GRAPE CONSUMPTION IMPROVES JOINT FLEXIBILITY AND REDUCES PAIN ASSOCIATED WITH KNEE OSTEOARTHRITIS

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BY

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To the Dean of the Graduate School:

I am submitting herewith a dissertation written by Casey Tiernan entitled "Grape Consumption Improves Joint Flexibility and Reduces Pain Associated with Knee Osteoarthritis." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Nutrition.

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DEDICATION

To my husband, Tim, and our children Johnny and Samantha. You have all three sacrificed a great deal for me to walk this path, and it would not have been possible without your love, support, and encouragement. May you each remember that in doing so you have allowed me to pursue and achieve a long-awaited dream. I look forward to taking my turn in supporting each of you in pursuing your own hopes and dreams.

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ABSTRACT

CASEY TIERNAN

GRAPE CONSUMPTION IMPROVES JOINT FLEXIBILITY AND REDUCES PAIN ASSOCIATED WITH KNEE OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common disease of joints, with a complex etiology. Pain and stiffness lead to loss of mobility that often requires invasive therapies. A growing interest in natural treatments suggests a role in OA therapy for foods with bioactive compounds. Grape polyphenols have anti-inflammatory properties that may influence OA outcomes. The purpose of this study was to assess the effect of grape consumption on self-reported pain, joint range-of-motion (ROM) and biochemical markers of inflammation (C-reactive protein [CRP]) and cartilage metabolism (insulin-like growth factor-1 [IGF-1], human cartilage glycoprotein 30 [YKL-40]) in individuals with self-reported knee OA. Using convenience sampling, 72 men and women with knee OA were recruited through local orthopedic clinics, primary care physicians, a senior recreation facility, the Texas Woman's University campus, and other locations in the community. The treatment group (n =35, 27 female) consumed 47 g of freeze-dried grape powder (FDGP) daily for four months. The placebo group (n = 37, 28 female) consumed a comparable placebo. The FDGP group had a significant decrease in pain related to activity from baseline to end of treatment in comparison to placebo (-5.3 vs. -2.1, $p < 0.05$). At midpoint, both groups had a significant reduction in total knee

symptoms and impact on quality of life (QOL) that was only evident in the FDGP group at the end of study. Furthermore, this improvement benefitted female participants more so than males. FDGP consumption resulted in gender specific changes in IGF-1 compared to the placebo group. Males in the FDGP group had a significant increase in IGF-1 from baseline compared to males in the placebo group (1.6 and 19.9 ng/mL in FDGP; 6.8 and 2.0 ng/mL in placebo for baseline and final, respectively), and females in both FDGP and placebo group (5.1 and 3.2 ng/mL FDGP; 4.9 and 8.9 ng/mL placebo for female baseline and final, respectively), $p < 0.05$). There was no change in overall ROM, CRP, or YKL-40 between FDGP and placebo groups. These results suggest consumption of whole grapes with their bioactive constituents may be a natural alternative to reducing pain and improving symptoms associated with OA.

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CHAPTER I

INTRODUCTION

Osteoarthritis (OA) is a complex chronic disease that reflects age-related degeneration of joint tissue in response to mechanical stress or injury. Normal repair and inflammatory responses follow and contribute to a cycle of further stress and damage (Arden, 2006; Shen et al., 2012). It is the most common arthritic disease world-wide, and nearly 27 million adults in the United States (US) aged 25 and older have clinical OA of at least one joint (Arden, 2006). Osteoarthritis treatment is expensive; costing Western countries approximately 1% to 2% of their gross domestic product (Hunter, 2011).

Age, gender, and ethnic differences are apparent in OA. Prevalence increases with age, with an overall incidence of hand OA at 27.2% which increases to $\geq$ 80% in the older age groups. Women report a higher frequency of OA in the hands and knees than men in the age group of 50 and older. A major population-based study showed OA to be significantly more prevalent in non-Hispanic African Americans as compared to non-Hispanic whites or Mexican-Americans at 52.4%, 36.2%, and 37.6%, respectively (Lawrence et al., 2008).

Non-pharmacological OA therapies are first-line approaches that include lifestyle changes, physical and occupational therapy, the use of assistive and supportive devices, water-based and strengthening exercises, and other measures (Zhang & Jordan, 2008). If symptom relief is not achieved then pharmacotherapy is initiated. Oral analgesics such as

acetaminophen may be used for mild or moderate OA cases, and non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and naproxen are used to treat more severe pain. However, NSAID use is linked to a greater risk of adverse events such as gastrointestinal discomfort, bleeding, and cardiovascular risks (Laine, White, Rostom, & Hochberg, 2008; Zhang & Jordan, 2008). Surgical joint replacement or arthroplasties are effective and appropriate treatment options when all other OA therapies have failed. Other procedures such as arthroscopic debridement may provide short-term symptom relief in patients where arthroplasty is contraindicated or has failed (Zhang & Jordan, 2008).

People are increasingly opting for alternative and naturally-derived OA treatment options such as glucosamine and chondroitin supplementation (Clegg et al., 2006; Zhang & Jordan, 2008). However, research demonstrating their efficacy has yielded conflicting results. The Glucosamine Unum In Die (once-a-day) Efficacy (GUIDE) trial of 318 adults showed improved pain and mobility scores with glucosamine supplementation, a higher response rate for glucosamine compared to acetaminophen and placebo, and no reports of adverse events (Herrero-Beaumont et al., 2007). In contrast, the National Institutes of Health (NIH) Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) in 1,583 older adults found no significant effect on pain reduction when combined glucosamine and chondroitin treatment was compared to a cyclooxygenase (COX)-2 inhibitor Celecoxib and a placebo (Clegg et al., 2006). Some subsequent studies have shown glucosamine and chondroitin to be ineffective (Vlad, LaValley, McAlindon, & Felson, 2007), but with a safety profile comparable to placebo (Häuselmann, 2001).

Overall, the literature supports the short-term use of glucosamine sulfate at 1,500 mg daily and chondroitin sulfate at 800 mg daily, with treatment to be discontinued if no benefit is apparent within six months (Zhang & Jordan, 2008).

The very foods we eat may represent another approach to prevention and treatment of OA. Many of our food sources are rich in polyphenols and other compounds with bioactive properties that may alter human health. Polyphenols are widely found in fruits and vegetables, such as isoflavones in soy; proanthocyanidins in apples, grapes and berries; catechins (flavanols) in green and other tea; and resveratrol in peanuts, berries, and grape skins (Barnes, 2008; Shen et al., 2012). They are associated with anti-inflammatory, antioxidant, cardioprotective, and chemopreventive properties (Barnes, 2008; Fang, Chen, & Yang, 2007; Frémont, 2000; Shen et al., 2012). There is evidence that dietary polyphenols provide benefit for inflammatory forms of arthritis, therefore they may also be beneficial for OA management (Elmali, Baysal, Harma, Esenkaya, & Mizrak, 2007; Shen et al., 2012). The mechanism of action appears to be down-regulation of the inflammatory cytokines, or by antioxidant or anti-inflammatory pathway signaling (Shen et al., 2012). In addition, cartilage tissue is estrogen receptor-positive. Polyphenols may bind and activate the estrogen receptor (Barnes, 2008; Gehm, McAndrews, Chien, & Jameson, 1997), or affect OA progression by mitigating chondrocyte inflammation and damage through directly or indirectly interacting with articular cartilage, bone, or synovium (Shen et al., 2012).

Specific grape polyphenols such as resveratrol also show anti-apoptotic, antioxidant, and anti-inflammatory traits that may influence OA outcomes. In human

primary articular cartilage stimulated by interleukin (IL) 1β, resveratrol has been shown to inhibit apoptosis through decreases in catabolic enzyme activity and suppression of ROS generation. *In vitro* studies have demonstrated that resveratrol reduces gene expression of vascular endothelial growth factor and COX-2, and down regulates matrix metalloproteinases (MMPs) associated with matrix degradation. Resveratrol has also been shown to protect major matrix proteins, proteoglycan, type II collagen, and aggrecan from the destructive effects of MMPs or inflammatory compounds such as COX-2 (Shen et al., 2012).

Opportunities in Research

First-line therapies for OA include lifestyle changes that may be time consuming and difficult to achieve for some patients (Hunter, 2011; O'Reilly & Doherty, 2001; Rannou & Poiraudeau, 2010; Zhang & Jordan, 2008). Progression of treatment to pharmacotherapy and surgery can be expensive and invasive, and it carries the potential for adverse risks (Laine et al., 2008; Zhang & Jordan, 2008). Yet research establishing the effectiveness of alternative nutraceutical treatments is conflicting (Clegg et al., 2006; Häuselmann, 2001; Herrero-Beaumont et al., 2007; Vlad et al., 2007). Evidence for the effectiveness of alternative OA treatments is needed.

There is *in vitro* and animal model evidence that bioactive polyphenols in darkly-colored fruits such as grapes have anti-apoptotic, antioxidant, and anti-inflammatory traits that may influence OA outcomes (Shen et al., 2012). However, this has yet to be demonstrated in human trials. There is an opportunity for research that shows whether

grape polyphenols affect OA parameters such as self-reported pain and joint movement in humans.

Non-adherence to OA treatment is estimated at 50-95% of patients, and it is the primary barrier to efficacy of interventions (Carr, 2001). It is conceivable that a whole food source of bioactive nutrients would be more acceptable to consumers, and potentially improve adherence to OA treatment and positively affect outcomes. There is no study to date assessing acceptability of a whole food grape product for OA treatment.

There is currently no known reliable biomarker or clinical measure for diagnosing OA (Hunter, 2011). Cartilage glycoprotein 39 (YKL-40), C-reactive protein (CRP), and insulin-like growth factor (IGF) -1 all hold promise as useful OA biomarkers, but that has not been fully determined (Mobasheri & Henrotin, 2010; Pagura, Thomas, Woodhouse, Ezzat, & Marks, 2005; Spector et al., 1997). More evidence is needed to establish the response of these biomarkers to OA treatments. In particular, there is no research to date that has examined the response of these biomarkers to grape polyphenols or whole grapes.

Objectives

The purpose of this study is to assess the beneficial effects of bioactive components in whole grapes on reducing or alleviating symptoms of knee OA. Specific aims include assessment of the effects of daily consumption of freeze-dried whole grape powder (FDGP) compared to placebo on: 1) the frequency and severity of self-reported knee pain; 2) the flexibility assessed by range of motion of the affected knee joint(s); and 3) selected serum biomarkers of inflammation and cartilage metabolism. It will be

assumed that a) participants can understand and complete the required surveys; b) participants will answer the survey questions truthfully and to the best of their knowledge; c) participants will adhere to the recommended treatment for the duration of the study; d) the primary objectives of the study are measurable; and e) the instruments employed to assess the specific aims are appropriate for collection of the study data.

Research Hypothesis

The central hypothesis of the study is that daily intake of 47 g of FDGP (with its bioactive constituents) will reduce or alleviate symptoms of OA. The principal aims of the study are that the treatment group receiving 47 g of FDGP daily will have: 1) decreased frequency of self-reported knee pain compared to the placebo group; 2) decreased severity of self-reported knee pain compared to the placebo group; 3) increased joint range of motion of the affected knee joint(s) compared to the placebo group; and improvements in serum biomarkers of inflammation or cartilage metabolism compared to the placebo group at the end of the study.

CHAPTER II

REVIEW OF LITERATURE

Epidemiology and Prevalence of Osteoarthritis

Osteoarthritis (OA) is most prevalent in the hands, knees, and hips, where it causes chronic pain and impairment of mobility (Arden, 2006). Often, OA in one joint will be accompanied by OA in a different joint site. In Caucasians this is particularly true for the link observed between hand and knee OA (Arden, 2006; Hunter, 2011). Nearly 27 million adults in the United States (US) aged 25 and older have clinical OA of some joint, which represents an increase in incidence from the estimated 21 million reported in 1995 (Lawrence et al., 2008).

Estimating OA prevalence is challenging due to the weak relationship between pathological structural changes and presence of symptoms (Duncan, Hay, Saklatvala, & Croft, 2006; Lawrence et al., 2008; Pereira et al., 2011). This may be partly explained by how differently people both experience and report pain (Hunter, 2011). Estimates of prevalence can also vary based on population age, whether studies use mild, moderate, or severe evidence of structural changes in their definition of OA, and the radiographic methods used to define that evidence (Duncan et al., 2006 Lawrence et al., 2008; Pereira et al., 2011). Radiographic criteria for defining OA is based on the Kellgren-Lawrence scale, which emphasizes the presence of bony formations at the joint site called osteophytes. Symptomatic OA is defined as having frequent joint pain on most days of the previous month, accompanied by radiographic evidence of OA in the affected joint

(Lawrence et al., 2008). Symptomatic OA is thought to be more clinically relevant than radiographic OA (Hunter, 2011).

Osteoarthritis is more frequently seen in the knees and hand than any other joint site (Hunter, 2011). Population-based studies show radiographic knee OA estimates ranging from 19.2% to 37.4%, while symptomatic knee OA is estimated at a much lower 4.9% to 16.7%. Hand OA estimates range from 27.2% for radiographic and 6.8% symptomatic, respectively (Lawrence et al., 2008). Hip OA is the least common form found in 3% to 5% of elderly US residents (Arden, 2006; Hunter, 2011).

Osteoarthritis poses a significant cost burden, both economically and socially. Western countries spend approximately 1% to 2% of their gross domestic product on arthritis care, with the majority of expense being related to total joint replacement (arthroplasty) (Hunter, 2011). In the US, more than 400,000 knee and hip joint arthroplasties are performed annually at a cost of $10 billion, and 95% of all total arthroplasties are performed on OA patients (Hunter, 2011; March & Bachmeier, 1997). OA stemming from traumatic injury accounts for 0.15% of total US healthcare direct costs, which translates to $3.06 billion yearly (Brown, Johnston, Saltzman, Marsh, & Buckwalter, 2006). Cost estimates can be imprecise as many OA patients are elderly and have comorbidities that contribute to their overall financial burden (March &Bachmeier, 1997).

In addition to direct economic costs, patients face indirect and intangible costs such as lost wages, declines in quality of life, loss of leisure and recreational ability, and

greater risks of depression, psychological problems, pain, and suffering (Felson et al., 2000; Hunter, 2011; March & Bachmeier, 1997).

Incidence of Osteoarthritis

Determination of OA incidence is fraught with challenges, primarily because there is no well-defined onset event. Estimates based on medical billing indicate overall OA incidence in the US to be 1,040 cases per 100,000 person-years (Sun, Gooch, Svenson, Bell, & Frank, 2007). A north-eastern US health maintenance organization calculated the overall age- and sex-standardized incidence of hand OA to be 100 in 100,000, with 240 in 100,000 for knee OA and 88 in 100,000 for hip OA. Incidence of hand, hip, and knee OA increases with age until approximately age 80 (Arden, 2006). Incidence is higher in women, particularly in women older than 50. Among women, incidence of knee OA is 2% yearly for cases that are radiographically diagnosed and 1% for those symptomatically diagnosed, respectively (Centers for Disease Control, 2011). Incidence is also higher in those with more depression, a higher BMI, previous injury, and regular participation in sporting activities (Cooper et al., 2000; Sun et al., 2007; Verweij, van Schoor, Deeg, Dekker, & Visser, 2009). Globally, OA incidence estimates vary widely from 0.1 to 22.3 per 1,000 person-years, depending on the methodology used to estimate. Incidence is expected to increase as obesity, a major risk factor for OA, is also globally on the rise (Verweij et al., 2009).

Classification of Osteoarthritis

Osteoarthritis can be broadly classified by 1) the site of joint involvement, 2) whether it is primary or secondary in nature, or 3) less commonly by the presence of

distinctive features that are inflammatory, erosive, atrophic, or destructive in nature (Arden, 2006). More specific to knee OA, it may vary by compartment. The patellofemoral compartment accounts for 11% of OA cases, and it may be more associated with the presence of bony nodules in the distal interphalangeal joints termed Heberden's nodes (Cooper, McAlindon, Coggon, Egger, & Dieppe, 1994; Hunter, 2011). When combined with the tibiofemoral compartment this makes up 41% of all incidence of symptomatic knee OA. Higher rates of OA are also seen in the medial tibiofemoral as compared to the lateral compartment, which could be partially explained by the heavier load bearing burden related to body weight in this compartment (Hunter, 2011). International Classification of Diseases, 9th Revision (ICD-9) diagnoses are based on a primary or secondary classification (Brown et al., 2006). Primary OA is idiopathic in nature with no identifiable cause, while secondary is attributed to a known trigger event such as an acute knee injury. Though the distinction between primary and secondary OA can be hard to distinguish, secondary causes of OA are frequently associated with a predisposition to OA and can be brought about by metabolic, anatomic, traumatic, or inflammatory triggers (Arden, 2006).

Biology of Articular Cartilage

Composition of Cartilage

Mature articular cartilage is divided into two major components: a network of cells (chondrocytes) and their surrounding gel-like extracellular matrix of collagen and elastic fibers set in chondroitin sulfate (Moskowitz, Howell, Altman, Buckwalter, & Goldberg, 2001). Normal articular cartilage thickness varies from one to two

millimeters, depending on age and joint site. In a large joint of a young person the thickness may approach five to seven millimeters (Moskowitz et al., 2001). Cartilage is arranged in four zones from the joint surface to the deepest subchondral bone later: superficial (Zone 1), transitional (Zone 2), radial (Zone 3), and the zone of calcified cartilage (Zone 4) (Goldring, 2012). Unlike most connective tissue, cartilage is avascular and lacks nerves outside of the membranous covering called the perichondrium. Therefore it grows slowly and recovers poorly from insult and injury, which is a contributing factor in OA when the articular cartilage present around the joints suffers damage (Tortora & Derrickson, 2006).

Chondrocytes

Two to five percent of cartilage volume is accounted for by the chondrocytes (Goldring, 2006), which are found within lacunae containing water, proteoglycans, and chondroitin sulfate that are dispersed throughout the matrix (Moskowitz et al., 2001). The chondrocytes function to synthesize and maintain the cartilage matrix and to support the active transport exchange of cations in the matrix (Goldring, 2006; Moskowitz et al., 2001). Chondroprogenitor cells synthesize type II, IX, and XI collagen that is specific to chondrocytes (Goldring, 2012). Immature chondroblasts are evenly distributed in the cartilage, and continually synthesize the matrix through a complex organelle system (Moskowitz et al., 2001). Once mature, the chondrocyte then remains as a resting cell and can either form articular cartilage or undergo endochondral ossification whereby the cartilage is gradually replaced by bone (Goldring, 2012). After maturity, chondrocytes

no longer undergo mitosis and characteristically tend to cluster in groups of two to four (Moskowitz et al., 2001).

Cartilage Metabolism

As an avascular tissue, cartilage shows very little metabolic activity. Any substances that are required for growth and repair must reach the cartilage by diffusion through the synovial fluid, which is a slow process (Tortora & Derrickson, 2006; Moskowitz et al., 2001). Additionally, cartilage repair is poor because mature chondrocytes are unable to produce matrix components in comparison to chondroblasts (Goldring, 2012). While cartilage is not a highly active tissue, the chondrocytes within do have an elevated metabolic rate to compensate for their sparse presence. Synovial fluid provides nutrients and oxygen to articular chondrocytes (Tortora & Derrickson, 2006), and it also removes unwanted microbes and built up waste products through phagocytosis (Tortora & Derrickson, 2006; Moskowitz et al., 2001). Chondrocytes mostly utilize glucose for their metabolic fuel (Goldring, 2006). Their metabolic activity varies depending on the zone of the cartilage, being relatively inactive in Zone 1, more active in Zone 2, less active in Zone 3, and senescent in Zone 4. The metabolic rate of chondrocytes also changes in response to chemical and mechanical stimuli. For instance matrix synthesis and amino acid uptake is altered in response to pH, oxygen tension, growth hormone, insulin, and thromboxanes. Exercise and articular cartilage loading may stimulate matrix synthesis, while immobilization of the joint decreases chondrocyte metabolism. Chondrocyte numbers peak and stabilize at about 30 years of age, but

become more metabolically active after that time with increased collagen and proteoglycan synthesis creating hypertrophy in the cell (Moskowitz et al., 2001).

Matrix and Collagen Framework

Extracellular matrix of the article cartilage is made of fibers and the "ground substance" or material surrounding the fibers, and is 65% to 80% water by volume (Tortora & Derrickson, 2006; Moskowitz et al., 2001). The fibers include proteins such as collagen, elastin, and reticular fibers that give strength and support to the tissue. The majority of the fibrous proteins in the matrix are parallel bundles of collagen that are strong, flexible, and resistant to stretching (Tortora & Derrickson, 2006). This collagen accounts for 10% to 30% of articular cartilage wet weight. Collagen is present in several different types, with type II being the major form present in articular cartilage (Arden, 2006; Moskowitz et al., 2001).

The ground substance surrounding matrix fibers primarily contributes to resiliency but also provides structural support. It is composed of water and polysaccharide compounds called glycosaminoglycans (GAGs), which in cartilage include hyaluronic acid and chondroitin sulfate. Proteoglycans are formed when GAGs other than hyaluronic acid attach to a core protein, projecting outward to attract and trap water, giving a gel-like property to the matrix (Tortora & Derrickson, 2006). Their negative charge makes them hydrophilic and they have the ability to take on water at up to greater than 50 times their own weight (Moskowitz et al., 2001; Tortora & Derrickson, 2006). The constant and repetitive loading of articular cartilage causes the matrix to change shape, giving a higher electric charge density to the negatively charged

proteoglycans. The proteoglycans subsequently relax and express their fluid into the joint cavity. When the joint load is removed, the fluid is reabsorbed, giving an elastic-like quality of resiliency to the cartilage that allows it to regain its shape after exposure to mechanical loading (Moskowitz et al., 2001). Aggrecan is the major form of proteoglycan present, consisting of 100 or more chondroitin sulfate chains and 20 to 50 keratan sulfate chains connected to the core protein. Aggrecan may combine with up to 200 additional aggrecan molecules and attach non-covalently to a hyaluronate molecule, immobilizing their aggregate structure in the matrix to give resistance to compression (Goldring, 2006; Moskowitz et al., 2001). As much as 50% of the dry weight of the cartilage, proteoglycans compose much of the remainder of the matrix. Their concentration increases at deeper layers of cartilage and is inversely proportional to that of collagen (Burr, 2004; Moskowitz et al., 2001).

Structural Organization of Cartilage

Cartilage zones vary in collagen fiber arrangements. This allows for resistance to tensile and shear stress at the surface and compressive mechanical stress in deeper zones (Burr, 2004; Goldring, 2012; Moskowitz et al., 2001). Zone 1 contains the highest concentration of collagen fibers which are arranged parallel to the surface of the joint. Zone 2 contains collagen in small fibers of four to 10 nanometers, arranged in a random fashion to give a lattice-work supportive structure for holding up larger fibers of 10 to 80 nanometers. The larger fibers are arranged in a sloping orientation to the joint. The radial zone or Zone 3 is named to reflect the radial arrangement of the collagen, with a tidemark present to mark the border between Zone 3 and the Zone 4. Zone 4 has the

lowest concentration of water of any of the cartilage zones, and is composed of hydroxyapatite crystals attached to thick collagen fibers. The fibers are arranged perpendicular to the synovial cavity and anchor the cartilage to the subchondral bone plate. Chondrocyte conformation changes throughout the cartilage zones, with flattened shapes seen in parallel layers at Zone 1, rounded forms in Zone 2, perpendicular columns in Zone 3, and small, less developed forms in Zone 4 (Moskowitz et al., 2001).

Changes in the Diseased Joint

Progression of OA

Progression of OA occurs slowly, and there is little correlation between radiographic evidence of progression and clinical outcomes, particularly for hip OA. The time period between immediate joint trauma and OA pathology can range from five to 15 years. Inflammation promotes formation of cysts and bony swelling that persist even during periods of remission. Hip OA is thought to progress from approximately three months to three years before the onset of advanced stage OA. Far less is known about the progression of knee OA. Radiographic studies have shown OA joints rarely improve, but often remain stable for many years. In contrast, some OA joints rapidly deteriorate and lose joint function. Symptoms can vary widely over the course of OA progression with equal occurrences of improvement, stasis, or decline (Arden, 2006).

Characteristic progression of OA affects the entire joint, including subchondral bone remodeling and formation of osteophytes through repair processes aimed at stabilizing the joint (Arden, 2006). Changes visible through radiographic assessment of the knee joint include joint space narrowing, osteophyte and cysts formation, subchondral

bone sclerosis, and other alterations in bone shape (Arden, 2006; Hunter, 2011). Muscles and ligaments may weaken and become lax, decreasing their ability to support the joint (Arden, 2006). Additional inflammation, joint pain, and loss of function can follow (Hunter, 2011). Risk factors for progression of hip and knee OA include obesity, malalignment of the joint, injury to the joint, and certain types of physical activity (Arden, 2006).

Cartilage Degradation

Immediate onset events of OA include chondrocyte necrosis, collagen rupture and GAG loss, and hemarthrosis or bleeding into the joint (Arden, 2006). The chondrocytes may also become activated and begin to proliferate and form clusters. They increase matrix protein and degradative enzyme production, possibly in an attempt to clear matrix fragments and repair the cartilage (Goldring, 2006; Goldring, 2012).

Cartilage is degraded by the action of several enzymes made in the synovium, chondrocytes, and leukocytes (Moskowitz et al., 2001). Two major targets of the cartilage destruction process in OA are type II collagen and aggrecan (Goldring, 2012). The pro-inflammatory MMP enzymes are heavily involved in the matrix degradation seen in OA. Members of the MMP family include collagenases (MMP-1 and MMP-13) and stromelysin (MMP-3) that degrade collagen and proteoglycans, respectively (Martel-Pelletier, 2004; Vincenti & Brinckerhoff, 2002). MMP-1 and MMP-13 are both prominent contributors to collagen degradation and the resulting inflammation in OA, but MMP-13 may also play a role in the remodeling of cartilage (Martel-Pelletier, 2004). MMP-13 is thought to be the critical MMP promoting cartilage degradation, though it is

expressed 200-fold lower than MMP-1 during OA and is not collagen-specific in its cleavage (Lauder et al., 2007). Initially the proteoglycan coating surrounding collagen offers some protection from MMP-1 and -13, but once set in motion, collagen degradation is irreversible (Goldring, 2012). Ultimately there is extensive loss of cartilage which exposes the surface of bone, creating the friction of bone against bone (Das & Farooqi, 2008; Tortora & Derrickson, 2006). MMP activity is stimulated by interleukin (IL) -1β and tumor necrosis factor alpha (TNF-α), causing the chondrocyte and synovium to release IL-6, which perpetuates the inflammation (Lauder et al., 2007). MMP members called aggrecanases (ADAMTS-1, ADAMTS-4 and ADAMTS-5) are also present in synovial fluid and can attack cartilage matrix and cause fragmentation of proteoglycans (Martel-Pelletier, 2004; Nagase & Kashiwagi, 2003). Other enzymes are also involved in OA pathogenesis, but generally as activators of MMPs (Martel-Pelletier, 2004).

The cartilage degradation that occurs with OA happens over the course of several months. After that time, inflammation will set in, with resulting leukocyte infiltration and apoptosis (Lotz, 2010). Some early signs of synovial inflammation may be detected with magnetic resonance imaging or ultrasound (Das & Farooqi, 2008; Hunter, 2011). As years pass, there is continued inflammation and remodeling of the joint tissue. Joint lubrication becomes deficient and the joint space can become fibrous (Lotz, 2010). The damaged collagen limits the ability of chondrocytes to regenerate the matrix, and there is senescence and apoptosis of the chondrocytes (Goldring, 2006).

Osteoarthritis Pathogenesis

Osteoarthritis pathology occurs in three major phases: 1) the irreversible breakdown of cartilage matrix by proteases and other catabolic enzymes, 2) the fibrillation and deterioration of articular cartilage with the resulting release of eroded fragments into synovial fluid, and 3) ingestion of the fragments by synoviocytes via phagocytosis, which produces more proteases and pro-inflammatory cytokines that create synovial inflammation. The subsequent release of the proteases promotes a continued cycle of inflammation (Lotz, 2010; Martel-Pelletier, 2004). The etiology of OA is not fully understood, but is thought to stem from the interaction of predisposing mechanical and systemic risk factors and a variety of trigger events (Hunter, 2011). Most cases appear to be instigated by mechanical stress, with injury triggering normal repair processes that promote bone growth in an attempt to stabilize the joint, resulting in osteophyte formation (Arden, 2006). Anabolic mediators such as IGF -1, transforming growth factor (TGF) -β and bone morphogenic proteins stimulate the cartilage growth and bone remodeling associated with repair attempts (Goldring, 2006; Hunter, 2011). Once established, OA disrupts the normal balance of both degradation and synthesis of joint tissue through increased stimulation of the pro-inflammatory pathway. (Hunter, 2011).

Inflammatory Mediators

Pro-inflammatory cytokines, primarily IL-1β and TNF-α, mediate the inflammation process triggered by proteases (Das & Farooqi, 2008; Martel-Pelletier, 2004). Their receptor activity is significantly increased during OA (Martel-Pelletier,

2004). These cytokines promote chondrocyte apoptosis and further the cartilage degradation process (Shen et al., 2012). TNF–α is thought to promote the inflammatory process and IL-1β the enzyme system. This leads to increased synthesis of degradative enzymes, and inhibition of enzyme inhibitors and matrix components that contribute to cartilage degradation (Martel-Pelletier, 2004).

Inflammatory changes are milder in OA when compared to other arthritic conditions such as rheumatoid arthritis (Hochberg et al., 1995). Nevertheless, the inflammation seen in OA is directly responsible for much of the structural degeneration and many of the clinical symptoms such as joint swelling, synovitis, and associated pain (Das & Farooqi, 2008). Symptomatic pain can be caused by osteophytes irritating sensory nerve endings, as well as pro-inflammatory compounds like prostaglandins, proteinases, and cytokines (Hunter, 2011). The synovial cavity of the joint contains densely packed sensory nerves, and inflammatory mediators such as IL-1β and TNF-α can stimulate the nerve fibers, causing an inflated pain response (Das & Farooqi, 2008; Hunter, 2011). Circulating cytokines may also promote release of prostaglandin E2 (PGE_2) and histamine from chondrocytes and increase the sensitivity of pain-receiving nociceptors (Hunter, 2011). The prostaglandin-endoperoxide synthase (or COX) enzyme mediates this prostaglandin synthesis from arachidonic acid. There are two isoforms of the COX enzyme, COX-1 being a "housekeeping" gene and COX-2 induced in inflammatory conditions (Subbaramaiah & Dannenberg, 2001).

Oxidative Stress

There is increasing evidence that oxidative and nitrosative stress play a role in OA pathogenesis. Highly unstable free radical molecules or reactive oxygen species (ROS) are produced in healthy tissue, but they must be continually eliminated by either antioxidants or ROS scavengers (Ziskoven et al., 2011). Superoxide dismutase, an ROS scavenger, has been found to be reduced in human and animal OA (Shen et al., 2012). Both ROS and nitrous oxide (NO) are required for normal chondrocyte activity. However, during OA the balance between their synthesis and degradation is disrupted, leading to accumulation of oxidative stress products that promote inflammation (Shen et al., 2012; Ziskoven et al., 2011). This imbalance is suggested to be a primary driver of OA progression and cartilage degradation. Upon exposure to inflammatory cytokines such as IL-1β and TNF-α, chondrocytes will induce production of NO via the NO synthase (NOS) enzyme with L-arginine being utilized as a substrate. Several isoforms of NOS have been found in OA joint tissue. Oxidative stress promotes sclerosis and bone resorption in subchondral bone, cartilage thinning in chondrocytes and the matrix, inflammation in synovial fluid, and fibrosis in the joint capsule. These changes lead to the presence of clinical symptoms such as pain, swelling, and stiffness (Ziskoven et al., 2011).

Osteoarthritis Risk Factors

Common risk factors include increasing age, female sex, higher bone density, certain ethnicities, genetic profile, obesity, prior joint trauma, and occupations with repetitive movements, squatting, and knee bending (Cooper et al., 1994; Das & Farooqi,

2008; Nguyen et al., 2011; Srikanth et al., 2005). These and other risk factors may also determine response to treatment and course of the disease (Arden, 2006; Felson, 2013).

Mechanical Risk Factors

The most well established and prominent risk factor for knee OA is obesity, though this relationship is weaker for hip and weakest for hand OA (Das & Farooqi, 2008; Felson, 2013; Hunter, 2011). Prevalence of OA in the elderly is 51.4% in those with a body mass index (BMI) less than 25, but as BMI rises to 40 and over the prevalence of OA increases to 100% in this population (Das & Farooqi, 2008). Undoubtedly, an important mechanism of action is via added weight bearing load, which leads to damage of cartilage, ligaments, and other support structures (Arden, 2006). Factors other than excessive joint loading can be important to OA risk as well, such as muscle strength (Arden, 2006; Verweij et al., 2009). Malalignment of joints, the presence of lesions, damage to the meniscus or bone, rupture of ligaments, and muscle weakness can all increase susceptibility to OA (Felson, 2013; Hunter, 2011; Urquhart et al., 2008).

Acute joint injuries are highly associated with development of OA in the injured joint (Arden, 2006). Aside from obesity, prior joint injury is also a modifiable risk factor in women and men (Hunter, 2011). Especially in males, repetitive heavy labor, squatting, and some activities of daily living influence OA risk (Arden, 2006; Felson et al., 2000). Possible mechanisms include greater damage to the menisci or ligaments, or the effect of repeated joint loading (Cooper et al., 1994). The results of the Framingham OA study

implicated occupational activities that entail kneeling, squatting, and lifting in 15% to 30% of knee OA cases in males (Felson et al., 2000).

While activities with excessive mechanical strain do increase OA risk, there is also a trend toward increased risk with low levels of mechanical strain (Verweij et al., 2009). Moderate levels of leisure activity such as walking or jogging do not appear to affect the risk of hip or knee OA, possibly due to frequent, moderate pressure promoting cartilage maintenance (Arden, 2006; Felson et al., 2007).

Systemic Risk Factors

As indicated by in the Framingham Cohort Study, prevalence increases with age, with an overall incidence of hand OA at 27.2% which increases to ≥ 80% in older age groups. In elderly populations this may not be the case, which may be due to the relative stability of cartilage with aging (Felson et al., 1987). However, it is not a disease that is confined to the elderly as two-thirds of those suffering from OA are younger than age 65 (Hunter, 2011). Proposed mechanisms for age-related changes include declines in chondrocytes and production of protective synovial fluid, thinning of cartilage, and loss of ligament length and flexibility (Tortora & Derrickson, 2006). Normal age-related changes also include slowed muscle function and neurological response, increased laxity of supporting ligaments, and thinning of the cartilage plate, all of which increase the vulnerability of the joint to injury (Arden, 2006).

Most of the difference in risk attributed to age is due to the higher proportion of women who have OA in older age groups. Prevalence increases with age for both radiographic and symptomatic OA, but women are more likely to present with

symptomatic knee OA (11.4% vs. 6.8%, p = 0.003) (Felson et al., 1987). Women report a higher frequency of OA in the hands and knees than men in the age group of 50 and older and tend to have more severe knee OA (Arden, 2006; Felson et al., 2000; Lawrence et al., 2008; Srikanth et al., 2005). Gender differences in OA prevalence are attributed to differences in estrogen status, though evidence is inconsistent in this area and stronger for radiographic OA than symptomatic OA (Felson et al., 2000).

Incidence of OA also varies considerably by ethnicity. The Johnston County OA and NHANES III studies reported OA more frequently in African American versus Caucasian adults (Lawrence et al., 2008). This may be attributable to higher rates of obesity in this group, though other studies suggest the link between obesity and OA is not completely clear (Arden, 2006). NHANES III showed OA to be significantly more prevalent in non-Hispanic African Americans as compared to non-Hispanic whites or Mexican-Americans at 52.4%, 36.2%, and 37.6%, respectively. In contrast, Johnston County reported comparable rates between African Americans and Caucasians (Lawrence et al., 2008). Hip OA prevalence is about 9% in Caucasian populations, with much lower rates seen in Asian and Indian populations (Hunter, 2011). There are observed differences in biomarkers of OA that also vary by ethnicity, which supports the idea that genetic and other biological differences may play a role (Felson et al., 2000). Genetic susceptibility may account for nearly half of the variability in susceptibility for hand, hip, and knee OA in women and hip OA in men (Arden, 2006; Felson et al., 2000, Jordan et al., 2003).

Diagnosis of Osteoarthritis and Biochemical Markers

Most epidemiological studies have used the Kellgren-Lawrence grading system to determine diagnostic criteria for OA. This system assigns a grade of 0-4 to a given joint site as compared to a radiographic atlas. Grading ranges from absence of OA features to severe OA with significant damage to the joint space and subchondral bone sclerosis, and the definite presence of osteophytes and pseudocysts (Arden, 2006). The Kellgren-Lawrence scale is not without limitations, as there is some inconsistency in in describing radiographic features and it places much emphasis on osteophyte presence (Arden, 2006). Joint space narrowing is also prone to false positives and difficulty in positioning of the subject (Spector et al., 1997). The American College of Rheumatology (ACR) has developed updated diagnostic criteria by comparing site-specific joint pain in clinically diagnosed OA to different arthritic or musculoskeletal diseases versus healthy controls. This criterion is based on presence of joint pain in the preceding month and is widely used in clinical studies (Aden, 2006).

Unlike other chronic diseases such as hyperlipidemia and hypertension, there is no reliable biomarker or clinical measure for diagnosing OA (Hunter, 2011). Relying on the use of radiography to assess joint space width does not take into account metabolic aspects of OA pathology that occur prior to structural damage (Henrotin, 2012). Although few studies have examined their link to structural damage in OA, biomarkers may be of use to detect changes in matrix synthesis and breakdown throughout the course of OA progression. This is particularly the case as degradative byproducts are secreted into joint fluid, blood, and urine (Bruyere et al., 2006).

YKL-40 may be a promising biomarker for evaluating joint catabolism (Mobasheri & Henrotin, 2010). It is highly present in the joint tissue in both rheumatoid arthritis and OA, particularly at superficial and mid-zone cartilage layers. YKL-40 has an unknown function and is generally not present in normal cartilage. Levels present in the synovium are about 10-fold higher than that in serum, but either can be detected using radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Volck et al. (2001) determined the distribution of YKL-40 in a group of 39 adults with OA who were awaiting total arthroplasty. In the synovium, YKL-40 was present in macrophages and synovial lining cells where it was positively associated with the degree of synovitis as shown histologically and by MRI. It was also present in chondrocytes. While YKL-40 levels were lower in the OA group as compared to a separate rheumatoid arthritis group, the results still suggest that YKL-40 is involved in arthritic pathophysiology (Volck et al., 2001). Bruyere et al (2006) conducted a year-long prospective study of 62 older adults with primary knee OA and found that YKL-40 was correlated with synovial membrane and joint effusion volumes seen on MRI. The authors found no correlation between baseline values and changes in cartilage volume or thickness at the study's end. However, OA pathology occurs over a number of years and the relationship between biomarkers and early stages of joint destruction is not established (Bruyere et al., 2006).

Production of CRP is stimulated by cytokines such as IL-6. Spector et al (1997) examined a subset of middle-aged women from the United Kingdom Chingford Study to determine the role of CRP in the onset and progression of early-stage OA. The authors found CRP levels were significantly higher in the 105 women with knee OA compared to

740 without (median 2.4 mg/L vs. 0.7 mg/L, $p<0.001$). CRP was also higher in the women who progressed in OA compared to those who did not (median 2.6 mg/L vs. 1.3 mg/L, $p=0.006$). Their findings implicate small increases of CRP early in OA may predict progression over the next four years. Mild-to-moderate OA will elicit low levels of CRP in an acute phase response, which could be difficult to detect. CRP increases to a greater extent in more classically defined inflammatory diseases, infection, or trauma where it can be seen from 10 mg/L to over 400 mg/L (Spector et al., 1997).

IGF-1 promotes growth and differentiation of the chondrocytes, which is rare in most growth factors. Whether levels are decreased in OA has not been established. However OA patients share several characteristics in common with those who tend to have low serum IGF-1, e.g., excess adipose tissue and reduced feelings of physical ability. Males have consistently been shown to have higher levels of serum and synovial IGF-1 than females, and levels of serum growth hormone and IGF-1 tend to decline around 15% for each decade of age (Pagura et al., 2005).

Current Treatments and Therapies for OA - Lifestyle

Because OA is a complex, multifactorial disease, its treatment by nature will also be complex and multifactorial (Arden, 2006). Clinical recommendations should attempt to influence modifiable risk factors linked to pain with a combination of pharmaceutical and non-pharmaceutical intervention, and then tailor the intervention to the individual patient's needs. General recommendations promote the use of non-pharmacologic therapies as a first-line treatment, progressing to drug treatment and finally surgery if

adequate symptom relief is not achieved. (Das & Farooqi, 2008; Hochberg et al., 1995; Hunter, 2011; Jordan et al., 2003).

Non-pharmacological approaches that may be beneficial include weight management and diet education, physical and occupational therapy, aerobic and muscle-strengthening exercises, water-based exercises, the use of assistive and supportive devices, heat and/or cryotherapy, transcutaneous electrical nerve stimulation, and acupuncture (Rannou & Poiraudeau, 2010; Zhang & Jordan, 2008). Patient education and support is important for the success of lifestyle changes, particularly with weight reduction, physical and occupational therapy, and the use of assistive devices (Hochberg et al., 1995; Jordan et al., 2003).

There is good evidence that weight reduction when indicated is helpful for OA management, and this is reflected in most OA treatment guidelines (Hunter, 2011; O'Reilly & Doherty, 2001). The literature suggests the use of a combination of diet and moderate physical activity aimed at reducing at least 5% of body weight results in up to 30% improvement in pain and function (Hunter, 2011). The Framingham OA study found women who lost 11 lbs. on average decreased their risk for OA by 50% (Felson et al., 2000).

Exercise programs can not only facilitate weight loss efforts, but also increase muscle strength, endurance, and aerobic capacity. Muscle weakness in itself is a risk factor for OA, most likely due to the decreased knee joint stability and shock-absorption properties. A recent clinical trial that examined strength training in lower extremities for knee OA showed those receiving strength training had decreased OA progression when

compared to the control group. Prevention of knee injury may prevent as much as 25% of knee OA in men and 14% in women, and muscle-strengthening programs have demonstrated reduction of anterior cruciate ligament (ACL) injury risk by up to 60% (Hunter, 2011). Patients with knee OA often demonstrate quadriceps weakness attributed to disuse atrophy from deliberately unloading painful joints. Quadriceps strengthening is widely employed as an OA therapy, and is also seen as promoting overall functional improvement and well-being (Das & Farooqi, 2008; Felson et al., 2000; Hochberg et al., 1995; O'Reilly & Doherty, 2001; Urquhart et al., 2008). Other low-impact aerobic activities that are particularly suited to OA are walking, biking, swimming, and aquatic activity (Hunter, 2011).

Current Treatments and Therapies for OA - Medications

Pharmacotherapy is the second line of treatment when non-pharmacological treatments fail to effectively relieve symptoms (Zhang & Jordan, 2008). The primary intent of pharmacological treatment is a reduction in pain that allows maximizing the regain of mobility, with the least toxic and least expensive medicines being the first line of treatment (Das & Farooqi, 2008). In cases where patients have pain that is unresponsive to pharmacotherapy or when certain OA medications are contraindicated, weak opioids such as tramadol (alone or combined with acetaminophen, codeine, or propoxyphene) and narcotic analgesics may be considered. If additional opioids or narcotics are used all non-pharmacological treatments should also be continued (Zhang & Jordan, 2008). To date, there is no pharmacological agent approved for disease modification in OA, though promising preclinical efforts have examined glucosamine

and chondroitin, sodium hyaluronan, doxycycline, MMP inhibitors, bisphosphonates, calcitonin, diacerein, and avocado/soybean unsaponifiables (ASUs) (Ameye & Chee, 2006; Häusselmann, 2001; Hunter, 2011; Jordan et al., 2003; Lopez, 2012).

Acetaminophen

Pharmacotherapy begins with the use of oral analgesics such as acetaminophen at up to 4 g daily for mild or moderate cases, progressing to the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or naproxen for more severe pain (Das & Farooqi, 2008; Jordan et al., 2003; Zhang & Jordan, 2008). However, analgesics have a limited ability to relieve symptoms and carry the risk of adverse effects. Adverse risks associated with their use are compounded by years of treatment (Goldring, 2006; Henrotin, Lambert, Couchourel, Ripoll, & Chiotelli, 2011; Hunter, 2011; Towheed, 2002). Acetaminophen has been a core recommendation for many years based on relative efficacy and safety. Recently this has been called into question, particularly with long-term use, due to evidence of potential GI, liver, and renal toxicity at recommended doses (Das & Farooqi, 2008; Laine et al., 2008; Zhang & Jordan, 2008). Acetaminophen is known to have lower efficacy than NSAIDs, and non-compliance can become a concern as treatment requires more pills daily (Das & Farooqi, 2008).

Non-Steroidal Anti-Inflammatories

NSAIDs are the most commonly used agent for OA symptoms with improvements reported in pain management and patient compliance (Das & Farooqi, 2008; Laine et al., 2008). Across several clinical OA trials, different NSAIDs had a similar efficacy with about a 30% reduction in pain and 15% improvement in joint

function (Hunter, 2011; Towheed, 2002). There is ample evidence from short-term trials that non-selective NSAID use is linked to a greater risk of adverse events compared to acetaminophen (Das & Farooqi, 2008; Zhang & Jordan, 2008). Potential events include gastrointestinal discomfort, bleeding, perforation, and peptic ulcers. The risk of these occurrences rises with increasing age, polypharmacy, and possibly the duration of therapeutic treatment (Laine et al., 2008, Zhang & Jordan, 2008). Therefore non-selective NSAIDs should be utilized at the lowest effective dose for the shortest period of time possible (Das & Farooqi, 2008; Laine et al., 2008; Zhang & Jordan, 2008).

NSAIDs that selectively inhibit the COX-2 enzyme have fewer adverse gastrointestinal effects, but were found to increase the risk of myocardial infarction and other cardiovascular events by nearly two-fold when compared to placebo and naproxen (Laine et al., 2008; Zhang & Jordan, 2008). NSAIDs of any kind can also increase rates of hypertension, edema, and congestive heart failure. However, this is less likely to occur with lower dose NSAID use. All patients using long-term NSAID therapy should be monitored for increased blood pressure and edema in the first month of use (Laine et al., 2008).

The selective COX-2 inhibitor rofecoxib was withdrawn from the US market in 2004 due to a demonstrated increase in the relative risk of thrombotic cardiovascular events such as myocardial infarction and stroke. While these concerns were not consistently raised with other COX-2 inhibitors like celecoxib and valdecoxib, the consensus is that all NSAIDs may raise cardiovascular risk, and in the US all prescription NSAIDs must carry a “black box” warning label outlining the risk of gastrointestinal

bleeding and cardiovascular events (Laine et al., 2008; Zhang & Jordan, 2008). However, NSAIDs of any kind have been found to increase cardiovascular risk so caution should be used for patients with cardiovascular risk factors (Das & Farooqi, 2008; Zhang & Jordan, 2008). COX-2 inhibitors in particular should be contraindicated in cases of ischemic heart disease or stroke (Zhang & Jordan, 2008).

Injectable Treatments

Therapy directly injected into the synovial cavity could be a useful alternative for treating pain-related symptoms, and has been in use for more than 50 years (Hunter, 2011; Zhang & Jordan, 2008). Intra-articular (IA) injections can allow for maximum drug concentration at the joint site without having system-wide exposure to the drugs and their side effects (Lotz, 2010). One such treatment is IA injections of corticosteroids, which show efficacy in symptom-relief in moderate to severe hip or knee OA that has not been responsive to oral analgesics or NSAIDs, or when there are signs of inflammation present (Hunter, 2011; Jordan et al., 2003). However, the evidence for effectiveness in hip OA is limited and overall safety determinations for frequency of injections has not been established. For this reason, IA injections of corticosteroids are typically capped at four injections annually. Side effects are usually not serious, but can include pain flares, crystal deposition and synovitis, hemarthrosis, joint sepsis, and atrophy of articular cartilage (Zhang & Jordan, 2008). Other potential agents that show promise for IA injection include inhibitors of caspases, cytokines, and proteases, and stimulators of growth factors, antioxidants, and lubricators (Lotz, 2010). Injections of hyaluronic acid may be helpful in cases of mild to moderate OA, with effectiveness reported in about

60% of patients. The mechanism of action is not known, but the effect has been seen within several weeks of starting therapy and continuing for six to 12 months. Hyaluronic acid injections may be a good option for those patients who do not tolerate other forms of therapy (Das & Farooqi, 2008).

Current Treatments and Therapies for OA - Surgical

Surgery is seen as a last resort treatment when adequate pain relief has not been achieved with other lifestyle changes and therapies. Common procedures available include arthroplasty or total joint replacement, arthroscopic irrigation and debridement, and joint fusion (Das & Farooqi, 2008).

Arthroplasty

While surgical arthroplasties are a measure of last resort, they are widely regarded as effective and appropriate options (Das & Farooqi, 2008; Zhang & Jordan, 2008). Joint arthroplasties can be beneficial and cost-effective procedures for reducing or eliminating significant OA symptoms or functional impairment that decreases quality of life. In terms of restoring joint function to normal, hip arthroplasty is more effective than knee arthroplasty (Zhang & Jordan, 2008). Poor outcomes have been seen in the elderly, patients who have more severe pain or mental health scores, and those with musculoskeletal comorbidities like back pain or OA in the untreated hip or knee (Das & Farooqi, 2008; Zhang & Jordan, 2008). Osteotomy, hip resurfacing, and surgery to preserve joints may be considered in young adults who have symptomatic hip OA or dysplasia, which may delay the need for joint replacement by up to 10 years (Das & Farooqi, 2008). However, surgery is not always a final solution. Myles et al. (2002)

examined a group of 42 older individuals with knee OA before and after total knee replacement. Only 2% of subjects fully recovered from the deficits in knee function and range-of-motion (Myles, Rowe, Walker, & Nutton, 2002).

Joint Fusion

Joint fusion or arthrodesis may be an alternative when knee joint arthroplasty fails, often after infection at the surgical site. Success rates have improved over the past two decades, and arthrodesis should be considered in the case of multiple failed revisions, infection, or serious comorbidities or complications. Typically post-fusion results include stabilization of the joint, remission of pain, and some limited functional impairments with activities such as stair climbing and rising after long periods of sitting. Fusion is contraindicated if the contralateral hip or knee has undergone arthrodesis, or if there is OA in the ipsilateral hip or ankle. Patients may experience complications such as peroneal nerve palsy, thrombophlebitis, and shortening of the leg by 2.5 to 6.4 cm (Zhang & Jordan, 2008).

Current Treatments and Therapies for OA - Nutraceutical

Because traditional therapy can be expensive, invasive, and it carries a significant risk of adverse effects, people are increasingly opting for alternative and naturally-derived treatments. The use of biological compounds such as glucosamine and chondroitin supplements has gained popularity as an OA treatment in recent years (Clegg et al., 2006; Zhang & Jordan, 2008).

Glucosamine

Glucosamine is an amino sugar that is naturally present in cartilage, so is thought to stimulate proteoglycan synthesis. Supplemental forms are either made from shellfish exoskeletons or synthetically (Huskisson, 2008). Treatment effect is slow to appear, generally taking several weeks (Huskisson, 2008, Lopez, 2012).

There have been numerous trials of glucosamine, most using a 1,500 mg dose but with variations in delivery and form of glucosamine used. Results have been conflicting, but with some reduction in Lequesne pain index scores and good response rates with no adverse effects (Huskisson, 2008).

The Glucosamine Unum In Die (once-a-day) Efficacy (GUIDE) trial of 318 adults compared 1,500 mg glucosamine sulfate and three grams acetaminophen daily to placebo for knee-OA symptom relief. The results showed a significantly improved Lequesne score at six months with glucosamine compared to placebo (-3.1 vs. -1.9, respectively, $p=0.032$), and a similar improvement in Western Ontario and McMaster Osteoarthritis Index (WOMAC) scores. The response-rate was higher than both acetaminophen and placebo at 39.6%, 33.3%, and 21.2%, respectively. There were no adverse events reported (Herrero-Beaumont et al., 2007).

In contrast, a high-profile study of glucosamine sulfate by Rozendaal et al. (2008) found glucosamine ineffective. The study was conducted over the course of two years in a group of 222 Dutch subjects with hip OA. Treatment with 1,500 mg glucosamine sulfate was no better than placebo in achieving symptom relief or preventing OA progression. The authors noted that the study was designed to use the form and dosage of

glucosamine though to be more effective, did not receive industry funding, used improved measurement techniques, and included both subjective pain measures as well as radiographic measures of joint space narrowing (Rozendaal et al., 2008).

Evaluating clinical trials of glucosamine treatment is challenging due to the many confounding factors present. Different studies use varying levels of elemental glucosamine in their treatment, with most not offering information on the bioavailability of the treatment used. There is also a large placebo effect and different response levels depending on disease severity. Many glucosamine preparations are combined with other active ingredients like manganese. Also, subject use of anti-inflammatory or pain medication during the study may be a confounding factor (Huskisson, 2008). Vlad et al. (2007) conducted a meta-analysis of glucosamine studies to determine the basis for the heterogeneity seen in clinical trials. The authors concluded that the heterogeneity was entirely explained by the heterogeneity of industry-funded trials that used non-commercial glucosamine preparations and had inadequate allocation concealment in results with positive trials. While the study was not large enough to draw firm conclusions, it is worth noting that of effect sizes were only 0.05 to 0.16 in non-industry studies but 0.47 to 0.55 in industry-sponsored studies (Vlad et al., 2007).

Differences in glucosamine efficacy might be attributed in some part to the preparation used. A recent review of the Cochrane database indicated an effect size of 0.44 (95% CI 0.18-0.70) for glucosamine sulphate and 0.06 (95% CI -0.08-0.20) for glucosamine hydrochloride (Jordan et al., 2003; Zhang & Jordan, 2008). Clinical studies conducted before 1990 tended to be small, poorly designed, and of short term. More

recent larger studies have attempted to correct this and have found a modest to large effect on pain and joint function. However, refined search techniques show that effect sizes suffer greatly if studies are limited to those seen as higher quality. For instance, median effect sizes for pain were shown in one meta-analysis to be 0.57 for glucosamine sulphate and 1.37 for chondroitin sulphate. This diminished to 0.26 and 0.37, respectively, when studies were limited to more recent and higher quality trials. Overall, many contend that there is poor scientific evidence that glucosamine or chondroitin are efficacious for OA therapy (Häusselmann, 2001).

Chondroitin

Chondroitin is a GAG that is also found in cartilage proteoglycans. Its mechanism of action is thought to be through stimulation of cartilage repair and/or inhibiting degradation, as well as promoting joint viscosity. Supplemental forms are based on bovine and shark cartilage. Studies of chondroitin have used dosages ranging from 800 to 1,200 mg daily, with benefits shown in Lequesne index and pain reduction but not patient or physician clinical assessments compared to placebo (Huskisson, 2008; Mazieres, Combe, Phan Van, Tondut, & Grynfeltt, 2001).

Combination Glucosamine and Chondroitin Therapy

Both clinical studies and pharmacological data indicate co-therapy with both substances has a synergistic effect in OA treatment (Huskisson, 2008). The National Institutes of Health (NIH) sponsored the Glucosamine/Chondroitin Arthritis Intervention Trail (GAIT) study in 1,583 older adults (mean age 59, 64% women) who had symptomatic radiographic OA of the knee (Clegg et al., 2006; Das & Farooqi, 2008).

This study took into account design flaws of previous glucosamine and chondroitin studies, which include small samples, insufficient masking of study agents, failure to carry out intention-to-treat analysis, and possible bias due to manufacturer funding. Overall, prior studies tended to recruit low knee pain subjects and did now show improvements in WOMAC scores while other studies show benefit when using outcomes other than WOMAC (Clegg et al., 2006). The GAIT study compared treatment with glucosamine hydrochloride at 1,500 mg/d and chondroitin sulphate at 1,200 mg/d, individually and combined, against placebo and celecoxib at 200 mg daily for six months. The primary outcome was a 20% reduction or greater in WOMAC pain score at 24 weeks, with secondary outcomes being WOMAC stiffness and function scores among others (Huskisson, 2008). The results showed no significant effect on pain reduction when compared to celecoxib and placebo. However, the validity of the data may be questionable as the placebo rate was an exceedingly high 60% and a sub-analysis in those with moderate to severe knee pain did show a significantly higher rate of response with combination treatment compared to placebo (79.2 vs. 54.3%, $p = 0.002$). This sub-group also experienced a significant decrease in joint swelling, joint effusion, or both with chondroitin treatment alone (Clegg et al., 2006; Das & Farooqi, 2008). In addition, the GAIT study used glucosamine hydrochloride, unlike other studies that showed response to glucosamine sulfate (Das & Farooqi, 2008).

A double-blind crossover study by Leffler et al. (1999) in a group of 20 young men with knee OA also compared the combination therapy at the same daily dosages, with added manganese ascorbate at 228 mg daily. The authors found significant

improvement in pain assessment ($p = 0.05$) (Leffler, Phillipi, Leffler, Mosure, & Kim, 1999). Another study also found combination therapy with a higher dose of the glucosamine, chondroitin, and manganese to be more effective than placebo, but in mild to moderate knee OA rather than severe (Huskisson, 2008).

In contrast, Messier et al. (2007) evaluated glucosamine and chondroitin therapy combined with exercise for knee OA symptom relief and found neither was better than placebo. This randomized controlled trial tested 1,500 mg glucosamine hydrochloride and 1,200 mg chondroitin sulfate for 12 months in a group of 89 adults aged 50 and older. The authors found no difference in WOMAC scores, pain, or mobility compared to placebo (Messier et al., 2007). Overall, the literature supports the short-term use of glucosamine sulfate at 1,500 mg daily and chondroitin sulfate at 800 mg daily. The Osteoarthritis Research Society International (OARSI) group recommends that treatment with either be discontinued if no benefit is apparent within six months (Zhang & Jordan, 2008).

Vitamins and Minerals

Nutrients present in the very foods we eat may represent a promising approach to prevention and treatment of chronic diseases such as OA, and one that is the least expensive, free of potential adverse effects, and least questionable in content compared to medications and nutrient supplements (Ameye & Chee, 2006; Barnes, 2008; Lopez, 2012; Shen et al., 2012). It has long been hypothesized that intake of antioxidants such as vitamins C and E might counteract the oxidative damage of cartilage and joints in OA. This is particularly true for vitamin C as it is essential for normal collagen synthesis

(Ameye & Chee, 2006; Arden, 2006; Lopez, 2012). The Framingham Study showed higher vitamin C intake in older men and women was shown to be associated with a three-fold reduction in progression of radiographic knee changes in OA, as well as reduced frequency of knee pain when compared to those with the lowest tertile of intake (Arden, 2006; Felson et al., 2000). The literature suggests that benefits may be obtained with 250 to 300 mg of vitamin C three times daily, which would be adequate for saturation of tissue and lymphocytes while remaining within the tolerable upper limit of 2,000 mg per day. Minerals such as selenium, zinc, and copper are required cofactors for antioxidant reactions, and support the antioxidant activity of vitamins C and E. Copper and manganese are important cofactors for collagen crosslinking in cartilage and bone and also play an important role (Lopez, 2012).

Other nutrients involved in bone metabolism may also influence OA. High dietary and serum vitamin D has been shown to protect modestly against symptomatic knee OA progression, though this has not been shown radiographically (Arden, 2006; Felson et al., 2000). However, progressive radiographic hip OA is more prevalent in elderly women with low serum vitamin D (Arden, 2006). There is epidemiologic and case-controlled data linking adequate vitamin D status to reduction in the risk of certain autoimmune diseases such as multiple sclerosis and type I diabetes mellitus. The relationship is present, but weaker, for other types of chronic diseases like rheumatoid arthritis, OA, type II diabetes mellitus, hypertension, and stroke. The Framingham Study examined both dietary intake of vitamin D and serum levels of vitamin D in relation to the progression of OA, finding that both were associated with knee OA. However, a 10-

year follow up study did not find a relationship between serum vitamin D or vitamin D intake and loss of joint space or cartilage. Serum vitamin D has been shown to be significantly and independently associated with BMD in primary knee OA. Because vitamin D deficiency and knee OA are on the rise, supplementation in this population could theoretically be beneficial (Das & Farooqi, 2008). Suggested supplementation should be with more bioavailable cholecalciferol (vitamin D3) from animal products within the tolerable upper limit of 4,000 IU daily, accompanied by serum assessment of 25(OH)D six to ten weeks into treatment. Target serum levels are above 45 ng/mL (Lopez, 2012).

Polyphenols

Many food sources are rich in polyphenolic and other natural compounds with bioactive properties that may alter human health. Flavonoids are a sub-class of polyphenols that are present in spices, fruits, and vegetables. Examples include curcumin in turmeric, isoflavones in soy, proanthocyanidins in apples, grapes and berries, and catechins in green and other teas. Resveratrol is a non-flavonoid polyphenol found in peanuts, berries, and grape skins (Barnes, 2008; Lopez, 2012; Shen et al., 2012).

Polyphenols are associated with anti-inflammatory, antioxidant, cardioprotective, and chemopreventive properties so have been widely studied for their potential use in OA therapy (Barnes, 2008; Bhat & Pezzuto, 2002; Castillo et al., 2000; Fang et al., 2007; Frémont, 2000; Lopez, 2012; Hsieh & Wu, 1999). Results have shown many positive effects on OA both *in vitro* in chondrocytes and tissue explants, as well as *in vivo* in animal models, and a limited number of human studies (Shen et al., 2012).

The literature highlights four different target areas that may positively impact OA progression through the use of dietary polyphenols. The first approach is through decreased matrix degradation. This occurs via increased matrix synthesis of type II collagen, GAGs, aggrecans, and anabolic cytokines, as well as increases in the MMP inhibitor called tissue inhibitor of metalloproteinase (TMP)-1. The second approach is by decreased inflammatory activity. Polyphenols may reduce stimuli from inflammatory mediators such as COX-2 and PGE_2, as well as down-regulate inflammatory cytokines like IL-1β, IL-6, IL-8, and TNF-α (Lopez, 2012; Shen et al., 2012). Decreased synthesis of inflammatory proteins such as CRP also contributes to decreased inflammatory activity. The third area of impact is from decreased oxidative activity and damage. Polyphenols may decrease activity of inducible NOS and NO, and increase activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. A fourth area for therapy is by decreasing cell apoptosis of caspases thus increasing chondrocyte proliferation. The resulting reduction in joint stiffness, pain, and inflammation from these actions could be a mechanism for improving joint mobility and overall OA management (Shen et al., 2012).

Approximately two-thirds of dietary polyphenols in humans are attributed to flavonoid consumption, especially flavan-3-ols such as catechins, proanthocyanidins, and anthocyanidins (Dell'Agli, Busciala, & Bosisio, 2004). Normal mixed diets of adults typically contain about 200 to 400 mg of flavonoids daily, while clinical trials are usually set at 150 mg to 1,500 mg daily dosages (Lopez, 2012). However, the bioavailability and metabolic pathways of polyphenols such as resveratrol need to be better understood

before firm conclusions can be drawn about the link between dietary consumption and health benefits (Dell'Agli et al., 2004; Elmali et al., 2005; Frémont, 2000; Rennie, Hughes, Lang, & Jebb, 2003). Dietary polyphenols should also be considered as adjuncts to current treatments, possibly with a goal of boosting efficacy and lowering treatment doses to decrease the risk of side effects and toxicity of drugs such as NSAIDs (Shen et al., 2012).

Curcumin. Curcumin is the primary component of the yellow spice turmeric. It has been shown *in vitro* to inhibit COX-2 and decrease chondrocyte apoptosis, and several clinical studies have found it comparable to ibuprofen for pain reduction and improvements in symptoms and joint function as assessed by WOMAC scores, treadmill performance and serum levels of IL-1 and -6 (Henrotin et al., 2011; Lopez, 2012; Shen et al., 2012). However, at high doses it does have a potential for toxicity (Ameye & Chee, 2006; Shen et al., 2012).

Soy isoflavones. Genistein is an isoflavone found in soy that acts as a phytoestrogen and may modulate estrogen receptor (ER) expression. Cartilage tissue is ER positive, with ER-β expression rising post-menopause. Altered estrogen metabolism is linked to OA, so it is believed genistein or soy may relieve some degree of OA symptoms and potentially improve cartilage health. Studies on phytoestrogen are limited, but genistein has been shown to inhibit COX-2 and NO production in primary human chondrocytes. Genistein also increases synthesis of GAGs, but can have negative effects at higher doses. In a monkey model for knee OA there has been no significant effect

shown from soy isoflavones on cartilage lesions or cartilage component levels (Shen et al., 2012).

Arjmandi et al. (2004) conducted the first human study that tested soy isoflavones as a treatment for OA of the knee in 135 middle-aged adults. The results showed 40 grams of soy protein daily with 88 mg soy isoflavones significantly improved range-of-motion and ability to engage in activities of daily living as compared to a milk-protein placebo. Over the course of the three month study the treatment group also reported improved work performance and productivity, and significantly reduced self-reported pain and use of pain medication. However, the effect was mostly present in men with no reduction in pain medication use and a shorter duration of effect in women. The sex-related differences were potentially related to estrogen levels in women via the interaction of estrogen receptors in cartilage and estrogenic activity of isoflavones. Biomarkers were assessed by change from baseline, with men in the soy group having significantly reduced YKL-40 and increased IGF-1 levels than those in the milk group ($p < 0.05$) (Arjmandi et al., 2004).

Green tea polyphenols. Epigallocatechin gallate (EGCG) is the primary polyphenol present in green tea that has demonstrated anti-inflammatory and anti-oxidant activity. Both *in vitro* and *in vivo* studies imply that EGCG may reduce synovial hyperplasia, cartilage degradation, and bone resorption through modulation of multiple target points (Shen et al., 2012). Potential mechanisms of action include epigenetic modifications, inhibition of transcription factors, and suppression of pro-inflammatory mediators (Fang et al., 2007; Henrotin et al., 2011; Lopez, 2012). In human and bovine

chondrocytes EGCG treatment has resulted in decreased expression and production of inflammatory mediators such as NO, COX-2, PGE_2, TNF-α, MMPs and aggrecanases, as well as activation of the nuclear transcription factor kappa beta (NF-κβ) and transforming growth factors (Ahmed et al., 2002; Ahmed, Wang, Lalonde, Goldberg, & Haqqi 2004; Andriamanalijaona et al., 2005; Rasheed, Anbazhagan, Akhtar, Ramamurthy, Voss, & Haqqi, 2009; Singh, Ahmed, Islam, Goldberg, & Haqqi, 2002; Singh, Ahmed, Malemud, Goldberg, & Haqqi, 2003). Similar effects have also been shown in bovine and human cartilage explants and human synovial cells (Adcocks, Collin, & Buttle, 2002; Ahmed et al., 2004; Huang, Tseng, Lee, Su, & Lee, 2010). Green tea polyphenols added to the drinking water of arthritic mice have promoted a reduction in COX-2 and TNF-α in arthritic joints (Shen et al., 2012). A green tea extract was also used in arthritic rats and resulted in reduced NO production and presence of inflammatory cells in the synovium, and a subsequent decrease in joint degradation (Sobhi, Mohamad, Mehana, & Abdel-Raheim, 2007). Compounds present in green tea other than EGCG may also contribute to demonstrated effects, such as the tannin prodelphinidin that has shown anabolic and anti-inflammatory activity in human chondrocytes (Shen et al., 2012). EGCG is unlikely to pose a toxic burden when consumed in normal amounts. In mice EGCG has been shown to elevate plasma homocysteine, but only at a single high dose equal to 20-35 cups of green tea (Fang et al., 2007). In human chondrocytes EGCG has been found to be non-toxic (Singh et al., 2002).

Resveratrol and grape polyphenols. Resveratrol is a stilbene (i.e., non-flavonoid) polyphenol found in several fruits and plants. In grapes it is synthesized upon

exposure to UV light or in response to fungal infection. The *trans* isomer is the major form found in red wine, present in amounts of about 0.1 to 15 mg/L. While the bioavailability of resveratrol in humans is uncertain, it has been shown in rat models to be absorbed rapidly and is easily detectable in tissue and plasma, particularly the heart, liver, and kidney (Frémont, 2000; Ray et al., 1999). Plasma resveratrol levels peak at 30 minutes post-consumption, and return to baseline at one hour (Williams, Sutherland, Whelan, McCormick, & de Jong, 2004). Average wine consumption in humans is thought to allow for resveratrol concentrations linked to health benefits in red wine, particularly over the long-term. (Frémont, 2000). Resveratrol has an excellent safety profile and is not toxic, even at high doses of 3,000 mg/kg in a rat model (Bhat & Pezzuto, 2002).

Resveratrol has demonstrated anti-carcinogenic, anti-apoptotic, antioxidant, and anti-inflammatory properties (Csaki, Keshishzaden, Fischer, & Shakibaei, 2008; Gehm et al., 1997; Henrotin et al., 2011; Shen et al., 2012). In human primary articular cartilage stimulated by IL-1, resveratrol inhibits apoptosis by 1) decreasing caspase-3 activity followed by cleavage of the DNA repair enzyme poly (ACP-ribose) polymerase or PARP (Csaki et al., 2008; Shen et al., 2012) and 2) suppressing the mitochondrial ROS and p53 tumor suppressor protein that activate caspase-3 and apoptosis. Resveratrol also decreases production of IL-1 and TNF-α, thus preventing activation of NF-κβ, a regulatory agent in apoptosis. *In vitro* studies have demonstrated resveratrol reduces gene expression of vascular endothelial growth factor and COX-2, and down regulates the MMPs associated with matrix degradation. In addition, resveratrol has been shown to

protect major matrix proteins, proteoglycan, type II collagen and aggrecan from MMPs or inflammatory compounds such as inducible NOS and COX-2 (Csaki et al., 2008; Henrotin et al., 2011; Shen et al., 2012).

Dietary flavonoid consumption has been inversely linked to cardiovascular risk in several cohort studies. Resveratrol in particular has a similar effect to anti-aging calorie restriction diets in terms of decreased atherosclerosis and inflammation (Delmas, Jannin, & Latruffe, 2005). In human studies, low and moderate doses of red and white wine have been shown to modulate lipid metabolism, and inhibit LDL oxidation and platelet aggregation (Blanco-Colio et al., 2000; Ceriello et al., 2001; Delmas et al., 2005; Frémont 2000; Rein et al., 2000; Williams et al., 2004). *In vitro* studies indicate the mechanism of effect in red wine polyphenols is through short-term NO-mediated vasorelaxation, and long-term effects such as increased NOS and inhibition of platelet aggregation (Dell'Agli et al., 2004). Studies with male Sprague Dawley rats have shown resveratrol treatment promoted greater protection against pro-inflammatory compounds and improved recovery from oxidative stress compared to controls, with results attributed to free radical scavenging (Ray et al., 1999; Shigematsu et al., 2003). The authors note it is difficult to determine the amount of red wine consumption in humans that would promote a similar outcome, as serum resveratrol will depend on digestion and absorption in the intestine (Shigematsu et al., 2003).

Animal models of arthritis have shown resveratrol to be effective when injected intra-articularly at 5-100 uM dosages. Elmali et al. (2005) investigated IA resveratrol treatments in the cartilage and synovium of an OA rabbit model. Treatment was given at

10 uM/kg daily for two weeks. Resveratrol treated rabbits had significantly reduced cartilage degradation scores (1.7 vs. 2.8, $p = 0.016$) and loss of proteoglycans (1.2 vs. 2.3, $p = 0.016$), but no differences in synovial inflammation. The results suggest that, at least in early stage OA, resveratrol applied via IA injections may have a protective effect in cartilage (Elmali et al., 2005). A follow up study also showed a reduction in synovial NO, chondrocyte apoptosis, cartilage destruction, and loss of proteoglycans as compared to controls. The authors do note that the resveratrol is applied directly to the joint, so it remains unclear whether dietary resveratrol would elicit a similar effect (Elmali et al., 2007).

Gene-regulating transcription factors that are affected by resveratrol may be important for understanding the OA inflammatory response. NF-κβ is a ubiquitous cytoplasmic transcription factor, i.e., it responds to environmental triggers, and is important in regulating more than 150 genes. It is critical to regulation of genes involved in inflammatory and immune responses, such as inducible NOS, COX-2, and adhesion molecules (Barnes & Karin, 1997; Roman-Blas & Jimenez, 2006; Shakibaei, Csaki, Nebrich, & Mobasheri, 2008). It is stimulated by antioxidants, cytokines, viruses, certain proteins, and other factors (Barnes & Karin, 1997). Lauder et al. (2007) determined in three different OA cell models that inhibition of NF- κβ was associated with reductions of IL-6, MMP-1, and MMP-3 (Lauder et al., 2007). It is also weakly inhibited by resveratrol, IL-10, aspirin, and antioxidants (Barnes & Karin, 1997; Blanco-Colio et al., 2000; Elmali et al., 2005; Elmali et al., 2007, Shakibaei et al., 2008; Shakibaei, Buhrmann, & Mobasheri, 2011). Subbaramaiah and Dannenberg studied whether

resveratrol could block synthesis of the NF- κβ-regulated COX-2 enzyme, thus reducing circulating levels. The authors treated a previously described cancer cell line with resveratrol concentrations ranging from 0 to 20 uM and found suppression of detectable COX-2 mRNA and synthesis (Subbaramaiah & Dannenberg, 2001).

An important study by Shakibaei et al. (2011) found that treatment with 100 uM resveratrol suppressed inflammatory signaling and apoptosis in IL-1β-stimulated human articular chondrocytes, possibly through the NF-κβ pathway. Resveratrol inhibited nuclear translocation of and activation of NF-κβ, and gene expression of vascular endothelial growth factor, MMP-3, MMP-9, and COX-2, all of which are regulated by NF-κβ. To further test this relationship, they examined the NF-κβ signaling pathway and found resveratrol increased phosphorylation and suppressed the degradation of the NF-κβ inhibitor IκBα. Therefore the inhibitory effect of resveratrol appears to be mediated by phosphorylation and degradation of IκBα (Shakibaei et al., 2008). A follow up study with bone-derived cells also resulted in inhibition of NF-κβ, suppression of IκBα kinase and IκBα phosphorylation and degradation (Shakibaei et al., 2011).

Compounds other than isolated resveratrol may have an effect on immune and inflammatory changes in OA. Sharma and Katiyar showed that diets containing grape seed proanthocyanidins inhibited immune suppression from UVB exposure in mice (Sharma & Katiyar, 2006). A single-blind, crossover trial by Zern et al. (2005) examined the daily intake of 36 grams of a lyophilized whole grape powder for four weeks in a small group of postmenopausal women (n = 20). The powder was rich in resveratrol and other grape compounds, with the dosage being equivalent to 200 g or 1.5 cups of fresh

grapes daily. Supplementation resulted in plasma triglyceride reduction of 6% in postmenopausal and 15% in premenopausal women ($P<0.01$), along with lower plasma LDL, apoB and apoE levels in the grape powder group ($p<0.05$). There was no change in LDL oxidation, but the treatment group did have a significantly decreased whole-body oxidative stress load. There was no change in IL-6 or CRP levels in either group, but TNF-α significantly decreased in both groups. The authors feel the decrease in TNF-α may have been due to decreased lipid peroxidation, and noted there was a non-significant trend toward decreased CRP in both groups. They also noted that CRP release in the liver is stimulated by IL-6 and there may not have been a large enough decrease in IL-6 to affect CRP (Zern et al., 2005).

Nutritional research related to OA is a budding field with much uncertainty. Most of the available literature is focused on pharmacokinetics or single nutrient and target interventions (Ameye & Chee, 2006). OA research examining the intake of bioactive components in whole foods is lacking. Many plant and nutrient extracts may be harmful in therapeutic doses, so whole foods are a more desirable approach. Grape polyphenols have anti-inflammatory properties that may influence OA outcomes (Henrotin et al., 2011). The literature suggests consumption of whole grapes with their bioactive constituents may be a natural alternative to reducing pain and improving symptoms associated with OA. In addition, consumption of diets rich in polyphenol sources is consistent with dietary recommendations that promote 8-10 daily servings of colorful fruits and vegetables (Barnes, 2008).

CHAPTER III

METHODOLOGY

Participants

The Texas Woman's University Institutional Review Board (IRB) approved the study methodology prior to recruitment. Independently living and freely mobile participants with mild to moderate self-reported knee OA were recruited through local orthopedic clinics, primary care physicians, a senior recreation facility, the Texas Woman's University campus, and other locations in the community. Screening for the purpose of identifying qualified participants was conducted using a brief questionnaire establishing medical history, current medications and supplements taken, and the proposed study inclusion or exclusion criteria. Qualified participants agreed to avoid any new medication regimen known to influence OA during the course of the study, such as COX-2 selective NSAIDs. Inclusion criteria included no history of severe liver disease, kidney disease, or other disease affecting their OA, and no supplemental sources of glucosamine hydrochloride or sulfate, chondroitin sulfate, grape powder, or grape seed extract.

Eligible participants signed a consent form and were randomly assigned to the FDGP or placebo group for a period of four months. Treatment aliquots of FDGP were packaged and provided by the California Table Grape Commission, and the dosage was based upon previously published work. The FDGP group consumed a total of 47 g daily of standardized FDGP. The placebo group served as a control and consumed 47 g of a

daily placebo treatment consisting of equal parts fructose and dextrose, equal in appearance and energy content to the FDGP. At the initial study visit participants were shown how to mix the treatment with water and consumed their first treatment at that time. Participants and researchers were blinded to the treatment, which was dispensed as a two-month supply at baseline and midpoint study visits. Participants received an honorarium paid in part at study midpoint (two-months) and final visits (four-months).

Research Design and Instruments

Primary outcome measures for this randomized, placebo-controlled trial were self-reported pain, stiffness, and flexibility of the affected knee joint(s). The WOMAC protocol and a modified questionnaire based on combined McGill Pain Questionnaire and SF-36 Health Survey forms were used to assess pain and stiffness. These questionnaires were based on limitation of movement, frequency and duration of pain, and pain associated with tasks, and were previously validated and used in the literature (Bagge, Bjelle, & Svanborg, 1992; Escobar et al, 2007; Stratford, Kennedy, Woodhouse, & Spadoni, 2007). Questionnaires were scored using a five-point Likert scale. Joint flexibility was assessed in triplicate using a 180° flexible goniometer according to the Neutral Zero Method described by Cave and Roberts (Cave & Roberts, 1936) and approved by the American Academy of Orthopaedic Surgeons. Range of motion parameters (flexion and extension) were also assessed in triplicate for greater precision and validity of measurements.

Secondary outcome measures included CRP and cartilage-specific biomarkers YKL-40 and IGF-1. Serum CRP has been shown to be elevated in severe cases of knee

OA, and may be an independent marker of disease progression (Spector et al., 1997). CRP was assayed using a human *in vitro* enzyme-linked immunosorbent assay (ELISA) kit by Ray Biotech, Inc. (Norcross, GA). YKL-40 is secreted by cartilage chondrocytes and is specific to cartilage metabolism. Production of YKL-40 may be mediated by cytokines and growth factors that regulate chondrocyte function, particularly during joint inflammation as seen in OA (Bruyere et al., 2006; Conrozier et al., 2000; Garnero et al., 2001). Serum YKL-40 was assayed using an ELISA kit by Quidel Corporation (San Diego, CA). Serum IGF-1 is a biomarker of cartilage metabolism thought to be sensitive to treatment interventions (Pagura et al., 2005). Serum IGF-1 was assayed using a human *in vitro* ELISA kit by RayBiotech, Inc. (Norcross, GA).

Data Collection

Data collection and measurements occurred in three visits to a university health clinic: baseline, midpoint (two months), and final (four months). At the baseline visit participants were given a verbal and written description of the study protocol and the opportunity to ask questions prior to signing the consent form. They were counseled to maintain their normal patterns of diet, physical activity, and body weight throughout the study. Anthropometrics were measured using standard techniques. Height and weight were obtained at baseline, and weight at final visit. Pain and joint flexibility were assessed at baseline, midpoint, and final visits by use of the described questionnaires and flexible goniometer.

Overnight fasting venous blood was collected at baseline and final visits only by a trained phlebotomist. Samples were centrifuged at 1500 g for 15 minutes to separate

serum within two hours of collection. The serum was stored at -70°C until analysis was performed according to the kit manufacturer's protocols. The concentration of serum biomarkers were determined using a log-log curve, and the values reported as ng/mL (IGF-1 and YKL-40) and mg/mL (CRP).

For the IGF-1 and CRP ELISA analyses, the samples were incubated in duplicate along with the kit standards in a 96-well plate at room temperature for 2.5 hours. All incubations occurred with gentle shaking. Each IGF-1 sample (125 µL) was diluted 2-fold prior to analysis. Each CRP sample (2 µL) was diluted 15,000-fold prior to analysis. The wells were washed and treated with 100 µL of biotinylated antibody and incubated for 1 hour at room temperature. The wells were again washed, and then 100 µL of streptavidin solution was added prior to incubation at room temperature for 45 minutes. After the final wash, 100 µL of substrate reagent was added to each well, and incubated enclosed in foil at room temperature for 30 minutes. Fifty µL of stop solution was added to each well, and the optical density (OD) read immediately at 450 nm.

For the YKL-40 analysis, 20 µL of each sample, standard, and control were added to each well. Samples were not diluted prior to the assay. One-hundred µL of a capture solution was added to each well, and the plate then incubated at room temperature for 1 hour. Wells were washed and treated with 100 µL of enzyme conjugate prior to a second incubation at room temperature for 1 hour. Wells were again washed, and then treated with 100 µL of substrate solution before a final 1 hour incubation at room temperature. After adding 100 µL of stop solution the OD was read immediately at 405 nm.

Compliance to treatment was assessed at midpoint and final visits through packet counts of the assigned treatment and collection of the treatment tracking calendars that were distributed at baseline and midpoint visits. Participants were also contacted randomly by phone throughout the course of the study to promote compliance and answer any questions or concerns about ongoing treatment.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 19.0 was used for statistical analysis. Descriptive statistics such as frequency, means, and standard deviations were used to summarize demographic data and determine the normality of the distribution of the response variables. Repeated measures ANOVA was used to detect overall patterns in trends and differences by group, gender, and age for body weight, pain, stiffness, flexibility, CRP, YKL-40, and IGF-1. This was followed by paired and independent sample T-tests to determine differences between groups. Non-parametric tests (Mann-Whitney U, Wilcoxon signed rank) were conducted to examine where differences occurred by time point and within the different groups. Independent variables were derived from demographic information such as age and gender. Dependent variables included joint pain, stiffness and flexibility, as well as CRP, YKL-40, and IGF-1 concentrations. The initial sample size (n = 70) was calculated to detect differences with 0.87 power between FDGP and placebo group for pain, joint flexibility, and stiffness with an allowable attrition rate of 12% to 15%. The sample size used in this study (n = 72) was adequate to permit detection of a difference between the treatment groups for these variables, but dictated that an intent-to-treat analysis be used with missing data

replaced in order to maintain study power. Statistical significance was determined at $p<0.05$. An attrition rate of 12% to 15% from the beginning sample was allowed for.

CHAPTER IV

CONSUMPTION OF WHOLE GRAPE POWDER REDUCES JOINT PAIN AND INFLUENCES SERUM BIOMARKERS IN INDIVIDUALS WITH SELF-REPORTED KNEE OSTEOARTHRITIS (OA)

A Paper to be Submitted for Publication to the Journal *Nutrition and Aging*

Casey Tiernan

Abstract

Background: Osteoarthritis (OA) is associated with joint pain and stiffness leading to loss of mobility. There is growing interest in use of natural therapies in joint pain management. Consumption of grapes, a rich source of polyphenols, may reduce inflammation and influence OA outcomes.

Objective: Examine the effect of grape consumption on self-reported knee pain, joint range-of-motion (ROM), and biochemical markers of inflammation (C-reactive protein [CRP]) and cartilage metabolism (insulin-like growth factor-1 [IGF-1], human cartilage glycoprotein 30 [YKL-40]) in individuals with knee OA.

Methods: Qualified participants (n=72) were randomly assigned to consume 47 g of freeze-dried grape powder (FDGP) (n =35, 27 female, 8 male) or a comparable placebo (n=37, 28 female, 9 male) daily for 4 months.

Results: The FDGP group had a significant decrease in activity-related pain from baseline to end of treatment compared to placebo (-5.3 vs. -2.1, $p < .05$). At midpoint, both groups had a significant reduction in total knee symptoms and impact on quality of life

(QOL). This improvement was evident only in the FDGP group at the end of study. FDGP resulted in gender specific changes in IGF-1 compared to placebo group. Males in the FDGP group had a significant increase in IGF-1 from baseline compared to males in the placebo group (1.6 and 19.9 ng/mL in FDGP; 6.8 and 2.0 ng/mL in placebo for baseline and final, respectively, $p < .05$). There was no change in ROM, CRP, or YKL-40 between FDGP and placebo groups.

Conclusions: FDGP consumption with its bioactive constituents can be a natural alternative to improving symptoms associated with OA.

Key Words: anthocyanins, C-reactive protein (CRP), grape, human cartilage glycoprotein 30 (YKL-40), insulin-like growth factor-1 (IGF-1), joints, nutraceuticals, osteoarthritis (OA), pain, polyphenols, range of motion.

Introduction

Osteoarthritis (OA) is a complex chronic disease that generally reflects age-related degeneration of joint tissue in response to mechanical stress or injury (1). Normal repair and inflammatory responses follow and contribute to a cycle of further joint stress and damage (2-4). It is the most common arthritic disease world-wide, posing a significant cost burden, both economically and socially (3, 5-7). Pain and stiffness can be a daily occurrence among OA sufferers, with associated symptoms leading to loss of mobility and disability (2).

Pathological structural changes in OA and the presence of symptoms are only weakly related (8-10). This may be partly explained by how differently people both experience and report pain (7). Radiographic criteria for defining OA is based on the

Kellgren-Lawrence scale, which emphasizes the presence of bony formations at the joint site called osteophytes. Symptomatic OA is defined as having frequent joint pain on most days of the previous month, accompanied by radiographic evidence of OA in the affected joint (9). Symptomatic OA is thought to be more clinically relevant than radiographic OA (7).

Prevalence increases with age for both radiographic and symptomatically diagnosed OA, but women are more likely to present with symptomatic knee OA (11.4% vs. 6.8%, $p = 0.003$) (11). Women report a higher frequency of OA in the hands and knees than men in the age group of 50 and older (9). Most of the difference in risk attributed to age is due to the higher proportion of women who have OA in older age groups (11). Women report a higher frequency of OA in the hands and knees than men in the age group of 50 and older and tend to have more severe knee OA (2, 6, 9, 12). Gender differences in OA prevalence are attributed to differences in estrogen status, though evidence is inconsistent in this area and stronger for radiographic OA than symptomatic OA (6).

Because traditional therapy can be expensive, invasive, and carry the risk of significant adverse effects, people are increasingly choosing alternative and naturally-derived treatments (13-14). Research demonstrating the efficacy of alternative OA treatments like glucosamine and chondroitin supplementation has yielded conflicting results (13-17). Food sources rich in polyphenols and other compounds with bioactive properties represent another approach to prevention and treatment of OA. These polyphenols include resveratrol, which is found in peanuts, berries, and grape skins (3,

18). Intra-articular (IA) injection of resveratrol has been shown to significantly reduce cartilage degradation and proteoglycan loss in a rabbit model (3, 19), but it is unclear whether dietary resveratrol would elicit a similar effect (3).

There is evidence that dietary polyphenols provide benefit for inflammatory forms of arthritis, therefore they may also be advantageous for OA management (3, 20). The mechanism of action appears to be down-regulation of inflammatory cytokines, or by antioxidant or anti-inflammatory pathway signaling (3). Polyphenols may affect OA progression by mitigating chondrocyte inflammation and damage by directly or indirectly interacting with articular cartilage, bone, or synovium (3).

In summary, the literature suggests consumption of whole grapes rich in bioactive constituents may be a natural alternative to reducing pain and improving symptoms associated with OA. Therefore, the purpose of this study was to assess the beneficial effects of bioactive components in FDGP in reducing or alleviating symptoms of knee OA. The central research hypothesis of the study is that daily intake of 47 g of FDGP (with its bioactive constituents) will reduce or alleviate symptoms of OA. The principal aims of the study are that the treatment group receiving 47 g of FDGP daily will have: 1) decreased frequency of self-reported knee pain compared to the placebo group; 2) decreased severity of self-reported knee pain compared to the placebo group; 3) increased joint range of motion of the affected knee joint(s) compared to the placebo group; and improvements in serum biomarkers of inflammation or cartilage metabolism compared to the placebo group at the end of the study.

Materials and Methods

Study Design and Participants

The Institutional Review Board (IRB) of Texas Woman's University approved the study protocol prior to initiation of the study. Participants recruited for the study were independently living and freely mobile adults aged 45-80 years with mild to moderate self-reported physician-diagnosed knee OA from Denton, Texas and surrounding Dallas-Metroplex areas. Qualified participants (n=90) took no supplements containing glucosamine, chondroitin, or grape; did not use cyclooxygenase (COX)-2 selective non-steroidal anti-inflammatory drugs (NSAIDs); and avoided beginning any new drug or supplement regimen during the course of the study. Participants were randomly assigned to consume the whole grape powder (47 g FDGP daily) or a placebo powder (47 g daily) similar in appearance and energy content to FDGP for a period of four months. Treatment aliquots of FDGP (one 47 g packet daily) were provided and packaged by the California Table Grape Commission. Participants and researchers were blinded to study treatment.

Data collection occurred during three visits to the Texas Woman's University Institute for Women's Health. At the baseline visit anthropometrics and knee joint ROM were measured, overnight fasting blood was collected, and pain questionnaires were administered. During the midpoint visit (2 months) knee joint ROM measurements and pain questionnaire administration were repeated. At the final visit (4 months) anthropometric and knee joint ROM measurements, overnight fasting blood collection, and administration of pain questionnaires were repeated.

Pain Assessment and Joint ROM

Self-reported joint pain and stiffness were evaluated at baseline, midpoint, and final visits using a modified 5-point Likert scale questionnaire based on the McGill Pain Questionnaire (21) and the SF-36 Health Survey (22). Sub-analyses were conducted to determine mean scores for activity-related pain, difficulty with activities of daily living (ADLs) and sports/recreation attributable to pain, and QOL factors related to pain symptoms. Lower scores reflected reduced impact on scale items. Joint flexibility was determined by ROM assessment at baseline, midpoint, and final visits with a 180° flexible goniometer using the Neutral Zero Method (23).

Biomarkers of Cartilage Metabolism and Inflammation

Overnight fasting venous blood was collected at baseline and final visits and centrifuged at 1500 g for 15 minutes to separate serum within two hours of collection. The serum was stored at -70°C until analysis was performed according to the manufacturer's protocols. Serum insulin-like growth factor 1 (IGF-1) was measured using a human *in vitro* enzyme-linked immunosorbent assay (ELISA) kit from Raybiotech, Inc. (Norcross, GA). One-hundred twenty-five µL of each serum sample was diluted 2-fold prior to analysis. Serum cartilage glycoprotein 39 (YKL-40) was evaluated using an ELISA kit from Quidel Corporation (San Diego, CA). Samples were not diluted for analysis. Serum C-reactive protein (CRP) was measured using an ELISA kit by Ray Biotech, Inc. (Norcross, GA). Two µL of each serum sample was diluted 15,000-fold prior to analysis.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 19.0 was used for statistical analyses. Descriptive statistics such as frequency, means, and standard deviations were used to summarize demographic data and determine the normality of the distribution of the response variables. Repeated measures ANOVA was used to detect overall patterns in trends and differences by group and gender for dependent variables. This was followed by paired and independent sample T-tests to determine differences between groups. Non-parametric tests (Mann-Whitney U, Wilcoxon signed rank) were conducted to examine where differences occurred by time point and within the different groups. Independent variables included treatment group and gender. Dependent variables included joint pain, stiffness and flexibility, as well as CRP, YKL-40, and IGF-1 concentrations. The initial sample size (n = 70) was calculated to detect differences with 0.87 power between FDGP and placebo group for pain, joint flexibility, and stiffness with an allowable attrition rate of 12% to 15%. The sample size used in this study (n = 72) was adequate to permit detection of a difference between the treatment groups for these variables, but dictated that an intent-to-treat analysis be used with missing data replaced in order to maintain study power. Statistical significance was determined at $p<0.05$.

Results

A total of 121 people were screened to determine eligibility for inclusion in this study. Of the 90 who were eligible, 72 participants began the study. The study population was primarily Caucasian and female (n=55) ranging in age from 45 to 80 years (mean age=59.8) with an average BMI of 30.61. A total of 16 participants

withdrew after beginning the study, for a total dropout rate of 15.28% at midpoint and 22.2% at final visit. Three participants withdrew due to complaints of gastrointestinal discomfort or disliking the taste or texture of the grape drink. Two were asked by their physicians to leave the study due to uncontrolled health conditions aside from OA. One person left the study to accommodate a travel schedule, and another left after having a total knee replacement during the course of the study. The remaining nine participants did not report for one or more follow up visits. Table 1 summarizes the demographic characteristics of the participants.

Range of Motion

There were no significant differences between the FDGP and placebo groups for ROM parameters at baseline. No significant changes in overall degrees of ROM were seen for FDGP or placebo groups (Table 2). However, the FDGP group had significantly lower scores at midpoint and final visits compared to baseline for knee extension in both knees (baseline, midpoint, and final scores of -3.74, -5.48, and -5.90 for right knee; -4.87, -5.99, and -6.32 for left knee, respectively, $p<0.05$). The placebo group increased extension scores in both knees at midpoint and in the left knee at final visit compared to baseline, though this was not statistically significant (Table 3).

Joint Pain and Stiffness Symptoms

In the FDGP group, midpoint and final Likert-scale scores for impact on total knee symptoms and QOL were significantly lower than baseline (baseline, midpoint, and final 12.69, 11.37, and 11.23 for total symptoms; 18.71, 15.80, and 14.71 for QOL, $p<0.05$, Table 4). The placebo group also had significant reductions in these categories

from baseline to midpoint, however the effect plateaued and did not persist into the final visit (baseline, midpoint, and final 12.03, 10.76, and 11.11 for total symptoms; 17.78, 15.78 and 15.30 for QOL, Table 4). When females were analyzed separately by age and treatment group, reported total knee symptoms were significantly lower at the final visit compared to baseline for the FDGP group (Figure 1).

Likert-scale scores for all pain measures were significantly lower at midpoint and final compared to baseline for females across both FDGP and placebo groups, and male scores were lower only at midpoint for pain related to activities of daily living (ADL) (35.59 vs. 41.71, $p<0.05$, Table 5). In the gender group by treatment analysis, females in FDGP and placebo groups experienced decreases in pain subscales across all measures (Table 6). Males across both groups reported no significant changes in total symptoms, total stiffness, activity-related pain, or impact on daily living (Table 7).

When difference from baseline was compared between different groups, the FDGP group had a significantly greater change in activity-related pain from baseline to midpoint compared to placebo group (-5.29 vs. -2.08, $p<0.05$, Table 8, Figure 2). This significance was maintained after analysis by treatment group and gender, extending significance to the final time point for females (Table 9, Figure 3). This effect was not found in males (Table 9). In FDGP and placebo groups, female difference scores from baseline were greater at midpoint for sports and recreation-related pain compared to males (-1.55 vs. -1.24, $p<0.05$, Figure 4).

Biomarkers

There was no significant difference between baseline and final serum IGF-1, CRP, or YKL-40 between the FDGP and placebo group, though non-significant increases in all biomarkers were seen at the final time point in both groups (Table 10). Intake of FDGP led to gender specific changes in IGF-1 compared to placebo group that were significant for the males only (male baseline 1.62 ng/mL, final 19.86 ng/mL, $p < .05$, Table 11, Figure 5). Males overall had a statistically greater increase in IGF-1 from baseline compared to females (Table 10). For females, serum IGF-1 rose significantly in the placebo group but not the FDGP group (baseline 4.86 ng/mL, final 8.93 ng/mL). There was a non-significant decline in IGF-1 in the female FDGP group (Table 11). Final IGF-1 values were significantly higher for males than females (10. 04 vs. 6.01 ng/mL, Table 10).

Discussion

The outcomes of this study demonstrate daily consumption of FDGP may reduce symptoms of pain and possibly impact a biomarker of cartilage metabolism in individuals with self-reported knee OA. Consumption of FDGP led to significant decreases in total knee symptoms and pain related to activity, along with improvement in quality of life. Although both groups experienced decreases in reported OA symptoms, the effect was more pronounced and prolonged in the FDGP group compared to placebo. Such was also the case with improvement in quality of life. In males, FDGP consumption was also accompanied by a significant increase in IGF-1, a biomarker of cartilage function. Whereas in females, a greater decrease in activity-related pain was observed when compared to placebo.

To our knowledge, no studies have been conducted administering FDGP or other forms of whole grape supplements in individuals with OA. However, a similar reduction in OA symptoms has been reported with the use of soy powder with its isoflavones (a class of polyphenols) (24). Arjmandi et al. (2004) observed significant decreases in self-reported OA symptoms and use of pain medication, but the effects were primarily seen in men. The gender differences observed in our study as well as those of Arjmandi et al. may be due to differences in the estrogen/antiestrogen action of soy versus grape polyphenols, perceptions of pain by gender, or in the types of physical activity pursued by different genders (24-25).

Polyphenols and flavonoids from sources other than grapes and soy may also affect pain and joint function in OA (3, 15, 26-29). Consumption of a tart cherry beverage rich in polyphenols has been reported to decrease Western Ontario University and McMaster Universities Arthritis Index (WOMAC) scores by 15%, which was accompanied by decreases in joint pain and stiffness scores in 58 adults with OA (26). Studies with pine bark flavonoids and curcumin have also reported improved WOMAC and joint function scores combined with decreased use of pain medication in OA populations (27-28).

Further evidence of an FDGP effect on OA symptoms was demonstrated with a significantly greater change in self-reported activity-related pain compared to placebo. This finding could have been driven in part by the large number of female participants. Such was not the case in males, and this may be explained by a smaller sample size of male participants in this study. Very interestingly, the women in the FDGP group started

the study with significantly greater activity-related pain than the placebo group at baseline. O'Connor et al. (2013) utilized the same grape powder product as the present study for six weeks to determine the effect on fitness, muscle injury, and perceived health in recreationally active young adults without OA. In contrast to our results, they found no effect on perceived pain or physical functioning using the SF-36 questionnaire. Differences in the age and fitness level of the study populations may account for the discrepancy in these findings compared to the present study (30).

In male participants, FDGP consumption resulted in a significant increase in IGF-1 in comparison to the placebo treatment. These results are in agreement with those of Arjmandi et al. (2004) where soy with its isoflavones resulted in similar increases in IGF-1 in male participants, but not in the females (24). Levels of serum growth hormone and IGF-1 tend to decline around 15% for each decade of age, which makes the reported increase in this group of older males particularly remarkable (31-32). Healthy women have been shown to have lower IGF-1 values compared to males (31, 33), as was seen at the gender level in this study. Yet IGF-1 status in women is not entirely predictable (31, 33). Women with end-stage OA have more marked decreases in serum and synovial IGF-1 relative to age-matched controls and male OA counterparts. This may be in part due to greater adiposity and reduced physical function (31). Dietary factors have also been shown to affect IGF-1 levels in women, and there is evidence that IGF-1 is decreased with exposure to oral versus transdermal estrogen replacement (33-34). We did not take into account the dietary polyphenol intake or hormonal status of the women in the present study, which may explain the gender differences in IGF-1 levels.

C-reactive protein levels are elevated in women with knee OA (35-36). However, the magnitude of the rise in CRP is much greater in more classically defined inflammatory diseases, infection, or trauma (35). We did not observe any change in CRP levels in the FDGP or placebo groups. Our sample mean for CRP (2.12 mg/L) fell below ranges reported in other OA studies (26, 35-37), and may have been insufficient to detect changes in CRP. Similarly, studies that have used the same grape product as the current study to determine the effect on cardiovascular risk factors reported no significant difference in CRP between treatment and placebo (38-40). However, when resveratrol was added to a grape extract a significant decrease in high-sensitivity (hs) CRP was observed in a triple-blind randomized-controlled trial of preventative cardiac care. These authors also tested a conventional grape extract with no added resveratrol, which failed to elicit significant changes in hsCRP (41). The current study addressed symptomatic OA, but it is worth noting that large cohorts have failed to show a correlation between CRP and radiographic OA (1, 42). Like IGF-1, CRP is known to increase with exposure to oral hormone replacement therapy, which was not evaluated in this study (43).

YKL-40 is a hydrolase that plays a role in cartilage metabolism. While its exact function is not well understood, it is believed to be representative of cartilage degradation and is a promising biomarker of degradative tissue changes in OA (44-47). Serum levels of YKL-40 are elevated in rheumatoid arthritis (RA) and, to a lesser-degree in OA (47). In the study by Arjmandi et al. (2004), men in the soy treatment group were shown to have significantly reduced YKL-40 at the end of the study compared to placebo group (24). We did not observe any significant changes in YKL-40 due to FDGP or placebo

treatment. It is possible that gender differences contributed to a lack of significant change in YKL-40 in our treatment group, which was primarily female and had a smaller number of males than Arjmandi et al. (2004) (24).

Unlike other chronic diseases such as hyperlipidemia and hypertension, there is no reliable biomarker or clinical measure for diagnosing OA (7). OA patients often seek treatment at later stages of the disease, yet biomarker changes across OA progression are not fully understood (46, 48). As was the case for our study, it is not always possible to stratify patients according to the stage of OA. It may be that other biomarkers involved in cartilage metabolism such as cartilage oligomeric matrix protein (COMP), type II collagen C-telopeptide fragments (CTX-II), hyaluronic acid, and collagen matrix metalloproteinases (MMPs) would be more representative of a treatment effect of FDGP in symptomatic OA (46). However, due to limitations of our study we were unable to evaluate these markers.

Range of motion, a measure of joint flexibility, was not found to differ between FDGP and placebo treatment. This finding is in contrast to that of soy isoflavones which significantly improved ROM, work performance, productivity, and ability to engage in ADLs in comparison to the casein-based placebo (24). It is possible that the casein used in their placebo had different effects on ROM parameters than that of the grape placebo used in the present study, which was composed of equal parts fructose and dextrose. There were unexpected decreases in knee extension for the FDGP group in the current study. Manual goniometry is the most commonly used method of ROM analysis used in physical therapy settings (49-50), and was chosen in the present study for its low cost,

minimal invasiveness, and ease of use. However, it is known to be prone to variability on the order of 5% to 10% (50). The reliability of manual goniometry can be influenced by differences in instrument, measurement procedures, joint and body regions, and patient types. Other variables that can affect ROM outcomes include edema, pain, adhesions, strength deficits, and muscle hypertrophy (49). In the present study, some participants reported feeling increases in knee pain with successive ROM measurements, while others described feeling less pain and greater movement with each successive measurement. ROM measurements also tended to vary based on reports of recent physical activity, injury, weather changes, and clothing worn by the participants on the day of measurement. Future studies might consider using electrogoniometry to decrease variability in ROM measurement. It is reported to be objective, and both faster and less expensive than conventional gait analysis. However, the equipment is fragile and requires a trained clinical staff member to operate (50).

BMI plays a major role in the onset of OA due in large part to the effect of increased weight bearing on the joints (1, 5, 7). The effect of FDGP could be influenced by variations in BMI across the genders participating in this study. This could potentially affect OA symptoms, pain, and ROM to a different degree of magnitude. The current study was primarily composed of obese females, which have a higher incidence of OA (5, 7, 11). Due to our sample size, we were unable to randomize treatment based on BMI. An abnormal distribution of BMI between treatment groups could have been a factor in our lack of significant findings for some measures. The use of larger study groups with

more males and varied ethnicities, along with a longer treatment period, could provide a greater ability to stratify OA patients by age, gender, BMI, and other variables.

Grape polyphenols such as resveratrol have demonstrated antiapoptotic, anti-oxidant, and anti-inflammatory properties (3, 19, 51). Compounds in grapes other than isolated resveratrol may also affect immune and inflammatory changes in OA (52-56). However, the bioavailability and metabolic pathways of polyphenols needs to be better understood before strong conclusions can be drawn about the link between dietary consumption and health benefits (20, 39, 57-59). The results of our study lend support to the growing consensus that consumption of FDGP with its bioactive constituents may be a natural alternative to reducing pain and improving symptoms associated with OA.

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Tables and Figures

Table 1. Baseline Participant Demographics

	n	**% of Sample**	**Mean±SD[a]**
Gender			
Male	17	23.6	61.4 ±10.6
Female	55	76.4	59.4 ±10.3
Total Sample	72	100	59.8 ±10.3
Age			
45-55 years	32	44.5	50.19 ±3.31
56-65 years	16	22.2	60.63 ±2.70
66-75 years	17	23.6	69.88 ±2.67
76+ years	7	9.7	77.86 ±1.57
Total Sample	72	100	59.8 ±10.3
Body Mass Index (BMI)			
Normal (18.5-24.9)	12	23.1	22.72 ±1.61
Overweight (25-30)	15	28.8	27.12 ±1.50
Obese (>30)	25	48.1	36.48 ±4.41
Total Sample	52	100	30.61 ±6.73

[a] SD = standard deviation

Table 2. Knee Joint Range of Motion (ROM) in Degrees

Group	***n***	**Right Knee**		**Left Knee**	
		Baseline	Final	Baseline	Final
		<---------- *Mean ± SD*[a] ---------->			
FDGP[b]	35	112.23 ±15.8	108.11 ±14.0	111.20 ±13.4	108.84 ±11.3
Placebo	37	109.36 ±14.2	109.67 ±17.7	108.48 ±14.5	108.83 ±15.9
Female	55	110.41 ±15.7	107.53 ±17.2	109.14 ±14.4	106.72 ±14.0
Male	17	111.88 ±12.9	113.37 ±9.7	111.94 ±12.6	115.66 ±10.9

[a] SD = standard deviation

[b] FDGP = freeze dried grape powder group

Table 3. Mean Knee Extension in Degrees by Treatment Group

Group	*n*	Right Knee			Left Knee		
		Baseline	Midpoint	Final	Baseline	Midpoint	Final
		<---------- *Mean ± SD*[a] ---------->					
FDGP[b] Group	35	-3.74 ± 3.5	-5.48 ± 4.3*	-5.90 ± 4.3*	-4.87 ± 3.5	-5.99 ± 4.9*	-6.32 ± 4.0*
Placebo Group	37	-4.72 ± 4.1	-3.46 ± 3.0	-4.76 ± 5.3	-4.37 ± 4.3	-3.88 ± 4.0	-3.79 ± 3.3

* significantly different from baseline, $p<0.05$

[a] SD = standard deviation

[b] FDGP = freeze dried grape powder group

Table 4. Self-Reported Knee Pain and Symptoms by Treatment Group[a]

Symptom	FDGP[b] Group (*n=35*)			Placebo Group (*n=37*)		
	Baseline	Midpoint	Final	Baseline	Midpoint	Final
	<---------- *Mean ± SD*[c] ---------->					
Total Symptoms	12.69 ± 3.2	11.37 ± 3.5*	11.23 ± 3.9*	12.03 ± 3.3	10.76 ± 3.0*	11.11 ± 3.2
Total Stiffness	5.83 ± 1.6	5.00 ± 1.5*	5.11 ± 1.9*	5.54 ± 1.4	4.70 ± 1.4*	4.86 ± 1.3*
Activity-Related Pain	21.51 ± 4.8	17.51 ± 5.0*	16.23 ± 5.0*	18.59 ± 5.5	17.32 ± 5.1	16.51 ± 4.2*
Sports/ Recreation Pain	17.31 ± 3.7	15.34 ± 4.3*	13.83 ± 5.1*	16.59 ± 5.0	15.59 ± 5.3	13.95 ± 4.6*
Impact on Daily Living	43.06 ± 11.1	34.09 ± 9.0*	33.40 ± 10.5*	40.05 ± 13.0	34.41 ± 10.4*	34.32 ± 9.9*
Impact on Quality of Life	18.71 ± 4.3	15.80 ± 5.1*	14.71 ± 4.4*	17.78 ± 4.8	15.78 ± 4.6*	15.30 ± 5.2

* significantly different from baseline, $p<0.05$

[a] Reported as mean Likert-scale score for each symptom measure

[b] FDGP = freeze dried grape powder group

[c] SD = standard deviation

Table 5. Self-Reported Knee Pain and Symptoms by Gender[a]

Symptom	Females (*n=55*)			Males (*n=17*)		
	Baseline	Midpoint	Final	Baseline	Midpoint	Final
	<---------- *Mean ± SD*[b] ---------->					
Total Symptoms	12.36 ± 3.2	11.24 ± 3.2*	10.95 ± 3.3*	12.29 ± 3.6	10.47 ± 3.4	11.88 ± 4.1
Total Stiffness	5.65 ± 1.4	4.82 ± 1.4*	4.95 ± 1.5*	5.76 ± 1.6	4.94 ± 1.6	5.12 ± 2.0
Activity-Related Pain	20.25 ± 5.5	17.35 ± 5.1*	15.95 ± 4.3*	19.24 ± 4.9	17.65 ± 4.9*	17.76 ± 5.4
Sports/ Recreation Pain	16.65 ± 4.6	15.11 ± 4.8*	13.31 ± 4.8*	17.88 ± 4.0	16.65 ± 4.9	15.76 ± 4.5
Impact on Daily Living	41.45 ± 12.2	33.84 ± 9.7*	32.91 ± 10.1*	41.71 ± 12.1	35.59 ± 9.7	37.00 ± 10.2
Impact on Quality of Life	18.18 ± 4.8	15.56 ± 4.6*	14.69 ± 4.5*	18.41 ± 3.9	16.53 ± 5.6	16.06 ± 5.7

* significantly different from baseline, $p<0.05$

[a] Reported as mean Likert-scale score for each symptom measure

[b] SD = standard deviation

Table 6. Self-Reported Knee Pain and Symptoms by Treatment and Gender, Females[a]

Symptom	FDGP[b] Group Females (*n=27*)			Placebo Group Females (*n=28*)		
	Baseline	Midpoint	Final	Baseline	Midpoint	Final
	<---------- *Mean ± SD*[c] ---------->					
Total Symptoms	12.67 ± 3.3	11.52 ± 3.6*	10.74 ± 3.2*	12.07 ± 3.1	10.96 ± 2.8*	11.14 ± 3.4*
Total Stiffness	5.74 ± 1.5	4.96 ± 1.4*	4.89 ± 1.8*	5.57 ± 1.4	4.68 ± 1.4*	5.00 ± 1.2*
Activity-Related Pain	21.89 ± 5.0	17.19 ± 4.7*	15.63 ± 4.3*	18.68 ± 5.6	17.5 ± 5.4*	16.25 ± 4.3*
Sports/ Recreation Pain	17.15 ± 4.0	15.11 ± 4.1*	13.63 ± 5.3*	16.18 ± 4.9	15.11 ± 5.4*	13.00 ± 4.4*
Impact on Daily Living	43.93 ± 11.3	33.81 ± 9.4*	32.67 ± 9.9*	39.07 ± 12.8	33.86 ± 10.2*	33.14 ± 10.4*
Impact on Quality of Life	18.74 ± 4.6	15.15 ± 4.6*	14.22 ± 3.9*	17.64 ± 4.9	15.96 ± 4.5*	15.14 ± 5.0*

* significantly different from baseline, $p<0.05$

[a] Reported as mean Likert-scale score for each symptom measure

[b] FDGP = freeze dried grape powder group

[c] SD = standard deviation

Table 7. Self-Reported Knee Pain and Symptoms by Treatment and Gender, Males[a]

Symptom	FDGP[b] Group Males (*n*=8)			Placebo Group Males (*n*=9)		
	Baseline	Midpoint	Final	Baseline	Midpoint	Final
	<---------- *Mean ± SD*[c] ---------->					
Total Symptoms	12.75 ± 3.3	10.88 ± 3.2	12.88 ± 5.4	11.89 ± 4.1	10.11 ± 3.8	11.00 ± 2.5
Total Stiffness	6.13 ± 1,9	5.13 ± 1.6	5.88 ± 2.2	5.44 ± 1.4	4.78 ± 1.6	4.44 ± 1.7
Activity-Related Pain	20.25 ± 4.1	18.63 ± 5.9	18.25 ± 7.0	18.33 ± 5.6	16.78 ± 4.0	17.33 ± 3.9
Sports/ Recreation Pain	17.88 ± 2.5	16.13 ± 5.1	14.50 ± 4.9*	17.89 ± 5.2	17.11 ± 4.9*	16.89 ± 4.0*
Impact on Daily Living	40.13 ± 10.6	35.00 ± 8.2	35.88 ± 13.0	43.11 ± 13.8	36.11 ± 11.4	38.00 ± 7.7
Impact on Quality of Life	18.63 ± 3.5	18.00 ± 6.3*	16.38 ± 5.5*	18.22 ± 4.4	15.22 ± 4.9*	15.78 ± 6.1*

* significantly different from baseline, $p<0.05$

[a] Reported as mean Likert-scale score for each symptom measure

[b] FDGP = freeze dried grape powder group

[c] SD = standard deviation

Table 8. Difference Scores From Baseline for Self-Reported Knee Pain and Symptoms by Treatment[a]

Symptom	FDGP[b] Group (*n=35*)		Placebo Group (*n=37*)	
	Midpoint	Final	Midpoint	Final
	<---------- *Mean ± SD*[c] ---------->			
Total Symptoms	-1.31 ± 3.6	-1.46 ± 3.8	-1.27 ± 2.6	-0.92 ± 3.5
Total Stiffness	-0.83 ± 1.7	-0.71 ± 1.9	-0.84 ± 1.4	-0.68 ± 1.4
Activity-Related Pain	-4.00 ± 5.3	-5.29 ± 5.4*	-1.27 ± 5.2	-2.08 ± 6.1
Sports/ Rec. Pain	-1.97 ± 3.7	-3.49 ± 4.8	-1.00 ± 4.2	-2.65 ± 4.4
Impact on Daily Living	-8.97 ± 10.5	-9.66 ± 11.6	-5.65 ± 9.7	-5.73 ± 12.4
Impact on Quality of Life	-2.91 ± 4.5	-4.00 ± 4.5	-2.00 ± 5.0	-2.49 ± 6.0

* significantly different from baseline, $p<0.05$;
[a] Reported as mean Likert-scale score for each symptom measure
[b] FDGP = freeze dried grape powder group
[c] SD = standard deviation

Table 9. Difference Scores From Baseline for Self-Reported Knee Pain and Symptoms by Treatment and Gender[a]

Symptom	FDGP[b] Females (*n=27*)		Placebo Females (*n=28*)		FDGP[a] Males (*n=8*)		Placebo Males (*n=9*)	
	Midpoint	Final	Midpoint	Final	Midpoint	Final	Midpoint	Final
	<---------- *Mean ± SD*[c] ---------->							
Total Symptoms	-1.15±3.6	-1.93±3.4	-1.11±2.8	-0.93±3.5	-1.88±2.5	0.13±4.9	-1.78±2.1	-0.89±3.9
Total Stiffness	-0.78±1.5	-0.85±1.7	-0.89±1.5	-0.57±1.4	-1.00±2.4	-0.25±2.6	-0.67±1.1	-1.00±1.6
Activity-Related Pain	-4.70±5.0*	-6.26±4.6*	-1.18±6.0	-2.43±6.0	-1.63±5.9	-2.00±5.4	-1.56±4.1	-1.00±6.1
Sports/ Rec. Pain	-2.04±3.6	-3.52±4.8	-1.07±4.4	-3.18±4.2	-1.75±4.2	-3.38±5.1	-0.78±3.5	-1.00±5.0
Impact on Daily Living	-10.11±10.0	-11.26±10.4	-5.21±10.5	-5.93±12.1	-5.13±12.0	-4.25±14.3	-7.00±7.1	-5.11±14.3
Impact on Quality of Life	-3.59±4.0	-4.5±4.2	-1.68±5.4	-2.50±5.8	-0.63±5.3	-2.25±5.5	-3.00±3.0	-2.44±6.8

* significantly different from baseline, $p<0.05$;
[a] Reported as mean Likert-scale score for each symptom measure
[b] FDGP = freeze dried grape powder group
[c] SD = standard deviation

Table 10. Serum Biomarkers

Group	*n*	IGF-1[a] (ng/mL)		CRP[b] (mg/mL)		YKL-40[c] (ng/mL)	
		Baseline	Final	Baseline	Final	Baseline	Final
		<---------- *Mean ± SD*[d] ---------->					
FDGP[e]	35	4.31 ± 7.15	7.04 ± 15.01	1.74 ± 1.75	2.06 ± 1.75	72.02 ± 56.71	75.31 ± 61.18
Placebo	36	5.95 ± 12.01	7.07 ± 9.27	2.49 ± 2.58	3.03 ± 2.98	71.60 ± 49.76	74.43 ± 31.30
Female	54	5.51 ± 9.35	6.01 ± 7.83	2.15 ± 2.05	2.65 ± 2.17	67.54 ± 56.80	73.40 ± 50.44
Male	17	3.97 ± 11.65	10.40 ± 21.16*	2.02 ± 2.77	2.24 ± 3.36	85.37 ± 36.14	79.53 ± 40.51

* significantly different from baseline, $p<0.05$

[a] Insulin-like growth factor 1

[b] C-reactive protein

[c] Human cartilage glycoprotein 30

[d] SD = standard deviation

[e] FDGP = freeze dried grape powder group

Table 11. Serum Biomarkers for Treatment by Gender

Group	*n*	IGF-1[a] (ng/mL)		CRP[b] (mg/mL)		YKL-40[c] (ng/mL)	
		Baseline	Final	Baseline	Final	Baseline	Final
		<---------- *Mean ± SD*[d] ---------->					
FDGP[e] Female	27	5.10 ± 8.0	3.24 ± 2.5	2.01 ± 1.9	2.33 ± 1.8	73.18 ± 61.9	73.38 ± 62.9
Placebo Female	25	4.86 ± 8.8	8.93 ± 10.5**	2.40 ± 2.3	2.91 ± 2.4	57.79 ± 47.1	72.78 ± 35.8
FDGP[e] Male	8	1.62 ± 0.9	19.86 ± 28.8*	0.83 ± 0.5	1.13 ± 1.1	68.46 ± 36.8	81.83 ± 58.5
Placebo Male	8	6.81 ± 17.1	2.03 ± 1.4	3.41 ± 3.6	3.36 ± 4.7	96.74 ± 29.6	77.97 ± 17.8

* significantly different from baseline, $p<0.05$

** significantly different from baseline in female-only analysis by treatment group , $p<0.05$

[a] IGF-1 = insulin-like growth factor 1

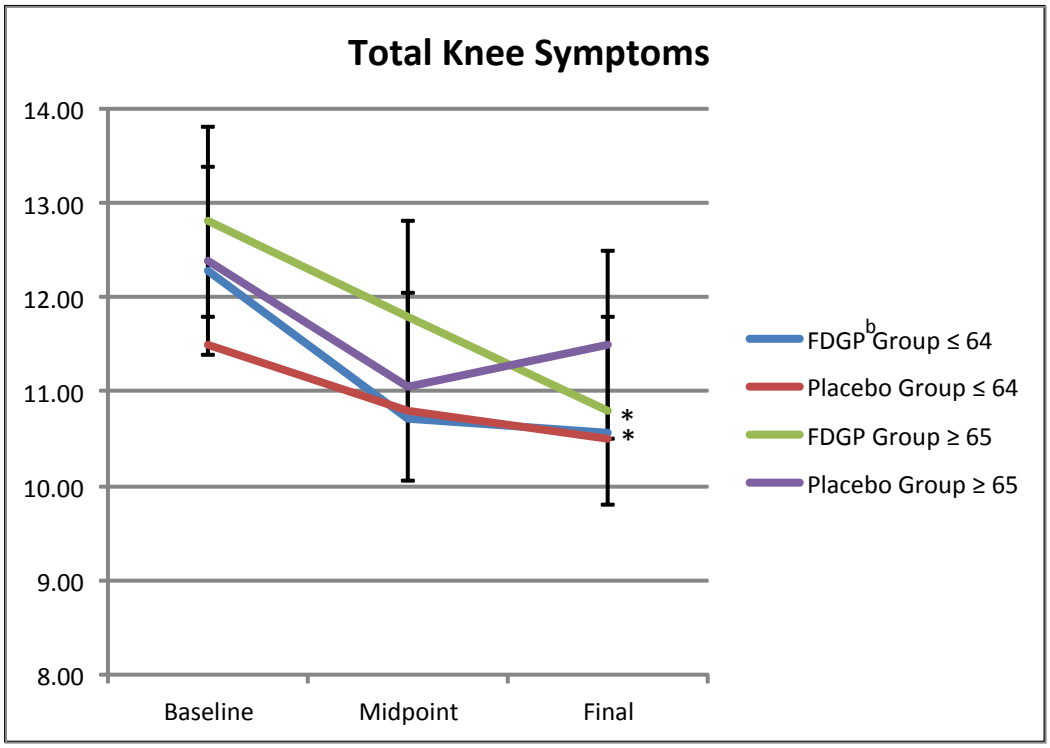
[b] CRP = C-reactive protein

[c] YKL-40 = human cartilage glycoprotein 30

[d] SD = standard deviation

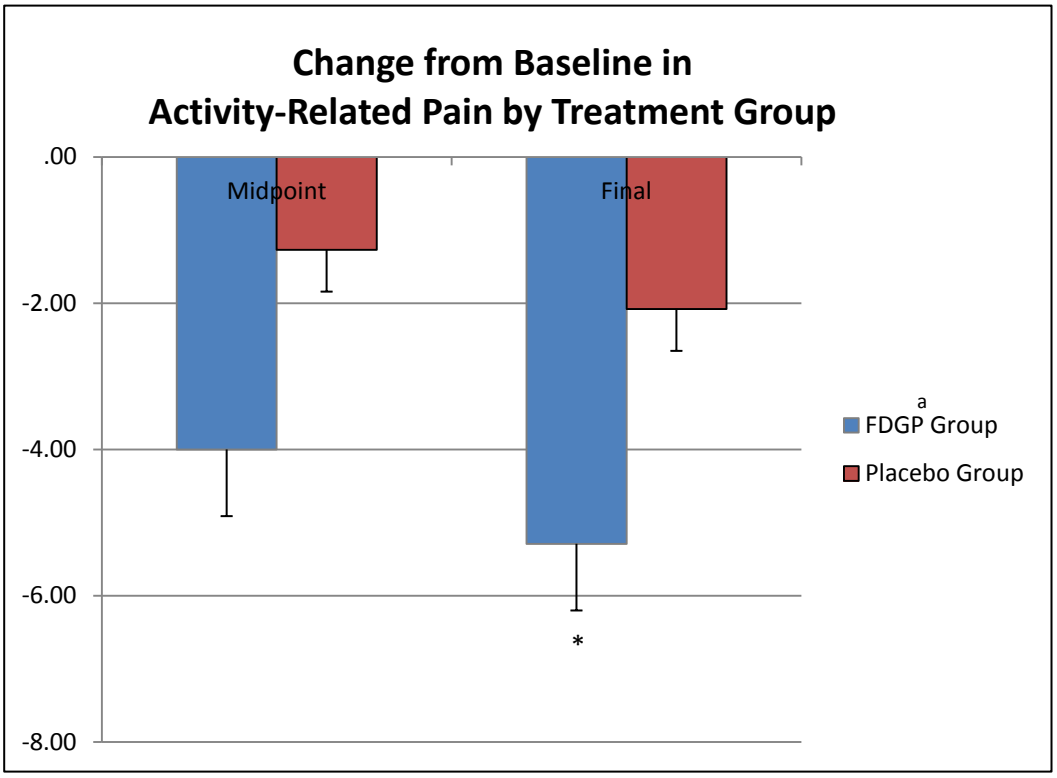
[e] FDGP = freeze dried grape powder group

Figure 1. Self-Reported Total Knee Symptoms by Treatment and Age Group, Female-Only Analysis[a]



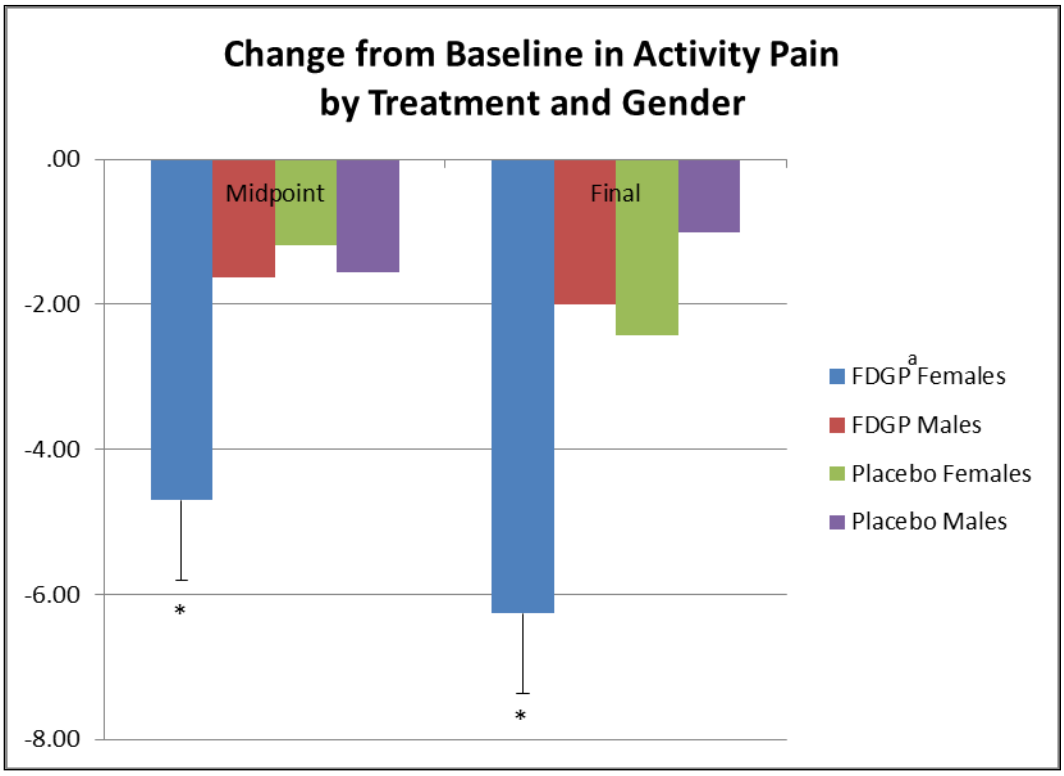
* significantly different from baseline, $p<0.05$
[a] Reported as mean Likert-scale score for each symptom measure
[b] FDGP = freeze dried grape powder

Figure 2. Difference Scores from Baseline for Self-Reported Activity-Related Pain by Treatment Group



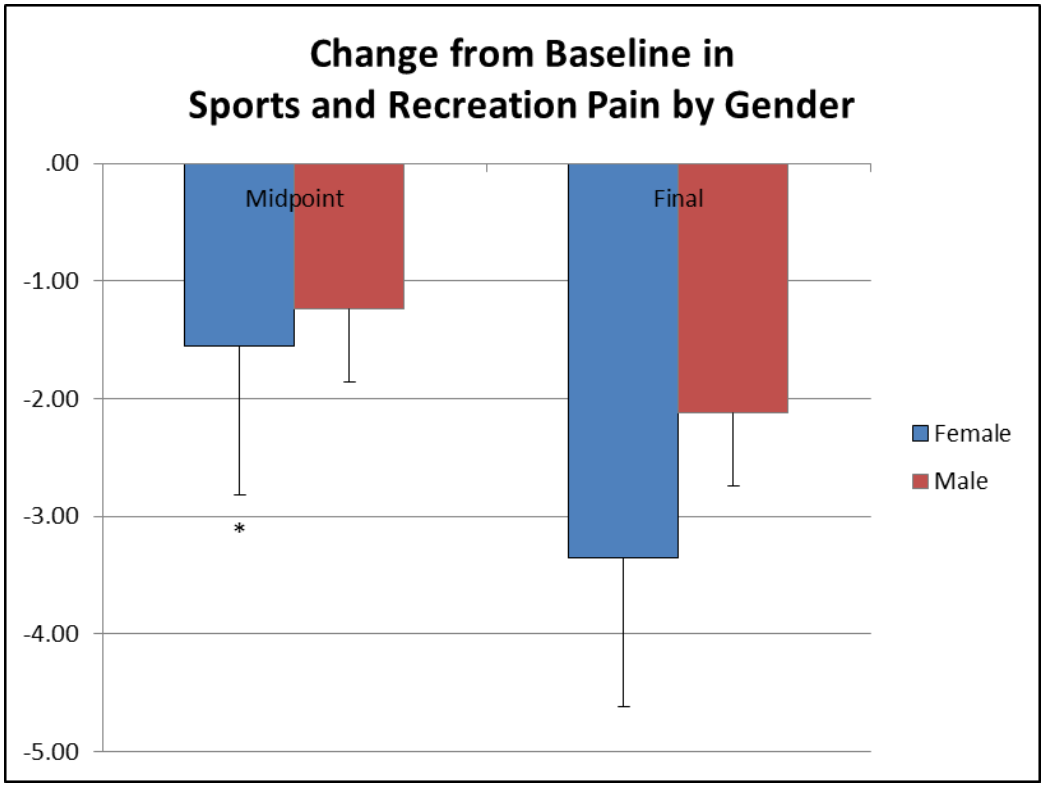
* significantly different from placebo group, $p<0.05$
[a] FDGP = freeze dried grape powder

Figure 3. Difference Scores from Baseline for Self-Reported Activity-Related Pain by Treatment and Gender



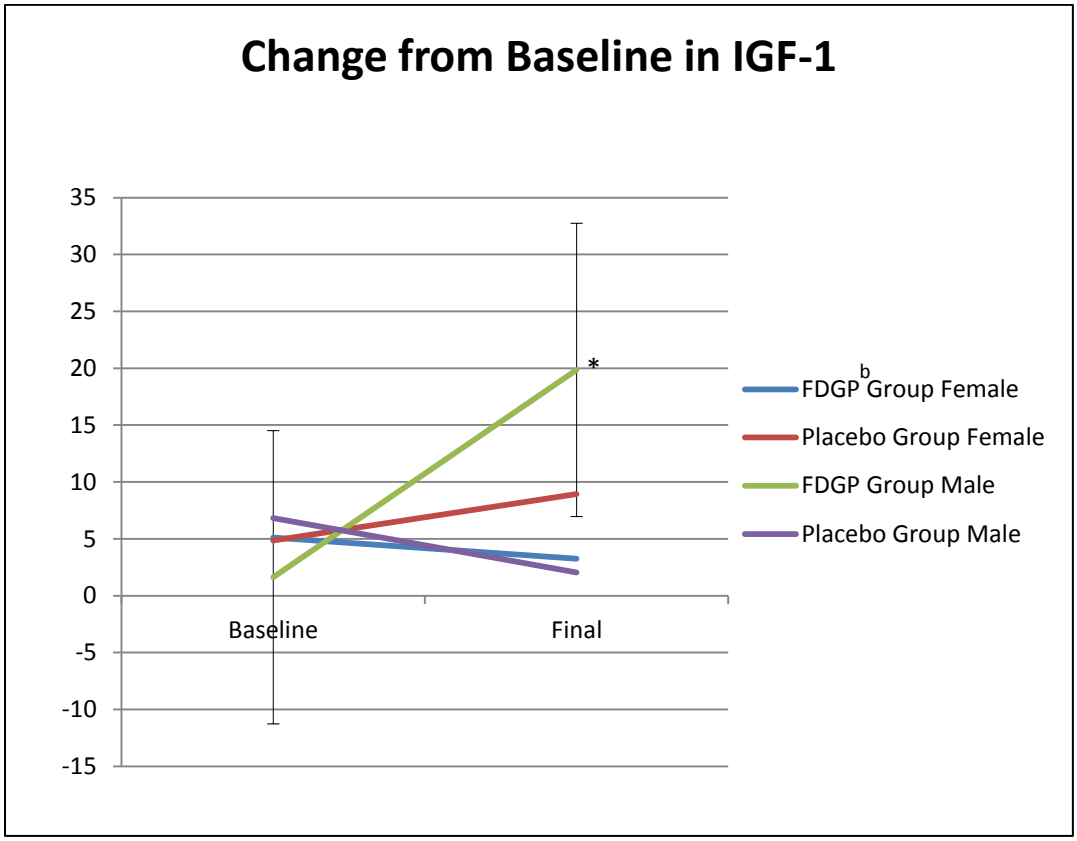
* significantly different from males (FDGP, placebo) and placebo group females, $p<0.05$
[a] FDGP = freeze dried grape powder group

Figure 4. Difference Scores from Baseline for Self-Reported Sports and Recreation Pain by Gender



* significantly different from males, $p<0.05$

Figure 5. Difference from Baseline in Serum IGF-1[a] by Treatment Group and Gender



* significantly different from females (FDGP and placebo), placebo males
[a] insulin-like growth factor 1
[b] FDGP = freeze dried grape powder

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

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

RECRUITMENT FLYER

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 C

ELECTRONIC RECRUITMENT STATEMENT

Email Version

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.

Pioneer Portal Version: Seeking participants for Knee Pain Study

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. 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.

APPENDIX D

SCREENING QUESTIONNAIRE

Screening Questionnaire

ID: Sex: Age:	
Telephone(s): e-mail:	
Do you smoke?: ____ Yes _____ No Cigarettes per day _____	
Medical condition you are taking medicine for:	
Hypertension ___ High cholesterol ___ Kidney disease ____ Lung disease ____ Diabetes ___ Heart disease ____ Liver disease ___	
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

CONSENT TO PARTICIPATE IN RESEARCH

Participant Initials _____

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 mobility associated with self-reported knee osteoarthritis. We will ask the following questions:

a) Will eating whole grape powder for 4 months improve joint mobility?
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 mobility. 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 mobility. 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 mobility. 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.

Participant Initials _____

Time Commitment

The study will last 4 months. The participant's time commitment during the three visits includes completion of screening questionnaire (~20 minutes), consent form (30 minutes), blood draw during start and end of study (-30 minutes), baseline, 2 months, and end of study measurements and questionnaires (~ 4 hours) Total time commitment for each participant is approximately 5 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 Participant

Participant Initials _____

phlebotomist will clean your arm with alcohol before taking blood and she will use a new needle. This will minimize the possibility of infection.

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 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:

_____
_____
_____

APPENDIX F

MODIFIED MCGILL KNEE PAIN SURVEY QUESTIONNAIRE

Modified McGill Knee Pain Survey Questionnaire

Symptoms

These questions should be answered thinking of your knee symptoms during the last week.

S1. Do you have swelling in your knee?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S2. Do you feel grinding, heat clicking or any other type of noise when your knee moves?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S3. Does your knee catch or hang up when moving?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your knee fully?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S5. Can you bend your knee fully?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

Stiffness

The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the eases with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

S7. How severe is your knee stiffness after sitting, lying or resting later in the day?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Pain

Which Statement describes your pain in the last month?

1. Rarely present- Have pain every few days or weeks
2. occasionally present for brief periods, a few seconds to a few minutes
3. occasionally present- have pain once to several times per day, lasting a few minutes to an hour
4. often present, but am pain free for most of the day

5. often present, but have pain free periods lasting for one to several hours
6. usually present but have short periods without pain
7. always present, intensity varies
8. Always present, always the same intensity

P1. How often do you experience knee pain?

Never	Monthly	Weekly	Daily	Always
☐	☐	☐	☐	☐

What amount of knee pain have you experienced the last week during the following activities?

P2. Twisting/pivoting on your knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P3. Straightening knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P4. Bending knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P9. Standing upright

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A1. Descending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A4. Standing

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A8. Going shopping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A9. Putting on socks/stocking

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A11. Taking off socks/stocking

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A12. Lying in bed (turning over, maintaining knee position)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A14. Sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A16. Heavy domestic duties (moving heavy boxes, scrubbing floor, etc.)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A17. Light domestic duties (cooking, dusting, etc.)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, sports and recreational activities

The following questions concern you physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the last week due to you knee.

SP1. Squatting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP2. Running

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP3. Jumping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP4. Twisting/pivoting on you injured knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP5. Kneeling

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Quality of life

Q1. How often are you aware of your knee problem?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Q3. How much are you troubled with lack of confidence in your knee?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Q4. In general, how much difficulty do you have with your knee?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

How much distress or discomfort have you had in the past month because of pain?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

How has the pain interfered with your normal work habits in the past month (including work both in and outside the home)?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Compared to the past month, how would you rate your health in general now?

1. much better now than 3 months ago
2. somewhat better now than three months ago
3. about the same as three months ago
4. somewhat worse now than three months ago
5. much worse now than three months ago

Have you drunk alcohol in the past month to relieve pain?

Yes ☐ No ☐

In the past month, have you taken medicine for pain relief?

1. no
2. yes- less than one time per week
3. yes- several times per week
4. yes- one or two times per day
5. yes- three or four times per day
6. yes- five or more times per day

If you have taken medication for the pain in the past month, did you take it:

When Needed ☐ Regularly ☐

During the past month, has your pain interrupted your sleep?

Not at all ☐ 1-2 nights ☐ 3-5 nights ☐ every night ☐

Do the following items increase or decrease your pain?

Items	Increase your pain	Decrease your pain	Not Applicable
Liquor			
Stimulants such as Coffee			
Eating			
Heat			
Cold			
Damp			
Weather changes			
Message or use of a vibrator			
Pressure			
No movement			
Movement			

APPENDIX G

RANGE OF MOTION MEASUREMENT FORM

Date: _____, 2012

Extension

Position: supine

Alignment

- align fulcrum with lateral epicondyle (center hole with outer knee)
- align stationary arm with greater trochanter (hip – have participant indicate first, then check placement)
- align mobile arm with lateral malleolus (ankle)

Measure: extension (knee is straight). Keep goniometer aligned, stationary arm fixed and mobile arm moving. In active they move themselves, in passive we move them.

- If goniometer reads 0° participant has full extension, record
- If goniometer bends in a V-shape participant has hyperextension, record as positive number with 0° extension
- If goniometer bends in a peaked shape record measurement as a negative (e.g. -6°)

Right Knee

	Trial 1	Trial 2	Trial 3
Active extension			
Passive extension			
Hyperextension?			

Left Knee

	Trial 1	Trial 2	Trial 3
Active extension			
Passive extension			
Hyperextension?			

Flexion

Alignment: bring knee into flexed position (thigh perpendicular to bed). Keep goniometer aligned as for extension.

Stabilize: support thigh, gravity will bring knee into flexion

Measure: record degrees of flexion

Right Knee

	Trial 1	Trial 2	Trial 3
Active flexion			
Passive flexion			

Left Knee

	Trial 1	Trial 2	Trial 3
Active flexion			
Passive flexion			

Height:_____

Weight:_____