THE EFFECT OF FREEZE DRIED WHOLE RASPBERRIES ON PAIN, JOINT FLEXIBILITY, AND INFLAMMATION IN INDIVIDUALS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

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ABSTRACT

THE EFFECT OF FREEZE DRIED WHOLE RASPBERRIES ON PAIN, JOINT FLEXIBILITY, AND INFLAMMATION IN INDIVIDUALS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

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Objective: The purpose of this study was to examine the effect of freeze dried whole raspberries on pain, joint flexibility, and inflammation in individuals with symptomatic knee osteoarthritis (OA).

Methods: In this double-blind and randomized trial, a total of 63 men and women were recruited and placed into either a treatment group (raspberry powder) or placebo group (powder without raspberry) for a period of 4 months. The raspberry group (n = 34) was given 35 grams of freeze dried whole raspberry powder daily. Participants were instructed to mix the raspberry powder in 10-12 oz of water, and to consume it within 5 minutes. The placebo group (n = 29) consumed 35 grams of placebo powder mixed with 10-12 oz of water. The placebo group was given a powder that was of similar appearance and energy content as the raspberry powder, but without the raspberry content. At baseline, midpoint (60 days), and final (120 days) visits, demographic data including height, weight, and blood pressure (systolic and diastolic) were obtained. At each study visit, participants filled out the Western Ontario McMaster Osteoarthritis Index (WOMAC) questionnaire to evaluate pain, stiffness, and difficulty in activity associated with knee OA. Range of motion testing was conducted using a goniometer during each

study visit to assess joint flexibility. Additionally, an overnight fasting blood specimen was collected at each study visit to assess biomarkers of inflammation.

Results: A total of 44 participants completed the study with a drop-out rate of approximately 30%. No significant changes in weight or BMI were observed in either group. A significant decrease in systolic and diastolic blood pressure was seen among the raspberry group at final visit compared to baseline. No changes in systolic or diastolic blood pressure were noted in the placebo group. Total WOMAC score as well as its subgroups (pain, stiffness, and difficulty performing daily activities) were significantly decreased at final visit compared to baseline in the raspberry group. In the placebo group, a significant decrease in stiffness was observed at final compared to baseline. There were no significant changes in total WOMAC score or its subgroups between the two groups. There were no significant changes in overall ROM scores in the raspberry or placebo groups. At midpoint visit, right knee extension was significantly less in the raspberry group than in the placebo group. There was also a significant decrease noted in right knee extension in the raspberry group at midpoint compared to baseline. A significant decrease in left knee extension was observed in the raspberry group at midpoint compared to baseline. No differences were observed in right knee flexion. However, there was a significant increase in left knee flexion in the raspberry group at final compared to baseline. For inflammation, no significant changes were witnessed among the pro-inflammatory (TNF-α, IL-1β, IL-6) or anti-inflammatory biomarkers (IL-10, IL-4, IL13). However, slight changes were noted in some of these biomarkers. There was a slight decrease in IL-6 concentrations in the raspberry group at final time-point, although not significant. Additionally, there was a slight increase in IL-6 concentrations in the placebo

group at final compared to baseline. There was a decrease in IL-10 concentration in both the raspberry and placebo group at final in comparison to baseline.

Conclusion: The findings of this study suggest that daily incorporation of whole raspberries can reduce pain, stiffness, and difficulty to perform daily activities. There was also a small, albeit not significant, improvement in joint flexibility in the knees. We did not observe any changes in the biomarkers of inflammation. Improvement in pain, stiffness, difficulty doing daily activities, and joint flexibility associated with consumption of raspberry may lead to an increase in overall quality of life in individuals with symptomatic knee OA.

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CHAPTER I

INTRODUCTION

Osteoarthritis (OA) is a disease characterized by pain, stiffness, decreased quality of life, and decline in physical function. It can be classified as either radiographic or symptomatic (Zhang & Jordan, 2010). Radiographic OA employs the use of radiograph images in which structural changes can be detected. These changes are detected using the Kellgren-Lawrence grading tool to assess severity of OA (Zhang & Jordan, 2010). Symptomatic OA refers to a combination of radiographic images and disease-related symptoms linked to OA (Zhang & Jordan, 2010). This condition is the most common joint disorder in the United States of America. The prevalence of knee OA seems to increase every decade, most likely due to the aging of the population and increased prevalence of obesity. As a result, it is likely that the number of individuals with symptomatic OA may rapidly increase in the upcoming decade (Deshpande et al., 2016). It is deemed a disease distinguished by habitual loss of articular cartilage due to wear and tear of the affected joint over time as well as an inflammatory disease of the synovial joints. Increased inflammation usually causes gradual progression of the disease, further decreasing functionality and quality of life (Deshpande et al., 2016).

According to the National Health and Nutrition Examination Survey (NHANES III), approximately 37% of individuals over the age of 60 have radiographic knee OA and 12.1% of individuals in the same age group have symptomatic knee OA (Lawrence et al.,

2007). According to the CDC, OA affects approximately 30 million US adults (CDC, n.d.). Women over the age of 60 are more likely to develop the disease. This may be due to a hormonal imbalance that occurs as estrogen levels decrease once menopause begins (Hame & Alexander, 2013). In this age group, approximately 13% of women and 10% of men have symptomatic knee OA (Zhang & Jordan, 2010). According to the Johnston County Osteoarthritis Project, the lifetime risk for developing symptomatic knee OA is approximately 45%. Risks increase to approximately 60.5% among individuals who are classified as obese (Murphy et al., 2008). Based on the National Health Interview Survey (NIHS), the number of patients with doctor-diagnosed arthritis is expected to reach 67 million by 2030. A majority of these cases are expected to be classified as symptomatic knee OA (Neogi, 2013).

Current treatment and therapies for OA include pharmacological, non-pharmacological, and dietary interventions. Since pain is the noteworthy symptom associated with OA, pain medications are often prescribed to patients. Acetaminophen, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly prescribed to patients, but can lead to complications such as chronic constipation or GI bleeding. They are the most commonly used medication for pain reduction among patients with OA. Approximately 65% of patients with OA are prescribed NSAIDs (Gore, Tai, Sadosky, Leslie, & Stacey, 2012). Surgical intervention, such as total knee replacement, can be indicated for individuals with severe OA. From 1999 to 2008, total knee replacement procedures in the US have nearly doubled among the overall population and tripled among individuals between the ages of 45 and 64. Approximately

54% of knee OA patients will eventually need a total knee replacement in their lifetime (Losina & Katz, 2013).

Non-pharmacological therapies and dietary interventions are also used for treatment options. Individuals are now using dietary supplementation to help with symptoms and inflammation. Physical therapy and exercise as well as weight loss have also been used to reduce OA-related symptoms. Recent studies have demonstrated potential benefits of dietary polyphenols associated with chronic musculoskeletal diseases such as OA. Polyphenols are known to have a multitude of properties including anti-inflammatory, anti-arrhythmic, and anti-carcinogenic roles. In relation to OA, the anti-inflammatory properties of polyphenols are especially important in combating rising levels of inflammatory biomarkers responsible for the disease progression. Polyphenols are also known for their antioxidant activity. These compounds are able to protect the cells against oxidative damage by scavenging free radicals, directly providing chondroprotective effects on the afflicted joints (Scalbert, Johnson, & Saltmarsh, 2005).

Berries, such as blueberries, strawberries, and blackberries, are a significant source of polyphenols. The major class of polyphenols among berries is anthocyanins. Anthocyanins are considered flavonoids, and are found to have strong anti-inflammatory and antioxidant properties (Han, Shen, & Lou, 2007). Studies have shown a beneficial relationship between anthocyanins and OA. Numerous clinical trials have suggested that consumption of fruits such as blueberries or strawberries are linked to a decrease in pain, stiffness, and increased function among patients with OA. Studies have also shown a decrease in inflammatory biomarkers. Raspberries are another significant source of polyphenols (Han et al., 2007). Much like other berries, raspberries may play a protective

role against the progression of OA. In an animal model of antigen-induced arthritis, raspberry extracts resulted in inhibition of inflammation as well as protected damage to the cartilage tissue (Schell, Betts, Lyons, & Basu, 2019). These studies suggest that raspberries may potentially have a protective role in individuals with OA. There are currently no human trials investigating the role of raspberry consumption and its effects on pain, stiffness, function, joint flexibility, and inflammation in individuals with OA. This study investigated the effects of daily raspberry consumption in men and women with symptomatic OA.

Hypothesis and Specific Aims

Hypothesis

Consumption of 35 grams of freeze-dried raspberry powder daily by individuals with symptomatic knee OA will reduce pain, improve joint flexibility, and decrease inflammation.

Specific Aims

Aim 1: To examine the effects of freeze-dried whole raspberry in comparison to a placebo powder on pain in men and women with symptomatic knee OA.

Aim 2: To evaluate the effects of freeze-dried whole raspberry in comparison with a placebo powder on joint flexibility in men and women with symptomatic knee OA.

Aim 3: To evaluate the effects of freeze-dried whole raspberry in comparison with a placebo powder on selective serum biomarkers of inflammation in men and women with symptomatic knee OA.

CHAPTER II

REVIEW OF LITERATURE

Osteoarthritis

OA is the most common cause of arthritis among the elderly in the world. This disease is also one of the most prevalent source of chronic disability in the United States of America. OA can occur among many different joints such as the fingers, neck, lower back, and weight-bearing joints such as the hips, knees, and feet ("Osteoarthritis," n.d.). However, the most common site of OA is in the knee joint. There is currently no cure for OA. Specific to knee OA, the three compartments of the joint that are affected include the medial, lateral, and patellofemoral (Lespasio, Piuzzi, Husni, Muschler, & Guarino, 2017). Major symptoms of this disease include severe joint pain, stiffness, swelling, and difficulty performing daily activities. Although the pathophysiology of this disease is still under investigation, it is clear that OA is a condition that is multifactorial in origin. Inflammation, age, family history, obesity, synovitis, joint shape, weight-bearing activities, and trauma are all factors that can result in this debilitating disease (Deshpande et al., 2016). A combination of these factors can lead to an increased risk for disease development (Deshpande et al., 2016). OA can be characterized by a gradual loss of articular cartilage in synovial joints. It is further distinguished by the formation of osteophytes (bone spurs), change in bone shape, and inflammation (Dieppe & Lohmander, 2005). Typically, cartilage gives bones the ability to glide over each other

without friction. However, as cartilage begins to degrade, friction between the bones increases. This can result in pain, swelling, inflammation, and problems with moving the affected joint. OA can be classified into two categories: radiographic and symptomatic. Radiographic OA refers to changes in bone structure or loss of cartilage determined via radiograph images. Symptomatic OA is refers to a combination of radiographic images and symptoms related to the disease.

The prevalence of OA increases in persons over the age of 65 (Felson et al., 1987). According to NHANES III, the rate of radiographic knee OA among US adults between 1991 and 1994 was 37.4%. Also, the incidence of symptomatic knee OA among US adults in the same time period was 12.1% in this age category (Lawrence et al., 2007). Radiographic knee OA was present more in women than men. Women were also found to have greater changes in Kellgren-Lawrence scores associated with classifying the degree of OA. The progression of symptomatic OA did not differ by sex. Both radiographic and symptomatic knee OA were significant among individuals with a high body mass index (BMI > 30), greater age, non-Hispanic/Black ethnicity, and among men who worked in manual labor occupations (Deshpande et al., 2016). According to the CDC, OA affects approximately 30 million adults in the United States. Approximately 15.1 million of these adults live with symptomatic knee OA (CDC, n.d.). According to Murphy et al., the lifetime risk of developing symptomatic OA is approximately 45% (Murphy et al., 2008). Frequency of OA increases with each decade due to the aging of previous generations. With aging, higher obesity rates, and more knee injuries, the prevalence of OA is expected to increase in the future (Deshpande et al., 2016).

Economic Burden

The condition of OA is accompanied by a high economic burden due to its effects on disability and expense of treatment. In 1997, economic costs of musculoskeletal disorders in five countries (Australia, Canada, France, United Kingdom, and United States) were analyzed. This analysis showed that OA was the most common musculoskeletal disorder and was found to have a rising trend in financial burden. In 1997, the total medical expenditure for arthritis was $233.5 billion. This increased further by 2003 to $321.8 billion (Bitton, 2009). According to the United States Bone and Joint Initiative, this cost once more increased between 2004 and 2014 to reach levels of $486.4 billion (United States Bone and Joint Initiative, 2015). OA has played a major role in cost of hospital visits and stays over the years. In 2013, there were approximately 20.7 million ambulatory care visits related to OA. Additionally, there were approximately 2.95 million in-patient hospitalizations associated with OA. Medical costs per person for OA have averaged $11,052 per year between 2008 and 2014 (United States Bone and Joint Initiative, 2015). Additionally, the cost for total knee replacements has increased by nearly five times from 1998 to 2013. As of 2003, California leads the nation with the highest cost attributable to arthritis at $12.1 billion (United States Bone and Joint Initiative, 2015). Greater disability is linked to greater medical costs among individuals with OA. In a study conducted by Gupta et al., individuals with higher WOMAC scores had approximately 3.5-times the reported costs of those with lower WOMAC scores (Gupta, Hawker, Laporte, Croxford, & Coyte, 2005).

The effects of OA extend to employment and the amount of days lost from work. Evidence suggests that employed individuals with OA have higher medical costs than

those without OA (Berger, Hartrick, Edelsberg, Sadosky, & Oster, 2011). From 2013-2015, 180.9 million total lost workdays were reported among individuals with OA (United States Bone and Joint Initiative, 2015). A 2012 study conducted by Sandell found that OA was the leading cause of total lost work among any other chronic disorder (Sandell, 2012). Between 2008 and 2011, earning losses due to OA were estimated at approximately $80 billion per year (United States Bone and Joint Initiative, 2015).

In 2012, over 1 million joint arthroplasties were conducted in the United States. This total cost approximated $18.8 billion ("Osteoarthritis," n.d.). In 2013, the average cost for a total knee arthroplasty (TKA) was approximately $20,293. Additionally, average cost of TKA revisions in the same year was approximately $26,388 (Losina & Katz, 2013). A TKA revision is performed if hardware from the initial surgery becomes loose, or if the patient has additional problems with the knee including infection or fractures (Cross, 2015).

Risk Factors

OA is considered a multifactorial condition. Risk factors for OA include age, gender, obesity, genetics, diet, and occupation. Other risk factors include joint injury, malalignment, or abnormal loading of the joints. Age is one of the leading risk factors for OA. It has been found that OA associated with age may be linked to oxidative damage, habitual cartilage thinning, muscle weakening, and reduced proprioception (Litwic, Edwards, Dennison, & Cooper, 2013). Age-related sarcopenia may also play a role in the progression of OA. This stems from increasing weakness in the joint. Weakness in the joint can be a result of muscle atrophy from disuse. Some data also suggests that weakness is related to arthrogenic muscle inhibition, which refers to a lack of knee

extension due to knee trauma (Palazzo, Nguyen, Lefevre-Colau, Rannou, & Poiraudeau, 2016). Approximately 37% of individuals with radiographic OA are older than 60 years. In the same age group, prevalence of symptomatic knee OA is approximately 12% (Lawrence et al., 2007). Although OA is typically considered a disease most prevalent among the elderly, there is an increasing number of younger adults who are now being diagnosed with OA. The largest growth in TKR rates has been among individuals who are younger than 60 years. This can be due to a variety of different factors including increased obesity rates or injury (Deshpande et al., 2016).

Studies explaining differences in the prevalence of OA between genders have shown that women have a greater likelihood of having OA than men. Among persons who are greater than 60 years of age, symptomatic knee OA occurs in approximately 13% of women and 10% of men (Zhang & Jordan, 2010). The sex difference in knee cartilage volume becomes greater after 50 years of age among women, which suggests that menopause might play a role in OA. Though not fully understood, there may be underlying hormonal factors associated with menopause that may contribute to the progression of OA. This could be due to hormonal imbalance that occurs as estrogen levels decrease after menopause begins (Hame & Alexander, 2013). Some studies have suggested a protective effect with hormone replacement therapy in women with radiographic knee OA. In a study conducted by Spector et al., findings indicated that the use of hormone replacement therapy could reduce the risk for OA (Spector, Nandra, Hart, & Doyle, 1997). Despite these results, more research must be done in order to examine this association with OA. Other explanations for greater sex difference in knee cartilage

after 50 years of age include reduced volume of cartilage, bone loss, and reduced muscle strength mainly among women (Johnson & Hunter, 2014).

Obesity is defined as having BMI > 30 kg/m^2. Obesity has a strong association with knee OA. Evidence suggests that risk for OA or rapid progression of OA is increased by three-fold if one is classified as obese (Deshpande et al., 2016). Overweight, which is defined as having BMI > 25 kg/m^2 is also associated with knee OA, though less than associated than obesity (Silverwood et al., 2015). For every 1-lb increase in weight, approximately 2-4 lbs of pressure are put on the knees (Lespasio et al., 2017). Therefore, it is evident that obesity can play a huge role in the progression of OA. Approximately 24.6% of cases involving patients with new-onset knee pain were associated with overweight or obesity (Silverwood et al., 2015). OA is also associated with metabolic syndrome. Metabolic syndrome refers to a cluster of conditions that may increase risk for diseases such as cardiovascular diseases, diabetes mellitus, or even stroke. Abnormalities present within metabolic syndrome are directly associated with systemic inflammation. This increased inflammation may encourage the progression of OA (Litwic et al., 2013). Serum leptin has been suggested as a bridge between obesity and knee OA. Leptin is an adipokine produced by adipose cells, and its main function is to suppress hunger. Higher levels of serum leptin are associated with obesity. In a study conducted by Fowler-Brown et al. (2014), patients with knee OA and greater BMI were found to have higher serum levels of leptin. In the same study, it was found that leptin was found in the synovial fluid especially among individuals with greater BMI. In correlation with greater BMI, this finding suggests that circulating leptin may seep into the joint space. Leptin receptors have been found on human chondrocytes. Additionally, leptin is found to be secreted by

chondrocytes. This secretion is increased among patients with knee OA (Fowler-Brown et al., 2014).

Genetics and diet both also play a role in the incidence of OA. Genetic factors make up approximately 40% of all knee OA cases (Palazzo et al., 2016). Low dietary levels of vitamin D, vitamin K, and vitamin C have also been linked to OA. Although more studies are needed to evaluate these claims, some research has suggested that vitamin D deficiency is associated with increased joint space narrowing or cartilage loss (Felson et al., 2006). Low levels of vitamin K are associated with OA attributed to the decreased presence of vitamin K-dependent proteins found within the joint tissues. As a result, joint tissue is exposed to deposition and mineralization of calcium, which is associated with damaged cartilage (Shea et al., 2015). Low levels of Vitamin C have also been associated with increased pain and reduced physical function (Hung et al., 2017).

Among all the joints in the body, the knee is the most frequently injured joint. Injury or over-tearing of the anterior cruciate ligament (ACL) has led to early-onset knee OA in approximately 13% of cases (Palazzo et al., 2016). The ACL is one of four ligaments in the knee that connect the femur bone to the tibia bone. The prevalence of knee OA increases to between 21% and 40% when a ruptured ACL is combined with damaged cartilage, subchondral bone, collateral ligaments, or ruptured menisci (Palazzo et al., 2016). Adolescents or young adults who experience ACL injuries are prone to acquire knee OA before the age of 40 years (Øiestad et al., 2010). Abnormal loading and overuse of joints are frequently observed among individuals who have occupations that require squatting, kneeling, prolonged lifting, and standing. Intense physical activity can play a role in the progression of OA. These types of physical activity are typically highly

repetitive and have high-impact on the joints. Studies suggest a 1.6 time increase in the risk of knee OA among individuals with labor-demanding occupations (Zhang & Jordan, 2010). Physical activity is beneficial for building muscle and gaining strength, but can be dangerous if there is excessive loading on the joints. However, in a study conducted by Barbour et al. (2013) using data from the Johnston County Osteoarthritis Project, individuals who met physical guidelines developed by the Department of Health and Human Services did not have increased risk for knee OA.

Mental Health and OA

OA is a condition linked to adverse effects on the mental health of individuals. It has been hypothesized that mental disorders such as anxiety and depression can deepen symptoms of OA. In fact, studies show that about 20% of individuals with OA also have depression. In a study conducted by Nazarinasab, Motamedfar, and Moqadam (2017), mental health status was analyzed among those who are diagnosed with OA. Among participants, depression was the most prevalent mental health condition, and studies show that prevalence of mental disorder is higher among patients with disease duration less than 6 months. This can possibly be due to initial stress associated with the diagnosis of this disease (Nazarinasab, Motamedfar, & Moqadam, 2017). Another study conducted by Veronese et al. found that individuals with lower limb OA (including knee OA) had a greater likelihood of developing depressive symptoms (Veronese et al., 2016). Some studies have analyzed relationships between depression and structural changes. A study conducted by Rathbun, Yau, Shardell, Stuart, and Hochber (2016) showed a greater likelihood of osteophyte formation in depressed participants compared to non-depressed participants. Another study by Demakakos et al. (2013) found that individuals with

elevated depressive symptoms have a slower gait speed. Patients with depression may have less motivation to seek medical attention. Additionally, severity of pain or decreased function may result in greater depressive episodes (Demakakos et al., 2013).

Anxiety can also be caused by disease symptoms, particularly pain. In a study conducted by Scopaz et al., patients with knee OA and high anxiety had greater limitations in physical function. One explanation for this phenomenon is that symptoms may increase emotional triggers. In the case of OA, increased pain may lead to higher psychological stress and anxiety. Another explanation could be that the expectation of feeling pain when doing certain activities may induce anxiety (Scopaz, Piva, Wisniewski, & Fitzgerald, 2009). Additionally, individuals suffering from chronic illness such as OA have a greater likelihood of having suicidal ideations and/or committing suicide (Kye & Park, 2017).

Pathophysiology

OA is characterized by a progressive deterioration and loss of articular cartilage. It also features changes in the structure and function of the affected joint. Examples of structural change include joint space narrowing, osteophyte formation, and hypertrophic bone changes (Ashkavand, Malekinejad, & Vishwanath, 2013). OA is a complex disease and is accompanied by a variety of different processes at a cellular level. Chondrocytes are the cells found in the connective tissue of cartilage. Interaction between chondrocytes and the extracellular matrix (ECM) are vital in maintaining cartilage tissue (Garcia-Carvajal et al., 2013). Chondrocytes maintain the ECM with the help of collagen, proteoglycans, and non-collagenous proteins. In normal cartilage, turnover of the matrix is common and frequent (Garcia-Carvajal et al., 2013). Normal cartilage involves a

balanced cycle between synthesis and degradation of the ECM. During development, anabolic compounds such as insulin-like growth factor I (IGF-I) and transforming growth factor beta (TGF-beta) are stimulated. This stimulates synthesis. Inflammatory cytokines, such as IL-1β and TNF-α, are also produced to cause cartilage degradation. In the OA-related joint, both synthesis and degradation are enhanced. Inflammatory cytokines increase synthesis of matrix metalloproteinases (MMPs) and decrease activation of MMP inhibitors. MMPs have the ability to prevent synthesis of the ECM from occurring. This in turn leads to breakdown of the cartilage. As OA progresses, degradation of the ECM happens faster than synthesis (Sandell & Aigner, 2001).

Diagnosis

Diagnosis of OA utilizes a patient's history, conducting a physical assessment, and performing the proper imaging studies (Lespasio et al., 2017). The process usually begins with obtaining a detailed history from the patient. Typically, patients complain of pain or stiffness related to the afflicted joint. These complaints and descriptive symptoms are most important in identifying OA. Patients may also complain of decreased functionality and ability to perform daily activities. After obtaining the patient history, a physical examination is performed by the clinician. During this phase of diagnosis, it is imperative to pay close attention to areas with increased pain and tenderness. Patients who claim to have symptomatic OA may not actually have signs of OA in the joint. Patients' symptoms can be a result of another underlying disease or disorder. These criteria for diagnosis is important for deciding which treatment options would be the most suitable (Balint & Szebenyi, 1996). Despite modern technological advances, the radiograph continues as the most accessible tool for diagnosing OA. Radiograph images

are used to observe the formation of osteophytes and joint space narrowing. The Kellgren-Lawrence grading system is used to diagnose severity of disease. A score of 0 indicates no radiographic signs of OA, while a grade of 4 indicates large osteophytes, marked joint space narrowing, and bony deformity (Braun & Gold, 2012).

Osteoarthritis and Pain

One of the most noteworthy symptoms of OA is pain. In most cases, this symptom is what propels individuals to seek medical treatment and interventions. Pain is normally perceived as a sensation that plays a protective role. Pain indicates that something is wrong. For example, when an individual touches a hot stove, they immediately feel a sensation of pain indicating that they must withdraw their hand. This type of pain is called nociceptive pain (Neogi, 2013). However, when pain persists, this can be a sign of further disorder in the body. As pain becomes more severe in the progression of OA, individuals begin to experience a decrease in quality of life as well as hindered mobility and functionality. The pain experience among individuals with OA has been assessed through many research studies. In a qualitative study conducted by Hawker et al. (2008), individuals with hip or knee OA identified two types of OA pain: intermittent pain that was severe or intense and persistent pain that was described as in the background or aching. The intermittent but severe pain had a greater impact on quality of life. This pain negatively affected mood and prevented individuals from participating in social or recreational activities due to the fear that pain would be triggered. Participants in this study also reported disturbances in sleep onset and sleep management (Hawker et al., 2008).

There are multiple tools used to assess pain in individuals with OA. A visual analog scale (VAS) or a numeric rating scale (NRS) is used for assessment of pain intensity over time. These scales question pain or stiffness in or around the knee. Validated questionnaires used for pain assessment over time are the Western Ontario and McMaster University Arthritis Index (WOMAC) or the Knee Injury and Osteoarthritis Outcome Score (KOOS). WOMAC and KOOS also do assess pain associated with specific activities of daily living (Neogi, 2013). WOMAC was developed in 1982 at the Western Ontario and McMaster Universities. It is a questionnaire divided into three categories: pain, stiffness, and physical function ("WOMAC Osteoarthritis Index," n.d.). KOOS was developed in the 1990s. KOOS is similar to WOMAC, but is split into five different categories instead of three: pain, other symptoms, function in daily living, function in sports and recreation, and knee related quality of life ("Knee Injury and Osteoarthritis Outcome Score," n.d.).

Though the etiology of OA is not entirely understood, researchers are focusing their investigations to determine the sources and mechanisms for the associated pain with this condition. There are three mechanisms that are thought to be involved in pain-related OA: local pathological processes in the joint leading to pain, neuronal mechanisms, and general factors. Local pathological processes and structural changes that occur in the affected joint can play a major role in the cause for the pain. Loss of articular cartilage is the key change and can be seen in radiographic images as osteophyte formation and joint space narrowing. Loss of articular cartilage is also associated with development of sclerosis and osteophytes in an attempt to repair the joint (Eitner, Hofmann, & Schaible, 2017a). There is a correlation between radiographic knee OA and prevalence of pain. In a

study conducted by Neogi et al. (2009), there was a positive association between greater pain scores and a higher Kellgren-Lawrence grade. This can be due to the formation of osteophytes and increased joint space narrowing in the joint. OA is now considered a disease of the entire joint, including the ligaments, menisci, synovium, and ligament capsules (O'Neil & Felson, 2018). MRI scans are used to identify which structural changes are most associated with pain. Studies suggest that pain is associated with structural factors including bone marrow lesions, synovitis, and periarticular lesions (O'Neil & Felson, 2018).

It is known that patients with OA experience nociceptive pain when first having symptoms. However, as the disease progresses, there may be neuronal mechanisms that come into play. Neuropathic pain occurs as a consequence of disease that affects the somatosensory system, thus resulting in pain that goes beyond nociceptive pain. Therefore, it is possible that the nerves innervating the affected OA joint will be damaged (Eitner et al., 2017a). In a study conducted by Hochman, French, and Gillian (2007), 34% of individuals with knee OA reported burning, intense pain, numbing, and tingling in the affected joint. This indicates neuropathic pain (Hochman, French, & Gillian, 2007). Though neuropathic pain may be overlooked sometimes, it is still important to understand because its pathology and the choice of treatment may differ from that of nociceptive pain.

As mentioned before, there are multiple risk factors associated with OA. These risk factors may also have an impact on the prevalence of pain. According to Berenbaum, Griffin, and Liu-Bryan (2016), individuals with OA are typically older, overweight or obese, and suffer from metabolic syndrome or diabetes. Obesity is one of the major risk

factors for OA. Individuals who are obese have increased load on their joints due to excessive weight. Additionally, individuals who are obese may have a higher level or presence of inflammatory mediators in the body. Diabetes mellitus is another risk factor for OA. Research suggests that patients with diabetes mellitus have increased production of pro-inflammatory cytokines. In a study conducted by Eitner et al, pain intensity was assessed among participants with knee OA who were diabetic and non-diabetic (Eitner et al., 2017b). The study showed that patients with diabetes mellitus experienced higher pain intensity and frequency. The study also showed that patients with diabetes mellitus were more likely to have severe synovitis and higher concentrations of the pro-inflammatory cytokine IL-6. This could explain why patients with a combination of diabetes mellitus and OA have greater intensity in pain (Eitner et al., 2017b).

Osteoarthritis and Joint Flexibility

Knee OA is one of the most debilitating diseases in the world and is associated with decreased functionality and range of motion. Range of motion refers to the full potential of movement in a joint. Range of motion measures range of flexion and extension and is measured in degrees. A goniometer is typically used to assess range of motion. Decreased range of motion is linked to disability. In a study by Dunlop et al, joint impairment was identified as predictor for disability. In this study, range of motion was one factor used to assess joint impairment (Dunlop, Hughes, Edelman, Singer, & Chang, 1998). In another study conducted by Bergstrom et al. (1985) among Swedish population, there was a strong correlation between restricted knee motion and disability. In a study by Steultjens, Dekker, Baar, Oostendorp, and Bijilsma (2000), correlation between disability and range of motion was assessed. It was found that restricted joint

motion was a determinant of disability. Although reduced range of motion is associated with OA, other factors could also be an indication with such phenomenon. These factors include decreased strength in the muscle or pain during motion. Despite the limited research on the relationship between range of motion and OA, it is clear that reduced joint flexibility is correlated with disability and reduced function.

Osteoarthritis and Inflammation

OA was once considered a disease primarily caused by loss of articular cartilage due to overuse. However, researchers now understand that OA is initiated by a multitude of factors. The prevalence of synovitis and pro-inflammatory compounds in the body has been shown to play a significant role in OA. The synovium, also known as the synovial membrane, is a soft tissue that lines the inside of the joints. The synovium is responsible for producing synovial fluid, which provides lubrication to the joint. In OA, there is an influx of leukocytes in the synovium. Studies also suggest an increase in macrophages and T-cells being recruited in the synovium (Mathiessen & Conaghan, 2017).

Inflammatory processes occur in the affected OA joint. These processes are activated through a group of inflammatory mediators called cytokines. These cytokines are known to disturb catabolic and anabolic processes within the ECM in the affected joint. This results in gradual degeneration in articular cartilage. Inflammatory cytokines are largely responsible for the catabolic processes that occur in the affected OA joint (Wojdasiewicz, Poniatowski, & Szukiewicz, 2018). The most noteworthy inflammatory cytokines are IL-1β, TNF-α, IL-6, IL-15, IL-17, and IL-18. IL-1β, TNF-α, and IL-6 will

be the primary inflammatory cytokines of focus. IL-1β is one of the major inflammatory cytokines involved in OA. It has the ability to induce inflammatory reactions as well as catabolic processes. Patients with OA have increased levels of IL-1β in the synovium as well as synovial fluid and cartilage (Wojdasiewicz et al., 2018). IL-1β is bound by a membrane receptor named IL-1R1. There is an increase in expression of this receptor in patients with OA. The binding of IL-1β and IL-1R1 results in a cascade of reactions that ultimately produce other cytokines, chemokines, inflammatory mediators, and enzymes. IL-1β effects the metabolism of cells and the ECM. Research shows that IL-1β blocks chondrocytes and interferes with the production of structural proteins. IL-1β also plays a role in the synthesis of enzymes from a group of metalloproteinases (MMPs). This group of enzymes can have a destructive impact on cartilage. Using its own secretions, IL-1β is able to stimulate the production of other cytokines such as TNF-α, IL-6, and IL-8. As the disease progresses, IL-1β has the ability to produce increased levels of reactive oxygen series (ROS), which can also be destructive to the affected joint (Wojdasiewicz et al., 2018).

Along with IL-1β, TNF-α is another major inflammatory cytokine that is involved in OA. Just as with IL-1β, there is an increased concentration of TNF-α in the synovium, synovial fluid, and cartilage. TNF-α binds to two receptors called TNF-R1 and TNF-R2. The binding of TNF-α to TNF-R1 has a greater impact on loss of articular cartilage. Two different signaling complexes are activated when TNF-α is bound to TNF-R1. In Complex I, several pathways are activated which stimulate inflammatory responses and prevent apoptosis. Complex II is associated with cell disintegration. Progranulin is a

ligand that incorporates both TNF-R1 and TNF-R2. This compound has anti-inflammatory properties and is elevated in patients with OA (Wojdasiewicz et al., 2018).

IL-6 in another compound that increases inflammatory responses in OA-affected joints. Like other cytokines, IL-6 decreases the production of structural proteins and increases the production of enzymes from the MMPs group. IL-6 creates changes in the subchondral bone layer and promotes the formation of osteoclasts thus resulting in bone resorption (Wojdasiewicz et al., 2018).

Anti-inflammatory cytokines are also involved in the pathogenesis of OA. These cytokines include IL-4, IL-10, and IL-13. Activity of IL-4 is brought about by a receptor system dedicated to both IL-4 and IL-13. IL-4 is activated through two receptor complexes: type 1 complex and type 2 complex. Type 1 complex enables attachment of IL-4 while type 2 complex enables attachment of both IL-4 and IL-13. IL-4 has the ability to inhibit proteoglycan degradation in articular cartilage. Proteoglycan is an important structural protein involved in cartilage formation. The action of IL-4 is a direct result of inhibiting secretion of MMPs metalloproteinases. Individuals with OA have an increased concentration of IL-4 in the synovial fluid and cells. Research shows that IL-4 has protective effects on chondrocytes. Despite these protective effects, chondrocytes have shown a decrease sensitivity to IL-4 in OA. This can explain why rapid degeneration of cartilage takes place. IL-4 is associated with a decrease in inflammatory cytokines such as IL-1β, TNF-α, IL-6, prostaglandins, and COX-2 enzyme expressions (Wojdasiewicz et al., 2018).

IL-10 is activated by first binding to the receptor IL-10R. This results in a cascade of events that stimulate the synthesis of products with gene expressions dependent on IL-

10. Like IL-4, IL-10 also has chondroprotective effects. IL-10 is involved in the production of type II collagen and aggrecan, both of which are structural components of cartilage. IL-10 also has an inhibiting ability on MMPs metalloproteinases (Wojdasiewicz et al., 2018).

IL-13 is activated through a receptor system that is utilized by both IL-13 and IL-4. IL-13 is noted to have anti-inflammatory and chondroprotective effects. IL-13 has been shown to have inhibitory effects on the production of inflammatory markers including IL-1β, TNF-α, and MMP-3 in the synovium. As a result of IL-13, there is a reduced rate of binding between IL-1β and its receptor in synovial fibroblasts (Wojdasiewicz et al., 2018).

Pharmacological Treatments

Various methods of pharmacological treatments are used to treat OA. These methods include use of pain medications, total knee replacements, intra-articular injections, and lavage and debridement. One of the main symptoms of OA is pain. It is common for doctors to prescribe pain medications for their patients. This is typically the first line of defense to tackle symptoms. Acetaminophen, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed pain relievers. In fact, 65% of patients with OA are prescribed NSAIDs (Gore et al., 2012). While these medications are helpful in reducing pain, they may cause adverse side effects. For example, use of pain medications can result in constipation as well as more serious gastrointestinal complications including bleeding (Yusuf, 2016).

The use of intra-articular glucocorticoid and intra-articular hyaluronic acid injections have been found to alleviate inflammation and pain associated with OA. Intra-articular glucocorticoid injections have been noted to decrease inflammation. Glucocorticoids have the ability to hinder collagenases that facilitate cartilage damage in OA. This type of injection is also notable in patients who have synovitis. Typically, the effects of intra-articular glucocorticoid injections become present after 1 week and last for up to 4-6 weeks. Hyaluronic acid is a fluid that lubricates the joints. Patients with OA often have lower concentrations of hyaluronic acid in the body. Administration of intra-articular hyaluronic acid injections have been linked to decrease in pain and improvement in function. Both of these types of injections are comparable and are indicated in patients who have failed exercise and other pharmacological treatments and are hesitant to get a total knee replacement surgery (Yusuf, 2016).

Individuals with severe OA may undergo a partial or total knee replacement. This invasive surgery consists of using metal and plastic hardware to cap the bottom of the femur and the top of the tibia. The goal of this procedure is the replace the weight-bearing surfaces of the knee joint. Total knee replacements are viewed as a definitive treatment for knee OA. Patients who undergo a partial or total knee replacement have a significant decrease in pain and stiffness as well as improved function. In a study conducted by Skou (2016), patients who underwent the total knee replacement surgery followed by a 12-week nonsurgical treatment program had significantly less pain and improved function in daily activities compared to patients who only completed a 12-week nonsurgical treatment program (Skou, 2016). Despite the known benefits, total knee replacement surgery is not conducive for everyone. When deciding if a patient is eligible

for total knee replacement surgery, clinical symptoms, functionality, and other medical history is taken into consideration. Patients who are eligible for surgery usually have severe pain and limited functionality. Eligible patients also have radiographic evidence of large osteophyte formation or significant joint space narrowing. Patients who undergo total knee replacement can be at risk for deep vein thrombosis or infection. Therefore, patients who are classified as morbidly obese may be contraindicated for surgery. Additionally, patients who have pain but are still able to perform activities of daily living should focus on pain management and conservative treatments before considering surgery (Yusuf, 2016).

Patients may choose to undergo arthroscopic lavage and debridement. During this procedure, the affected joint undergoes a washout in order to remove any debris. Additionally, surgical tools are used to remove damaged cartilage (Medical Advisory Secretariat, 2005). Joint lavage has been associated with small changes in pain over 3 months. However, the extent of pain reduction associated with joint lavage is not significant enough to classify joint lavage as a good treatment for OA (Yusuf, 2016).

Non-Pharmacological Treatments

There are multiple nonsurgical therapies available for patients with knee OA such as diet therapy, dietary supplementation, weight loss, physical therapy, exercise, and aquatic therapy. These treatment plans are designed to help patients lose weight, build muscle, and gain strength. Individuals who are overweight or obese are at higher risk for worsening OA. Therefore, dietary intervention for weight loss can significantly reduce

this risk. Individuals who have been able to lose weight have noted a decrease in pain and improvement in functionality (Yusuf, 2016).

Glucosamine sulfate and chondroitin sulfate are two supplements that have been linked to improvement in pain, stiffness, and function in patients with OA. Both glucosamine and chondroitin are produced in the body and play a role in cartilage repair. Therefore, it is suggested that these compounds can perform the same function in supplemental form. Research on glucosamine and chondroitin has been varied. In a meta-analysis conducted by Wandel et al. (2010) in which 10 large scale OA trials were reviewed, there was no clinical effect of chondroitin, glucosamine, or the two combined. However, in another meta-analysis conducted by Zhu et al. (2018), supplementation of glucosamine, chondroitin, or a combination of the two resulted in a slight reduction in pain.

Physical therapy and exercise are used to help strengthen the joint while reducing pain symptoms and severity. Exercises must be individualized as those who have intense pain in the affected joint may benefit from isometric exercise. This type of exercise is movement without involving the affected joint. This is usually recommended for patients who have significant pain and reduced functionality in the affected joint (Yusuf, 2016). Aquatic therapy, also known as hydrotherapy, has had noteworthy success in patients with knee OA. Water buoyancy reduces the compressive load that joints have to sustain. Therefore, this type of therapy may be beneficial for individuals who have excessive pain when exercising on land. The effect of this therapy, however, has been minor and has not shown clinical significance in patients with knee OA (Yusuf, 2016).

Dietary Interventions

For many individuals, management of symptoms associated with OA occurs using dietary interventions in which certain nutrients and supplements are incorporated into the diet to aid with symptoms. Various nutrients have been linked to improvement in pain among individuals with OA. Furthermore, individuals who focus on dietary interventions with weight loss have experienced a decrease in symptoms and improvement in functionality.

As mentioned before, obesity is one of many risk factors for OA. Patients who are clinically obese have an increased load on the affected joints, which can result in pain and stiffness. Weight loss through dietary interventions and exercise has shown significant outcomes related to symptoms of OA and inflammation. In the IDEA Clinical Trial, participants were separated into three groups: diet group, exercise group, and diet + exercise group. Primary outcomes for this 18-month trial included knee joint compressive force, and plasma IL-6 levels. Researchers also assessed pain, function, mobility, and quality of life. The diet + exercise group experienced the most weight loss and the greatest reduction in IL-6 levels. This group also had the greatest decrease in pain scores and greater improvement in mobility by the end of the study. There was no significant difference in quality of life between the groups (Messier et al., 2004). In the Arthritis, Diet, and Activity Promotion Trial (ADAPT), the same interventions were studied. In this 18-month clinical trial, participants adhered either to long-term exercise, weight loss, or to a combination of both. Participants in this study were classified as either overweight or obese. By the end of the study, participants who performed a combination of exercise and

weight loss had better improvements in pain, function, and mobility (Messier et al., 2013).

Researchers have analyzed the relationship between amount of weight loss and symptom improvement in individuals with knee OA. In a study conducted by Atukorala et al. (2016), participants enrolled in an 18-week Osteoarthritis Healthy Weight for Life weight loss program. Weight-change categories were >10%, 7.6-10%, 5.1-7.5%, 2.6-5%, and <2.5% of body weight loss. Symptoms of OA that were assessed included pain and functionality. There were significant differences among symptoms between each category. Researchers found that participants required at least a 7.7% change in body weight in order to have minimal improvement in function. While improvement in pain and function was seen in all categories, the most significant improvement was seen in participants who lost greater than 10% body weight. This study proves that there is a dose-relationship between weight and symptoms of OA (Atukorala et al., 2016).

Different types of nutrients and nutritional supplements have been researched in order to investigate if they can be used as treatment for OA. Vitamin D supplementation has been linked to OA due to the role that vitamin D plays in inflammation and bone quality. Vitamin D is an important immunoregulator in the reduction of inflammation. When vitamin D binds to vitamin D receptors (VDR) in the body, vitamin D response elements (VDRE) are activated. These elements have the ability to block transcription in nuclear transcription factors such as NF-AT (nuclear factor of activated T-cells) and NF-kB. As a result, cellular response to inflammatory cytokines such as TNF-α and IL-1 are hindered, resulting in decreased inflammation. Additionally, several studies have shown that there is an increased amount of VDRs on damaged cartilage. This upregulation of

vitamin D signaling communicates to the body that there is damage and deterioration at that joint (Garfinkel, Dilisio, & Agrawal, 2017). Vitamin D plays an important role in bone metabolism. Vitamin D affects calcium metabolism, osteoblast activity, and bone density. Therefore, low vitamin D may result in poor response to changes from OA, further promoting the disease (McAlindon & Felson, 1997). In a study conducted by McAlindon et al, Vitamin D levels were assessed among individuals who participated in the Framingham Heart Study and had completed knee radiography. Lower levels of vitamin D were linked to risk of OA progression, loss of cartilage, loss of joint space, and osteophyte growth (McAlindon, 1996). High levels of vitamin D are also linked to better functionality. In a study conducted by Hung et al. (2017), high levels of supplemental Vitamin D were associated with better function.

Research has now shown associations between different types of dietary fat and OA progression. In a study conducted by Lu et al. (2014), dietary fat and progression of knee OA was evaluated. It was found that there is a positive correlation between total fat and saturated fatty acid intake and radiographic progression of knee OA. Increased intake of total fat was linked to decreased joint space width seen on radiographic images. This could possibly be due to the fact that dietary fat is associated with weight gain, putting increased load on the joints (Lu et al., 2014). Another study by Wang et al. (2009) concluded that a positive relationship exists between SFAs and bone marrow lesions. Higher intake of polyunsaturated fatty acids and monounsaturated fatty acids were associated with reduced progression of knee OA. Some PUFAs, especially omega-3 fatty acids, are known to have anti-inflammatory effects on the body, which could be the reason why increased intake is linked to a decrease in knee OA progression. Research has

repeatedly demonstrated that omega-3 PUFAs have the ability to lower inflammatory levels in the body. In a study conducted by Ferrucci et al. (2006), omega-3 PUFAs were associated with lower inflammatory markers such as IL-6 and TNF-α. This study also showed a correlation between omega-3 PUFAs and anti-inflammatory markers such as IL-10. As mentioned before, these inflammatory markers play a key role in the pathophysiology of OA. Therefore, the effect of omega-3 PUFAs on these inflammatory markers shows promising options for treatment (Ferrucci et al., 2006).

Vitamin C and vitamin E have also been linked to OA due to their antioxidant effects. It is known that the presence of reactive oxygen species (ROS) are associated with increased inflammation. Research has suggested that ROS plays a role in pathogenesis for OA because OA-affected joints are known to produce these species. Oxidative stress that occurs from ROS promotes destruction of cartilage. Inflammation brought about by this oxidative stress has the ability progress into OA over time (Ziskoven et al., 2010). Chondrocytes found on the affected joint can produce ROS as well. In fact, a study by Henrotin et al. (1993) analyzed the production of hydroxyl radicals from human chondrocytes and found that human chondrocytes do have the capability to form ROS. The human body's defense against ROS includes antioxidant enzymes such as superoxide dismutase, catalase, and peroxidases. Additionally, vitamin E, vitamin A, and vitamin C have antioxidant properties that especially play a key role in extracellular space (McAlindon & Felson, 1997). Nutrient intake was assessed among individuals who participated in the Framingham Osteoarthritis Cohort Study. Data showed a significant reduction in risk of OA progression in participants who consumed a high intake of vitamin C. A reduction in risk of OA progression was also seen for vitamin

A and vitamin E however, this data was less significant (McAlindon et al., 1996). Despite the antioxidant activity associated with vitamin E, some studies have shown that vitamin E may not be effective for OA. In a study conducted by Brand, vitamin E was assessed and it's potential to relieve symptoms. Pain, stiffness, and function were assessed. The study found that vitamin E was not helpful in management for symptomatic knee OA (Brand, 2001). In another study by Wluka, Stuckey, Brand, and Cicuttini (2002), the relationship between vitamin E and volume of cartilage loss was assessed. This study also found that vitamin E did not have any benefit in managing knee OA and did not affect the volume of cartilage loss.

Dietary Polyphenols

Dietary polyphenols consist of a group of metabolites obtained from fruits, vegetables, tea, wine, chocolate, etc. This group of secondary metabolites has the ability to perform a wide array of biological functions that are beneficial to human health. Such functions include their role as an antioxidant, anti-inflammatory, anti-apoptosis, anti-carcinogenic, and anti-microbial compound. They are also known to have cardioprotective and neuroprotective effects. One of the key functions of dietary polyphenols involves actions against inflammation. Polyphenols are widely known to scavenge reactive oxygen species (Han et al., 2007).

There is an abundance of dietary polyphenols that are present in the human diet. There are over 8000 dietary sources of polyphenols with a majority of them associated with fruits and vegetables. Examples of dietary polyphenols are diferuloylmethane (curcumin), stilbenes, phenolic acids, tannins, and flavonoids. Flavonoids are the most abundant class of polyphenols and are further broken down into anthocyanins and

anthoxanthins. Anthocyanins can be found in colorful flowers and fruits. Fruits such as berries would be classified as anthocyanins. Anthoxanthins are colorless and can be further divided into several categories (Han et al., 2007).

Curcumin, a major component of turmeric, is a spice from the plant Curcuma longa. Curcumin is known to have antioxidant and anti-inflammatory properties (Shen et al., 2012). The benefits of curcumin related to osteoarthritis has been seen in both cell model and intervention studies. In an in vitro study, curcumin was shown to protect chondrocytes against the effects of IL-1β. This means that curcumin could block IL-1β signaling which is a known process in the pathogenesis of OA. Curcumin's ability to block the signaling of IL-1β may be due to its ability to inhibit NK-kappa B, which is important in IL-1β activation (Schulze-Tanzil, Mobasheri, Sendzik, John, & Shakibaei, 2004). In a different cell study, curcumin demonstrated inhibition of inflammatory mediators such as nitric oxide, prostaglandin (PGE_2), IL-6, IL-8, and MMP-3 in chondrocytes (Mathy-Hartet et al., 2009). In a clinical study on a Thai population, 2 grams of curcumin supplementation per day showed similar effects on pain relief in comparison to 800 mg of ibuprofen (Kuptniratsaikul, Thanakhumtorn, Chinswangwatanakul, Wattanamongkonsil, & Thamlikitkul, 2009).

Epigallocatechin Gallate (EGCG) is a polyphenol from green tea that is known to inhibit the effects of multiple inflammatory mediators including IL-8, nitric oxide, and PGE_2. ECGC inhibits mitogen-activated protein kinase (MAPK), activator protine-1 (AP-1), and JNK activation (Singh, Ahmed, Malemud, Goldberg, & Haqqi, 2003). These compounds are vital in the signaling of cytokines that regulate inflammation particularly in chondrocytes (Singh et al., 2003). EGCG also protects chondrocytes by inhibiting the

production of TNF-α, MMP-1, and MMP-13. As a result, chondrocytes are protected by the catabolic degradation of its matrix protein (Ahmed, Wang, Lalonde, Goldberg, & Haqqi, 2003).

Resveratrol is a compound found in grapes, berries, peanuts, and wine. Resveratrol has anti-inflammatory and anti-apoptotic effects. In a study conducted by Dave et al. (2008), chondrocyte and cartilage explants were taken from patients with OA. Resveratrol was shown to inhibit catabolic effects of IL-1β and block the synthesis of PGE_2 (Dave et al., 2008). It is also shown to suppress NF-κb by blocking signaling from TNF-α and IL-1β (Manna, Mukhopadhyay, & Aggarwal, 2000). In a rabbit study conducted by Elmali et al. (2005), it was shown that intra-articular injections of resveratrol might protect cartilage against progression of the disease. In a clinical trial, 500 mg of resveratrol for 90 days resulted in reduced pain severity and inflammatory biomarkers in patients also being treated with Meloxicam, an anti-inflammatory drug (Marouf, Hussain, Ali, & Ahmmad, 2018).

Nobiletin is a citrus flavonoid found in oranges and other citrus fruits. Nobiletin has been shown to have anti-inflammatory properties due to its ability to prevent matrix degradation of cartilage. In human synovial fibroblasts, Nobiletin was found to activate tissue inhibitor of metalloproteinase-1, a MMP inhibitor (Lin et al., 2003). In another study, daily intraperitoneal injection of Nobiletin in collagen-induced arthritis mice was found to prevent cartilage destruction by inhibiting the gene expression of enzymes that cause destructive effects on cartilage such as ADAMTS-4 and ADAMTS-5 (Imada et al., 2008).

Pomegranates contain high levels of polyphenols such as tannins and anthocyanins. Pomegranate protects the cartilage by inhibiting matrix degradation enzymes and NF-κb (Shukla, Gupta, Rasheed, Khan, & Haqqi, 2008). Human chondrocytes were treated with pomegranate polyphenols and stimulated with IL-1β. The polyphenols in pomegranate were found to inhibit COX-2 action and prostaglandin synthesis (Rasheed, Akhtar, & Haqqi, 2010). In a clinical trial, individuals with OA who consumed pomegranate juice for 6 weeks had increased glutathione peroxidases and decreased levels and matrix metalloproteinases. Patients also had improved function and decreased stiffness (Ghoochani, Karandish, Mowla, Haghighizadeh, & Jalali, 2016).

Raspberries, Berries, and OA

Raspberries are a fruit that come from genus Rubus of the rose family. Raspberries are a rich source of polyphenols including anthocyanins and various phenolic acids. Berries, in general, are known to have anti-inflammatory and anti-oxidant activity. Blueberries are noteworthy in their anti-osteoarthritic effects. In a study conducted by Du et al. (2019a), individuals with symptomatic knee OA were asked to consume 40 grams of freeze-dried blueberry powder vs placebo for four months. It was found that daily consumption of blueberries resulted in reduced pain, stiffness, and difficulty doing daily activities as well as improvement in gain performance (Du et al., 2019a). Like blueberries, strawberries also have potent antioxidant effects. In a study conducted by Schell et al., participants with radiographic knee OA were asked to consume 50 grams of freeze-dried strawberry powder each day. Compared to the placebo group, individuals who consumed the strawberry powder had a decrease in inflammatory markers such as MMP-3, IL-6, and IL-1β. Participants in the treatment group also reported reduced pain

(Schell et al., 2017). A similar study conducted by Basu et al. (2018) further confirmed the anti-inflammatory properties of strawberries. In this randomized cross-over study, participants who were clinically obese were given strawberry supplements for 12 weeks, followed by a 2-week washout and another trial of placebo supplements for 12 weeks. The study concluded that strawberry supplementation resulted in decreased TNF-α and lipid peroxidation products (Basu et al., 2018).

There are currently not many studies that observe the effect of raspberries on various components of OA. The anti-inflammatory effects of raspberries are shown in a study conducted by Schell et al. In this cross-over study, participants consumed a raspberry and placebo supplement for four weeks. Raspberry supplementation was associated with lower IL-6 and TNF-α levels (Schell et al., 2017). In a rat study conducted by Jean-Gilles et al. (2011), rats with antigen-induced arthritis were given raspberry extract for 30 days. Rats who received higher doses of the raspberry extract had lower incidence and severity of pain. Further analysis found significant inhibition of inflammation, cartilage damage, and bone resorption induced by raspberry extract. This study shows promising results, indicating effects raspberries have in cartilage protection and inflammation (Jean-Gilles et al., 2011). To date, no studies have shown the effects of raspberries on pain related to knee OA. Currently, there are no studies investigating the role of raspberries in inflammation related to knee OA. Therefore, this study investigated whether consumption of freeze-dried raspberry powder can decrease some inflammatory markers, increase some anti-inflammatory markers, improve joint flexibility, and reduce pain in patients with symptomatic knee OA.

CHAPTER III

METHODOLOGY

Study Design

Using a double-blind, systematically randomized, placebo-controlled study design, a total of 63 participants were recruited for the study. Of the 63 participants, 46 were women and 17 were men. Participants were between the ages of 45 and 79, and had self-reported symptomatic knee OA. Participants were recruited through Texas Woman's University in Denton, Texas as well as various communities and independent-living homes from the DFW Metroplex Area. The use of social media platforms such as Facebook were also used to recruit participants. In order to be eligible for the study, participants agreed to discontinue taking medications such as COX-inhibitors, glucosamine sulfate, glucosamine hydrochloride, and chondroitin sulfate supplement. These medications may influence symptoms associated with knee OA. Additionally, participants agreed to avoid consuming raspberries and other types of berries throughout the course of the study.

Participants who met the inclusion/exclusion criteria at initial screening were systematically randomized and placed into one of the two groups: the raspberry powder (n = 34) and the placebo powder group (n = 29). The raspberry group received 35 grams of freeze-dried raspberry powder daily. Subjects were supplied with a scoop, a blender bottle, and two opaque containers that contained the powder. Subjects were given two

opaque containers at baseline visit, and another two containers at midpoint visit. Each container held enough powder to last approximately one month. Therefore, two containers were expected to last the 2-month period between study visits. Participants were informed that two scoops of powder equated to 35 grams. They were then instructed to mix two scoops of powder in 10-12 ounces of water using the blender bottle. Subjects were advised to consume the drink within a span of 5 minutes. Anyone who was concerned about blood glucose control was advised to split the total amount of 35 grams of powder into two portions per day. The placebo group received 35 grams of placebo powder, which was matched in appearance and energy to the raspberry powder. Both groups were asked to consume the powder each day for 4 months. Study protocols were reviewed and approved by the Institutional Review Board at Texas Woman's University.

Recruiting and Inclusion/Exclusion Criteria

Subjects were contacted and recruited via email and Facebook. Once participants showed interest in the study, initial screening was conducted via phone. The screening was comprised of short questions including demographic data, contact information, medical history, a list of current medications/supplementations, smoking history, and whether the potential participant was on a special diet or had any known food allergies. Inclusion criteria included healthy and mobile individuals who were between the ages of 45 and 79 and experienced symptoms of knee pain. Exclusion criteria included uncontrolled diabetes, heart disease, liver disease, lung disease, and kidney disease. Other exclusion criteria included anyone who smoked greater than one pack of cigarettes per day, who have had knee replacement surgeries on both knees, who have had injections in both knees within 3 months of starting the study, and who were unwilling to discontinue

taking COX-inhibitors, glucosamine sulfate, glucosamine hydrochloride, and chondroitin sulfate supplements throughout the study period. Potential participants with a raspberry allergy were also excluded from the study.

Baseline, Midpoint, and Final Measurements

Qualified subjects were required to visit the study site on three separate occasions throughout the course of the four months. These visits occurred at baseline, midpoint (2 months), and final (4 months). Each visit was done at the Pioneer Clinic in Woodcock Hall at Texas Woman's University in Denton, Texas. Participants were initially emailed an appointment reminder. During the baseline visit, participants were given a written consent form that detailed each aspect of the study protocol. If participants agreed with study protocol and rules, they were then asked to sign the consent form. Research protocols began once the consent form was signed. At each visit, procedures performed included obtaining anthropometric measurements (height, weight, and blood pressure), providing a blood sample, performing a range of motion test, and completing the WOMAC questionnaire. Blood pressure was obtained while subjects were sitting upright. A blood pressure monitor was used on the non-dominant arm. Participants were required to fast overnight for each visit.

Treatment Compliance

At the end of baseline and midpoint appointments, participants were given a calendar to track compliance of daily powder consumption. Participants were asked to mark each day on the calendar that the powder was consumed. Additionally, they were

asked to mark each day pain medications were taken as a result of knee pain. Participants were asked to bring back their calendars to midpoint and final visits in order to assess for treatment compliance. They were also asked to return both of the opaque containers that contained the powder in order to assess how much powder was left over after the 2-month period. Subjects had access to the email addresses and phone numbers of the study coordinators. Therefore, any questions or concerns regarding study protocols could be addressed throughout the four months.

Blood Collection and Analysis of Inflammatory Markers

Fasting venous blood samples were collected at baseline, midpoint, and final visits by a trained phlebotomist. Blood specimens were centrifuged for 15 minutes to separate plasma. The specimens were then aliquoted into microcentrifuge tubes and stored at -80°C for analysis of IL-4, IL-1β, TNF-α, IL-6, IL-13, and IL-10 inflammatory makers. Human High Sensitivity T Cell Magnetic Bead Panel Multiplex ELISA kits from Millipore were used for analysis of inflammatory markers. These kits utilized Luminex xMAP technology, which allowed for assessment of multiple inflammatory markers. This technology internally color-codes microspheres with two fluorescent dyes. Bead sets of 500 5.5 µm polystyrene microspheres or 80 6.45 µm magnetic microspheres are created and coated with specific capture antibodies. Once the bead captures the analyte in the test sample, a biotinylated detection antibody is added followed by a Streptavidin-PE conjugate. The latter is known as the reporter molecule and makes the analyte visible for detection. As a result of fluorescent reporter signals, microspheres are detected and concentrations of each analyte are obtained. Luminex 200 software was used to analyze the data.

Assessment of Pain

Pain was assessed using the WOMAC questionnaire. Participants were asked questions about knee pain. The questionnaire was administered during baseline, midpoint, and final appointments and asked about pain, stiffness, and difficulty performing daily activities related to knee pain. Questions asked in the WOMAC questionnaire pertained to specific activities or events such as walking up/down the stairs or taking a bath. The WOMAC questionnaire is an effective measurement tool, and is used by many different healthcare professionals. In an analysis conducted by McConnell et al., the WOMAC questionnaire was found to be reliable, valid, and responsive tool for assessment of pain (McConnell, Kolopack, Davis, 2001).

Assessment of Joint Flexibility

Joint flexibility was assessed through range of motion testing in both knees. Range of motion was evaluated using a 180-degree goniometer. Measurements of active extension and flexion were gathered for each participant at baseline, midpoint, and final appointments. Measurements were taken while participants laid in a supine position. Each test was done in triplets for accuracy.

Statistical Analysis

The minimum total sample size needed was 50 participants in order to conduct analysis with alpha = 0.5 and power = 0.87, along with moderate effect size. Descriptive statistics were calculated for all variables, means, standard deviations, minima, and maxima for all continuous variables. Frequencies and percentages were used to calculate categorical variables. Distributions of the response variables were assessed to determine

if statistical tests of hypothesis based on the assumption of normality were being met. Outliers were investigated for error. Independent sample *t*-tests were used to evaluate baseline differences of dependent variables. Repeated measures (ANOVA) were conducted to measure differences in pain, stiffness, difficulty doing daily activities, inflammatory biomarkers, and joint flexibility between treatments at baseline, midpoint, and final. *P*-values < 0.05 were considered statistically significant. Men and women were not separated into groups for statistical analysis, as the current study was not assessing gender differences, but differences between and within the raspberry and placebo groups.

CHAPTER IV

RESULTS

Demographics

A total of 113 individuals were contacted by researchers and screened for the study. Of the 113 potential participants, a total of 63 men and women met the study criteria and were scheduled for a baseline visit. As the study progressed, 19 participants (3 men and 16 women) withdrew from the study for reasons including gastrointestinal complications from the powder, lack of compliance, and life events. One participant restarted glucosamine supplements due to pain, and another participant received a cortisone injection during the study that resulted in withdrawal. A total of 44 participants completed the study. Demographic data related to study screening, participation, and dropout rates are shown in Figure 1.

At baseline, there was no significant difference in body weight between the raspberry and placebo groups. There was also no significant difference in body weight within both groups between baseline, midpoint, and final visits (see Figure 2). There was also no significant difference in BMI between the groups as well as within the groups at baseline, midpoint, and final visits (see Figure 3). At each time point, there was no significant difference in systolic blood pressure between the two groups. In the raspberry group, there was a significant decrease in systolic blood pressure at final visit in comparison to baseline ($p = 0.022$). Within the placebo group, there was no significant

difference in systolic blood pressure at any time point (see Figure 4). There was no significant difference in diastolic blood pressure between both groups at any time point. Within the raspberry group, there was a significant decrease in diastolic blood pressure at final compared to baseline ($p = 0.021$). No changes were observed in the placebo group (see Figure 5). Although not significant, it was observed that there was an insignificant difference in BMI and systolic blood pressure observed at baseline between the raspberry and placebo group. BMI was insignificantly greater in the placebo group, and blood pressure was insignificantly greater in the raspberry group.

Pain, Stiffness, and Difficulty in Daily Activity Questionnaire

The WOMAC questionnaire was used to assess pain, stiffness, and difficulty performing daily activities. There were no significant differences in total WOMAC score between the groups at any time point. In the raspberry group, there was a significant decrease in total WOMAC score at midpoint over baseline ($p = 0.020$). There was also a significant decrease in total WOMAC score overall at final over baseline ($p = 0.004$) in the raspberry group. There were no significant differences in total WOMAC score in the placebo group at any time point (see Figure 6).

In the raspberry group, there was a significant decrease in pain at midpoint over baseline ($p = 0.016$) and final over baseline ($p = 0.002$). There were no significant differences in pain within the placebo group at any time point (see Figure 7). No significant differences in pain were noted between the two groups at any time point.

A significant decrease in stiffness was observed in the raspberry group at midpoint over baseline ($p = 0.005$) and at final over baseline ($p = 0.004$). A significant

decrease in stiffness was also observed in the placebo group at final over baseline (p = 0.032; see Figure 8). There were no significant differences in stiffness between the two groups at any time point.

Within the raspberry group, a significant decrease in difficulty performing daily activities was noted at midpoint over baseline (p = 0.046) and final over baseline (p = 0.006). There were no significant differences in difficulty performing daily activities within the placebo group at any time point (see Figure 9). There were no significant changes between the groups at any time point in difficulty performing daily activities.

Joint Flexibility (Range of Motion)

Range of motion was measured in degrees using a goniometer. Parameters included active extension and flexion, which were measured in both knees in triplicate. Measurements were taken while participants were in a supine position. Active knee extension occurs as an individual straightens their rested leg. Lesser degrees of active knee extension signify a better ability to actively extend and straighten the knee. Therefore, a decrease in active knee extension over time signifies improvement. Knee flexion refers to the bending of the knee joint. Greater degrees of flexion signify a greater amount of bending. Therefore, an increase in flexion over time signifies improvement. There were no significant changes in overall right knee and left knee range of motion between and within the two groups at any time interval (see Figures 10 and 11). However, right extension was noted to be significantly less in the raspberry group over the placebo group at midpoint (p = 0.042). Right knee extension was significantly reduced at midpoint over baseline (p = 0.022) in the raspberry group (see Figure 12). There was also a significant reduction in left knee extension at midpoint over baseline (p

= 0.007) in the raspberry group (see Figure 13). No significant differences were noted in right knee flexion between or within the groups at any time point (see Figure 14). However, left knee flexion was significantly greater at final over baseline (p = 0.045) in the raspberry group (see Figure 15).

Inflammation

Pro-inflammatory cytokines IL-6, IL-1β, and TNF-α were assessed. Additionally, serum anti-inflammatory cytokines IL-4, IL-10, and IL-13 were also analyzed. There were no significant differences between the groups at any time point among any of the six biomarkers. In addition, there were no significant differences in any biomarkers within the groups at any time point (see Table 1). Despite there being no significant changes overall, slight changes were observed among some of the cytokines. However, results are varied as IL-6 showed a slight decrease in the raspberry group and an increase in the placebo group from final in comparison to baseline. Concentrations of IL-10 were decreased from baseline in both raspberry and placebo groups at final evaluation. The same results were noted with concentrations of IL-13. Additionally, concentrations of IL-4 were slightly decreased in the raspberry group and increased in the placebo group at final from baseline.

CHAPTER V

DISCUSSION

The findings of this clinical study demonstrated that consumption of whole freeze-dried raspberry powder for a period of 4 months resulted in reduction in pain, stiffness, and difficulty performing daily activities. There was also improvement in joint flexibility related to knee extension and flexion in the raspberry treatment group. Despite these positive findings related to joint function, our results did not show any significant changes in markers of inflammation in individuals consuming the raspberry powder. A plethora of research studies has examined the positive relationship between polyphenol intake and risk of chronic disease (Shen et al., 2012). Research has indicated that polyphenols have antioxidant activity and are able to scavenge free radicals in the body. Polyphenols are associated with a decrease in inflammation and can be found in a wide variety of food and beverages. Examples of these include green tea, wine, citrus fruits, cumin, pomegranate, and other berries such as blueberries and strawberries (Han et al., 2007).

A validated questionnaire, WOMAC was utilized to assess for pain, stiffness, and difficulty performing daily activities. Our study findings indicated that the consumption of whole freeze-dried raspberry powder for 4 months resulted in decreased pain, stiffness, and difficulty performing daily activities. In the raspberry treatment group, a significant

decrease in overall WOMAC score indicated improvement in the symptoms of OA across the study time points in comparison to baseline. No changes in overall WOMAC score was observed in the placebo group. In a study conducted by Du et al. (2019a), similar findings were observed in individuals with symptomatic knee OA who consumed 40 grams of blueberry powder daily for 16 weeks. The study found a decrease in overall WOMAC score in the blueberry group, but no significant changes between the blueberry and placebo groups (Du et al., 2019a). In a cross-over study investigating tart cherries, Schumacher et al. (2013) demonstrated the efficacy of tart cherry in reducing pain in individuals with knee OA. The improvement in WOMAC scores was only within the tart cherry treatment group (Schumacher et al., 2013). Our study findings associated with whole raspberry assessing pain, stiffness, and limited functionality is promising. While there may not have been significant differences between the two groups, improvement in symptoms were only observed within the raspberry group, while no changes were observed in the placebo group. This indicates that consumption of the powder may have had positive effects related to pain, stiffness, and difficulty performing daily activities.

Joint flexibility as represented by assessment of range of motion (ROM) did not change in either the raspberry or the placebo group. However, some improvement was observed in knee extension and flexion measurements. In the raspberry-treated group, improvement in right knee active extension, left knee active extension, and left knee flexion was noted. Improvement in ROM associated with polyphenol intake has been observed in other studies. In a study by Jensen et al, participants with OA were given a fruit juice blend consisting of acai pulp, pomegranate, wolfberry, camu camu, passion fruit, aronia, acerola, and billberry. This fruit blend was consumed every week for 12

weeks. Results demonstrated an improvement in ROM in multiple areas of the body as well as reduction in pain and improvement in functionality (Jensen et al., 2011). A study conducted by Du and colleagues evaluated the effects of freeze-dried blueberry powder on joint flexibility in individuals with symptomatic knee OA. Participants were randomized and given either whole freeze-dried blueberry powder or a control powder. Results showed a slight increase in overall range of motion in both right and left knees in the blueberry group (Du et al., 2019b). Though not statistically significant, the raspberry group in our study had improvement in overall left knee ROM, whereas the placebo group had a decline in overall right knee ROM. Improvement in knee extension and flexion can be linked to greater functionality and ability to perform activities of daily living. With better joint flexibility in the knees, individuals may find it easier to do simple tasks such as bending over or walking down the stairs. The ability to complete such tasks can significantly improve quality of life in individuals with knee OA. The study results give some hopeful indications that the polyphenols from the raspberry have a positive effect on joint flexibility.

No significant differences in pro-inflammatory biomarkers (TNF-α, IL-1β, and IL-6) were observed. These cytokines have been linked to destruction of cartilage, synovitis, and pain with progression and severity of the condition. Polyphenols are noted to have anti-inflammatory properties. In a 12-week study by Schell et al., participants with radiographic knee OA consumed 50 grams of freeze-dried strawberry powder. At the end of the study, significantly lower levels of IL-6 and IL-1β were found in the strawberry group vs. the placebo group (Schell et al., 2017). In an animal study by Zhang and Zeng (2019), rats were injected with monosodium iodoacetate in order to induce knee

OA. They were then randomized into experimental groups, one that included treatment with curcumin. It was found that concentrations of IL-6, IL-1β, and TNF-α were significantly lower in rats with OA treated with curcumin (Zhang & Zeng, 2019). Rasheed et al. (2009) investigated the effects of EGCG (epigallocatechin-3-gallate), a green tea polyphenol, on the expression of advanced glycation end produced-induced TNF-α in human chondrocytes. Results showed that expression of TNF-α as well as MMP-13 was inhibited by EGCG in human chondrocytes. This research suggests that EGCG-derived compounds may possibly inhibit cartilage degradation and inflammation in humans with OA (Rasheed et al., 2009). In our study, no significant changes were noted. A slight increase in TNF-α was noted at final over baseline in both groups. Additionally, it was observed that IL-6 was increased at final over baseline in the placebo group and decreased at final over baseline in the raspberry group. The results associated with the pro-inflammatory markers may have been influenced by the dietary habits of participants at home.

It is well known that intake of certain types of foods are associated with an increase in inflammation. In fact, a study by Lopez-Garcia et al. (2004) found that women who ate a Westernized diet (high in red meat, sweets, desserts, and refined grains) compared to a diet high in fruits and vegetables had a greater concentration of inflammatory markers such as IL-6 and CRP. The same group found similar results with trans-fatty acid consumption (Lopez-Garcia et al., 2005). In the current study, dietary intake of participants had not been monitored or controlled throughout the course of the study. Future studies may need to implement practices to monitor or control diet. Also,

additional inflammatory markers that are more sensitive to the inflammatory response should be measured.

While significant changes were not observed among the inflammatory markers measured in the current study, there may have been a decrease in other markers of inflammation such as prostaglandins (PGE_2). This phenomenon could potentially be the reason why a significant reduction in pain was seen in the raspberry group. Prostaglandins are lipid molecules derived from arachidonic acid. They control homeostatic functions in the body, but can also mediate inflammatory response (Ricciotti & Fitzgerald, 2011). In OA cartilage, prostaglandin (PGE_2) has the ability to enable inflammation while contributing to the destruction of the ECM (Shen et al., 2012). Evidence suggests that polyphenol intake can lead to inhibition of prostaglandin production. In an animal model, OA-induced rabbits were fed pomegranate fruit extract for eight weeks. Results showed a decrease in plasma levels of prostaglandins (Akhtar, Khan, Ashruf, & Haqqi, 2017). In an in vivo study, human cartilage explants and human chondrocytes were cultured in the absence or presence of IL-1β either with or without curcumin. It was found that curcumin inhibited IL-1β-induced production of PGE_2. These findings suggest that curcumin can prevent the production of inflammatory mediators such as PGE_2 (Mathy-Hartert et al, 2009). In future studies, this inflammatory marker may provide valuable and positive results related to inflammation and raspberry intake in individuals with knee OA.

No significant differences in anti-inflammatory biomarkers (IL-10, IL-4, and IL-13) were observed. Few studies have examined the anti-inflammatory effects of polyphenols while also measuring concentrations of IL-10, IL-13, and IL-4. A study by

Nikniaz, Ostradrahimi, Mahdavi, Ebrahimi, and Nikniaz (2014) examined the effects of Russian olives, a source of flavonoids, on serum levels of inflammatory cytokines in females with knee OA. In this 8-week study, participants were randomized into intervention and placebo group. The intervention group received 15 mg of Russian olive fruit powder each day. At the end of the study, it was shown that supplementation with Russian olive fruit powder decreased concentrations of TNF-α and MMP-1 while enhancing IL-10 (Nikniaz, Ostadrahimi, Mahdavi, Ebrahimi, & Nikniaz, 2014). In another study conducted by Karlsen et al. (2007), Medox, a bilberry and black currant extract, was given to participants daily for 3 weeks. Results showed decreased concentrations of IL-4 and IL-13 as well as IL-8, suggesting that the anthocyanin properties in the extract may aid in treatment in chronic inflammatory diseases. Lack of significance in data from the current study differs from previous studies examining the effects of polyphenols on anti-inflammatory cytokines. It is possible that other factors could have influenced the concentrations of IL-10, IL-4, and IL-13. Such factors include dietary intake, physical activity, or other sources of inflammation in the body.

Some of the limitations associated with this clinical trial included the dropout rate. We experienced a higher dropout due to gastrointestinal complications associated with the powder and noncompliance. This could have affected the clinical significance and findings of the study, especially the outcomes related to inflammatory markers. Having a larger sample size would provide for a more robust effect to differentiate significance in outcomes. Additionally, the study was based on participant compliance with the raspberry or placebo powder. Participants were given calendars to track compliance. However, compliance was self-reported. Participants were asked to bring

back their treatment bottles at midpoint and final visits. When bottles and calendars were brought back, it was noted that some participants had missed taking their powder on some days. If participants missed more than 20% of days over 2 months, they were unable to continue with the study. In future studies, protocols should be implemented to better track compliance. In addition to noncompliance, diet was not monitored over the course of the study. Although participants were asked not to consume raspberry products over the 4 months, no other dietary requirements were established for the study. In the future, participants may need to be placed on controlled diets along with raspberry or placebo intake. Dietary intake can be monitored using diet recall questionnaires.

In summary, overall conclusions based on our outcomes indicate that consumption of raspberry had a favorable impact on reducing symptoms with modest improvement in joint flexibility in individuals with symptomatic knee OA. This research demonstrates that whole raspberry consumption leads to a reduction in pain, stiffness, and difficulty performing daily activities. It also indicated positive findings between whole raspberry consumption and knee extension and flexion. Although this study did find valuable results for symptoms of OA and joint flexibility, there were no significant changes in pro-inflammatory or anti-inflammatory biomarkers. Future studies should incorporate larger sample sizes, better protocols to track compliance, and monitor dietary intake. Additionally, a cross-over study design may be beneficial in assessing comparison between treatment and placebo groups. Using a cross-over design for a chronic condition such as OA can possibly provide a better indication on whether whole raspberry powder is actually benefitting the participants.

	Raspberry (Mean)						Placebo (Mean)					
	TNF-α	**IL-1β**	**IL-6**	**IL-10**	**IL4**	**IL-13**	**TNF-α**	**IL-1β**	**IL-6**	**IL-10**	**IL4**	**IL-13**
Baseline	3.90 ± 0.41	0.91 ± 0.27	13.52 ± 9.76	16.28 ± 3.97	16.28 ± 36.20	9.67 ± 5.65	3.09 ± 0.36	1.06 ± 0.24	19.28 ± 8.73	18.68 ± 3.55	96.01 ± 32.38	11.66 ± 5.05
Midpoint	3.58 ± 0.31	0.76 ± 0.15	14.36 ± 9.58	14.34 ± 3.21	14.34 ± 39.64	9.49 ± 5.37	3.13 ± 0.28	0.97 ± 0.13	17.87 ± 8.56	16.66 ± 2.87	95.54 ± 35.55	10.36 ± 4.77
Final	4.10 ± 0.42	0.92 ± 0.21	9.14 ± 9.27	15.46 ± 3.17	15.46 ± 41.91	7.41 ± 5.25	3.40 ± 0.38	1.01 ± 0.19	21.95 ± 8.29	16.64 ± 2.84	96.73 ± 37.49	12.47 ± 4.70

Table 1. *Effects of Raspberry vs. Placebo on Concentrations of Pro-Inflammatory and Anti-Inflammatory Cytokines.* Mean ± SEM. *N* = 16 for the raspberry group, *N* = 20 for the placebo group. No significant differences within or between groups at any time point.

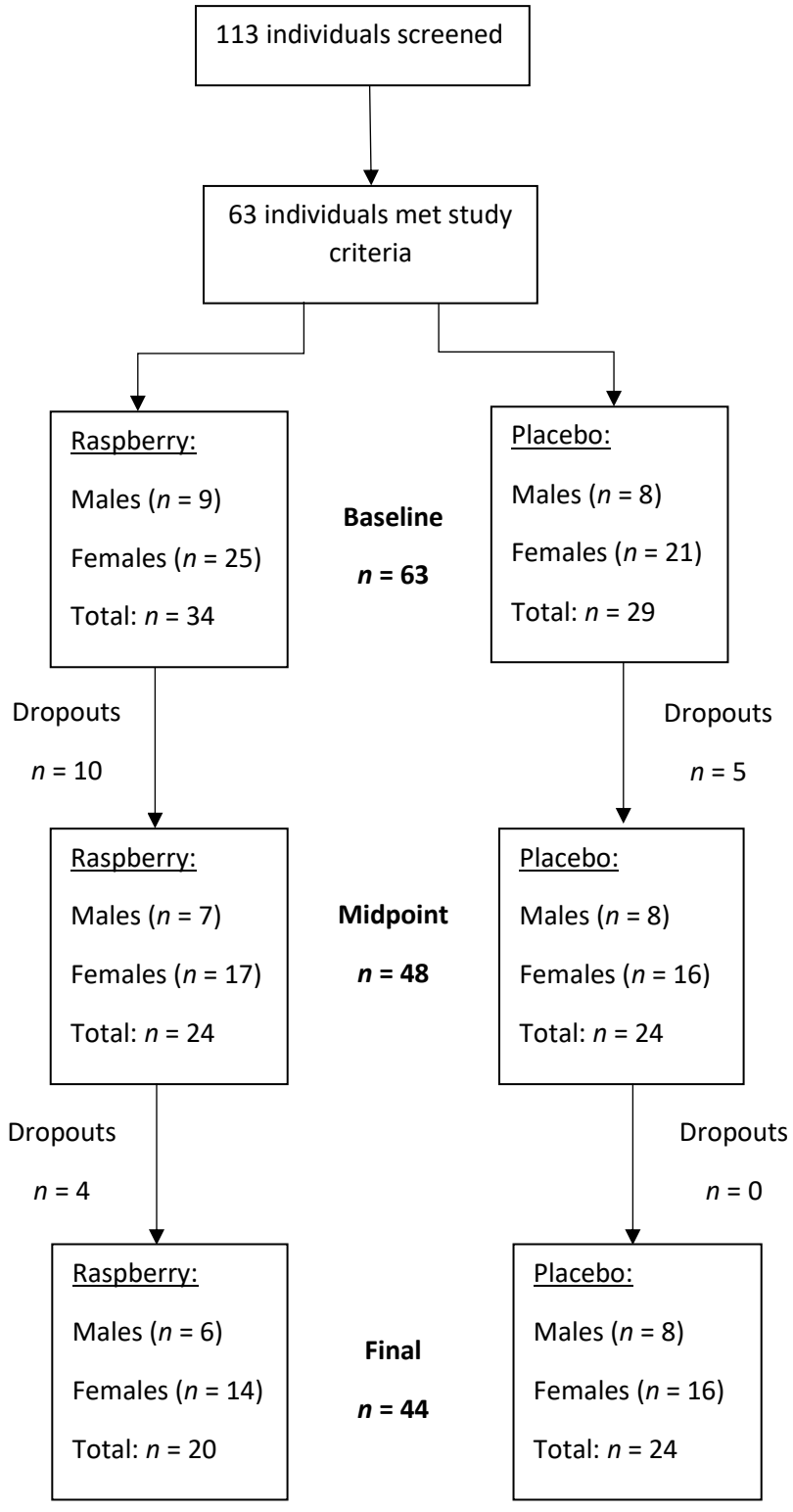

Figure 1. Participant Screening and Dropouts.

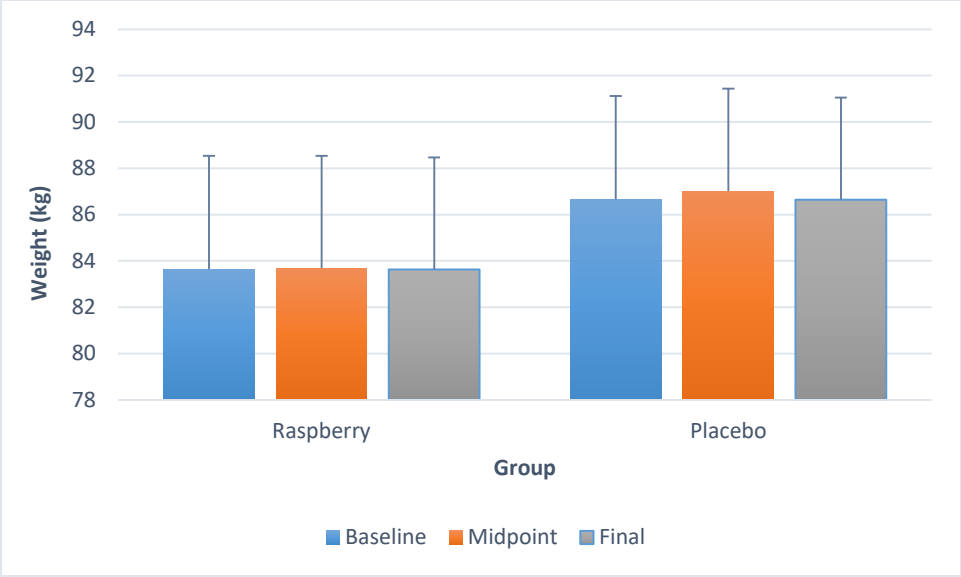

Figure 2. Effects of Raspberry vs. Placebo on Weight. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group. No significant changes between and within groups at any time point.

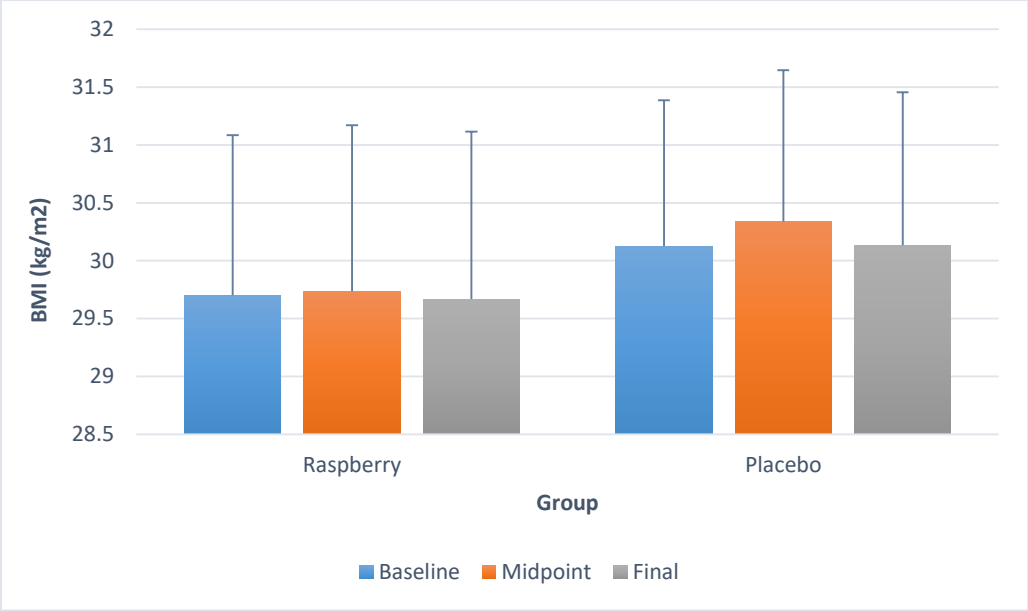

Figure 3. Effects of Raspberry vs. Placebo on BMI. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group. No significant changes between and within groups at any time point.

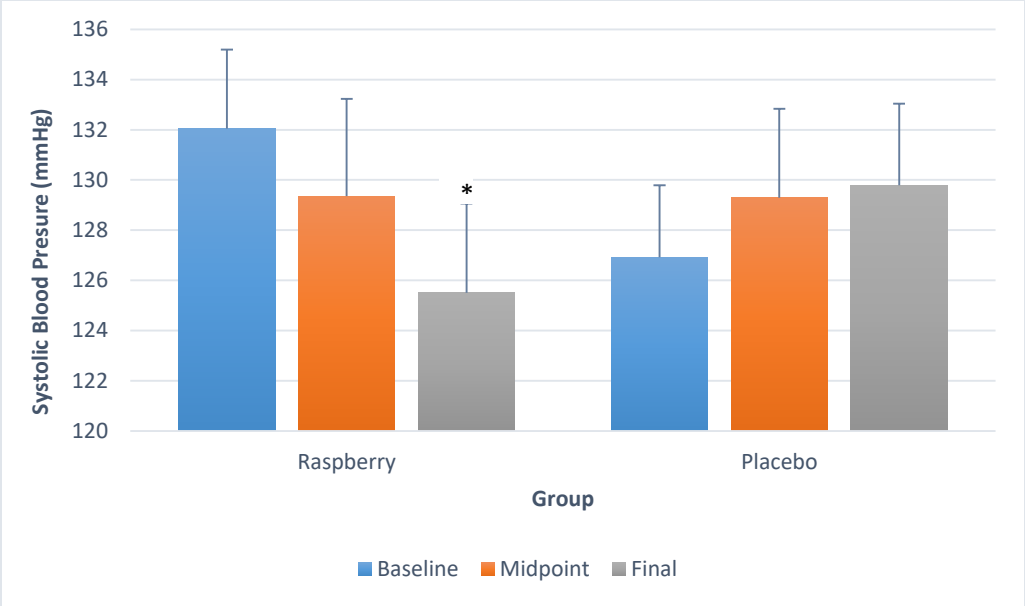

Figure 4. Effects of Raspberry vs. Placebo on Systolic Blood Pressure. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

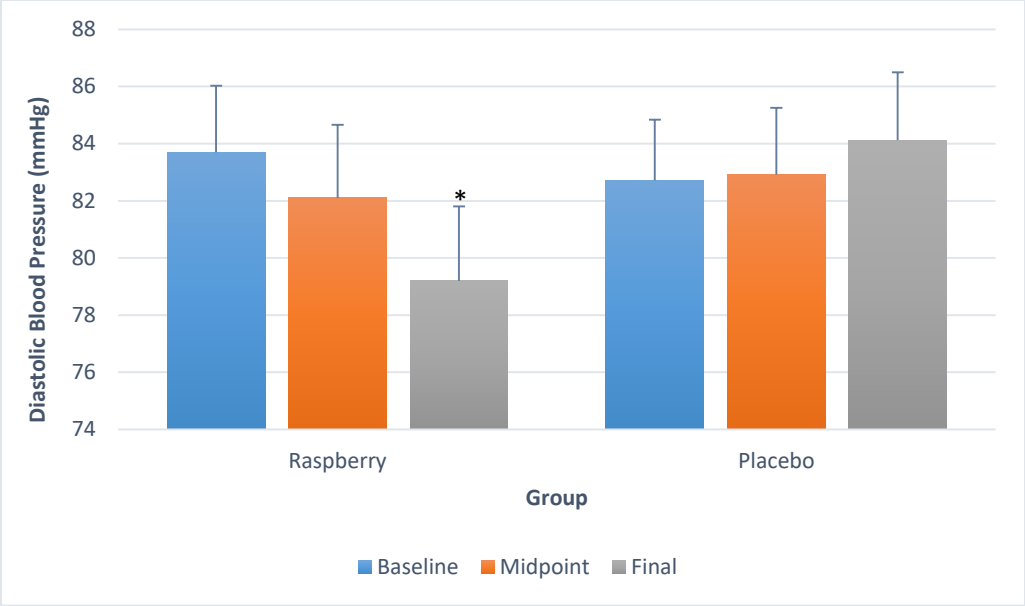

Figure 5. Effects of Raspberry vs. Placebo on Diastolic Blood Pressure. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

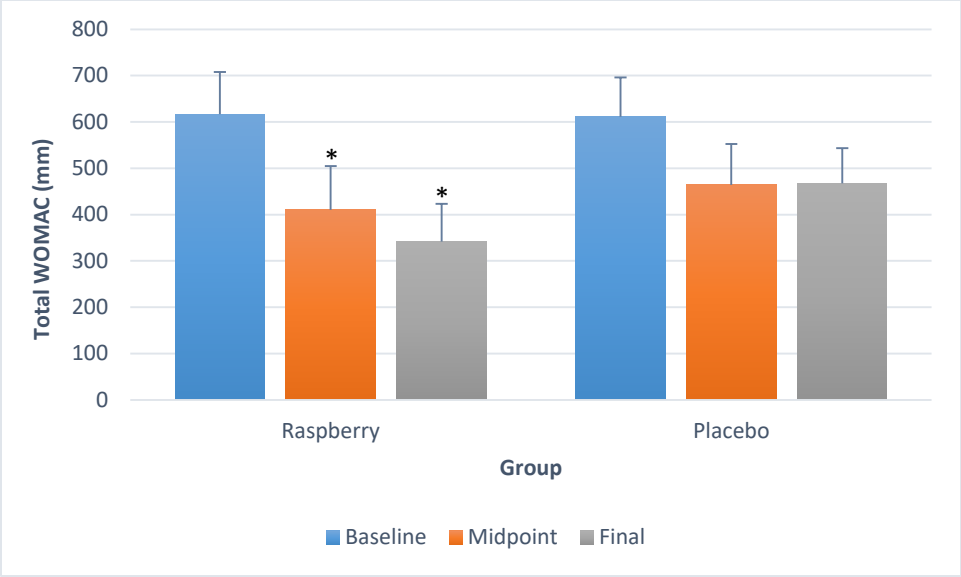

Figure 6. Effects of Raspberry vs. Placebo on Total WOMAC. Mean ± SEM. N = 20 for the raspberry group, N = 24 for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

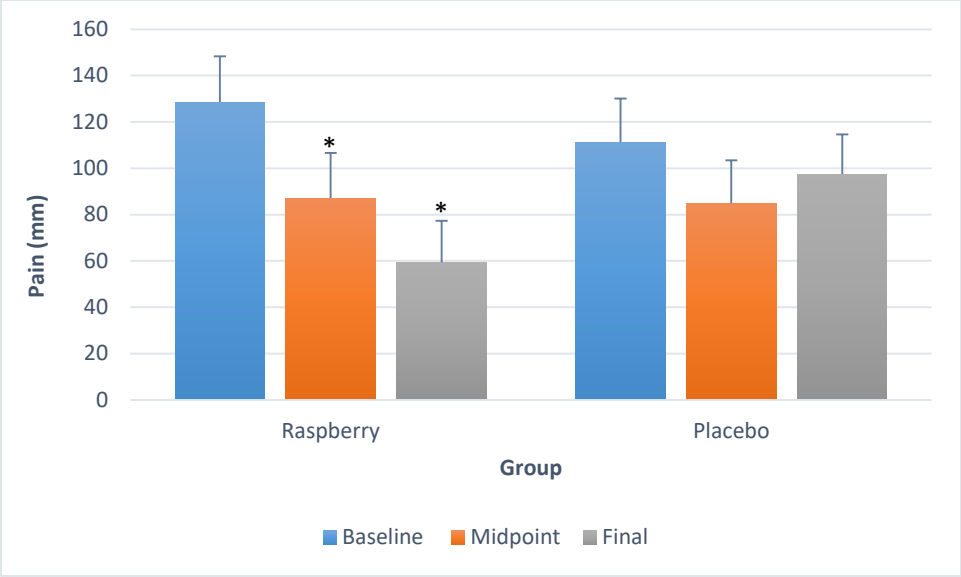

Figure 7. Effects of Raspberry vs. Placebo on Pain Intensity. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

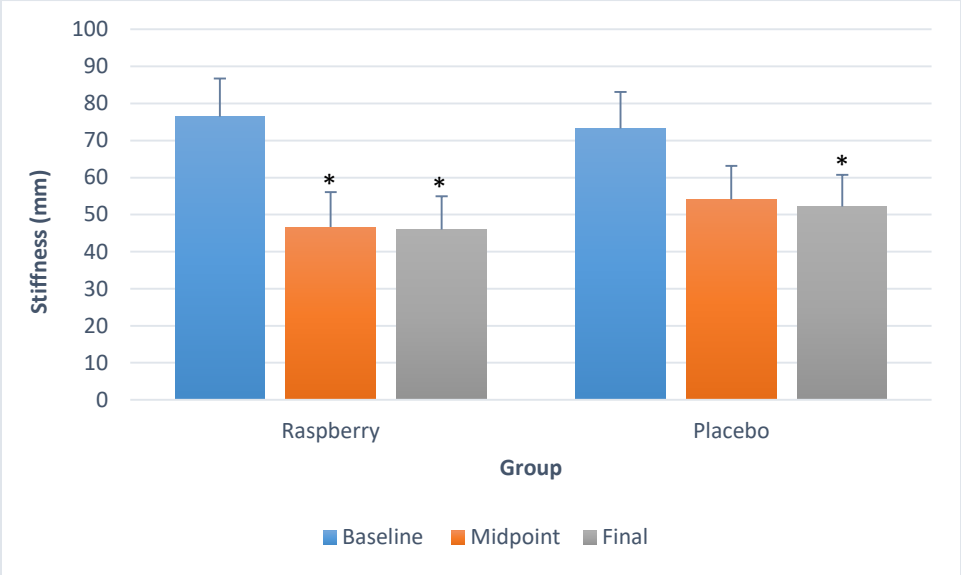

Figure 8. Effects of Raspberry vs. Placebo on Stiffness. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 24 for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

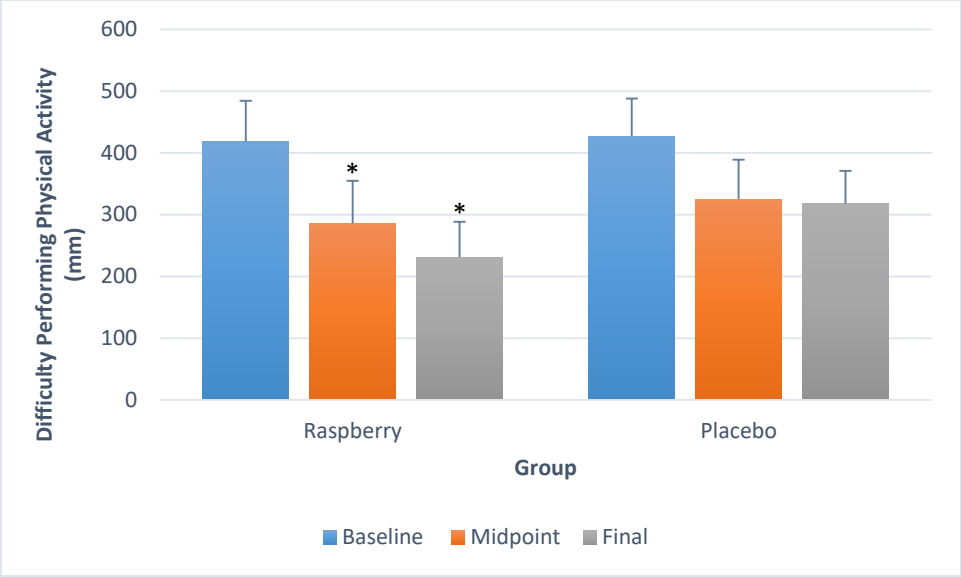

Figure 9. Effects of Raspberry vs. Placebo on Difficulty Performing Daily Activity. Mean ± SEM. $N = 20$ for the raspberry group, $N = 24$ for the placebo group.

* Significance as compared to baseline ($p < 0.05$) in the same group

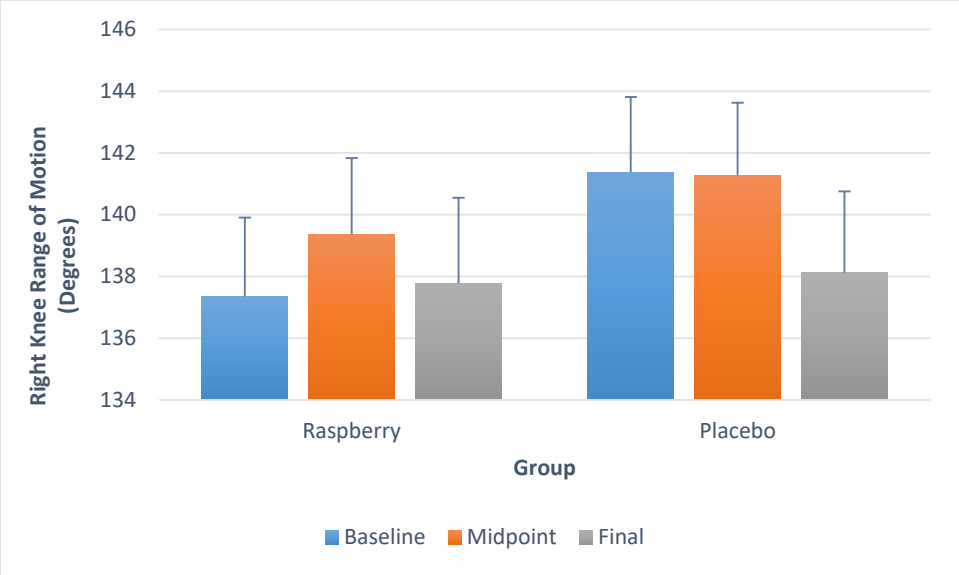

Figure 10. Effects of Raspberry vs. Placebo on Right Knee Range of Motion. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 22 for the placebo group. Outliers omitted. No significant changes between or within groups at any time point.

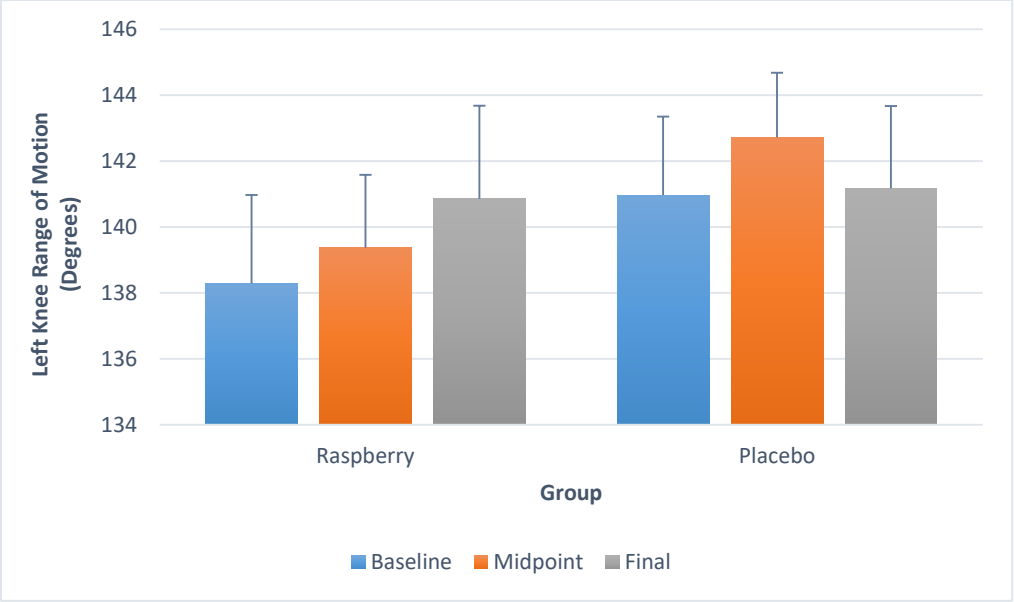

Figure 11. Effects of Raspberry vs. Placebo on Left Knee Range of Motion. Mean ± SEM. *N* = 18 for the raspberry group, *N* = 23 for the placebo group. Outliers omitted. No significant differences between or within groups at any time point.

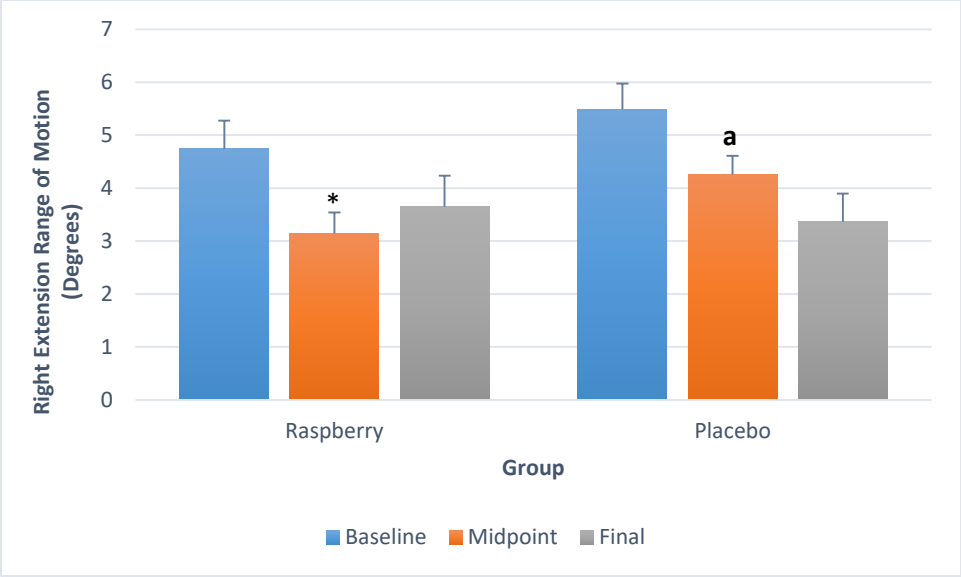

Figure 12. Effects of Raspberry vs. Placebo on Right Extension Range of Motion. Mean ± SEM. N = 18 for the raspberry group, N = 22 for the placebo group. Outliers omitted.

* Significance as compared to baseline ($p < 0.05$) in the same group

a denotes significant difference between placebo vs. raspberry at midpoint

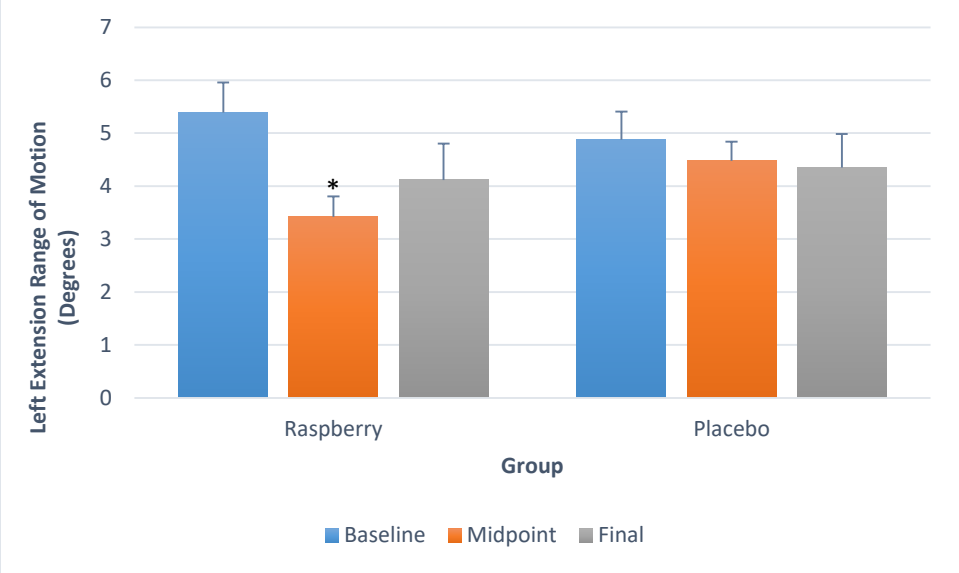

Figure 13. Effects of Raspberry vs. Placebo on Left Extension Range of Motion. Mean ± SEM. $N = 20$ for the raspberry group, $N = 23$ for the placebo group. Outliers omitted.

* Significance as compared to baseline ($p < 0.05$) in the same group

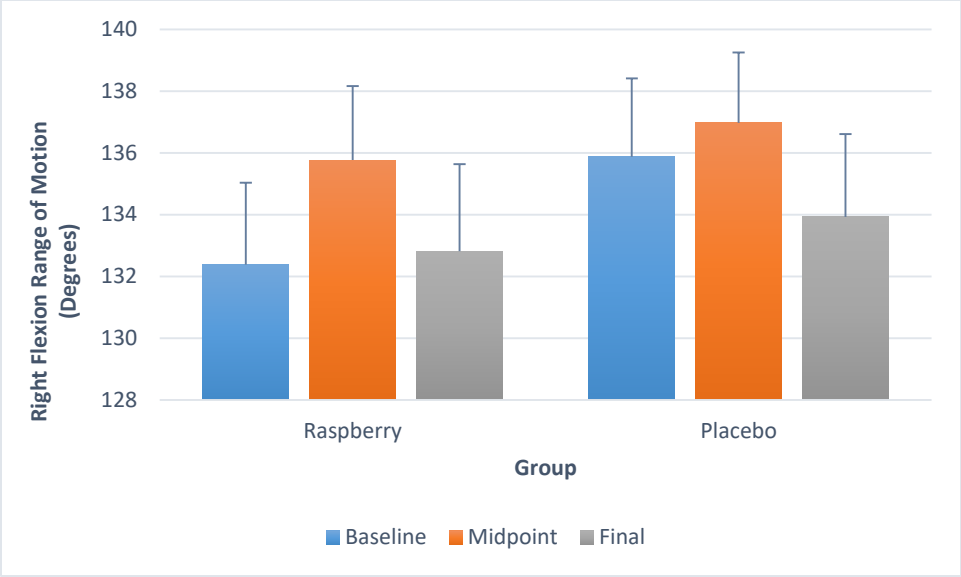

Figure 14. Effects of Raspberry vs. Placebo on Right Flexion Range of Motion. Mean ± SEM. *N* = 20 for the raspberry group, *N* = 22 for the placebo group. Outliers omitted. No significant differences within or between groups at any time point.

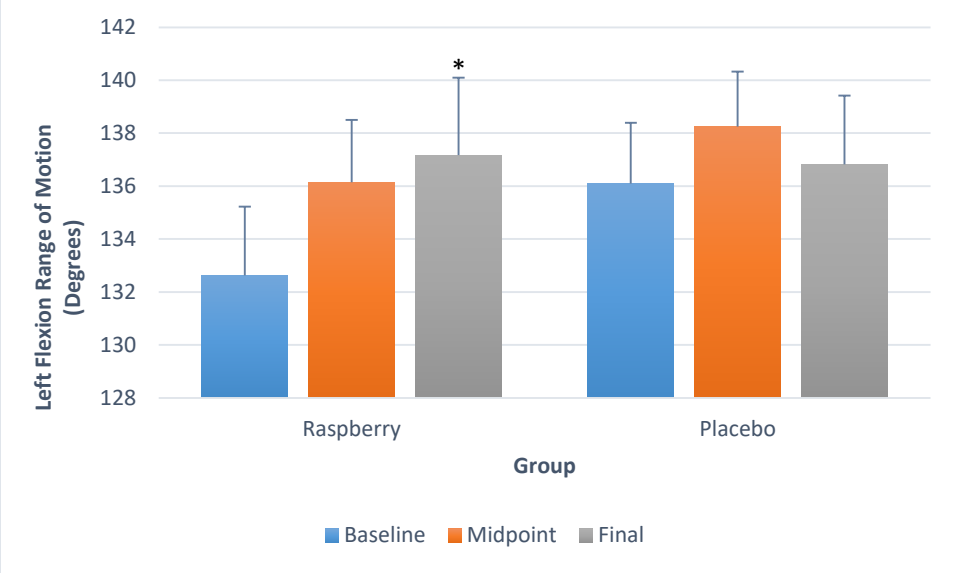

Figure 15. Effects of Raspberry vs. Placebo on Left Flexion Range of Motion. Mean ± SEM. N = 18 for the raspberry group, N = 23 for the placebo group. Outliers omitted.

* Significance as compared to baseline ($p < 0.05$)

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APPENDIX A

IRB APPROVAL LETTER

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DATE: September 7, 2018

TO: Dr. Shanil Juma
Nutrition & Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Approval for Effect of Freeze Dried Red Raspberry Powder on Range of Motion, Pain Symptoms, and Cartilage/Inflammatory Markers in Individuals with Symptomatic Knee Osteoarthritis (Protocol #: 20153)

The above referenced study was reviewed at a fully convened meeting of the Denton IRB (operating under FWA00000178). The study was approved on 9/6/2018. This approval is valid for one year and expires on 9/6/2019. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

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.

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 adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. Shane Broughton, Nutrition & Food Sciences

APPENDIX B

RECRUITMENT FLYER

Who?

Ages 45-79
Healthy and Mobile Adults
Willing to consume juice with or without whole red raspberry powder for 4 months

Yes?

If you said yes to the above, then you may be eligible to participate in a 4-month research study to look at the beneficial effects of raspberry in improving joint function and reducing pain associated with knee osteoarthritis.

Contact

If interest, please contact:
Shanil Juma, PhD Department of Nutrition and Food Sciences
sjuma@twu.edu
940-898-2704

Additional Criteria

There will be blood draws, measurement of joint flexibility, questionnaires, and range of motion measurement at the start, midpoint (2 months) and at the end (4 months) of the study.

Total Time Investment:
Approximately 3 hours 45 minutes over 3 office visits.

Benefits

- Promotion of knee joint health
- Measurements of height, weight, pain, stiffness assessment, and range of motion.
- Blood marker of cartilage health evaluation.

- Upon completion, you will receive a compensation of $100 for your time in partial payments of $50 at midpoint (2 months) and final follow-up visit

APPENDIX C
INFORMED CONSENT

Texas Woman's University

Consent to Participate in Research

Study Title: Effect of Freeze Dried Red Raspberry Powder on Range of Motion, Pain Symptoms, and Cartilage/Inflammatory Markers in Individuals with Symptomatic Knee Osteoarthritis

Investigators: Shanil Juma, PhD 940-898-2704(sjuma@twu.edu)

Young-Hoo Kwon, PhD 940-898-2598(ykwon@twu.edu)

Parakat Vijayagopal, PhD 940-898-2709(pvijayagopal@twu.edu)

Explanation and Purpose of Research

We are asking you to participate in a research study at Texas Woman's University. The purpose of the study is to find out if consumption of freeze dried red raspberry powder for 4 months will improve pain, stiffness and flexibility associated with self-reported knee osteoarthritis. We will ask the following questions:

a) Will consuming freeze-dried red raspberry powder for 4 months improve joint flexibility?
b) Will consuming freeze-dried red raspberry powder to reduce pain and stiffness in the knee joint?
c) Will consuming freeze-dried red raspberry powder for 4 months positively impact blood and urine biomarkers of inflammation and cartilage health?

Research Procedures

For this study, the baseline visit will first involve obtaining consent for your participation in this study. As part of the consent, you agree that you will not initiate any new therapies associated with the osteoarthritis of the knee during the duration of the treatment period. If you do decide to initiate a new therapy, please contact the principal investigator to determine if you still qualify to continue participating in this study.

First Visit

During the first visit you will be asked to arrive at the study site fasted (not to eat any food overnight or at least 10 hours). A phlebotomist (person taking the blood) will draw 15 milliliters (approximately 1 tablespoon) of your blood from one of the veins of your arms. You will be provided with a snack and drink (cookies, crackers, and orange juice). This will be followed with

a spot urine collection. You will be asked to provide a urine specimen after the first morning void. A trained personnel of the same gender will take your height and weight measurements. Filtered water and a light snack will be available for you at the study site. You will be asked to complete a physical activity questionnaire regarding your activity habits over the past week. You will complete a questionnaire regarding pain and stiffness. A measurement of knee motion (flexibility) will be done in a lying down position on a patient table and repeated three times during this visit by a trained personnel of the same gender associated with the study. A gait analysis to evaluate walking parameters will be done by trained personnel with instructions to walk short distance (30 feet) at usual speed and fastest speed. Each walking speed will be repeated three times with a 30 second rest between each walk. At the end of the baseline visit, you will be randomly assigned to a treatment based on chance, like a flip of a coin. Neither you nor the researcher chooses your assigned treatment group. You will have an equal chance of being in either group. You will be provided a 60 day supply of either the study treatment (freeze dried red raspberry powder packaged in pouches) or a control (comparative placebo freeze-dried powder without red raspberry). The daily dosage of placebo and raspberry powder (30 grams) will be reconstituted by you using 12 ounces of cold water and mixed in a blender bottle that will be provided to you as part of the study.

Midpoint Visit

At the 60 day visit (midpoint), you will again be asked not to eat any food overnight (10 hours). A trained female personnel will take your height and weight measurements. A phlebotomist (person taking the blood) will draw 3 table spoons of your blood from one of the veins of your arms. You will be provided with a snack and drink (cookies, crackers, and orange juice). This will be followed with a spot urine collection. You will be asked to provide a urine specimen after the first morning void. A trained personnel of the same gender will take your height and weight measurements. Filtered water and a light snack will be available for you at the study site. You will be asked to complete a physical activity questionnaire regarding your activity habits over the past week. You will complete a questionnaire regarding pain and stiffness. A measurement of knee motion (flexibility) will be done in a lying down position on a patient table and repeated three times during this visit by a trained personnel of the same gender associated with the study. Similar to baseline, a gait analysis will be done by trained research personnel. You will again be provided with a 60 day supply of either the study treatment (freezedried red raspberry powder in pouches) or a control (comparative placebo freeze dried powder without red raspberry). The daily dosage of placebo and raspberry powder (30 grams) will be reconstituted by you using 12 ounces of cold water and mixed in a blender bottle that was provided to you during the baseline visit of this study.

Final Visit

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 (1 tablespoons of blood will be obtained). You will be provided with snacks and filtered water. You will be asked to provide a spot urine specimen. A trained personnel of the same gender will measure height and weight. We will also ask you to complete a physical activity questionnaire regarding activity habits over the past week. You will complete a questionnaire regarding pain and stiffness. A measurement of knee motion (flexibility) will be done in a lying down position on a patient table and repeated three times during this visit by a trained personnel of the same gender associated with the study. A gait analysis to evaluate walking parameters will be done by trained personnel

Time Commitment

The study period is 4 months. Your time commitment includes initial screening questionnaire (~10 min), consent form (20 minutes), pain, stiffness, physical activity, and diet questionnaires (~30 minutes each during baseline, 2 months, and final), flexibility assessment (10 minutes each during baseline, 2 months, and final), gait assessment (10 minutes each during baseline, midpoint, and final), anthropometrics-height and weight (5 minutes each during baseline, 2 months, and final), and blood draw and spot urine (10 minutes each at baseline, midpoint and final). Your total time commitment for the entire study is approximately 3 hours 45 minutes over the three study visits.

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 the principal investigator will know your identity. All records will be stored in a locked filing cabinet in the principal investigator'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 freeze-dried red raspberry powder or the comparative placebo powder without red raspberry. If you do not like the randomized treatment, there is no penalty for not consuming it. You are free to quit the study at any time. Freeze dried red raspberry powder or the placebo powder without red raspberry is from a whole fruit source or equivalent to the sugar content of the whole fruit that has been custom prepared and packaged for our study. It has been previously used in other human clinical studies and is deemed safe for consumption and not harmful in any way.

Another possible risk to you as a participant in this study includes the discomfort of blood drawings. The phlebotomist will ask you about any concerns or previous issues with having a blood draw. If there are serious concerns or reactions to blood draw, we will ask you that you have the option to withdraw from participating in the study at any time. Blood draw may cause minor pain, bruising, discomfort, swelling, anxiety, infection or fainting. We will use a certified

expert for blood draw. This will minimize the possibility of pain, bruising, discomfort, swelling, infection, and anxiety. A light snack and water will be made available at the draw site to avoid fainting.

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

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

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

In addition to the risks above, you may experience anxiety or embarrassment related to height, weight, range of motion, and gait assessment. In order to minimize this risk, you will be assured of complete confidentiality before taking these measurements. All measurements will be taken only by experienced and trained personnel of the same gender in a private room. Anthropometrics (height and body weight) measurements will be conducted by trained personnel of the same gender. Blood draw will be done by a trained and experienced phlebotomist. Flexibility and gait analysis will be done with research personnel of the same gender who will describe the procedure and address any questions that you may have before the assessment is done.

The study treatment consists of whole red raspberry that has been freeze-dried into a powder and the comparative placebo powder containing sugar equivalent to the red raspberry treatment without red raspberry. If participants are allergic to raspberries or sugar found in raspberries he or she may consider not participating in the study. If any participant becomes allergic to either of the treatment powders used in the study, she can withdraw from the study at any time.

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

Participation Benefits

Your participation in this research study is completely voluntary, and you may discontinue your participation in the study at any time without penalty. As a participant in the study, you will receive the study powder for 4 months. You will also receive a cash incentive of $100.00, of which $50 will be paid at midpoint (60 days) and the remaining $50 after you complete the study. In addition, at completion of the study a summary of results as well as the results of your blood analysis will be mailed to you upon request. *

Questions Regarding the Study

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

_____ _____

Signature of Participant Date

Approved by the
Texas Woman's University
Institutional Review Board
September 6, 2018

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 D

SCREENING QUESTIONNAIRE

Screening Tool

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? _____Yes __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: Red Raspberry powder or powder without Red Raspberry		

APPENDIX E

ANTHROPOMETRICS MEASUREMENT SHEET

Raspberry OA Study

Subject ID: _____ D.O.B _____

Initial Height _____ Initial Weight _____

Blood Pressure 1:

Blood Pressure 2:

Blood Pressure 3:

Average BP:

Midpoint Height _____ Midpoint Weight _____

Blood Pressure 1:

Blood Pressure 2:

Blood Pressure 3:

Average BP:

Final Height _____ Final Weight _____

Blood Pressure 1:

Blood Pressure 2:

Blood Pressure 3:

Average BP:

APPENDIX F

RANGE OF MOTION TRACKING SHEET

Extension

- 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.
  - 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°)

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- Extension

	Trial 1	Trial 2	Trial 3
Active extension			
Hyperextension?			

Right Knee- Flexion

	Trial 1	Trial 2	Trial 3
Active flexion			

Left Knee- Extension

	Trial 1	Trial 2	Trial 3
Active extension			
Hyperextension?			

Left Knee- Flexion

	Trial 1	Trial 2	Trial 3
Active flexion			

Comments: (clothing, restricted movement, pain, etc)

APPENDIX G

WOMAC QUESTIONNAIRE

WOMAC OSTEOARTHRITIS INDEX VERSION VA3.1

INSTRUCTIONS TO PATIENTS

In Sections A, B, and C questions are asked in the following format. Please mark your answers by putting an " ✗ " through the horizontal line.

EXAMPLES:

1. If you put your " ✗ " at the left-hand end of the line as shown below, then you are indicating that you feel **no** pain.

2. If you put your " ✗ " at the right-hand end of the line as shown below, then you are indicating that you feel **extreme** pain.

3. Please note:
   a) that the further to the right you place your " ✗ ", the **more** pain you feel.
   b) that the further to the left you place your " ✗ ", the **less** pain you feel.
   c) **please do not** place your " ✗ " **past either end of the line**.

You will be asked to indicate on this type of scale the amount of pain, stiffness or disability you have felt during the last 48 hours.

Think about your _____ (study joint) when answering the questions. Indicate the severity of your pain and stiffness and the difficulty you have in doing daily activities that you feel are caused by the arthritis in your _____ (study joint).

Your study joint has been identified for you by your health care professional. If you are unsure which joint is your study joint, please ask before completing the questionnaire.

Section A

PAIN

Think about the pain you felt in your _____ (study joint) caused by your arthritis during the last 48 hours.

(Please mark your answers with an " ✗ ".)

QUESTION: **How much pain have you had . . .**

		Study Coordinator Use Only
1. when walking on a flat surface?	No Pain — Extreme Pain	PAIN1 _____
2. when going up or down stairs?	No Pain — Extreme Pain	PAIN2 _____
3. at night while in bed? (that is - pain that disturbs your sleep)	No Pain — Extreme Pain	PAIN3 _____
4. while sitting or lying down?	No Pain — Extreme Pain	PAIN4 _____
5. while standing?	No Pain — Extreme Pain	PAIN5 _____

Section B

STIFFNESS

Think about the stiffness (not pain) you felt in your_____ (study joint) caused by your arthritis during the last 48 hours.

Stiffness is a sensation of **decreased** ease in moving your joint.

(Please mark your answers with an " ✗ ".)

6. How **severe** has your stiffness been **after you first woke up** in the morning?

No Stiffness ——— Extreme Stiffness

7. How **severe** has your stiffness been after sitting or lying down or while resting **later in the day**?

No Stiffness ——— Extreme Stiffness

Study Coordinator Use Only	
STIFF6	____
STIFF7	____

Section C

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities caused by your arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and take care of yourself.** (Please mark your answers with an " ✗ ".)

QUESTION: **How much difficulty have you had . . .**	Study Coordinator Use Only
8. when going down the stairs? No Difficulty — Extreme Difficulty	PFTN8 _____
9. when going up the stairs? No Difficulty — Extreme Difficulty	PFTN9 _____
10. when getting up from a sitting position? No Difficulty — Extreme Difficulty	PFTN10 _____
11. while standing? No Difficulty — Extreme Difficulty	PFTN11 _____
12. when bending to the floor? No Difficulty — Extreme Difficulty	PFTN12 _____
13. when walking on a flat surface? No Difficulty — Extreme Difficulty	PFTN13 _____

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities caused by your arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and take care of yourself.** (Please mark your answers with an " ✗ ".)

QUESTION: **How much difficulty have you had . . .**	Study Coordinator Use Only
14. getting in or out of a car, or getting on or off a bus? No Difficulty — Extreme Difficulty	PFTN14 _____
15. while going shopping? No Difficulty — Extreme Difficulty	PFTN15 _____
16. when putting on your socks or panty hose or stockings? No Difficulty — Extreme Difficulty	PFTN16 _____
17. when getting out of bed? No Difficulty — Extreme Difficulty	PFTN17 _____
18. when taking off your socks or panty hose or stockings? No Difficulty — Extreme Difficulty	PFTN18 _____
19. while lying in bed? No Difficulty — Extreme Difficulty	PFTN19 _____

DIFFICULTY PERFORMING DAILY ACTIVITIES

Think about the difficulty you had in doing the following daily physical activities caused by your arthritis in your _____ (study joint) during the last 48 hours. By this we mean **your ability to move around and take care of yourself.** (Please mark your answers with an " ✗ ".)

QUESTION: **How much difficulty have you had . . .**	Study Coordinator Use Only
20. when getting in or out of the bathtub? No Difficulty — Extreme Difficulty	PFTN20 _____
21. while sitting? No Difficulty — Extreme Difficulty	PFTN21 _____
22. when getting on or off the toilet? No Difficulty — Extreme Difficulty	PFTN22 _____
23. while doing heavy household chores? No Difficulty — Extreme Difficulty	PFTN23 _____
24. while doing light household chores? No Difficulty — Extreme Difficulty	PFTN24 _____