronic inflammatory insult to the cartilage of joints occurs (Calich et al, 2010; Dahesia et al., 2008; Goldring, 2010; Haudenschild et al., 2011).

**Statistical Analysis**

The sample size used in this study (72 adults) was adequate to permit detection of a difference between the treatment groups related to mobility with 0.87 power at significance level (alpha) = 0.05, allowing for 12-15% attrition rate over a 4 month treatment period for detecting changes in mobility.

Prior to conducting analyses, missing data points were computed utilizing an intent to treat (ITT) maximum likelihood estimation. Missing data points were only replaced if the majority of baseline measures were obtained for a given set of variables. All analyses were tested on both the raw data and the ITT data set.

In order to test for the main effect of time, group, gender, and age, a series of repeated measures analysis of variance (ANOVA) tests were conducted. Furthermore, in order to test the interaction effects of said independent variables, a series of factorial repeated measures ANOVAs were calculated. In order to detect where significant differences were, data was split by key variable combinations, and further ANOVAs were conducted (i.e., intervention group by gender, age by gender, etc.). Lastly, due to limited sample size and slight violations of normality, all analyses were confirmed using non-parametric equivalencies (i.e., Kruskal-Wallis and Wilcoxon's sign rank tests). All analyses were conducted using SPSS v. 19, data was reported as mean $\pm$ standard deviation, and significance was at the .05 level.

## Outcome Measurements

**Outcome Measure 1.** Changes in physical activity levels and mobility between FDGP and placebo groups were assessed by comparing levels of self-reported physical activity at baseline and at the end of the study (4 months) on the modified Five-City Project physical activity assessment questionnaire.

**Outcome Measure 2.** Fasting venous blood was collected at baseline and at the end of the study to assess changes in levels of IL-1β and COMP levels in the blood at baseline and at 4 months between FDGP and placebo groups.

# CHAPTER IV

## AGE-ASSOCIATED EFFECT OF FREEZE-DRIED GRAPE POWDER ON INFLAMMATORY MARKERS AND PHYSICAL ACTIVITY IN ADULTS WITH KNEE OSTEOARTHRITIS

To be submitted to *The Journal of Applied Gerontology*

Authors: Rebecca M. Small B.A., RDN, LD; Shanil Juma, PhD; Vicorine Imrhan, PhD, RD, LD; Rene Paulson, PhD; Casey Tiernen MS, RD, LD; and Parakat Vijayagopal, PhD

Departments of Nutrition and Food Sciences, Kinesiology, and Office of Research and Sponsored Programs, Texas Woman's University, Denton, TX

### Abstract

Osteoarthritis (OA) is the most common joint disease among US adults, leading to pain and disability. Treatment of OA is focused on symptom management since no cure has been found, and may involve expensive procedures including joint replacement and may lead many to seek natural dietary approaches. Grapes, rich in anti-inflammatory polyphenols may aid in management of OA symptoms. The purpose of this study was to assess the effect of daily consumption of freeze-dried grape powder on physical activity, and biochemical markers of inflammation and cartilage metabolism in individuals with self-reported knee osteoarthritis. A total of 72 men and women with knee OA were recruited and randomized into 2 groups. Group 1 (n=28, 21 female and 7 males) consumed 47 grams of freeze-dried grape powder (FDGP) daily for four months. Group 2 (n=28, 21 female and 7 males) consumed 47 grams of a comparable placebo. Serum specimens and self-reported physical activity were obtained at baseline and at four months. There was a significant decrease in very hard activity in the placebo group.

However, an increase in moderate activity was seen for those ≤64 yr. in the placebo group. Participants ≥65 yr. reported a significant decline in moderate and hard activities ($P<.05$) in both groups from baseline to final. A statistically significant increase in interleukin 1-beta (IL-1β) was observed in both groups, males greater than females. A greater increase occurred in the placebo group (637.33%) vs. FDGP (194.64%). Levels of IL-1β and cartilage oligomeric protein (COMP) increased more in those 65 years and older across both genders. In regards to cartilage turnover, both groups had a statistically significant increase in COMP (154.2% FDGP vs. 172.27% placebo). We showed that age has a significant impact on physical activity levels, inflammation, and cartilage metabolism in people with knee OA. FDGP supplementation may decrease age-related inflammation, decline in physical activity and cartilage matrix breakdown in those with OA.

## Age-Associated Effect of Freeze-Dried Grape Powder on Inflammatory Markers and Physical Activity in Adults with Self-Reported Knee Osteoarthritis

Osteoarthritis (OA) is the most common joint disease occurring among one-in-five US adults and may reduce mobility as a result of joint disfiguration and pain (CDC, 2010). OA is among the leading causes of disability among US adults and leads to decreased health-related quality of life as well as increased mortality rates and may play a role in reduced physical activity among one-third of US adults (CDC, 2010; Dunlop et al., 2011; Hochberg, 2008; Hoogeboom, 2013; Murphy, 2012; White et al., 2013).

Current treatment is aimed at symptom management, as a cure for OA is still to be discovered, leading many to resort to surgery to replace the affected joint(s). (Ringdahl, 2011; Sinusas, 2012). Treatment of arthritis costs about 128 billion dollars annually, 4,741 dollars per patient, with 5 percent of healthcare costs incurred by joint-replacements (CDC, 2010; Helmick et al, 2008, London et al, 2010). Non-pharmacologic treatment options may include: physical therapy, weight loss, walking aids, and insoles (Conaghan et al. 2008; Sinusas, 2012). Medications used to reduce inflammation and pain in OA include: Glucosamine and Chondroitin supplementation, non-steroidal anti-inflammatory (NSAIDs) medications (including COX-2 inhibitors) hyaluronic acid injections, and analgesics such as capsaicin cream and opiates (Sinusas, 2012). These medications, however, have negative side effects on the kidneys, gastrointestinal tract, and platelet aggregation, and have been shown to increase inflammatory cytokines in those with OA (Conaghan et al., 2008; Renda et al., 2006; Schumacher et al., 1996).

Symptoms and occurrence of OA increase significantly with BMI and metabolic syndrome (CDC, 2010; Dunlop, 2010; Elbaz, 2011; Engstrom et al., 2009). The overall lifetime risk of symptomatic knee OA increases to two in three in among adults who are obese compared to those who are of normal weight (Murphy, 2012; Yusuf, 2011). Overweight and obesity are thought to increase cartilage damage and misalignment of the knee joint as a result of increasing mechanical load on the knees as well as inflammation caused by adipose tissue (Gkretsi et al., 2011; Pottie, 2006; deBoer et al., 2012).

Inflammation is thought to play an integral a role in OA progression. The cyclooxygenase-2 (COX-2) pathway, part of the OA inflammatory process, has become a target of many drug therapies (Conaghan et al., 2008; Ou et al., 2012). Researchers have observed elevated serum levels of inflammatory cytokines and proteins including: interleukin -1 beta (IL-1β) and cartilage oligomeric matrix protein (COMP) among those with OA and are also involved in activation of the COX-2 pathway (Atkas et al., 2012; Denoble et al., 2010; Li et al., 2011; Marcu et al., 2010; Shahi et al., 2012; Sun et al., 2011; Verma & Dalal 2013; Zivanovic et al., 2011). Andriacchi et al. describe OA pathology in two phases, an initiation phase, stimulated by mechanical changes in the joint causing a shift in loading to an area of the joint not fit to bear that load. This is followed by the progression phase, in which metabolic pathways induce an inflammatory response, favoring catabolism of the extracellular matrix and adaptation of the subchondral bone (osteophyte formation) (Andriacchi et al. 2004; Goldring et al., 2010; Heijink et al., 2012).

Red grapes contain plant compounds known as polyphenols shown to reduce inflammation in OA and in other chronic diseases (Kovacic and Somanathan, 2010; Zhou and Raffoul, 2012). Resveratrol, one compound found in red grapes, has been shown to reduce reactive oxygen species (ROS) during inflammation and can inhibit the activity of COX-1 and COX-2 enzymes, cytochrome P450 complex, and quinone reductase protecting cells from destruction by stress, infection, and environmental toxins (Korolkiewicz RP et al., 2003, Liu et al., 2010; Mobasheri et al., 2012). In several animal

studies, resveratrol was shown to protect knee cartilage from inflammatory cytokines known to promote OA progression including IL-1β induced pathways (Liu et al., 2010; Shakibaei et al., 2007 & 2008; Shen et al., 2012; Wang, et al., 2012). Delphinidin reduced PGE production in human chondrocytes by inhibiting IL-1β induced COX-2 expression (Hasseb et al., 2013). Studies using antioxidants extracted from grape skin and pomace have have not shown a reduction in oxidative stress in obese mice (Zhou and Raffoul, 2012), suggesting a role for the use of whole grapes in anti-inflammatory treatment. Few studies to date have examined the effect of whole grape on osteoarthritis in humans *in vivo*.

Osteoarthritis is not caused or accelerated by regular moderate physical activity (Helmark et al., 2012). Instead, moderate exercise has been shown to be an effective strategy for increasing flexibility and reducing pain in those suffering from OA as regular use of a joint stimulates cartilage matrix synthesis. Whereas strenuous activity or a sedentary lifestyle may promote joint inflammation, degradation, and increase serum COMP levels. (Dunlop et al, 2010; Goldring, 2006; Helmark et al., 2012; Holla et al., 2012; Verwij et al., 2009). However, lack of mobility as a result of pain from OA or lack of muscle strength associated with aging can make exercise a daunting task (Andriacchi et al., 2004; Holla et al., 2012). In addition, several Cochrane Reviews have found the effects of exercise to be short-lived, indicating a need for additional strategies, such as dietary supplementation, to reduce overall inflammation (van Baar et al, 2001; Rutjes et al, 2009 & 2010).

Inhibiting the inflammatory response and subsequent metabolic pathways induced by chondrocytes is a target of drug and nutraceutical therapies for OA. Since elevated levels of COMP and IL-1β may be indicative of cartilage degradation as seen in OA, they were chosen for this study to measure inflammation of the knee before and after treatment versus placebo (Calich et al, 2010; Daheshia, 2008; Hunter et al, 2007). Therefore, the purpose of this study was to assess the effect of grape consumption on physical activity, biochemical markers of inflammation (IL-1β) and cartilage metabolism (COMP) in people with OA.

## Methodology

### Study Design

A randomized double-blinded placebo controlled clinical trial was conducted over a four month time period. A total of 72 mobile adult volunteers with self-reported mild to moderate degree of knee OA who met the inclusion criteria and provided consent to participate were included. Participants were randomly divided into two treatment groups. Group 1 (n=35) was given 47 g freeze-dried grape powder (FDGP) made from whole grapes and Group A (n=37) was given 47 g of a placebo treatment, as a control. The placebo powder consisted of equal parts fructose and dextrose, equivalent to FDGP in appearance and calorie content. Each group was informed to take 1 package of the treatment or placebo powder orally mixed with water or other liquid daily for 4 months.

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The participants agreed to abstain from initiation of new medications known to influence OA and were not on prescribed medications classified as COX-2 inhibitors. Participants also agreed to avoid taking supplements including: chondroitin sulfate, glucosamine sulfate, glucosamine hydrochloride powder, grape powder or grape seed extracts. Subjects who wished to participate and reported taking these supplements were required to discontinue the supplements and undergo a one-month washout period prior to beginning this study. The Institutional Review Board at Texas Woman's University approved this study (Appendix A).

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Serum levels for each of the inflammatory and specific cartilage biomarkers (IL1-β and COMP) were compared between baseline and final visits to evaluate change for each participant. Degree of change in the levels of IL-1β and COMP for participants was also compared between placebo and treatment group. Enzyme-linked immunosorbant assay (ELISA) kits were used to measure levels of inflammatory markers in collected serum samples from participants at baseline and final visits. Human recombinant IL-1β ELISA kits were sourced from RayBio Tech (Norcross, GA) and the human COMP ELISA Kit from BioVendor (Chandler, NC). The rationale for using IL-1β as a marker of inflammation is in its perceived involvement in OA pathology, increased serum levels suggest increased inflammation in the body. In order to locate the source of inflammation, serum COMP was measured as this cartilage protein is released into the blood after mechanical or chronic inflammatory insult to the cartilage of joints occurs (Calich et al, 2010; Dahesia et al., 2008; Goldring, 2010; Haudenschild et al., 2011).

**Statistical Analysis**

The sample size used in this study (72 adults) was adequate to permit detection of a difference between the treatment groups related to mobility with 0.87 power at significance level (alpha) = 0.05, allowing for 12-15% attrition rate over a 4 month treatment period for detecting changes in mobility.

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## Results

### Demographics of Study Participants

Seventy-two men and women adults (55 female, 17 males) of those recruited for this study met the inclusion criteria and consented to participate. Final participation after four months was 56 (42 female, and 14 males) with a drop out rate of 22.22 % (Table 1.1). The average age of participants was 59.8 years and total time treatment/placebo consumption averaged 16.7 weeks (Figure 1.1, 1.2). Average body mass index (BMI) of the study participants was 30.30 kg/m$^2$ (Table 1.2). Those who dropped out of the study reported dislike of taste and texture of product, increase in gas after initiation of study, travel/time constraints, knee surgery, or for unspecified reasons.

Table 1.1

*Demographics of the Study Participants*

| | Number of Participants Baseline | Number of Participants Final | Drop-out Rate | Average Weeks Treatment | Average Age |
|---|---|---|---|---|---|
| Placebo | 37 | 28 | 24.32% | 16.8 | 61.1 |
| FDGP | 35 | 28 | 20.00% | 16.6 | 58.5 |
| Overall | 72 | 56 | 22.22% | 16.7 | 59.8 |

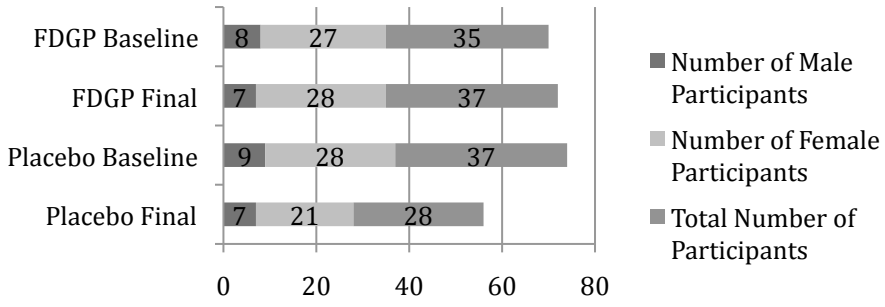


*Figure 1.1.* Demographics of the Study Participants.

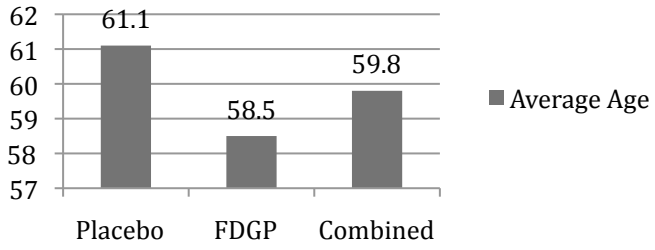


*Figure 1.2.* Average Age of Study Participants.

Table 1.2.

*Average Body Mass Index (BMI) in kg/m$^2$ of Study Participants*

| **FDGP & Placebo Combined** | | | |
|---|---|---|---|
| | *n* | *M* | *SD* |
| Baseline | 56 | 30.47 | 6.88 |
| Final | 56 | 30.30 | 6.50 |

| | **FDGP** | | | **Placebo** | | |
|---|---|---|---|---|---|---|
| | *n* | *M* | *SD* | *n* | *M* | *SD* |
| Baseline | 28 | 31.62 | 7.41 | 28 | 29.31 | 6.22 |
| Final | 28 | 31.11 | 6.90 | 28 | 29.49 | 6.08 |
| | **Female** | | | **Male** | | |
| | *n* | *M* | *SD* | *n* | *M* | *SD* |
| Baseline | 42 | 31.58 | 7.12 | 14 | 27.13 | 4.91 |
| Final | 42 | 31.40 | 6.74 | 14 | 27.01 | 4.44 |
| | **≤ 64 years** | | | **≥ 65 years** | | |
| | *n* | *M* | *SD* | *n* | *M* | *SD* |
| Baseline | 35 | 31.61 | 6.94 | 21 | 28.56 | 6.49 |
| Final | 35 | 31.82 | 6.83 | 21 | 27.76 | 5.1 |

**Effect of FDGP vs. Placebo Consumption on Physical Activity**

Participants were asked to report the number of hours slept on average per night at baseline and final appointments. Both the FDGP and placebo groups reported fewer hours of sleep at the end of the study than at baseline; a greater decline occurred in the placebo group (Fig. 2.1). Physical activity declined significantly in hard activity for both groups (FDGP -55.95%, placebo -52.22%) (Fig. 2.1). Moderate activity increased in the placebo group by 75.22% vs. 18.83% decrease in the FDGP group (Fig. 2.1). A decrease in very hard activity occurred from baseline to final in both the placebo and FDGP groups, with a greater change occurring in the placebo group (Fig. 2.1).

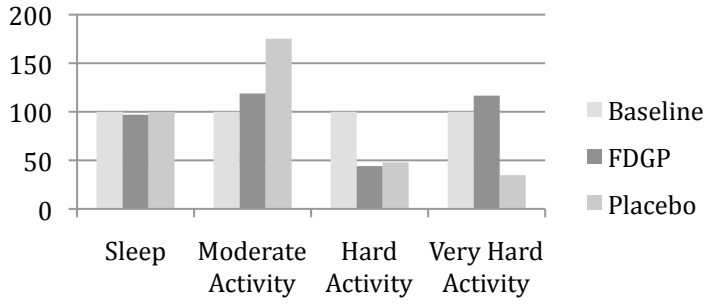


*Figure 2.1.* Percent Change in Physical Activity and Sleep between Placebo and FDGP.

Males experienced a significant decline in moderate activity from baseline to final visits (Fig. 2.2). Both males and females experienced a significant decline in hard activity from baseline to final visits; however, males reported a greater decline than females. The greatest decline occurred in males in the FDGP group, whereas females in the FDGP group experienced the smallest decline (Fig. 2.2).

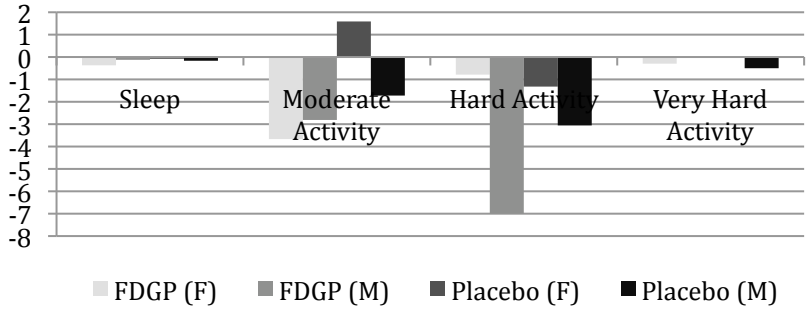


*Figure 2.2.* Difference in Physical Activity Levels and Sleep between Placebo and FDGP by Gender.

Participants ≤ 64 years experienced less of a decline in moderate activity (-0.1944) than those ≥ 65 years of age (-2.5) (Fig. 2.2). Both age groups reported a decline in hard activity, with those ≥ 65 years of age experiencing a greater decline (Fig. 2.3). Participants ≤64 yr. in the placebo group showed an increase in moderate activities compared to those ≥ 65 yr. in the same group (3.772 vs.-2.25). Whereas, those in both age groups in the FDGP group experienced decline in moderate and hard activities (Fig. 2.3). Those ≥ 65 yr. in the placebo group experienced the greatest decline in hard activity (Fig. 2.3).

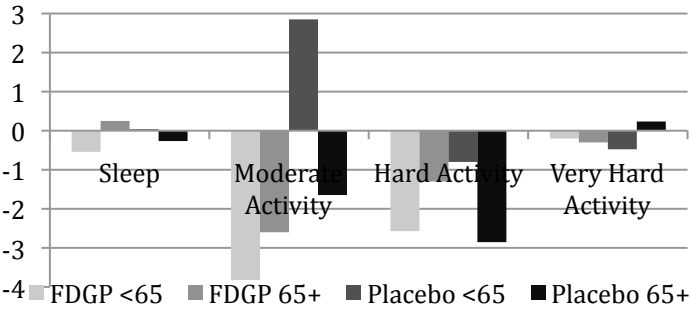


*Figure 2.3.* Difference in Physical Activity Levels and Sleep between Placebo and FDGP by Age.

Participants were asked to report in minutes per day and days per week, how often they participated in a variety of activities. After four months the FDGP group reported significant increases in power walking but decreased treadmill use. The placebo group reported significantly increased walking and weight aerobics in days per week (Fig. 2.4).

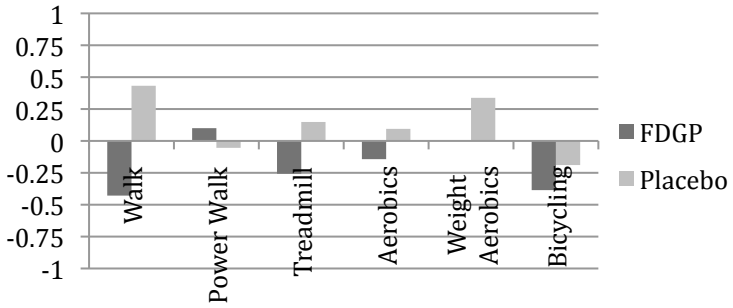


*Figure 2.4.* Difference in Physical Activity Levels (Days/Week) between Placebo and FDGP

Men reported the greatest decline in reported activities. In the FDGP and placebo groups men reported a significant decline in walking but an increase in weight aerobics (Fig. 2.5). In the FDGP group, men also reported a significant decrease in bicycling (Fig. 2.6). Females did not report significant change in these activities from baseline to final (Fig. 2.5, 2.6).

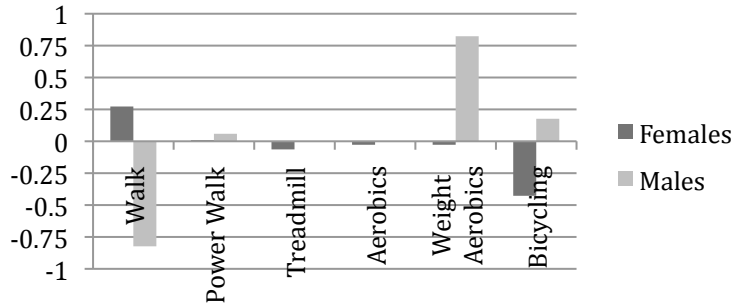


*Figure 2.5.* Difference in Physical Activity Levels (Days/Week) between Placebo and FDGP by Gender.

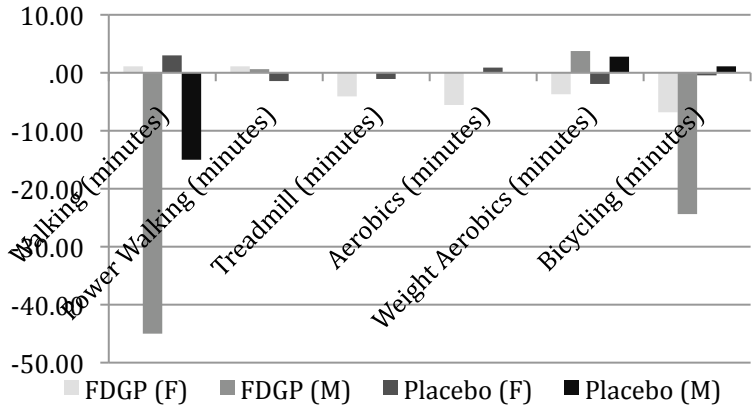


*Figure 2.6.* Difference Physical Activity Levels (Minutes/Day) between Placebo and FDGP by Gender.

Participants $\leq$ 64 reported a significant increase in aerobics and treadmill use from baseline to final (Fig. 2.7). Those in the placebo group < 64 years experienced a significant increase in treadmill use, and those in the same group >65 years reported more time in aerobics at the end of the study than those in the FDGP group (Fig. 2.7)

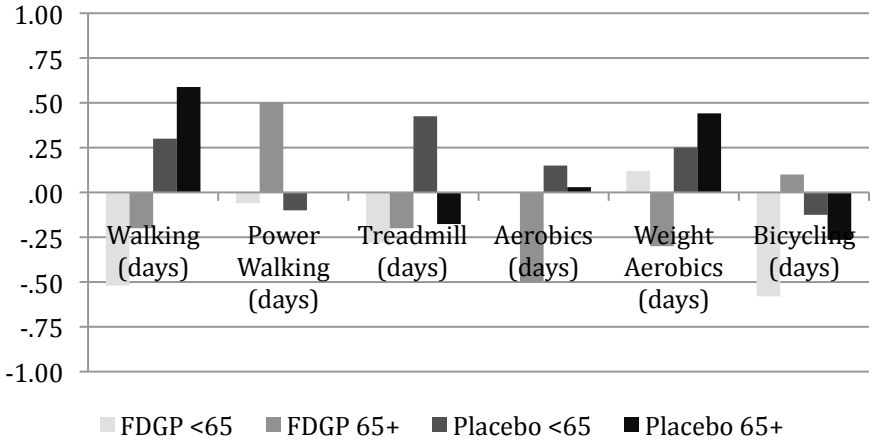


*Figure 2.7.* Difference in Physical Activity Levels (Days/Week) between Placebo and FDGP by Age.

**Effect of FDGP vs. Placebo Consumption on Serum IL-1β**

A statistically significant increase in serum IL-1β was observed in both groups compared to final visits (Table 2.1, Fig. 3.1). However, the placebo group experienced a greater increase in levels than FDGP (637.33% vs. 194.64%) (Table 2.2, Fig. 3.1).

Table 2.1.

*Overall Average Serum IL-1β (pg/ml) Baseline to Final*

| | *n* | *M* | *SD* |
|---|---|---|---|
| Baseline | 69 | 13.12 | 21.11 |
| Final | 69 | 31.07 | 55.89 |

Table 2.2.

*Average Serum IL-1β (pg/ml) Baseline to Final, FDGP and Placebo*

| | | **FDGP** | |
|---|---|---|---|
| | *n* | *M* | *SD* |
| Baseline | 35 | 11.80 | 22.44 |
| Final | 35 | 29.90 | 43.98 |
| | | **Placebo** | |
| Baseline | 34 | 14.49 | 19.88 |
| Final | 34 | 32.27 | 66.63 |

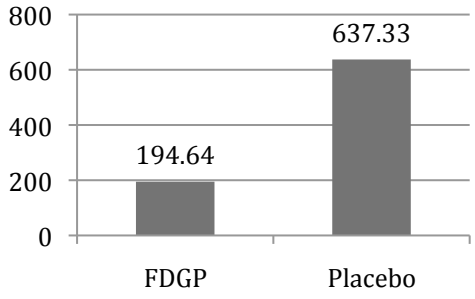


*Figure 3.1.* Percent change in Serum IL-1β between Placebo and FDGP.

Males and females in both groups experienced a statistically significant increase in serum IL-1β compared to final visits. When assessed by gender per group, the greatest increase occurred in the placebo group for males (Figure 3.2). Males in the FDGP group did not experience a significant change in serum IL-1β compared to placebo (1.5439 vs. 42.8525) (Figure 3.3).

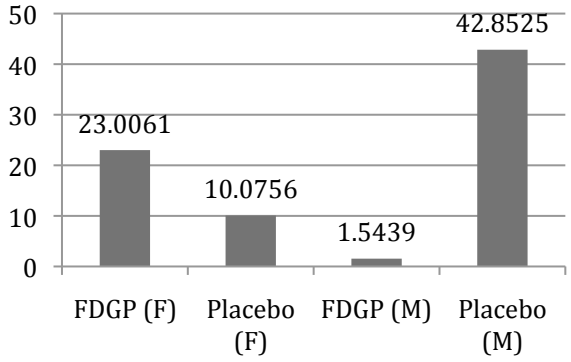


*Figure 3.2.* Change in Serum IL-1β between Placebo and FDGP by Gender.

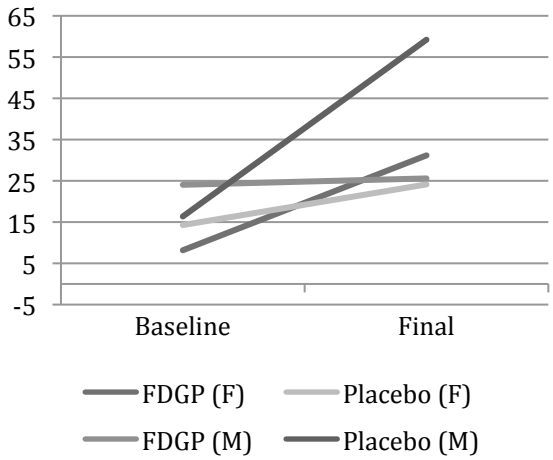


*Figure 3.3.* Serum IL-1β between Placebo and FDGP by Gender.

Participants ≥ 65 years of age had significant increase in serum IL-1β levels at final compared to baseline (Fig. 3.4). Those ≥ 65 years of age in both the FDGP and placebo groups experienced a significant increase in serum IL-1β at final compared to baseline (Fig. 3.4). Participants ≤ 64 years of age in the FDGP group experienced a significant increase serum IL-1β from baseline, whereas those in the placebo group did not (Fig. 3.4).

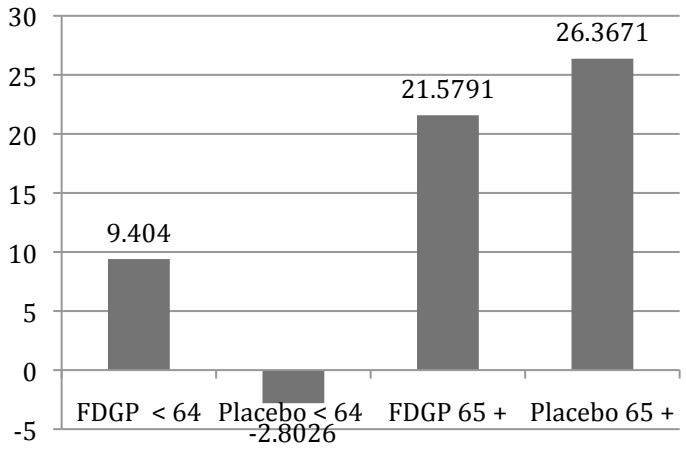


*Figure 3.4.* Change in Serum IL-1β between Placebo and FDGP by Age.

**Effect of FDGP vs. Placebo Consumption on Serum COMP**

Serum COMP in the FDGP and placebo groups both increased significantly from baseline to final visits (Table 3.1, 3.2, Fig. 4.1).

Table 3.1.

*Overall Average Serum COMP (ng/ml) Baseline to Final*

| | *n* | *M* | *SD* |
|---|---|---|---|
| Baseline | 69 | 825.05 | 360.84 |
| Final | 69 | 1119.31 | 494.47 |

Table 3.2.

*Average Serum COMP (ng/ml) Baseline to Final, FDGP and Placebo*

| | | | |
|---|---|---|---|
| | **FDGP** | | |
| | *n* | *M* | *SD* |
| Baseline | 35 | 805.72 | 348.23 |
| Final | 35 | 1120.03 | 524.18 |
| | **Placebo** | | |
| Baseline | 34 | 844.95 | 377.57 |
| Final | 34 | 1118.57 | 469.82 |

Males and females in both groups experienced a significant increased in serum COMP from baseline to final visits (Figure 4.2). Females in the FDGP group increased more than those in the placebo group, but the opposite occurred in males, though not statistically significant.

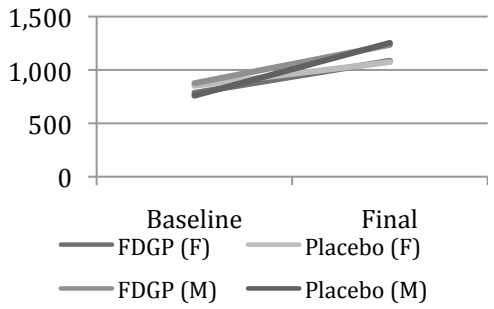


*Figure 4.1.* Serum COMP between Placebo and FDGP by Gender.

Participants who were ≤ 64 years of age (B: 712.75 vs. F: 822.18 ng/ml) and ≥ 65 years (B: 860.95 vs. F: 1242.29 ng/ml) had a significant increase in serum COMP from baseline to final visits (Fig. 3.3). Levels of serum COMP increased more in those ≥ 65 years in both groups than those ≤ 64 years (160.99% vs. 137.01%), however, the difference was not statistically significant (Fig. 4.3).

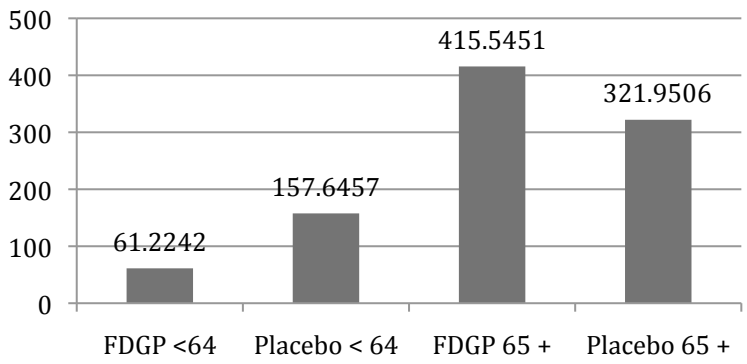


*Figure 4.2.* Serum COMP between Placebo and FDGP by Age.

## Discussion

Red grapes contain a variety of anti-inflammatory compounds including resveratrol, primarily located in the grape seed and skin (Zhou and Raffoul, 2012). Resveratrol, shown to reduce reactive oxygen species (ROS) during inflammation and to inhibit COX-1 and COX-2 enzyme activity, may play a role in the protection of cells from stress (Korolkiewicz RP et al., 2003, Liu et al., 2010; Mobasheri et al., 2012). The dosage (70 μg resveratrol) used in our study was consistent with that used in other studies (10 to 100 μg resveratrol) (Shakibaei et al., 2007 & 2008; Shen et al., 2012).

Moderate exercise has been shown to be an effective strategy for increasing flexibility and reducing pain in those suffering from OA, while strenuous activities may promote joint inflammation (Dunlop et al, 2010; Helmark et al., 2012). Few studies have been performed in humans to observe the effect of whole grape consumption on physical activity in adults with osteoarthritis. In our study, physical activity declined in the FDGP group from baseline to four months.

Due to budget restraints, the method used in our study for retrieving baseline and final levels of physical activity depended upon retrospective information provided by each participant and may have left room for memory recall error (Ayhan & Isiksal, 2005). In future studies, improved accuracy to measure changes in levels of physical activity may include a daily activity log, pedometer, or a physical activity test measured at baseline and final visits (Dunn-Lewis et al., 2011; Dunlop et al., 2010; Germanou et al., 2012).

In a randomized double-blind, placebo-controlled study, women improved in balance, while men performed better in a vertical jump and handgrip strength test after a 28 day crossover trial of a multi-nutrient supplement that included grape (Dunn-Lewis et al., 2011). This is consistent with reported increases in weight aerobics in men from both groups in our study. Both age and female gender have been associated with decreased physical activity as well as increased severity of symptomatic OA (Dunlop et al., 2010; Elbaz et al., 2011), and may have played a role in the reduction of activity reported in our study by females. Age played a significant role in physical activity changes observed in our study. Those who were $\leq$ 64 years reported increases in several activities regardless of treatment group, whereas those $\geq$ 65 years of age reported decline in most levels of physical activity. However, FDGP in those $\geq$ 65 years appeared to reduce the amount of decline in hard activity that was seen in the placebo group.

Symptoms and occurrence of OA increase significantly with BMI and metabolic syndrome (CDC, 2010; Dunlop, 2010; Elbaz, 2011; Engstrom et al., 2009). As the average BMI of the study participants was 30.30 $kg/m^2$ it may be expected that increased symptoms and inflammation associated with OA may be observed. Overweight and obesity (BMI > 25 $kg/m^2$) are thought to increase cartilage damage and misalignment of the knee joint as a result of increasing mechanical load on the knees and inflammation (deBoer et al., 2012; Gkretsi et al., 2011; Lee and Kean, 2012; Pottie, 2006). Increased adipose tissue, a metabolic tissue that also stores fat, especially in the central region of the body, has been associated with knee and hip OA as well as increased loss of joint cartilage (Gkretsi et al., 2011). Adipose tissue secretes adipokines such as adiponectin, resistin, leptin and may be responsible for the elevated expression of genes for IL-1β and TNF-α, inflammation of the synovium and cartilage matrix damage observed in those with OA and/or an increased BMI (deBoer et al., 2012; Gkretsi et al., 2011; Hotamisligil et al., 2006; Iwata et al., 2013).

A statistically significant increase in serum IL-1β was observed in both groups compared to final visits, however, those in the placebo group experienced a greater elevation than those in the FDGP group. In other studies, resveratrol was shown to inhibit the expression of COX-2 and the expression of iNOS, another marker of inflammation, in various animal studies and subsequent synthesis of NO and $PGE_2$ in articular chondrocytes via the inhibition of NF-kB even in the presence of IL-1β (Decendit et al., 2013; Liu et al., 2010; Ming et al., 2013; Wang et al., 2012). Ten *in vitro* and two *in vivo*

studies observing the effect of resveratrol on chondrocytes and in rabbits found that NF-kB activation and translocation, NO chondrocyte apopotosis, and cartilage degeneration were reduced as well as IL-1β induced pathways (COX-2 expression, apoptosis, and ROS production) (Shakibaei et al., 2007 & 2008; Shen et al., 2012).

In our study males and females in both groups experienced an increase in serum IL-1β compared to final visits (FDGP < placebo). Males in the FDGP group did not experience a significant change in serum IL-1β, whereas women in this group did. Our findings were consistent with the higher levels of IL-1β seen in women than in men reported by Lynch et al. (Lynch et al., 1994).

Age also appears to influence serum IL-1β as levels have been shown to increase with age as a result of increased inflammation from free radical oxidation and reduced T cell response (Fagiolo et al., 1993; Murray and Lynch, 1998;Wick et al., 1997). Previous increases observed in TNF-α in elderly versus younger people may reflect the elevated IL-1β seen in other studies as well as our own (Fagiolo et al., 1993). Participants in our study ≥ 65 years of age had significantly higher serum IL-1β levels at final compared to those ≤ 64 years regardless of treatment group and experienced a significant increase in serum IL-1β at final compared to those ≤ 64 years. However, those ≤ 64 in the FDGP group also experienced a significant serum IL-1β increase from baseline.

We found that serum COMP in the FDGP and placebo groups increased significantly from baseline to final visits. As serum IL-1β is known to mobilize matrix metalloproteases, it can be suggested that, in the presence of elevated levels of serum IL-1β, serum COMP might also be elevated (Shakibaei et al., 2007 & 2008; Shen et al., 2012). In two studies involving exercise in adults with knee OA synovial fluid COMP and serum COMP levels were reduced acutely after exercise (Helmark et al., 2012; Andersson et al., 2006). Our study observed long-term changes in physical activity.

Males and females, in our study, experienced a significant increase in serum COMP from baseline to final visits. Females in the FDGP group increased more than those in the placebo group, but the opposite occurred in males. Resveratrol, similar in structure to estrogen, is able to target cartilage (an estrogen receptor positive tissue) by binding to and activating ERα and ERβ. As a result varying levels of estrogen may interfere with resveratrol's ability to impact cartilage tissue directly (Delmas et al., 2005; Zhou and Raffoul, 2012). Further, Vilim et al., observed an increased release of COMP by osteoblasts in post-menopausal women in whom bone metabolism tends to be increased (Vilim et al., 2001).

Serum COMP increased more in those $\geq$ 65 years in both groups than those $\leq$ 64 years. Our findings were consistent with Clark et al.'s findings that people $\geq$65 years had significantly elevated serum COMP levels compared with those aged 45-64 (Clark et al., 1999). Correlation between age, OA severity, progression, and increased serum COMP observed in previous studies may result from increased collagen turnover due to elevated

oxidative stress observed in elderly adults (Andriacchi et al., 2004; Engelfriet et al., 2013; Holla et al., 2012). Among those ≤ 64 years in our study, serum COMP increased more in the placebo than in the FDGP group, suggesting a protective role for FDGP in those ≤ 64.

Additional sites of OA and severity are positively correlated with serum COMP (Clark et al., 1999). Measuring these parameters may have provided greater insight as to the effect of FDGP. Since symptoms and occurrence of OA increase significantly with BMI and metabolic syndrome (Dunlop, 2010; Elbaz, 2011; Engstrom et al., 2009), additional studies should be conducted to consider the effect of FDGP on knee OA in normal weight versus overweight and obese individuals as well as the role of varying dietary factors (Dean and Hansen, 2012; Kantor et al., 2012; Knott et al., 2011; Mooney et al, 2011; Peregoy et al., 2011).

## Conclusion

We showed that age has a significant impact on physical activity levels, inflammation, and cartilage metabolism in people with knee OA. FDGP supplementation may decrease age-related inflammation, decline in physical activity and cartilage matrix breakdown in those with OA.

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# CHAPTER V

## Summary, Conclusions, and Limitations

Osteoarthritis (OA) is the most common joint disease among US adults, leading to pain and disability. Treatment ranges from symptom management to joint replacement and may lead many to seek natural dietary approaches. Grapes, rich in anti-inflammatory polyphenols may aid in management of OA symptoms. Few *in vivo* studies to date have looked at the effect of whole grape consumption on physical activity levels in people with knee OA. The purpose of this study was to assess the effect of grape consumption on physical activity, biochemical markers of inflammation (IL-1β) and cartilage metabolism (COMP).

A decrease in moderate activity was observed in those ≤64 yr. in the FDGP group compared to placebo. Participants ≥65 yr., however, reported a significant decline in moderate and hard activities. A statistically significant increase in IL-1β was observed in both groups, males > females. A greater increase occurred in the placebo group vs. FDGP. Though both groups experienced a statistically significant increase in serum levels of IL-1β and COMP, a greater increase was observed in those ≥65 yr. We showed that age has a significant impact on physical activity levels, inflammation, and cartilage metabolism in people with knee OA. FDGP supplementation may decrease age-related inflammation, decline in physical activity and cartilage matrix breakdown in those with OA.

**Assumptions and Limitations**

The study was designed to recruit and randomize male and female adults with self-reported knee osteoarthritis. One limitation with recruitment and participation was the lack of gender balance with far fewer males in the study. This limits the applicability of our study findings to the general population. Radiographic assessment is recommended to determine degree of OA severity, however, due to limited funding and duration of treatment, we were unable to use this method of measuring change in knee pathology before and after treatment. Reliance on self-reported physical activity levels allows for subjective error among participants and may have resulted in inaccurate measures of baseline and final physical activity levels.

Daily consumption of treatment or placebo was based on participant compliance, as with other human studies. We were unable to find any other studies published using whole freeze-dried grape powder in a human study and so were unable to determine acceptability of the product prior to the study, resulting in dropout from our study. Extending the duration of treatment beyond four months and including a larger male representation would be recommended for future studies to determine acceptability of the product as well as long-term effectiveness.

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Appendix A

IRB Approval Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

**Institutional Review Board**
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378 FAX 940-898-4416
e-mail: IRB@twu.edu

December 19, 2013

Dr. Shanil Juma
Department of Nutrition & Food Sciences

Dear Dr. Juma:

*Re: Grape Consumption Improves Joint Mobility and Reduces Pain Associated with Knee Osteoarthritis (Protocol #: 16300)*

The request for an extension of your IRB approval for the above referenced study has been reviewed by the TWU Institutional Review Board (IRB) 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. 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 November 5, 2013. 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. If you have any questions, please contact the TWU IRB.

Sincerely,

Vicki L Zeigler

Dr. Vicki Zeigler, Co-Chair
Institutional Review Board - Denton

cc. Dr. Gay James, Department of Nutrition & Food Sciences

Appendix B

Participant Consent Form

Appendix C

**Texas Woman's University**
**Consent to Participate in Research**

Study Title: Grape Consumption Improves Joint Mobility and Reduces Pain Associated with Knee Osteoarthritis


| Investigators: | | | |
|---|---|---|---|
| | Shanil Juma, PhD | 940-898-2704 | sjuma@twu.edu |
| | Nancy DiMarco, PhD, RD | 940-898-2785 | ndimarco@twu.edu |
| | Young-Hoo Kwon | 940-898-2598 | ykwon@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 whole grape powder for 4 months will improve pain and flexibility associated with self-reported knee osteoarthritis. We will ask the following questions:

a) Will eating whole grape powder for 4 months improve joint flexibility?
b) Will eating whole grape powder reduce pain in the knee joint?

Research Procedures

For this study, we will ask you not to eat any food overnight (10 hours) and to appear the next day at a certain place in the university. A female researcher (for female participants) or a male researcher (for male participants) will take your height and weight 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).. 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 questionnaire regarding your food habits over the past week. You will complete a questionnaire regarding pain and flexibility. A measurement of knee motion will be done in a sitting position and repeated three times during this visit. You will be provided a two month supply of either the study treatment (grape powder) or a control (powder without grapes). At two months visit (midpoint), you will again be asked to complete a questionnaire regarding food intake, joint pain and flexibility. A repeated measurement of range of motion will also be done three times during the midpoint visit. You will get a 2 month supply of the food powder. At the end of the study (4 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. We will also ask you to complete a food frequency questionnaire regarding your food habits over the past week. You will complete a questionnaire regarding pain and flexibility. A measurement of knee motion will be done in a sitting position and repeated three times during this visit. At the end of the study, we will again take your height, weight, waist and hip measurements.

Time Commitment

The study period is 4 months. The study volunteer time commitment includes initial screening questionnaire (~10 min), consent form (25 minutes), pain, flexibility, and


Participant Initials _____

physical activty questionnaires (~30 minutes each during baseline, 2 months, and final), flexibility assessment (10 minutes each during baseline, 2 months, and final), anthropometrics-height and weight (5 minutes each during baseline, 2 months, and final), and blood draw (10 minutes each at baseline and final). Total time commitment for each participant is approximately 3 hours.

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 food powder. If you do not like the powder, there is no penalty for not eating it. You are free to quit the study at any time. Grape powder and powder without grapes is whole fruit that has been freeze-dried and has been 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. 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. 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 you are waiting. This will help you to overcome boredom and fatigue. 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.



Participant Initials _____

In addition to the risks above, you may experience anxiety or embarrassment related to height, weight and range of motion measurements. 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 male or female investigator in a private room.

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 the study powder for 4 months. You will also receive a cash incentive of $100.00, of which $50 will be paid at midpoint (60 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 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 C

Participant Recruitment Flyer and Email

TWU PORTAL RECRUITMENT EMAIL

Flyer and Email Version (includes time spent on study and loss of confidentiality statement)

Research volunteers needed! Do you have knee pain? If so you may be eligible to participate in a 4-month research study looking at effects of grape powder in improving joint function and reducing pain associated with knee osteoarthritis. If you are between 45-70 years old and otherwise healthy and mobile you may qualify. Participants will consume a grape powder or placebo for 4 months and undergo range of motion measurement, complete pain and physical activity questionnaires, and provide a blood specimen twice during the study. Estimated time commitment is 5 hours total including 3 visits to the study site. Benefits include nutrition and weight management education, body fat and composition assessment and $100 compensation for time spent on the study. If interested, please contact Dr. Shanil Juma at sjuma@twu.edu or 940-898-2704.

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

# Need Research Volunteers

# Do you have KNEE Pain?

- Are you between 45 – 79 years old
- Are you otherwise healthy and mobile
- Would you be willing to participate in a study where you may be asked to consume grape powder daily for 4 months

If you have answered **YES** to all of the above, then you may be eligible to participate in a 4 month research study to look at the beneficial effect of grape powder in improving joint function and reducing pain associated with knee osteoarthritis.

**Criteria** include meeting the requirements listed above and willing to consume either the grape powder or a similar powder without grapes for a period of 4 months. There will be blood draws at the start and at the end of the study. Pain and joint mobility will be assessed at start of study, midpoint, and end of study using questionnaires and range of motion measurement. The total time you need to spend for the study is 5 hours over 4 months involving 3 visits.

**Benefits** include: nutrition and weight management education, promotion of joint health, measurements of body fat, body composition, and range of motion. Upon completion, you will receive a compensated of $100 for your time

If interested, please email or call for more information:
Dr. Shanil Juma; Department of Nutrition and Food Sciences sjuma@twu.edu; 940-898-2704

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

Appendix D

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 ___ | | |
| 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: Grape Powder or Powder without Grape | | |

Appendix E

## PHYSICAL ACTIVITY QUESTIONNAIRE

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

1. On the average, how many hours did you sleep each night during the last five weekday nights (Sunday-Thursday)? _____hours

2. On the average, how many hours did you sleep each night last Friday and Saturday nights? _____hours

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

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

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

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

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

   *Please tell me to the nearest half-hour.* _____*hours*

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

   ____hours

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

or others like them?

Please tell me to the nearest half-hour. ____hours

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

   _____ hours

7. Now, let's look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour**.** (*Probe: Can you think of any other sports, job, or household activities that would fit into this category?*)

   _____ hours

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

   _____ hours

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

   _____ 1. More
   _____ 2. Less
   _____ 3. About the same

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

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

10. Are you engaged in any regular exercise?

Walk_____, Minutes per day_____, Days per week_____

Power Walk_____, Minutes per day_____, Days per week_____

Treadmill_____, Minutes per day_____, Days per week_____

Aerobics_____, Minutes per day_____, Days per week_____

Weight aerobics_____, Minutes per day_____, Days per week_____

Bicycling/Stationary bike_____, Minutes per day_____, Days per week_____

Tennis_____, Minutes per day_____, Days per week_____

Other, please specify:

_____, Minutes per day_____, Days per week_____

_____, Minutes per day_____, Days per week_____

_____, Minutes per day_____, Days per week_____

_____, Minutes per day_____, Days per week_____

## EXAMPLES OF ACTIVITIES IN EACH CATEGORY

***MODERATE ACTIVITY (3-5 METS)***

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

***HARD ACTIVITY (5.1 – 6.9 METS)***

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

***VERY HARD ACTIVITY (≥7.0 METS)***

| | |
|---|---|
| **Occupational Tasks:** | 1) Digging or chopping with heavy tools<br>2) Carrying heavy loads, such as bricks or lumber |
| **Sports** *(actual playing time)* | 1) Racket Sports: handball, racketball, squash, tennis<br>2) Soccer<br>3) Snow skiing (down hill and cross country)<br>4) Dancing: aerobics (high impact & intensity)<br>5) Jumping rope<br>6) Vigorous bicycling on hills<br>7) Jogging or running (≥8 mile/hr) |