THE EFFECT OF FREEZE-DRIED WHOLE BLUEBERRY POWDER ON JOINT FLEXIBILITY, MOBILITY, AND SERUM BIOMARKERS OF CARTILAGE METABOLISM IN SYMPTOMATIC KNEE OSTEOARTHRITIS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTERS IN SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCES

COLLEGE OF HEALTH SCIENCES

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MAY 2019

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Date of Defense

To the Dean of the Graduate School:

I am submitting herewith a thesis written by Amy L. Smith entitled "THE EFFECT OF FREEZE DRIED WHOLE BLUEBERRY POWDER ON JOINT FLEXIBILITY, MOBILITY, AND SERUM BIOMARKERS OF CARTILAGE METABOLISM IN SYMPTOMATIC KNEE OSTEOARTHRITIS." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters in Science with a major in Nutrition.

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We have read this thesis and recommend its acceptance:

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ACKNOWLEDGEMENTS

I would like to thank Dr. Juma for his guidance and support during the research process and the completion of my thesis. My committee members, Dr. Vijayagopal and Dr. Patterson were also instrumental in keeping me on track during the writing process. Dr. Vijayagopal a special thank you for your guidance in graduate school with support and suggestions with my teaching assistant duties. Your compassion for students is infectious. Although your physical distance is great, Dr. Patterson was able to make it insignificant with her attention to detail. I would also like thank several graduate students for their support and ever-ready presence, Chen Du, Jacquelynn Lucero, and Sanique South. Lastly, I would like to thank my family for their love, patience, and understanding the importance of accomplishing this feat.

ABSTRACT

AMY L. SMITH

THE EFFECT OF FREEZE DRIED WHOLE BLUEBERRY POWDER ON JOINT FLEXIBILITY, MOBILITY, AND SERUM BIOMARKERS OF CARTILAGE METABOLISM IN SYMPTOMATIC KNEE OSTEOARTHRITIS

MAY 2019

Objective: The purpose of this study was to examine the effect of freeze dried whole blueberry powder on joint flexibility, mobility, and serum biomarkers of cartilage metabolism in men and women ages 45-79 years old with symptomatic knee osteoarthritis.

Methods: A total of 63 participants were recruited and randomized into two groups. The freeze-dried blueberry powder (FDBP) group (treatment; n = 33) consumed a total of 40 grams of freeze-dried whole blueberry powder daily (packaged in 20 gram pouches to be consumed twice a day, to be equivalent to two servings of fresh blueberries) for 16 weeks. A control group (n = 30) consumed 40 grams of a powder daily for 16 weeks which closely matched the freeze-dried blueberry powder in appearance and energy content but devoid of blueberries. All of the following outcomes were assessed at baseline, midpoint (8 weeks), and final visits. Flexibility of the afflicted joint(s) using range of motion (ROM) measurements were assessed using a 360 degree goniometer based on neutral zero method. The ROM parameters, extension (active and hyper) and flexion were performed by the same trained individual at each visit in triplicates. Mobility was measured by the International Physical Activity Questionnaire

Long Form (IPAQ) and blood specimens assessed changes in biomarkers of cartilage metabolism.

Results: A total of 49 participants completed the study with an attrition rate of 22%. Range of motion increased slightly for both knees in FDBP group, while the placebo group had steady declines from baseline to final measurements. Physical activity measured in METs decreased for both groups; however, activity levels decreased more in the placebo than the FDBP group. Slight improvements in hyaluronic acid from baseline to midpoint were noticed but from midpoint to final, the concentrations slowly regressed. The FDBP group had an increasing trend in concentrations of IGF-1 with consistently stable concentrations of IGFBP-3, while the placebo group had declining concentrations of IGF-1 and increases in IGFBP-3 over the course of the study. The FDBP group had an overall decrease in concentrations of YKL-40 from baseline to the final evaluation, while the placebo group had a steady increase in concentration throughout the study period. Systolic and diastolic blood pressure decreased significantly in the FDBG from mid-point and final as compared to baseline measurements, while there were no changes observed in the placebo group. BMI increased significantly at final over baseline and midpoint in the placebo group, whereas the blueberry group maintained their body weight throughout the study.

Conclusions: The findings of the study suggest that blueberries may have a positive effect on joint health by preventing further breakdown of cartilage and

promoting repair. This was further supported by improvements in range of motion and maintenance of physical activity levels in the blueberry treatment group.

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CHAPTER 1
INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis, and is a disease involving the articular surfaces of the synovial joints resulting from either age-related degeneration or in response to injury (Escott-Stump, 2015). The area is marked by abnormal joint metabolism: cartilage degradation, increased subchondral bone remodeling, osteophyte formation (bone spurs), and inflammation (Chin & Pang, 2017). The Center for Disease Control and Prevention (CDC) estimates over 30 million adults are afflicted with OA in the United States alone (CDC, 2017). OA causes pain, aching, stiffness and reduction in motion of joints due to break down of cartilage (Deshpande et al., 2016). The affliction may be present in any joint but is typical in weight-bearing joints: knees, hips, spine, and hands (Deshpande et al., 2016; U.S. National Library of Medicine, 2017). In 2010, it was estimated that about 10 percent of men and 13 percent of women age 60 and older have symptomatic knee osteoarthritis in the United States (Zhang et al., 2010). OA is thought to be the most prevalent of all musculoskeletal pathologies worldwide, ranking fifth among all forms of disability (Arthritis Foundation, 2017).

In normal joints, cartilage is a slippery tissue on the ends of bones helping to absorb shock from movement. The loss of cartilage tissue results in the rubbing together of bones, causing permanent damage. The disease is prevalent in older populations due to normal “wear and tear” of the joint. However, continual stress on the joint will stimulate

an inflammatory response to attempt to repair the broken-down tissue. The inflammatory response can result in further degeneration of the tissue (Arden & Nevitt, 2006; Chin & Pang, 2017; Das, 2008). Swelling and pain in the area causes problems with movement and mobility. Economic burdens are distributed among the afflicted, employers, and caregivers. Direct costs include pharmacological and other forms of treatments. Earning losses from absenteeism as well as costs of short-term disability or workers' compensation also contribute to the overall cost of the disease (Chen, Gupte, Akhtar, Smith, & Cobb, 2012).

There is no known cure for OA, forcing treatments to focus on managing pain and improving function. Osteoarthritis requires complex, individualized approaches for treatment, including a combination of nonpharmacological therapies, pharmacological agents, and surgical procedures. Medical costs associated with arthritis in 2013 reached $140 billion, with OA being implicated as the most expensive condition for which privately insured patients were hospitalized, accounting for over $6.2 billion in hospital costs (CDC, 2017). Nonpharmacological treatments revolve around self-management programs including strengthening and low-impact aerobic exercises. Weight management is also a top recommendation to limit stress on afflicted joints to combat symptoms. Pharmacological agents include nonsteroidal anti-inflammatory drugs (NSAIDs) either oral or topical. Intraarticular (IA) corticosteroid injections, repositioning of bones, or joint resurfacing are some of the most common surgical procedures used in the management of OA (AAOS, 2013; NIH, 2016). Biological compounds such as glucosamine and chondroitin have been widely researched as alternative treatments and

have demonstrated potential for controlling symptoms, potentially inspiring others to investigate alternative treatments such as nutraceuticals (Clegg, 2008).

Blueberries have risen in popularity over the last few decades, particularly after 2010 when they were tagged a superfood gaining the name "Little Blue Dynamos" (U.S. Highbush Blueberry Council, 2018). They contain a category of phytonutrients called polyphenols, particularly anthocyanins. Polyphenols from a variety of foods demonstrate anti-inflammatory effects by modulating cell signaling and cytokines and acting as reducing agents, hydrogen donors, and singlet oxygen quenchers (Jayarathne et al., 2017; Gambino et al., 2018). Blueberries have been shown to have positive effects on cardiovascular and brain health, as well as improving the insulin response (Basu, et al., 2010; Shukitt-Hale, 2012; Stull, Cash, Johnson, Champagne, & Cefala , 2012). Blueberries in cancer research has also been widely studied (Yi, Fischer, Krewer, & Akoh, 2005; Adams, et al., 2010). This study will examine the effect of blueberry consumption on joint health using men and women with symptomatic knee osteoarthritis.

Hypothesis and Specific Aims

Hypothesis

The main hypothesis of this study is that the daily consumption of 40 grams of whole blueberry powder for 16 weeks will improve joint flexibility (range of motion), and mobility (physical activity), and serum biomarkers of cartilage metabolism (YKL-40, IGF-1, IGFBP-3, and hyaluronic acid) in men and women with active knee OA symptoms.

Specific Aims

Aim 1. To examine the effects of freeze-dried whole blueberry in comparison to a placebo powder intervention on symptoms associated with knee OA in both men and women. Primary measures will include assessing joint flexibility (range of motion) and mobility (physical activity) of the afflicted knee joint(s) before and after treatment.

Aim 2. To evaluate the effects of freeze-dried whole blueberry in comparison with a placebo powder intervention on selective serum biomarkers of cartilage metabolism before and after treatment.

CHAPTER II

REVIEW OF LITERATURE

Osteoarthritis: Definition and Epidemiology

OA, is a degenerative condition of the weight-bearing joints mainly due to age-associated breakdown of cartilage from constant or repeated use. The disease is characterized by progressive structural changes in joint tissue, particularly articular cartilage. Changes also occur in subchondral bone, the synovial membrane, and synovial fluid (Umlauf, Frank, Pap, & Bertrand, 2010). The loss of articular cartilage and structural changes result in debilitating pain, disability, and a significant reduction in quality of life in afflicted patients. OA is the most common form of arthritis and its associated high costs (Escott-Stump, 2015).

Although the etiology of the disease is greatly unknown, several non-modifiable factors have been linked to increased likelihood of disease development: increasing age, gender, and race. Older age, over 63.5 years, is independently associated with accelerated knee osteoarthritis (Driban et al., 2017). OA rates increase continually with age and according to projections by the United States Census Bureau, an aging population will have wide-range implications on the country (Prieto-Alhambra, et al., 2014). By 2050, the U.S. population aged 65 and over is projected to be nearly doubled from 2012, and forecast to reach an estimate of 83.7 million (Ortman, 2014). Nearly 20% of U.S. residents are projected to be 65 and older in 2030, an increase from 13% in 2010 and from 9.8% in 1970 (Ortman et al., 2014). Women have out-lived men for decades. According to data from the CDC, the differences in life expectancy between the sexes

from 1900 to 1975 increased from 2.0 years to 7.8 years (CDC, 2016). Although the gap in years lived between sexes slightly narrowed from 1975 to 2012, women still live longer than men. This trend is anticipated to continue as female life expectancy is projected to be longer than men in 2050 with over 55% of those 65 years and older as women, and even greater among those 85 years and over (Ortman et al., 2014).

Corresponding to longer life expectancies, studies over time have consistently demonstrated a higher risk for osteoarthritis among women (Felson et al., 1995; Srikanth et al., 2005; Driban et al., 2017). A meta-analysis of 12 studies, evaluating sex differences in prevalence of OA found a significant reduction in risk for males 55 years of age and older for knee osteoarthritis (Srikanth et al., 2005). The same analysis showed prevalent knee OA was significantly more severe in females versus males with radiographic definition of OA. However, before 50 years of age, the prevalence of OA in most joints is higher in men than in women, but after the age of 50, women are more affected with hand, foot, and knee OA (Felson et al., 2000). Short-term hormonal imbalances and fluctuations or permanent declines in hormone concentrations may contribute to the surge in osteoarthritis risk, which begins around the age of menopause. Changes in sex-specific hormones could affect osteoarthritis risk and may induce inflammatory factors, which play an important role in cartilage metabolism (Nevitt & Felson, 1996). Dramatic decreases in sex hormones are a marked aspect of menopause. The use of estrogen replacement therapy (ERT) in postmenopausal women is associated with lower than expected risk of knee and hip osteoarthritis (Nevitt & Felson, 1996).

These factors as well as others may be associated with the shift in incidence of OA between the sexes.

Racial differences in risk of development of OA have reported mixed results. In a recent study, racial or ethnic minorities were more likely to be younger and therefore less likely than non-Hispanic whites to have symptomatic knee OA (Deshpande et al., 2016). According to the Women's Health Initiative, more than 80% of women with self-reported OA were non-Hispanic white (over 50,000 individuals), and the next highest racial group was African Americans with 9.2% (Wright, Riggs, Lisse, & Chen, 2008). According to the Johnston County Osteoarthritis Project, of those with only knee OA, African Americans had significantly worse pain and function scores than Caucasian scores an effect that may be due to differences in BMI (Allen et al., 2009). The prevalence of OA in minorities is expected to rise because this population is afflicted with OA at a substantially younger age than the white population and the obesity rates observed in Hispanics nearly doubled as reported in the NHANES 2007-2008 to NHANES 2011-2012 data (Deshpande, 2016).

Rapid development of genotyping technology and recent inquiries into the human genome have revealed potential associations with genetic variants and osteoarthritis. Genome-wide association studies (GWAS) have successfully tested the association between single nuecleotide polymorphisms (SNPs) and OA susceptibility (Chu et al., 2017). ArcOGEN, a large-scale study, examined patients of European descent with severe OA, and identified five genome-wide significant loci for association with OA (Zeggini et al., 2012). Jeffries et al. (2016) and Zhao et al. (2017) suggested

hypomethylation of DNA in subchondral bone, articular chondrocytes, and overlying cartilage may contribute to the progression of OA. Zhao et al. showed hypomethylation in TRAF1, CTGF, and CX3CL1 genes in chondrocytes and have demonstrated consistent correlation with mRNA expression; suggesting epigenetic changes in methylation status of the identified genes may contribute to the pathology of OA (Zhao et al., 2017). A study conducted in the Chinese population found a potential association with knee osteoarthritis and single nucleotide polymorphism (SNP) rs4238326 in ALDH1A2 gene (Chu et al., 2017.).

Genetic disposition may contribute as a risk factor to the likelihood of developing OA. However, gene alterations across populations are slow, the recent rise in OA diagnoses suggests environmental and lifestyle factors are large contributors (Hunter, March, Sambrook, 2002 ;Vrezas, Elsner, Bolm-Audorff, et al, 2010). The rise in incidence of OA can be attributed to sedentary lifestyles and prevalence of high obesity. Less than 22% of adults in the US meet the Physical Activity Guidelines for both aerobic and muscle-strengthening activity, which may partially be a cause of increasing prevalence of OA and contributing to the worsening of symptoms (CDC, 2017; Dunlop et al., 2011). Data from the National Health and Nutrition Examination Survey from 2011-2014, showed the prevalence of obesity was just over 36% in U.S. adults, with higher numbers associated with middle-aged (40-59 years) and older adults (60 and over) than among younger adults aged 20-39 years of age (NCHS, 2015). A significant increase in obesity was observed in adults from 1999-2000 through 2013-2014, which corresponds to declines in physical activity. In data from 2011-2014, women were more likely than men

to be obese, with nearly 40% of adult women meeting obesity criteria based on body mass index (NCHS, 2015).

Findings from the Framingham study indicate a strong association with higher body mass index (BMI) and increased risk of OA, a 40% increase in risk for every 10-lb weight gain along with commensurate decrease in risk for weight loss (Felson et al., 1995). Groups were stratified to assess whether the effect of each risk factor on incident knee OA varied according to sex, and found effects were strongly and significantly present in women as compared to men (Felson et al., 1995). More recently, a study evaluating the effects of body composition on likelihood to have joint replacements due to osteoarthritis; indicated that body mass index, total fat mass, trunk fat mass, and waist circumference were strongly associated with a greater risk for knee replacement but not with hip replacement (Munugoda et al., 2018). Furthermore, Munugoda et al. (2018) suggested that obese participants have a two times higher risk of knee replacement compared to underweight and normal participants, concluding that the effect of higher BMI is more profound in the obese category (Munugoda et al., 2018). This corresponds with findings from an article published by Leyland et al. who previously stated that overweight and obese participants had a 40-100% greater risk of knee replacement as compared to those with normal BMI (Leyland et al., 2016). Overloading the knee could lead to cartilage breakdown and failure of other structural support as for each pound increase in body weight, the overall kinetic force across the knee in a single-leg stance increases by 2-3 pounds and could explain the increased risk in knee OA among overweight persons (Felson et al., 2000). Retaining fat-free mass with weight loss and

increasing skeletal muscle strength and function has been shown to improve functional capacity in individuals with OA (Toda et al., 1998; Slemenda, Brandt, & Heilman, 1997). The AAOS suggests weight loss for patients with symptomatic OA of the knee and a BMI $\geq$ 25 (AAOS, 2013).

Excessive or repetitive loading exerted on joint structures could also be detrimental to joint health. Ambulatory activity (AA), typical walking is the most common form of physical activity performed by older people (Munugoda et al., 2018). Researchers found for every 1000 steps/day increase from baseline, the risk of knee replacement increased by almost 10%; however, dose-dependent associations were not significant (Munugoda et al., 2018). Some occupations require workers to do repetitious tasks, which over-works the joints and causes fatigue to surrounding muscles that protect the joint. Data from the Framingham study suggested that job-related activities such as kneeling or squatting along with heavy lifting caused anywhere from 15% to 30% of knee OA in men; other activities including climbing stairs, walking on uneven ground, standing or sitting have been inconsistent in relation to OA risk (Felson et al., 2000).

Biomechanical factors may play a role in OA development and progression. High-heeled shoes have even been accused of predisposing wearers to degenerative changes by altering forces at the knee. Data from the Genetics of Osteoarthritis and Lifestyle (GOAL) case-control study, showed negative association between high heeled and narrow heeled women's shoes and lower limb OA (McWilliams et al., 2014). Stiletto high-heeled shoes, averaging 2.5 inches in height, have been shown to exaggerate knee torques during walking, specific torques that are thought to be relevant to the incidence of

knee OA (Kerrigan, Todd, & Riley, 1998). One of the same researchers found a significantly greater torque on the knee in those who wore shoes with a 1.5-inch-high heel as compared to control (Kerrigan et al., 2005).

The demands for traditional care for treatment of knee OA coincide with the rise in incidence; therefore, alternative therapies need to be explored. The conclusion may be drawn that older women are more at risk of developing osteoarthritis due to their longer life expectancies, higher rates of obesity prevalence, and lifestyle factors such as sedentary activity levels and habitual use of high heels.

Significance of the Problem

A secondary analysis of the National Health Interview Survey (NHANES) 2011-2012 using the Osteoarthritis Policy Model, calculated the number of persons living with symptomatic knee OA to be approximately 15.1 million persons in the United States; this is up from the previous evaluation of NHANES 2007-2008 that estimated 13.7 million Americans at least 25 years of age living with symptomatic knee OA (Deshpande, 2016). As one of the leading causes of disability among older adults, an estimated one in eight will be affected (Hunter, Schofield, & Callander, 2014). According to the Global Burden of Disease Study (GBD), OA of the knee accounts for 83% of total OA burden, with roughly 80% of persons with OA having some degree of movement limitations and a quarter requiring help with routine activities. Because of its prevalence and the frequency of disability that accompanies OA in both the hip and knee, it accounts for more trouble with climbing stairs and walking than any other condition (Guccione et al., 1994).

The problem is not only seen in the U.S. as the GBD revealed a 64.8% increase in global years lived with disability (YLDs) from 1990 to 2010 for those with OA (Vos, Flaxman, Naghavi et al., 2012). For all musculoskeletal disorders, OA is the second leading cause of disability worldwide with the top contributor being lower back pain (Storheim & Zwart, 2014). Globally, hip and knee OA are ranked as the 11th highest contributor to global disability (Vos, Flaxman, Naghavi et al., 2012).

Impacts and Cost

Osteoarthritis burden can be measured in direct costs such as financial costs of treatments, but also indirectly with absenteeism and reduced productivity. Pain, decreased quality of life, and physical disability also contribute to intangible costs of OA. In the Framingham study, patients with OA required assistance to carry out four of seven functional activities: stair climbing, walking a mile, housekeeping, and carrying bundles (Breedveld, 2004). Researchers using data from the US Medicare Expenditure Panel Survey (1996-2005) estimated that $185.5 billion in annual insurer expenditures were attributed to medical care for patients with OA in the US (Kotlarz et al., 2009). Both men and women with OA have greater annual expenditures (charged to insurers and out-of-pocket) as compared to those without OA. Most of direct costs of OA are usually attributed to hospital stays, with the bulk of hospitalizations for OA involving total joint replacement; over 1 million joint replacement procedures were performed in 2014 in the US alone, doubling over the last decade and projected to increase to over 3 million annually by 2030 (Hunter, et al., 2014). Many joint replacements are being performed in younger adults who will likely need revision procedures in the future. Length of hospital

stay and costs associated with revisions are over double than that of the initial surgery (Hunter et al., 2014).

Social and economic impacts of OA are also substantial; limited physical mobility can lead to anxiety and depression, favoring the onset of mental disorders. Poor sleep in the majority of older persons with OA is linked to fatigue and is known to have many negative effects on health including mental well-being (Hunter et al., 2014). A U.S. study found similar health-related quality of life scores related to depression and advanced cancer among patients with OA. (Groessl, Kaplan, & Cronan, 2003). Recent research by the U.S. Department of Health Policy and Management in conjunction with John Hopkins Bloomberg School of Public Health found failing to obtain effective treatment increased costs and limited benefits measured in quality-adjusted life years (QALYs) for all ethnicities studied. The same researchers also found those forgoing treatment for OA incur the highest loss of productivity; conversely, those that chose a nonsurgical treatment pathway had reduced levels of lost productivity (Karmarkar et al., 2017). As the U.S. population continues to age and experience obesity at alarmingly high rates, the physical and financial impacts of knee OA is likely to increase, partially because of the longevity of working careers and the substantial prevalence of osteoarthritis in middle-aged persons, OA causes a considerable burden in lost time at work and early retirement.

Treatment and Therapies

Osteoarthritis requires complex, individualized approaches for treatment that includes a combination of nonpharmacological treatments, pharmacological agents, and surgical procedures. Most treatment plans aim to manage pain and improve function. The

American Academy of Orthopaedic Surgeons (AAOS) and the American College of Rheumatology (ACR) both strongly recommend a self-management program to include strengthening and low-impact aerobic exercises consistent with national guidelines as the therapy of choice for OA (AAOS, 2013; Hochberg et al., 2012). Exercise interventions are predominantly conducted under supervision, usually by a physical therapist, where-as self-management interventions are led utilizing various healthcare providers as guides such as physicians, nurses, physical and occupational therapists, and health educators. Many studies have shown improvements in pain and function in those with OA with strengthening exercises versus no exercise to include weight-bearing, non-weight-bearing such as high-resistance strength, yoga, aquatic therapies, and aerobic walking (Baker, et al., 2001; McCarthy, et al., 2004). In 2008, the federal government published national guidelines for physical activity that generally emphasize the importance of aerobic conditioning and muscle and bone strengthening, regular activity, and balance exercises for older adults, that serve as a good basis for self-management therapy in OA.

Another strong recommendation from the AAOS and the ACR is the use of nonsteroidal anti-inflammatory drugs (NSAIDs) either oral or topical, but gastrointestinal tolerability may pose issues for long-term maintenance. AAOS specifically names Tramadol as a pharmacologic treatment with a high of recommendation based on high quality of supporting evidence; however, they were unable to recommend for or against the use of acetaminophen, opioids, or pain patches, stating evidence is inconclusive (AAOS, 2013).

Exercise and pharmacological agents are the primary focus for initial treatments, but if symptoms persist or become unbearable to the individual, the AAOS recommends, with limitations, procedural treatments such as use of intraarticular (IA) corticosteroids, repositioning of bones, or joint resurfacing (AAOS, 2013; NIH, 2016). IAs corticosteroids injections have been evaluated as pain relief treatments with mixed results, some are demonstrated to be superior as compared to a placebo, but another study found IA corticosteroid injections to be inferior to hyaluronic injections (Chao et al., 2010). Hyaluronic acid (HA) a polysaccharide containing glucosamine and glucuronic acid has been shown to improve pain and function in patients with knee OA (Aggarwal & Sempowski, 2004; Evanich, Evanich, Wright, & Rydlewicz, 2001; Wu, Shih, Hsu, & Chen, 1997).

Biological compounds represent newer potential therapies. Chondroitin sulfate, a complex carbohydrate, is a major component of cartilage that helps it retain water and thus aiding in cushion for impact of joint movements; glucosamine is an amino sugar produced by the body and found primarily in the fluid around the joints also providing lubrication for joint movement (Clegg, 2008) Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) results favored only a combination of glucosamine and chondroitin to be beneficial in patients with moderate to severe OA of the knee as compared to glucosamine alone, chondroitin alone, celecoxib, or placebo (Clegg, 2006). However, the AAOS does not recommend using glucosamine and chondroitin for patients with symptomatic OA of the knee due to a lack of supporting evidence and is based on lack of efficacy not on potential harm. Lack of evidence of long term effectiveness,

despite studies demonstrating fewer side effects related to the gastrointestinal system as compared with use of NSAIDs warrant further research to justify their wide spread use (Zhang et al., 2010). An increased interest in complementary and alternative treatments arises from limitations and side effects of established treatments. Nutraceutical therapies may provide complementary approaches to the treatment of OA. Because OA is a disease that often extends over decades, such agents must be safe to permit their use over extended time periods.

Cartilage and Joint Health

In order to understand potential origins of OA development it is important to review joint mechanics. Synovial joints allow for pain-free movement, with articular cartilage contributing the most functional capacities by forming the bearing surfaces. The cartilage has stiffness to compression, resilience, and exceptional ability to distribute loads, thereby minimizing peak stress on subchondral bone all the while maintaining durability over the human lifetime (Buckwalter, Mankin, & Grodzinsky, 2005). Unfortunately, the tissue has limited abilities to repair itself, and decline with age, hence the increased risk of progressive degenerative diseases in an aging population. It varies in thickness, cell density, matrix composition, and mechanical properties within the same joint but in all synovial joints it consists of the same components, extracellular matrix and a sparse population of cells known as chondrocytes (Buckwalter et al., 2005).

Chondrocytes are responsible for producing tissue that can provide normal, synovial joint function by synthesizing appropriate types and amounts of macromolecules and then organizing and assembling them into a framework. Chondrocytes synthesize

collagen, large aggregating proteoglycans, and noncollagenous proteins (Buckwalter et al., 2005). Collagens are distributed relatively uniformly and give cartilage its form and tensile strength; proteoglycans and noncollagenous proteins bind to the collagenous framework. The area fills with water and other molecules forming the extracellular matrix and synovial fluid. In OA, the collagen turnover rate increases, the proteoglycan content decreases, the proteoglycan composition changes, and the water content increases (Moreland, 2002).

Proteoglycans consist of a protein core and at least one glycosaminoglycan chain (long unbranched polysaccharide chains consisting of repeating disaccharides with an amino sugar); the major class of proteoglycans in articular cartilage is large aggregating molecules or aggrecans (Buckwalter et al., 2005). These aggrecans are noncovalently associated with a central protein filament, hyaluronic acid, and small link proteins (noncollagenous proteins) to form proteoglycan aggregates. These structures help anchor proteoglycans in the matrix, stabilizing the relationship between the proteoglycan and collagen network. Although structural function has been identified, their potential other roles in articular cartilage remain poorly understood, including responses to inflammation and osteoarthritis progression.

Although the roles the major structural components play in degenerative disease processes remains unknown, other processes have been studied. An overexpression of matrix-degrading enzymes results in a loss of matrix particularly at the cartilage surface. An increase in water content of cartilage in response to initial loss of matrix content and a decrease in proteoglycans and cleavage of collagen also advances the destruction

(Hooshamand, Juma, Khalil, Shamloufard, & Arjmandi, 2015; Umlauf et al., 2010). Tensile strength of the cartilage is compromised as a result of damages to the collagen network, altering biomechanical properties of the cartilage with a reduced stiffness; compensation by enhancing proliferation and synthesis of collagen from chondrocytes and proteoglycans is varied in efforts in individual zones of the cartilage (Umlauf et al., 2010). The yielding matrices are of different structure and more susceptible to erosion and loss of proteoglycans. Eventually, repair attempts are outmatched by degradation. Cartilage degradation enzymes are unregulated by the presence of proinflammatory cytokines, as well as by cyclic compressive loading and mechanical injury (Umlauf et al., 2010).

Chondrocytes are responsible for the synthesis, regeneration, and maintenance of the cartilage matrix, however chondrosenescence, the loss of a chondrocyte cell's power of division and growth, occurs naturally as a result of aging (Chin & Pang, 2017; Musumeci et al., 2015). They have a limited number of replications allowed during their lives, approximately 30-40 (Musumeci et al., 2015). Senescence compromises the ability of the cells to maintain and repair articular cartilage tissue; the loss of ability to repair and continual stress of the area lead to chondrocyte cell death, leading to irreversible cartilage damage and matrix depletion (Chin & Pang,, 2017). Mechanical damage can elicit a localized inflammatory response at the joint, marked by several pro-inflammatory markers including nitrite oxide (NO), further exaggerating cartilage tissue damage via oxidative stress, resulting in a self-destructive cycle (Chin & Pang, 2017). Several enzymes related to oxidative stress produce reactive oxygen and nitrogen species

resulting in an imbalance between oxidants and antioxidants resulting in oxidative damage that can lead to chondrocytic differentiation or apoptosis, all contributing to the progression of OA (Felson, 2000; Chin & Pang,, 2017). In combination with degradation of cartilage, clinical evidence from OA joints suggests that joint swelling, with its accompanying chronic inflammation, is caused by synovitis, retention of synovial fluid in the joint cavity and edema, resulting from circulatory disturbances (Basu, Schell, & Scofield, 2018).

The complex nature of disease progression is further complicated in early onset due to biosynthetic activity of the chondrocytes. Prior to the loss of cartilage mass and the onset of proteoglycan depletion, research has demonstrated an increase in these proteins, and major components of extracellular matrices of connective tissue. The increase in concentrations results in a thickening of the articular cartilage during early stages of OA. The mass production of proteoglycans, a result of the repair process attempting to keep pace with disease progression, produces molecules with abnormalities in structure, further disturbing the balance of homeostasis in the joint (Martel-Pelletier, Di Battista, Lajeunesse, & Pelletier, 1998). The repair process may be sufficient to maintain the joint for many years; however, the degradative process will eventually exceed the anabolic, resulting with cartilage loss. The scales are tipped after disturbances are shifted in the extra-cellular matrix (ECM). The synovial membrane becomes inflamed, favoring synthesis of proinflammatory cytokines, which also have an impact on cartilage homeostasis by favoring chondrocyte catabolism while simultaneously decreasing anabolism by the same cells. The fact that chondrocytes have been shown to exert

increased expression of inflammatory mediators and stress response factors, and that the number of cartilage macromolecules in synovial fluid released after cartilage destruction have significant inflammatory properties, combine to stress the need to interfere with inflammatory mediators and pathways as an approach to designing strategies for the treatment of OA.

Joint Flexibility (Range of Motion) (ROM)

Knee OA is associated with a characteristic pattern of discrete reductions in function involving transfer from seated or prone position to a standing position, these functions are crucial in activities of daily living and are dependent on flexibility of joints (Milesky et al., 2006). Joint flexibility, measured based on range of motion parameters such as flexion and extension of the knee joint affect gait and walking patterns, and potentially have effects on the individual's ability to perform physical activity. According to the CDC, range of motion measurements for the knee for the age range of 45-69-year old's are as follows: flexion ranging from 132-137 degrees and extension range of 0.5-1.2 degrees, respective of age (CDC, 2018).

Limited range of motion has been documented in individuals with knee OA, but these studies failed to control for variables such as age and gender (Brinkman & Perry, 1985; Stauffer, Chao, & Gyory, 1977). ROM measurements indicated that the affected knee OA group exhibited significantly less knee extension and knee flexion as compared to subjects without degenerative joint disease as demonstrated by Messier and associates. The study went further to provide evidence that older adults with OA of the knee had flexibility measures that differed significantly from age, mass, and gender-matched adults

with no evidence of lower extremity joint disease (Messier, Loeser, Hoover, Semble, & Wise, 1992).

As joint flexibility effects functional measures of daily activity such as walking, therapeutic interventions aimed to increase range of motion parameters could benefit those afflicted with knee OA. Evidence supporting interventions to increase joint flexibility are abundant such as with aquatic exercises and strength training through muscle rehabilitation, but limited evidence was found to support the use of dietary interventions as a means of improving range of motion in knee OA (Fisher, Pendergast, Gresham, & Calkins, 1991; Milesky et al., 2006; Wang et al., 2007).

Mobility and Physical Activity

The chronic degenerative nature of osteoarthritis acts as a primary cause of activity restriction and physical disability among older adults. Despite recommendations from the American Academy of Orthopaedic Surgeons (AAOS) and the American College of Rheumatology (ACR) to include self-management programs to include strengthening and low-impact aerobic exercises consistent with national guidelines as a top therapy for OA, most OA patients fail to obtain recommended amounts of daily physical activity (Fontaine, Heo, & Bathon, 2004; Wallis, Webster, Levinger, & Taylor, 2013). Evidence points to the fact that inactivity exacerbates pain symptoms and accelerates progression toward disability in older knee OA patients (Davis, Ettinger, & Neuhas, 1990; Leville, Fried, McMullen, & Guralnik, 2004). Further reductions in physical activity may be heightened if people with OA believe physical activity is not beneficial or even harmful for their joints (Holden, Nicholls, Young, Hay, & Foster,

2012). These unfounded beliefs can be dismissed with evidence from a comprehensive systematic review of 49 studies that found no evidence of serious adverse events, increasing pain, decreases in physical function, or progression of structural OA on imaging or increased TKR with long-term (3-30 months) therapeutic exercise in older adults with knee pain or OA (Quicke, Foster, Thomas, & Holden, 2015). In fact, one case control study concluded that increasing levels of regular physical activity was associated with lower risk of progression to TKR (Quicke et al., 2015).

Current physical activity guidelines for older adults recommend at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week, or a combination of equivalent amounts of moderate and vigorous activities (WHO, 2018). Aerobic activities should be in bouts of 10 minutes or more (WHO, 2018). The recommendations can be applied to older adults with disabilities however adjustments for each individual based on their exercise capacity and specific health risks or limitations may be needed.

Dependent on the stage of OA and amount of pain from OA, accomplishment of reaching weekly recommendations for physical activity may be unlikely, resulting in increase of OA progression. Adherence to physical activity interventions as medical management of knee OA has been proven to be of low likelihood (Sullivan, Allegrante, Peterson, Kovar, & MacKenzie, 1998; VanBaar et al., 2001). These finding suggest that many older knee OA patients quickly return to sedentary lifestyles following the end of structured exercise interventions and that the lack of adherence results in the reversal of

the benefits associated with implementing physical activity. Taking this into consideration, exercise as a first-line treatment for OA may not be feasible, bring about the need for additional strategies such as dietary interventions.

Biomarkers

Hyaluronic Acid

Hyaluronic acid (HA), also known as hyaluronan or hyaluronate, is a large linear glycosaminoglycan (GAG), and a major component of connective tissues including cartilage matrices as well as synovial fluid. Glycosaminoglycans, associated with articular cartilage, are chains of monosaccharides covalently bonded to protein monomer cores that remain separated by charge repulsion (Fox, Bedi, & Rodoe, 2009). HA is commonly associated with aggrecan, a proteoglycan, together representing a vital component of interfibrillar space of cartilage extracellular matrix (ECM); these large proteoglycan aggregates provide cartilage osmotic properties allowing the joint ability to resist compressive shock loads (Fox, Bedi, & Rodoe, 2009). HA is also responsible for the viscoelastic properties of synovial fluid, and the concentration of HA has previously been shown to be lower in OA joints than healthy ones (Moreland, 2002).

HA in an intraarticular injection in knee OA has been proven superior in improving knee pain and function as compared to a placebo (Huskisson & Donnelly, 1999; Petrella & Petrella, 2006). HA and nociception properties have been documented. Relief from pain may be due to the effects of HA on nerve impulses and nerve sensitivity (Moreland, 2002). The use of HA as a treatment lends to the notion that increased levels

of HA as a result of an alternative intervention would be beneficial in articular cartilage repair in OA.

IGF-1

Insulin-like growth factor-1 (IGF-1) belongs to a family of peptide hormones such as insulin, and is capable of stimulating cell proliferation, particularly protein and proteoglycans, but also able to prevent cell apoptosis. Proinflammatory cytokines activate the release of IGF-1 from chondrocytes; the peptide interacts with specific IGF membrane receptors (IGF-1R) to become activated. Chondrocytes can produce the peptide to act as both a paracrine and autocrine modulator to stimulate matrix synthesis and inhibit matrix degradation. Size and growth of osteophytes have been directly correlated to serum levels of IGF-1 in subjects with OA of the knee (Schouten, Van den Ouweland, Valkenburg, & Lamberts, 1993). Culture studies have found IGF-1 stimulates proteoglycan production in a dose-dependent manner, (Jenniskens et al., 2006; van Susante, Buma, van Beuningen, van den Berg, & Veth, 2000). Although IGF-1 expression and synthesis are increased in OA cartilage, chondrocytes are hyporesponsive to IGF-1 stimulation, which may be partially due to the presence of increased levels of a binding protein with a high affinity for the hormone (Martel-Pelletier et al., 1998). The same proinflammatory cytokines influencing the release of IGF-1 from chondrocytes also triggering the release of the binding protein. This phenomenon may account for the decrease in cartilage repair capacity found in advanced stages of the disease.

IGFBP-3

Insulin-like growth factor binding protein-3 (IGFBP-3) is the major contributor of protein to the IGFBP family as the major carrier of IGF and is secreted by various types of cells in the body. IGFBP-3 when bound to IGF-1 is responsible for transporting the growth factor from the blood stream to the target tissue (Hooshamand, Juma, Khalil, Shamloufard, & Arjmandi, 2015). IGFBP-3 has a higher binding affinity for IGF-1 than IGF-1R, meaning it can competitively conjugate free IGF-1 with IGF-1R and block IGF-1's cell proliferation effect (Martel-Pelletier, Di Battista, Lajeunesse, & Pelletier, 1998). Previous research has shown, despite elevated IGF-1R levels in OA joint cartilage as compared to normal cartilage, chondrocytes from the OA joints did not respond to the stimulus IGF-1 correlatively. The same researchers also found elevated expression of IGFBPs in OA cartilage. Female patients with OA also have significantly higher concentrations of IGFBP-3 in synovial fluid (Hooshamand et al., 2015). The failure to respond to the anabolic effects of IGF-1 may be explained by the increased presence of the binding protein thus leading to cartilage degeneration.

YKL-40

In the early 1990s, a newly discovered glycoprotein, YKL-40 was making headlines as a product of human osteosarcoma cell lines and was cloned and characterized as a major secretory product of articular chondrocytes and synovial fibroblasts from patients with rheumatoid arthritis (Johansen et al., 1996; Vaananen et al., 2014). Its name was based on its molecular weight (40 kDA) and its three N-terminal amino acids; tyrosine, lysine, and leucine; however, another common known name is

human cartilage glycoprotein-39 (Johansen et al., 1996). The amino acid sequence is similar to the chitinase protein family, thought to be related in response to allergies, but the glycoprotein has no chitinase activity, and the exact biological activities are still being identified (Johansen et al., 1996).

Despite continued research and development into the primary function of YKL-40 in the human body, some evidence has shown its production by articular cartilage, synovial membrane fibroblast and macrophages, synovial fluid neutrophils, and osteoblast, osteocytes in osteophytes (Volck et al., 2001; Kawasaki, Hasegawa, Kondo, & Iwata, 2001; Connor et al., 2000; Huang & Wu, 2009). Patients with late-stage osteoarthritis of the knee joint had significantly higher serum YKL-40 as compared levels in normal healthy adult subjects in both age ranges of 60-69 and 70-79 years (Johansen et al., 1996). Work done by Johansen et al. (1996) found a significant correlation between concentrations in synovial fluid and serum in patients with OA (early and late stages) whereas no relationship was seen in patients with injury of the knee. This indicates that serum YKL-40 in these patients reflects local synthesis of YKL-40 in the knee joint. The increase serum quantities of YKL-40 in patients with OA may be due to the heavy burden of biomechanical loads as seen in one study evaluating YKL-40 positive chondrocytes; these were seen in particular in superficial and middle layers of the cartilage, whereas chondrocytes from normal cartilage were mainly YKL-40 negative (Volck et al., 2001). The results of several studies propose YKL-40 as a factor associated with inflammation and catabolic process in OA joints as YKL-40 levels and proinflammatory cytokine levels were confirmed, as well as having an association with cartilage destruction

mediators such as extracellular matrix degrading MMP enzymes (Johansen et al., 1996; Connor et al., 2000; Vaananen et al., 2014).

Diet and Inflammation and Cartilage Metabolism

Chronic inflammation is thought to play a role in the development and progression of many disease states. The emergence of evidence to support the role of low-grade chronic inflammation in aging and age-related conditions suggest that inflammation could be an important link between aging and OA. The term "inflamm-aging" was coined by Franceshi et al. to describe the pro-inflammatory state that occurs with increasing age (Franceschi, et al., 2000). Inflamm-aging was originally proposed to be the result of an accumulation over time of increased various stressors on the immune system resulting in immunosenescence, but age-related endocrine, metabolic, and nutritional changes are now thought to promote a pro-inflammatory state. These include an increase in fat mass that can be a source of pro-inflammatory mediators and increasing levels of reactive oxygen species that promote metabolic changes that can have negative impact on cartilage tissue (Greene & Loeser, 2015). Long, Blake, Song, Lark, and Loeser (2008) found that articular chondrocytes in monolayer culture from older donors released higher levels of the cytokine, interleukin 7 (IL-7) as compared to younger donors, and that OA chondrocytes release higher levels of IL-7 than age-matched normal donors (Long, Blake, Song, Lark, & Loeser, 2008).

In order to help combat the inflammatory process and the potential damaging effect on tissue and organs, clinicians have recommended a diet rich in fruits and vegetables. In particular, the sources of these fruits and vegetables should include

different variety with the emphasis on pigment and color. The variety and various pigments will help supply different antioxidants that act in two ways: first, preventing the generation of excess free radicals, and thus avoiding oxidative damage to the cell; and second, after damage has occurred, preventing further cell degeneration, thus alleviating the progression of conditions caused by oxidative stress (Mancini et al., 2017). Studies have shown an inverse relationship between total antioxidant capacity of the diet and various disease conditions such as metabolic syndrome, hypertension, and type 2 diabetes (Bahadoran, Golzarand, Mirmiran, Shiva, & Azizi, 2012; Mancini et al., 2017; Montonen, Knekt, Jarvinen, & Reunanen, 2004). In recent years, the role of dietary antioxidants in arthritis management is increasingly being addressed by researchers in a number of reported studies.

Vitamin C is a well-documented dietary antioxidant found in higher quantities in plant-based foods, particularly fruits. In the longitudinal Framingham Knee OA Cohort Study, a threefold reduction in risk for progressive OA was observed in persons in the middle to highest tertile of vitamin C intake; the same individuals had reduced risk for knee pain during the study (Felson et. al., 2000). A more recent study examined the efficacy of vitamin C to prevent OA in vitro and in vivo with monosodium iodoacetate (MIA)-induced OA rats. The vitamin C role in prevention of OA progress included decreases in apoptosis and in the expression of proinflammatory cytokine (Chiu et al., 2017).

Vitamin E (VE), a fat-soluble vitamin, has a principle function as an antioxidant, where it is able to maintain membrane integrity of cells throughout the body (Gropper &

Smith, 2013). The protective mechanism by which VE prevents destruction of cell membranes is through its ability to prevent oxidation of fatty acids. The phenolic hydroxyl group structure of vitamin E provides hydrogen ions to free radicals before they can destroy cell membranes and other cell components (Gropper & Smith, 2013). A study in dogs with OA showed a reduction in inflammation joint markers and histological expression, as well as a trend in improving signs of pain in the treatment group using a high dose of vitamin E (Rhouma et al., 2013).

Selenium, a trace mineral, is an integral part of the enzyme glutathione peroxidase (GPX) which catalyzes the removal of hydrogen peroxides, which damage cellular membranes and other cell components including proteins and DNA (Gropper & Smith, 2013). An animal study investigating the protective effects of selenium and vitamin E on bisphenol A (BPA)-induced damage on the liver found administration of selenium and vitamin E through the diet in BPA treated rats ameliorated the negative biochemical parameters (Amraoui, Adjabi, Bouauza, et al., 2018). Another animal study found rats exposed to toxins enducing chondronecrosis of knee joint cartilage in a selenium deficient environment experienced more severe damage in a dose dependent manner (Guan et al., 2013).

In addition to clinical studies showing some protective effects of specific vitamins, and minerals, epidemiological data further substantiates the protective associations of a dietary pattern, such as the Mediterranean diet rich in a combination of dietary polyphenols derived from fruits, vegetables, olives and red wine in OA (Veronese et al., 2016). Studies are limited to European samples when considering the relation

between MeDiet and quality of life. The authors of this study investigated whether the MeDiet was associated with better quality of life in a large cohort of North Americans. They found a higher adherence to this dietary pattern was associated with lower frequency of pain, stiffness, disability, and depression as each of these outcomes is closely related to quality of life. Some research has been done probing the beneficial properties of polyphenol rich foods on inflammatory disease states such as cardiovascular, metabolic syndrome, diabetes, obesity, and certain cancers (Basu et al., 2010; Shukitt-Hale, 2012; Stull et al., 2012). A recently reported cross-sectional study from Korean National Health and Nutrition Examination Survey revealed higher intake of fruits and vegetables to be associated with lower prevalence of knee pain in older adults with knee OA (Han, Chang, Lee, & Lee, 2017).

Polyphenols

Many different types of antioxidants are present in food sources. Plant based foods, such as fruits, vegetables, teas, and herbs or spices have naturally occurring compounds, phytonutrients or phytochemicals, they have potential health benefits and are biologically active in the body. These chemicals are thought to promote health by eliciting an antagonistic response to oxidants that may reduce the risk for developing most or progression of various diseases. The largest groups of phytochemicals are polyphenols with more than 8,000 phenolic compounds identified in various forms (Erdman et al., 2007). Polyphenols are classified by chemical structure, denoted by the number of phenolic rings and the structural elements bound to the rings. Polyphenols arise from a common intermediate, phenylalanine, or a close precursor, primarily in

conjugated forms with one or more sugar residues linked to hydroxyl groups, or direct sugar linkages (Pandey & Rizvi, 2009). There are more than a dozen sub-classes; the most common are phenolic acids, flavonoids, stilbenes, and lignans. The largest sub-class, the flavonoids, is responsible for the attractive colors and pigments in fruits and plant leaves or flowers. Flavonoids share a common basic structure consisting of two aromatic rings bound together by three carbon atoms forming an oxygenated heterocycle (Pandey & Rizvi, 2009). Flavonoids are then further sub-classed, and include the isoflavones (genistein, daidzein, and equal) found in soybeans, legumes, nuts and milk (Gropper & Smith, 2013). Fruits, particularly berries are rich in flavonoid pigments such as anthocyanins and proanthocyanidins. Individual differences within each group arise from variations in the number and arrangement of the hydroxyl groups and the extent of alkylation and/or glycosylation (Pandey & Rizvi, 2009). Flavonoids can scavenge free radicals by donating a hydrogen atom from its phenolic hydroxyl group but also must be able to remain stable with its own unpaired electron (Gropper & Smith, 2013). A diet rich in servings of fruits and vegetables (10 servings for 15 days) has been shown to significantly correlate with fasting plasma antioxidant capacity measured as oxygen radical absorbance capacity (ORAC), meaning those with higher intake of plant-based foods, have higher circulating antioxidant capacity up to 3.0 mmol after which a plateau is reached (Cao, Booth, Sadowski, & Prior, 1998).

Polyphenols and Cartilage Health-Cell Studies

In-vitro studies are a common practice to test hypothesis in an exploratory manner with lower costs and risks than in vivo studies. Many in-vitro studies on antioxidants

have been explored the potential benefits of polyphenols (Mevel et al, 2016; Henrotin et al., 1998; Mauviel et al., 1989; Mauviel , Loyau , & Pujol, 1991). Olive and grape seed extracts prevented post-traumatic osteoarthritis damages and exhibited anti-inflammatory and chondroprotective actions in vitro (Mevel et al., 2016). Soybeans are also a rich source of polyphenols including proanthocyanidins and flavonoids (Malencic, Maksimovic, Popovic, 2008). Avocado soybean unsaponifiables (ASU) displayed anabolic, anticatabolic, and anti-inflammatory effects on chondrocytes in several in-vitro studies (Henrotin et al., 1998; Mauviel et al., 1989; Mauviel et al., 1991).

Polyphenols and Cartilage Health-Animal Studies

Polyphenols associated with fruits have been studied in various animal models including mice, rats, and rabbits. Collagen induced arthritis and mono-iodacetate (MIA) induced OA are most commonly used in animal models. MIA is a glycolytic inhibitor that causes behavioral, histological and biochemical changes that resemble human OA and its associated joint pain when injected (Basu et al., 2018). In a rat model of collagen-induced arthritis, treatment of animals with raspberry extract was demonstrated to improve the symptoms of arthritis including tissue swelling, osteophyte formation and decreased articular destruction when compared to untreated animals (Figueira et al., 2014). Similarly, an experimental model of OA using rabbits investigated extracts from pomegranates. Findings from this animal study demonstrated several chondroprotective effects of pomegranate extract such as decreasing expression of cartilage degradation enzymes, and decreasing synovial fluid and plasma levels of inflammatory interleukins and prostaglandins (Akhtar, Khan, Ashruf, & Haggi, 2017). From a study conducted in

Santa Catarina, Brazil gojiberries, blueberries, and cranberries given twice daily for 10 days have also been proven to reduce paw edema in a rat model (Nardi et al., 2016). Researchers suggested the results indicated the berries exhibited acute anti-inflammatory activity by reducing damage mediated by ROS and had compounds with activity more similar to non-steroidal anti-inflammatory drugs (Nardi et al., 2016). Procyanidin, another common polyphenol present in fruits and vegetables, showed promising effects by mitigating OA pathogenesis by suppressing signaling necessary for the production of proteolytic enzymes and preventing increased bone resorption and abnormal bone formation (Wang et al., 2016).

Polyphenols and Joint Health-Clinical Trials

Pomegranate juice (PJ) has grown in popularity and is known as a "superfood" for its high polyphenol content. A clinical trial with pomegranate extract supplementation for eight weeks was shown to significantly improve RA disease activity scores leading to less pain and joint swelling, as well as improved quality of life scores in adults (Ghavipour et al., 2017). Participants with knee OA in a 6-week treatment consuming 200 ml of PJ daily were found to have decreases in WOMAC-score and improvement in physical function and stiffness in comparison to a placebo group. Decreased enzymes responsible for cartilage breakdown and increases in antioxidant activity in the PJ treatment group were also observed (Ghoochani et al., 2016).

In a cross-over design study, adults with radiographic evidence of knee OA individuals were randomized to a reconstituted freeze-dried strawberry beverage or control beverage group, where participants consumed their respective beverage for 12

weeks, separated by a 2-week washout phase and crossed over to the other treatment. The primary outcomes were as follows: serum biomarkers of inflammation and cartilage degradation decreased after strawberry vs. control treatment, significantly reducing constant, intermittent, and total pain, as well as improvements in disability indices were observed with the strawberry treatment (Schell et al., 2017).

Blueberry

Blueberry plant is one of the few fruit bearing plants native to North America, with highbush, lowbush, and rabbiteye varieties constituting the three major species in the U.S. market (Su et al., 2017). Blueberries are a rich source of several polyphenols including anthocyanins, procyanins, flavonols, and phenolic acids (Basu et al., 2018; Su et al., 2017). A Chinese investigation to quantify blueberry polyphenol fractions found total content of phenolic acids from sixteen fresh BB samples belonging to highbush, and half-highbush varieties was mostly composed of chlorogenic acid (CGA) (Su et al., 2017). Major anthocyanins found in wild and cultivated blueberries are cyanidin, delphinidin, petunidin, paeonidin, pelargonidin, and malvidin, which are responsible for the distinctive blue pigment of the fruit (Diaconeasa, Leopold, Rugina, Ayyaz, & Socaciu, 2015). A potential mechanism contributing to declining functional mobility may be a reduced ability to mount an adequate antioxidant response to protect cells against the damage caused by excessive and unopposed production of reactive oxygen species. Figueira and colleagues concluded blueberries have a significantly high antioxidant capacity when compared to other fruits such as raspberry, blackberry, and gooseberry, as determined by the high scavenger capacity for reactive oxygen species (Figueria et al,

2016). One study showed that a diet rich in blueberries helped to support arterial structure by helping maintain healthy blood flow via reduced LDL oxidation, normal platelet aggregation, and endothelial function improvement (de Pascual-Teresa, Moreno, & Garcia-Viguera, 2010). Studies by Diaconeasa and associates indicated that berry juices exhibited high antiproliferative activity against tumor cells. A 24-hour treatment with blueberry purified anthocyanin-rich fractions (ARFs) revealed that cervical cells were very sensitive with exposure reducing cell viability from 100% to 45% (Diaconeasa et al., 2015).

Blueberry and Joint Health

In recent years, the role of dietary antioxidants in arthritis management has been of increasing interest by researchers. In a cross-sectional report among patients with rheumatoid arthritis (RA), commonly consumed foods were associated with varying levels of pain and RA symptoms; blueberries and strawberries were identified by the participants to be associated with improving RA symptoms and were ranked high on the list of 'anti-inflammatory foods' (Tedeschi et al., 2017). The emerging observational data support the role of blueberries in alleviating symptoms and the need for further clinical investigation. A CIA animal model demonstrated some promising effects of blueberry extract in knee joint and hind paws of rats (Figueria et al., 2016). They concluded that blueberries used in the study had a wide range of phenolic compounds, with quercetin as the most common flavonol identified and a significant portion of the extract consisted of anthocyanins (Figuera et al., 2016). Blueberry extract administered orally for 13 days was able to reduce edema formation induced by carrageenan but also delayed the

development of clinical signs on days 24-35 and improved the histologic and radiographic scores of the knee joint and hind paw (Figuera et al., 2016).

Vitamins and minerals have been studied for the benefits they play in inflammation mitigation and prevention of disease progression. Polyphenols have been widely studied for their anti-inflammatory properties in cell research, animal and human studies. The phenolic content of a variety of fruits has also been researched. However, no clinical human trials have been conducted to assess phenolic rich blueberry effects on range of motion, physical activity, and cartilage biomarkers in symptomatic knee osteoarthritis.

CHAPTER III
METHODOLOGY

Study Design

A randomized placebo-controlled pre-test post-test design study was designed with the intent to recruit a total of 63 volunteers, consisting of both men and women, ages 45-79 years with self-reported mild to moderate degree of pain associated with symptomatic knee osteoarthritis. Those who met the inclusion criteria and provided consent to participate in the study were included. Subjects were randomly placed into one of two treatment groups; the freeze-dried blueberry powder (FDBP) (n = 33) group consumed 40 grams of freeze-dried whole blueberry powder (packaged in 20-gram pouches to be consumed twice a day, equivalent to two servings of fresh blueberries) while the placebo (n = 30) group consumed 40 grams of a placebo powder, serving as a control. The placebo powder was equivalent to the FDBP in appearance and energy content, with equal parts fructose and dextrose. The participants were instructed to reconstitute each 20-gram pouch of either the placebo powder or FDBP in 10-12 ounces of water immediately before consumption.

Screening to Evaluate Inclusion/Exclusion Criteria

Subjects were recruited through Texas Woman's University, orthopedic clinics, private practices, and the local Denton and Dallas-Fort Worth Metroplex. A recruitment flyer and email announcement instructed interested parties to email or call the principal investigator for a brief screening questionnaire to assess qualification status. A second phone interview verified if the participant was within the age requirements of 45-79 years

of age with mild to moderate knee OA. Other questions inquired about smoking habits, medications, dietary supplements, diet restrictions, and food allergies. Race and ethnicity were not exclusion factors. Exclusion criteria included smoking more than 20 cigarettes daily, past medical history of severe liver, kidney, or other chronic or acute disease that affected their condition and ability to participate in the study. Subjects were excluded if they were currently on prescribed medications classified as COX-2 inhibitors. Additionally, participants were asked to refrain from taking supplemental sources of chondroitin sulfate, glucosamine sulfate, glucosamine hydrochloride powder, and blueberries or blueberry products.

Subjects reported to the Inst. For Women's Health on three occasions for data collection: baseline, Week 8 and Week 16. Once qualification was established, participants were emailed a copy of a Consent to Participate in Research Study form to review before their baseline appointment held at the Institute for Women's Health at Texas Woman's University in Denton, TX. The consent packet include: explanation and purpose of research, procedures, time commitment, potential risks, participation benefits, and contact information for potential questions. Baseline visits were scheduled, and participants were informed to arrive fasted (no food or drink for at least 8 hours) to provide an accurate blood and urine specimen.

Treatment Compliance

Calendars were supplied to each participant to aid in treatment compliance, providing a tracking system to help ensure daily consumption of blueberry or placebo powders. The calendars also provided a place to record over the counter pain medication

use. The calendars were provided at baseline with instructions to return at their midpoint appointment when a second calendar would be provided for the remainder of the study. Compliance was also monitored by requesting participants to return any unconsumed blueberry or control powders at study visits. Follow-up emails and phone calls were made to ensure compliance and address any concerns or issues related to the treatment and study.

Joint Flexibility (Range of Motion)

Flexibility of the afflicted joints were assessed using a 360-degree goniometer at baseline, midpoint, and at the end of the study using the net zero method. Active extension, degree of hyperextension, and flexion was measured from the supine position. The range of motion parameters were measured in triplicate by the same trained individual to address precision and validity and reduce variability.

Mobility Assessment

Mobility was assessed utilizing the International Physical Activity Questionnaire Long Form (IPAQ). Participants' responses were compared from baseline, midpoint, and final visits. The tool has been validated and utilized for assessment of physical activity in numerous studies associated with symptomatic knee osteoarthritis (Rosemann, Kuehlein, Laux, & Szecsenyi 2007; Van Poppel, Chinapaw, Mokkink, van Mechelen, & Terwee, 2010). The questionnaire requires the participant to quantify vigorous and moderate activities from the previous 7 days (IPAQ, 2002). IPAQ assesses physical activity undertaken across a comprehensive set of domains including: leisure time physical activity, domestic and gardening activities, work-related physical activity, and transport-

related physical activity. The IPAQ long form asks details about the specific activities undertaken within each of the four domains. Examples include walking for transportation and moderate-intensity leisure-time activity. The items in the long form were structured to provide separate domain specific scores of walking, moderate-intensity, and vigorous-intensity activity within each of the work, transportation, domestic chores, and gardening and leisure-time domains. Computation of the total scores for the long form requires summation of the duration (in minutes) and frequency (days) for all the types of activities in all domains. A measure of the volume of activity was computed by weighting each type of activity by its energy requirements defined in metabolic equivalents of task (METs) to yield a score in MET-minutes/week. The IPAQ was used to evaluate whether the level of physical activity was positively impacted by the FDBP in comparison to the placebo.

Blood and Urine Collection and Storage

Fasting venous blood samples were collected at baseline, midpoint, and final visits by a certified phlebotomist. Blood specimens were centrifuged at 1500x g for 15 minutes within 2 hours of collection. Plasma was separated and aliquoted in microcentrifuge tubes and stored at -70 degrees Celsius for future analysis.

Biochemical Analysis of Cartilage Markers

YKL-40 (glycoprotein-39) was assessed as a marker of cartilage degradation at baseline, midpoint, and end of the study. Enzyme-linked immunoabsorbent assays (ELISA) were the primary type of kits used. MicroVue Bone kits were acquired from Quidel and used to assess sample levels of YKL-40 at baseline, midpoint and at 16 weeks

(Quidel, San Diego, CA). The MicroVue YKL-40 assay was performed in three steps. First, samples were mixed in YKL-40 antibody coated plates. Secondly, the samples were subjected to alkaline phosphate conjugated rabbit anti-YKL-40, which binds to the immobilized biomarker from the first step. A chromogenic substrate solution then reacts with the bound enzyme causing a color change. The color intensity was then measured at an optical density of 405 nm, which is proportional to the concentration represented in the samples using a standard curve generated using linear regression analysis.

Elevated levels of IGFBP-3 would assess the inability to maintain joint homeostasis in OA patients. IGFBP-3 was measured with quantitative sandwich enzyme immunoassay technique from R&D Systems in Minneapolis, MN. Samples were diluted in IGFBP-3 assay buffer as concentrations were determined to be higher than assay standards. Samples, standards, and controls were added to microplates pre-coated with antibodies specific to the corresponding biomarkers. Any biomarker present would then adhere to the plate. A polyclonal enzyme-linked antibody added would then attach to the anchored biomarker. Substrate added facilitates color change to be read by spectrophotometer at 450 nm. Concentrations for IGFBP-3 were calculated by creating a four-parameter logistic curve-fit. Series of washes were added between steps to remove any unbound products.

IGF-1, a marker of anabolic processes associated with cartilage matrices, was measured using the quantitative sandwich enzyme immunoassay technique from R&D Systems in Minneapolis, MN. IGF-1 samples were pretreated to release attached binding

proteins immediately prior to following the instructed kit procedure. IGF-1 measures were determined by using software capable of generating log/log curve fit graphs.

Hyaluronic acid was used to assess anabolism associated with cartilage matrices. Hyaluronic acid levels in participant plasma was analyzed with sandwich ELISAs from TECOmedical Group using a microtiter plate coated with hyaluronic acid binding protein (HABP) and hyaluronan receptor protein (HRP) conjugated HABP for detection (TECOmedical Group, Switzerland). Levels of HA are detected once a substrate reaction occurred with the HRP conjugated HABP binds with HA in the sample. A color reagent reacted quantitatively with HA levels in samples and read at an optical density of 450 nm using quadratic calibration curve fitting software. All samples were prepared according to each ELISA kit instructions.

Statistical Analysis

A power analysis determined a minimum sample size of 50 participants was needed to conduct analysis with the alpha = 0.5, power = 0.87, and a moderate effect size. Descriptive statistics were calculated for all variables, consisting of means, standard error of means, minima, and maxima for all continuous variables, while frequencies and percentages were calculated for all categorical demographic variables. Distributions of the response variables were examined to determine if statistical tests of hypotheses based on the assumption of normality are met and parametric testing is appropriate. Extreme outliers were investigated for technical or clerical error. Baseline differences of dependent variables were tested using independent sample t-tests. Although dependent variables in each area are related to each other, the small sample size dictated the use of

repeated measures of ANOVA to be conducted to evaluate outcome differences of flexibility, physical activity, and cartilage biomarkers between groups over time at baseline, mid-point, and final. Most variables were not normally distributed, and some variables contained outliers, requiring analysis to be conducted with removal of these data points. Covariate analysis, including ANOVA and regression, were conducted to control for baseline differences. The data was analyzed using SPSS V 25.0.0.

Chapter IV

RESULTS

Demographics

A total of 116 individuals responded to the recruitment flyers and emails for this study. Of these, 103 people were initially screened to participate in the study. A total of 63 participants met the criteria and were scheduled for the baseline visit. After consenting to participate, individuals were randomized into either the blueberry or the placebo intervention groups for a period of 4 months. Over the course of the study, 14 individuals withdrew from the study due to various reasons such as taste or palatability of intervention, mild to moderate GI discomfort, or conflicts in study scheduling. A total of 49 participants completed the study. Demographic data associated with study participants is provided in Table 1 and Table 2.

There were no significant differences between treatment groups at baseline for body mass index (BMI). The BMI increased significantly at final over baseline and midpoint in the placebo group, whereas the blueberry group maintained their body weight throughout the study (see Figure 1).

In the blueberry group, systolic blood pressure differed significantly at mid-point and final visits as compared to baseline, demonstrating a significant reduction in systolic blood pressure over the course of the study (see Figure 2). There were no changes in the systolic blood pressure in the placebo group. Diastolic blood pressure was similarly affected throughout the course of the study. Within the blueberry treatment group, the diastolic blood pressure reduced significantly from baseline to mid-point and again to final (see Figure 3). No significant differences were noted in the placebo group or between the groups. The only significant differences between the blueberry and the placebo groups in regards to BMI, systolic and diastolic blood pressure at any time point was the significant difference between baseline measurements of systolic blood pressure readings, where the placebo group had a lower initial level.

Flexibility (Range of Motion)

Range of motion was measured using a goniometer using the net zero method. Flexion was measured from the supine position with a triplicate average used to assess flexibility of the knee. After analysis, no significant differences were noted between the blueberry group and placebo in regards to joint flexibility of either knee. However, a slight continual increase in range of motion was observed in both the right and left knees of the blueberry group, whereas a gradual decrease in flexibility was observed in both knees in the placebo group (see Figures 4 and 5).

Physical Activity

Mobility was assessed utilizing the International Physical Activity Questionnaire Long Form (IPAQ). MET minutes/week was calculated for each domain and then a

summation of all domains to arrive at total MET-minutes/week to be used in analysis. No significant differences were observed (see Figure 6). Despite no significant findings, the blueberry group overall had higher MET-minutes/week than the placebo group.

Cartilage Biomarkers

Plasma biomarkers that were evaluated included hyaluronic acid (HA), insulin-like growth factor (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and YKL-40. HA is associated with cartilage repair and growth. A non-significant increase in HA was observed from baseline to midpoint in the blueberry group. However, the levels of HA declined during the final time point. No changes in HA occurred in the placebo group (see Figure 7). IGF-1 a surrogate biomarker of cartilage favors formation. Despite the significantly higher concentrations of IGF-1 in the placebo group at baseline and mid-point as compared to the blueberry group, a steady decrease was observed in the placebo group over the duration of the study. Interestingly, an overall non-significant increase in the blueberry group was observed (see Figure 8). IGFBP-3 is known to compete for IGF-1 receptor. Preventing IGF-1 from activating the anabolic processes associated with cartilage formation. IGFBP-3 was significantly higher from baseline to final in the placebo group (see Figure 9). No significant differences in mean concentrations of IGFBP-3 were observed in the blueberry treatment group. YKL-40, a biomarker linked with inflammation and cartilage degradation, had no significant differences in either of the treatment groups (see Figure 10). A noteworthy observation is the non-significant decrease in concentration of YKL-40 in the blueberry group and an increase in the placebo group over the course of the study.

Chapter V

DISCUSSION

The findings of our research study demonstrated that freeze-dried blueberry powder consumption for a period of 16 weeks resulted in slight improvements in joint flexibility and had positive impact on selective biomarkers of cartilage metabolism in individuals with symptomatic knee OA. Plant based foods containing phytonutrients, particularly polyphenols, have recently been touted for their potential health benefits. Recent research has focused on examining their protective effects against inflammation and oxidative stress as it relates to chronic disease conditions. Studies have shown an inverse relationship between total antioxidant capacity of the diet and various disease states including metabolic syndrome, hypertension, and type 2 diabetes (Bahadoran et al., 2012; Mancini et al., 2017; Montonen et al., 2004). Studies using animal models have evaluated the effects of polyphenolic content of foods in relation to joint health. Components of the Mediterranean diet have been extensively studied, with favorable effects regarding joint oxidative stress and joint health (Manna et al., 1999; Turner et al., 2005; Cumaoglu et al., 2011; Veronese et al., 2016). A rise in berry popularity, particularly blueberries due to their phytonutrient content as one of the richest sources of antioxidants may show to have similar effects.

In the blueberry treatment group, range of motion increased slightly for both knees, while the placebo group had steady declines from baseline to final measurements. The ROM results are promising as joint flexibility, mainly the function involving transfer

from seated or prone position to standing are crucial in activities of daily living. Decrease in the functionality of the joints, particularly the knee, limits these activities resulting in decreased quality of life. Also, decreased flexibility also limits functionality and ability to perform other physical activities such as walking, strength training, and aerobic exercise, which are typically recommended in the management of OA symptoms. In the FDBP treatment group a trend of increased ROM was observed as compared to declining trends in ROM within the placebo group. These findings are similar to those of a study with pomegranate juice as the treatment group that had improvements in stiffness in patients with knee OA (Ghoochani et al., 2016). Similarly, a study with RA patients and pomegranate juice found significant improvement in joint swelling with the treatment group (Ghavipour et al., 2017). Improvement in disability indices were also seen in a study where participants consumed reconstituted freeze-dried strawberry beverage (Schell et al., 2017). These studies indicated that the effects may be attributed to the polyphenols found in these fruits and that the phenolic compounds present in blueberries may have a protective effect on joint health by inhibiting inflammation or stiffness of the area allowing for continued flexibility of the knee.

Physical activity measured in METs decreased for both groups; however, activity levels decreased more in the placebo than the blueberry treatment group. Ghoochani et al. found improvements in physical function in patients with knee OA who consumed pomegranate juice for 6 weeks (Ghoochani et al., 2016). Incorporating regular physical activity/exercise has been proven to promote improvements on functional capacity in individuals with knee OA (Rodrigues da Silvaet al., 2017). Furthermore, reduced PA is

frequently associated with gain in body weight, which aggravates the impact of OA especially on the weight-bearing joints such as the knee; decreased PA also leads to decreased muscle strength and stability of joints, which has been shown to be important for the onset and also the course of OA, especially at the knee joint (Rosemann, Kuehlein, Laux, & Szecsenyi 2007). This study's findings suggest blueberry consumption may help individuals with knee OA maintain their physical activity levels, which can improve their functional capacity.

In this study, the blueberry treatment group had slight improvements in serum hyaluronic acid levels from baseline to midpoint but this reversed back at the end of the study. The results suggest the treatment may only have a temporary effect on increasing HA to help repair degradation of cartilage and synovial fluid, characteristics of OA progression. HA production has previously demonstrated an ability to modulate inflammatory markers such as interlukin-1B (Olivotto et al., 2018). The small increase in serum HA in study participants from initiation of blueberry treatment consumption to midpoint, may have lasting effects on symptoms, despite the slight decline in concentration from midpoint to final.

IGF-1 appears to be one of the most important growth factors affecting the anabolism of the principle molecules found in cartilage, such as collagen and proteoglycans, but also strongly stimulates production of chondrocyte extracellular matrix components. Circulating IGF-1 is normally synthesized and secreted by the liver in response to growth hormone stimulation, however, with the presence of OA IGF-1 is also produced by chondrocytes (Martel-Pelletier et al., 1998). Despite the increased

localized expression and production of IGF-1, the diseased cells of OA become hypo-responsive to its paracrine and autocrine modulating capacity to stimulate matrix synthesis and inhibiting matrix degradation, partially due to the increased presence of insulin like growth factor binding protein (IGFBP) (Hooshmand et al., 2015). IGFBP has a strong affinity for IGF-1, as the main function of the binding protein is to transport the growth factor throughout the body. IGF-1 attached to the binding protein limits the bioavailability of the growth hormone. Both factors are stimulated by cytokines such as interleukin-1 and tumor necrosis factor (TNF-α), which are elevated in response to injury or disease (Hooshman et al., 2015). The cytokines may further contribute to disease progression in that they also stimulate activation of proteases that cleave bound IGF-1 from cell receptors, preventing repair (Martel-Pelletier et al., 1998).

Blueberries and other anthocyanin containing foods may help prevent, or at least slow the progression of inflammatory diseases such as OA by impacting growth factors that have a positive impact on tissue, organs, and systems (Joseph, Edirisinghe, & Burton-Freeman, 2014). In our study, the blueberry treatment group had an increasing trend in concentrations of IGF-1 with consistently stable concentrations of IGFBP-3, while the placebo group had declining concentrations of IGF-1 and increases in IGFBP-3 over the course of the study. These trends suggest that the bioactive constituents in blueberries positively impact the anabolic growth factors simultaneously inhibiting binding protein that may block the desired cartilage forming action, while in the placebo group, both the markers (IGF-1 and IGFBP-3) demonstrated classic tendencies of OA progression.

The blueberry treatment group had a non-significant decrease overall in concentrations of YKL-40 from baseline to the final evaluation, and the placebo group had a steady increase in concentration throughout the study period. As YKL-40 concentrations have previously been found in higher concentrations in the presence of proinflammatory factors, the declining trend of concentrations in the FDBP group and increasing concentration of the placebo group together suggest the known anti-inflammatory properties of blueberries as the influential mechanism. Treatments used in the management of OA that result in elevated levels of HA and IGF-1 are desirable as both biomarkers are associated with growth and repair. IGFBP-3 and YKL-40 have both been associated in higher concentrations in individuals with OA and are related to catabolic processes or prevention of anabolic repair mechanisms. Human cartilage glycoprotein- 39, or YKL-40, despite continued research and development into the primary function, has been documented to be produced by articular cartilage and surrounding joint tissues (Johansen et al., 1996; Volck et al., 2001). YKL-40 is also seen in significantly higher concentrations in serum of patients with late-stage OA as compared to normal healthy adults (Johansen et al., 1996; Volck et al., 2001). Furthermore, studies have suggested the association with concentrations of YKL-40 and proinflammatory cytokine levels as a potential factor influencing cartilage destruction (Johansen et al., 1996; Connor et al., 2000; Vaananen et al., 2014). Similar trends were seen in the present results, despite the lack of statistical significance between treatments.

The outcomes of this study in relation to the effect of blueberry on joint health are promising. Additional biomarkers to consider would be transforming growth factor-ß

(TGF-ß) fibroblast growth factors (FGFs), and bone morphogenetic proteins (BMPs) (Umlauf et al., 2010). Osteoarthritis is characterized by an eventual imbalance of degradation over repair, leading to more advanced stages of cartilage destruction; however, the stages of OA were not determined in this study. Additionally, differences between genders such as age of onset and hormonal differences were not accounted for. More in-depth screening questions or diagnostic tools such as radiographic images, magnetic resonance images (MRI), or optical coherence tomography (OCT) could help to identify disease progression stages (Braun & Gold, 2012).

We acknowledge that out study did have several limitations that could have direct impact on the outcomes. Future studies would benefit from investigating physical activity utilizing a more reliable tool. The higher dropout rate made determining significant changes harder to detect. The study was based on participant compliance with routine daily consumption of blueberry or placebo powder that was reconstituted in water. Side effects including palatability and GI disturbances led to several study participants withdrawing from the study. The ability to incorporate the powders into other foods may need to be considered to improve compliance and retention. Furthermore, the use of self-reported surveys to measure physical activity are less reliable because of over-reporting and moderate reproducibility (Munugoda et al., 2018). Categorical and continuous indicators of physical activity are possible from IPAQ but given the non-normal distribution of energy expenditure in many populations, it is suggested that the continuous indicator be presented as median minutes/week or median MET-minutes/week rather than means. The IPAQ tool was developed and tested for use in

adults, age range of 15-69, with this study including older age groups, and the fact that the tool was not intended to be used as an outcome measure in small-scale intervention studies, the viability of the tool may not have been as accurate as intended. The metabolic rates used in calculating MET-minutes for each domain are equivalent to kilocalories for a 60-kilogram person, and as many of the participants of this study with BMI classifications of obese, the likelihood of the efficacy of the tool is poor. Lastly, due to randomization, we were unable to distribute levels of disease state between the two groups making it more difficult to achieve statically significant results.

In summary, this study is the first to investigate whole freeze-dried blueberry powder as a treatment to mitigate joint damage and resulting quality of life activities due to progression of osteoarthritis of the knee. Our findings suggest that blueberries may have a positive effect on joint health by preventing further cartilage and surrounding joint tissue degradation, and promoting repair, resulting in improvements of range of motion while maintaining physical activity levels. An increase in ROM in both knees in the FDBP group was backed by the decreased presence of YKL-40 and consistent levels of IGFBP-3. The decreased levels of IGFBP-3 potentially allowed for increased levels of IGF-1. All these combined factors may contribute to halting or slowing cartilage degradation and possibly allow growth and repair in the affected area. The opposite was witnessed in the placebo group. Increases in cartilage destroying YKL-40 and increases of binders preventing repair all could be used to explain declines in ROM in the placebo groups. Future study designs should include larger sample size and potentially dose-dependent quantities of blueberries to see more significant changes in cartilage

biomarkers potentially resulting in increases in physical activity and improvement in flexibility as measured by range of motion. Also, evaluating the phenolic content of the blueberry samples would be interesting and lend to potential further studies in the field.

TABLE 1 PARTICIPANT SCREENING AND DROP OUT RATE

Participants Screened	Qualified and Initiated Study	Completed Study	Participant Drop Out	Drop Out Rate
103	63	49	14	22%

TABLE 2 DEMOGRAPHICS OF STUDY PARTICIPANTS

		Baseline	Mid-point	Final
Blueberry	Male	9	9	9
	Female (*n*)	24	19	18
	Total (*n*)	33	28	27
	Avg Age	57.67	57.54	56.44
Placebo	Male	7	7	7
	Female (*n*)	23	18	17
	Total (*n*)	30	23	22
	Avg Age	55.3	55.42	54.59
Total	Male	16	14	14
	Female (*n*)	47	37	35
	Total (*n*)	63	51	49
	Avg Age	56.54	56.18	55.61
Drop Rate **(%)**		N/A	19%	22%

Figure 1 Effect of Blueberry vs Placebo on Body Mass Index (BMI)

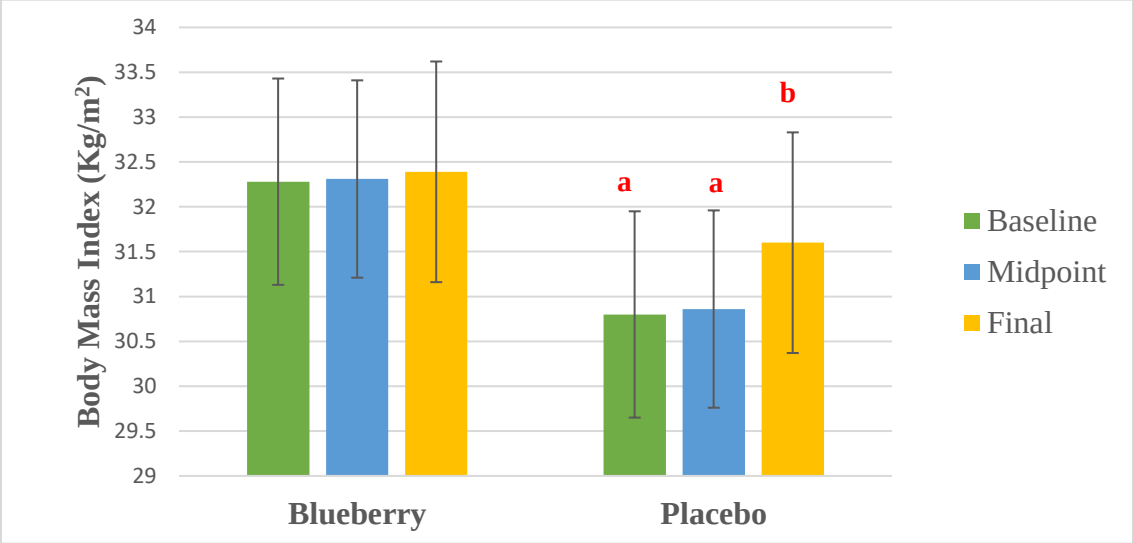

Figure 1 BMI. Mean ±SEM. *N* = 22 for blueberry, *n* = 21 placebo. Asterisk denotes significant difference ($p \leq 0.05$) from baseline. Letters denote means with significant differences across time points.

Figure 2 Effect of Blueberry vs Placebo on Systolic Blood Pressure

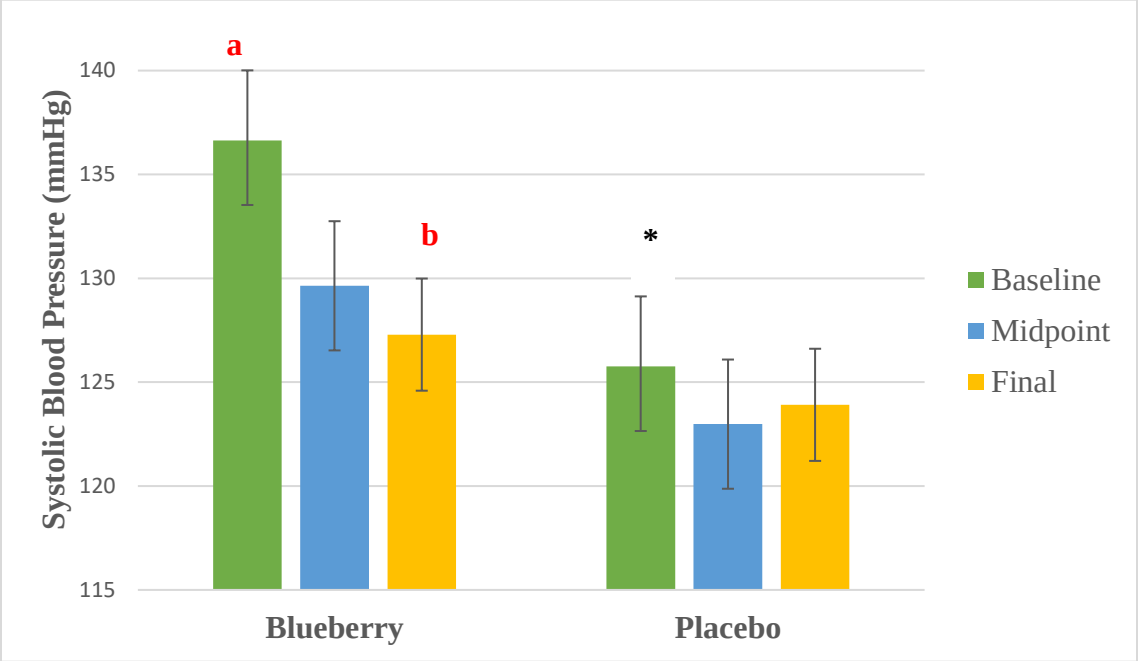

Figure 2 Systolic Blood Pressure. Mean ±SEM. *N* = 25 for blueberry, *n* = 22 placebo. Asterisk denotes significant difference ($p \leq 0.05$) from Treatment B. Letters denote means with significant differences across time points.

Figure 3 Effect of Blueberry vs Placebo on Diastolic Blood Pressure

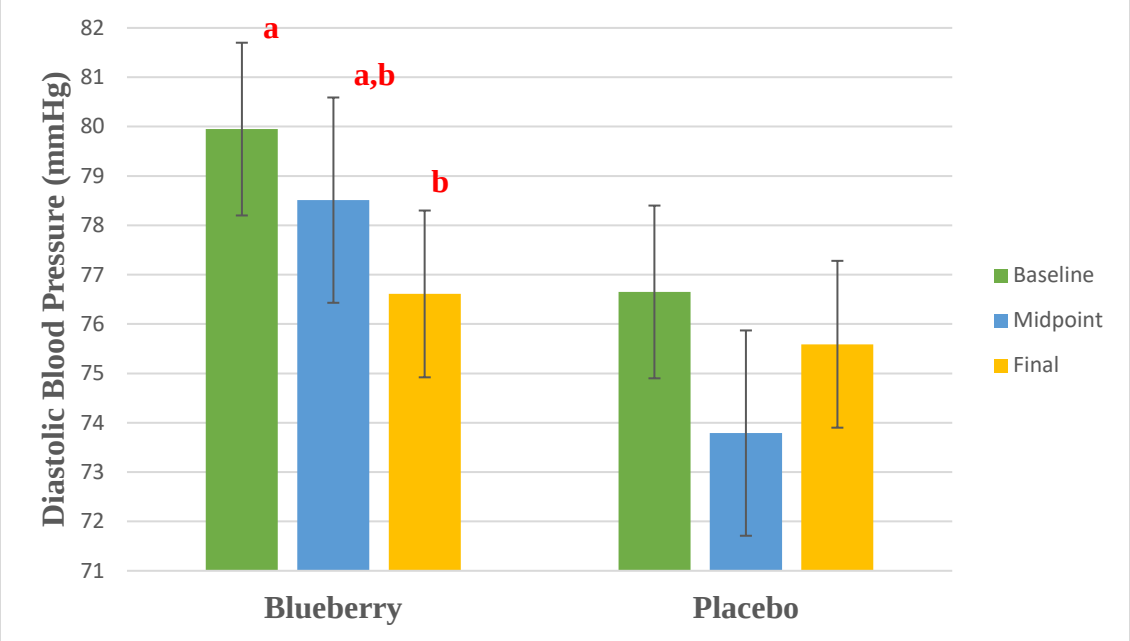

Figure 3 Diastolic Blood Pressure. Mean ±SEM. *N* = 25 for blueberry, *n* = 22 placebo. Letters denote means with significant differences across time points in the blueberry group.

Figure 4 Effect of Blueberry vs Placebo on Right Knee Range of Motion

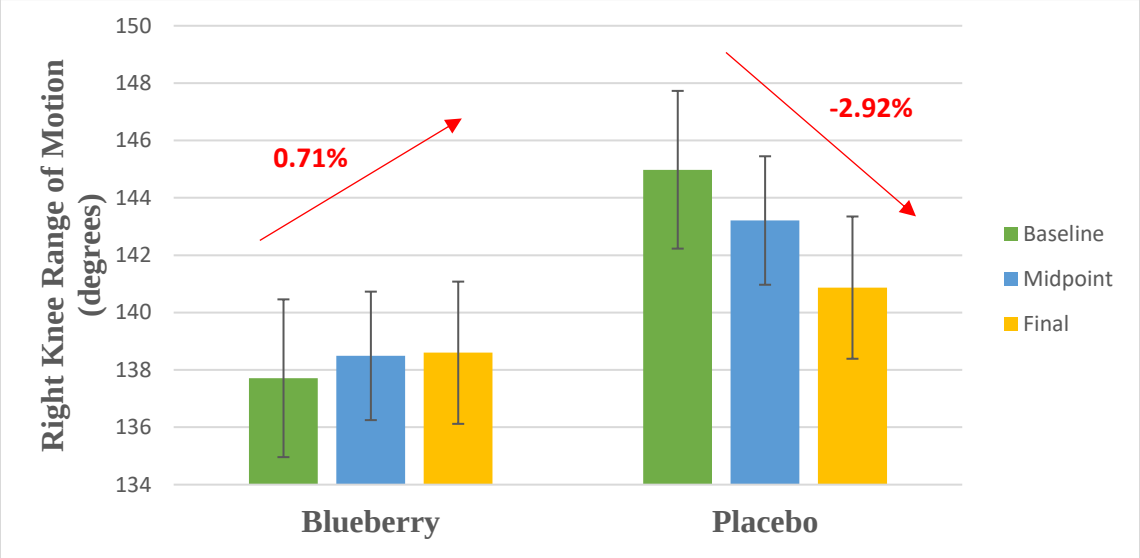

Figure 4 Right Knee Range of Motion. Mean ±SEM. *N* = 26 for blueberry, *n* = 21 placebo. No significant differences were observed. Blueberry group had a 0.71% increase from baseline to final. Placebo had a decrease in ROM of 2.92%.

Figure 5 Effect of Blueberry vs Placebo on Left Knee Range of Motion

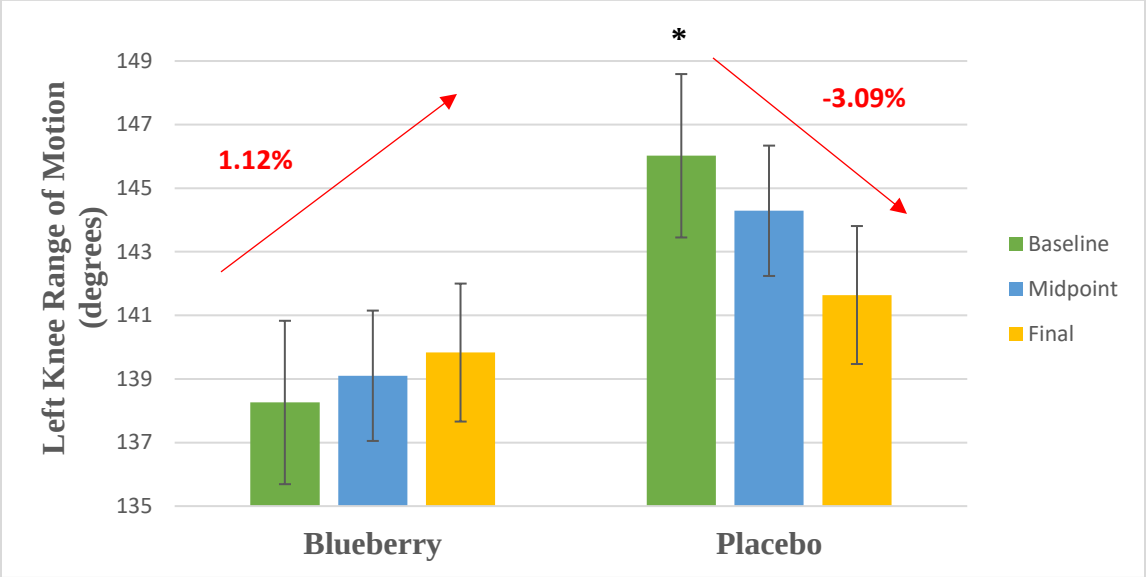

Figure 5 Left Knee Range of Motion. Mean ±SEM. *N* = 24 for blueberry, *n* = 21 placebo. Asterisk denotes significant difference ($p \leq 0.05$) from blueberry group at baseline. Blueberry group had a 1.12% increase from baseline to final. Placebo had a decrease in ROM of 3.09%.

Figure 6 Effect of Blueberry vs Placebo on Total Physical Activity

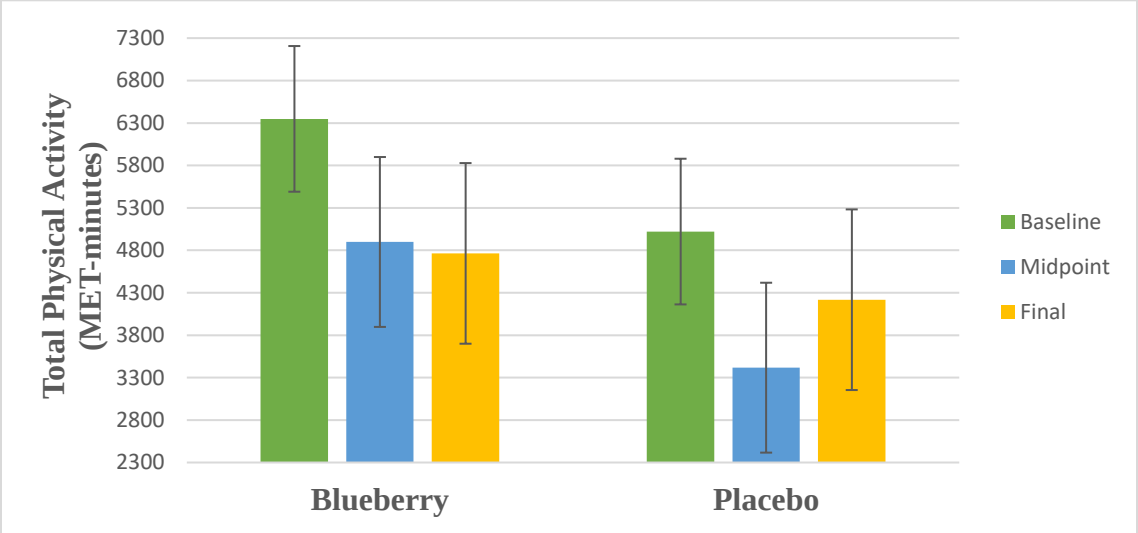

Figure 6 Total Physical Activity. Mean ±SEM. *N* = 30 for blueberry, *n* = 29 placebo. No significant differences were observed.

Figure 7 Effect of Blueberry vs Placebo on Plasma Hyaluronic Acid Levels

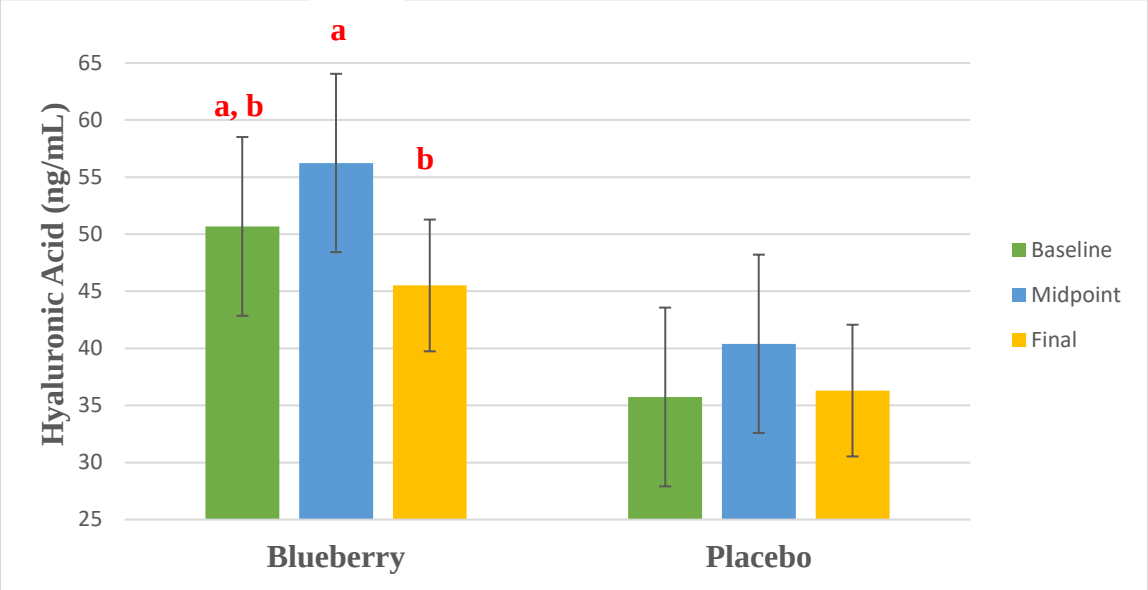

Figure 1.7 Hyaluronic Acid. Mean ±SEM. *N* = 27 for blueberry, *n* = 20 placebo. Letters denote means with significant differences across time points.

Figure 8 Effect of Blueberry vs Placebo on Plasma Insulin-Like Growth Factor 1 Levels

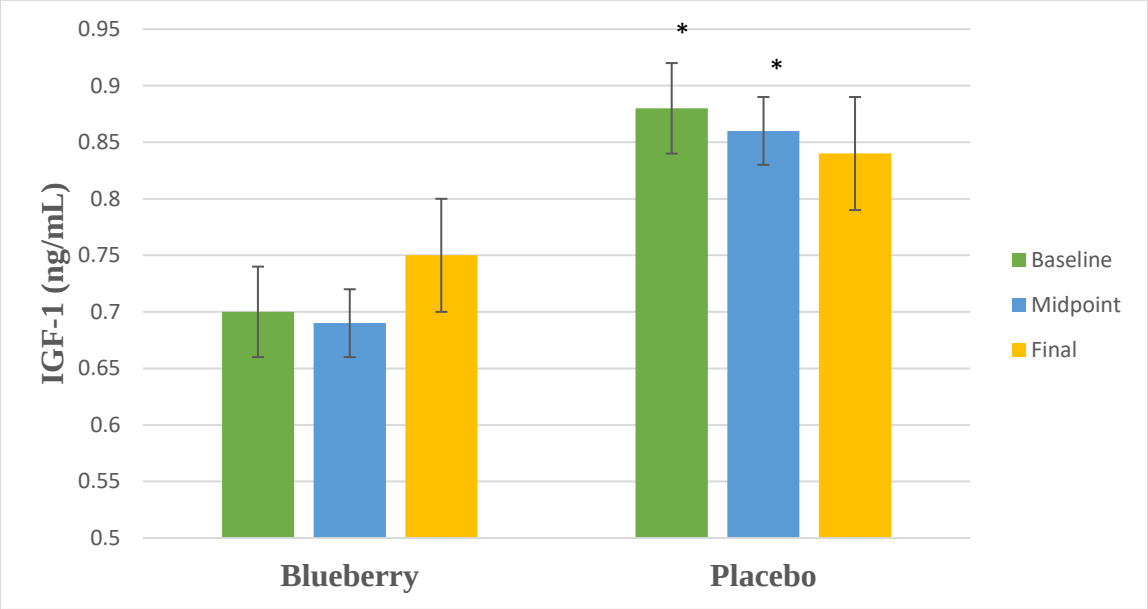

Figure 8 IGF-1. Mean ±SEM. *N* = 27 for blueberry, *n* = 19 placebo. Asterisk denotes significant difference ($p \leq 0.05$) from blueberry group.

Figure 9 Effect of Blueberry vs Placebo on Plasma Insulin-Like Growth Factor Binding Protein 3 Levels

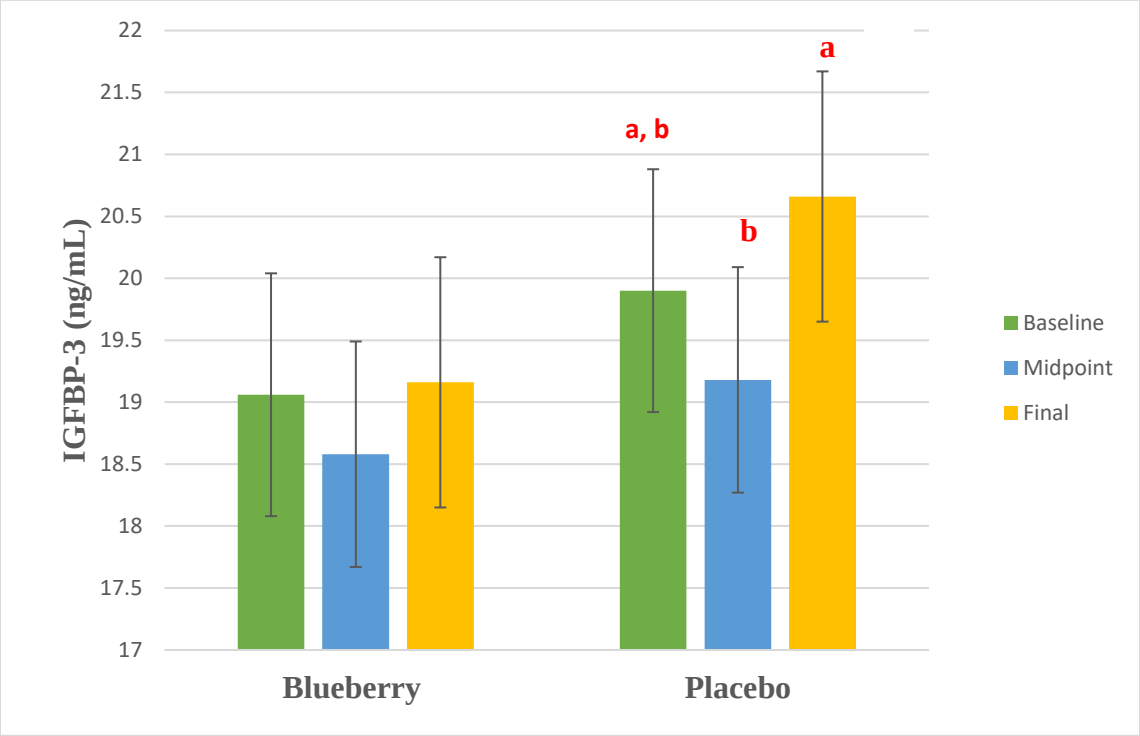

Figure 9 IGFBP-3. Mean ±SEM. *N* = 27 for blueberry, *n* = 22 placebo. Letters denote means with significant differences across time points.

Figure 10 Effect of Blueberry vs Placebo on Plasma Glycoprotein-39 Levels

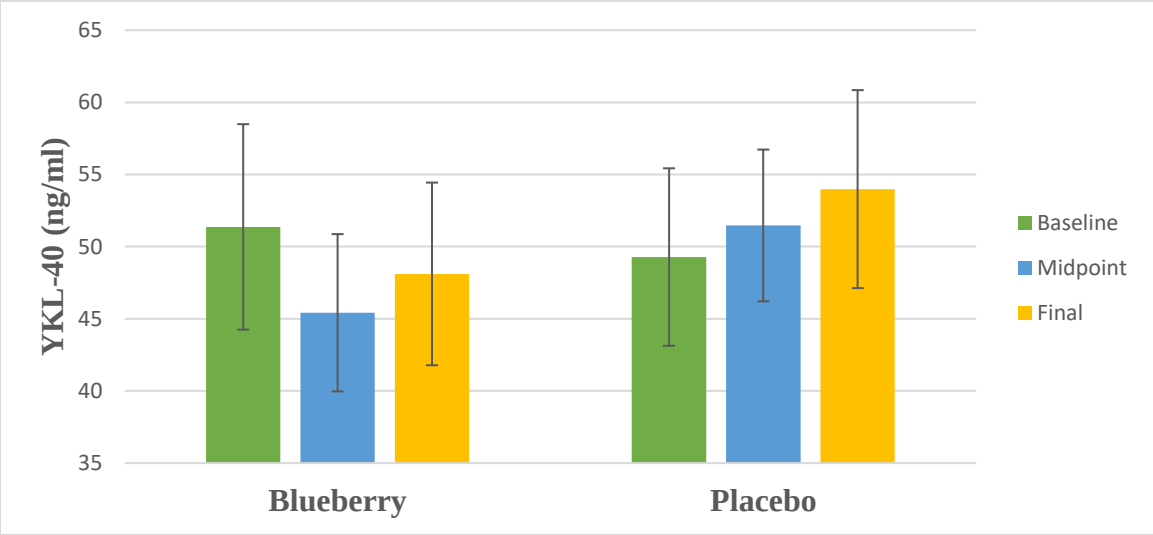

Figure 10 YKL-40. Mean ±SEM. *N* = 22 for blueberry, *n* = 21 placebo. No significant differences were observed.

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Appendix A

PARTICIPATION RECRUITMENT FLYER

Need Research Volunteers

Do you have KNEE Pain?

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

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

Criteria include meeting the requirements listed above and willing to consume either whole blueberry powder or a powder without blueberry for a period of 4 months. There will be blood draws at the start, midpoint (2 months) and at the end (4 months) of the study. Pain and joint flexibility will be assessed at start of study, midpoint, and end of study using questionnaires and range of motion measurement. The total time you need to spend for the study is 3 hours over 4 months involving 3 visits.

Benefits include: promotion of knee joint health, measurements of height and weight, pain and stiffness assessment and range of motion. Blood and urine markers of cartilage health will be evaluated. 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 visits.

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

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

Appendix B

SCREENING QUESTIONAIRE

Screening Questionnaire

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

Appendix C

INFORMED CONSENT

Texas Woman's University
Consent to Participate in Research

Study Title: Beneficial Effect of Whole Blueberry Consumption on Joint Flexibility, Mobility, and Pain Symptoms Associated with Knee Osteoarthritis

Investigators: Shanil Juma, PhD 940-898-2704 (sjuma@twu.edu)
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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 blueberry 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 blueberry powder for 4 months improve joint flexibility?
b) Will consuming freeze-dried blueberry powder to reduce pain and stiffness in the knee joint?

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.

During the baseline visit you will be asked to come fasted (not to eat any food overnight or at least 10 hours). A phlebotomist (person taking the blood) will draw 3 table spoons of your blood from one of the veins of your arms. We will then provide you with a snack and drink (cookies, crackers, and orange juice). This will be followed with a spot urine collection. A sterile specimen cup will be provided to collect a small urine specimen after the first morning void. A trained 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. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week. You will 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

Participant Initials _____

provided a 60 day supply of either the study treatment (freeze dried blueberry powder packaged in pouches) or a control (comparative placebo freeze-dried powder without blueberry). 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. We will then provide you with a snack and drink (cookies, crackers, and orange juice). A spot urine specimen will be obtained in a sterile specimen cup. Filtered water and a light snack will be available for you at the study site. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week. You will 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 (freeze-dried blueberry powder in pouches) or a control (comparative placebo freeze dried powder without blueberry). At the end of the study (6 months), you will be asked to come in for your last visit and not to eat any food overnight (10 hours) for a blood draw (3 tablespoons of blood will be obtained). You will be provided with snacks and filtered water. A spot urine specimen will be obtained. A trained research personnel of the same gender will measure height and weight. We will also ask you to complete a food frequency and physical activity questionnaire regarding your eating and activity habits over the past week. 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. The study volunteer 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). Total time commitment for each participant 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 Dr. Juma will know your identity. All records will be stored in a locked filing cabinet in Dr. Juma's office. The records will be shredded within 5 years of completion of the study. Your name or any other identifying information will not be included in any publication that

Participant Initials _____

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 blueberry powder or the comparative placebo powder without blueberry. 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 blueberry powder or the placebo powder without blueberry is from a whole fruit source or equivalent to the sugar content of the whole fruit that has been custom prepared and packaged for our study. It has been previously used in other human clinical studies and is deemed safe for consumption and not harmful in any way.

Another possible risk to you as a participant in this study includes the discomfort of blood drawings. The phlebotomist will ask you about any concerns or previous issues with having a blood draw. If there are serious concerns or reactions to blood draw, we will ask you that you have the option to withdraw from participating in the study at any time. Blood draw may cause minor pain, bruising, discomfort, swelling, anxiety, infection or fainting. We will use a certified expert for blood draw. This will minimize the possibility of pain, bruising, discomfort, swelling, infection, and anxiety. A light snack and water will be made available at the draw site to avoid fainting.

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

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

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

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 blueberry that has been freeze-dried into a powder and the comparative placebo powder containing sugar equivalent to the blueberry treatment without blueberries. If participants are allergic to blueberries or sugar found in blueberries 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

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

PROTOCOL APPROVAL LETTER

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

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

DATE: March 4, 2016

TO: Dr. Shanil Juma
Nutrition & Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Approval for Beneficial Effect of Whole Blueberry Consumption on Joint Flexibility, Mobility, and Pain Symptoms Associated with Knee Osteoarthritis (Protocol #: 18955)

The above referenced study was reviewed at a fully convened meeting of the Denton IRB (operating under FWA00000178). The study was approved on 3/4/2016. This approval is valid for one year and expires on 3/4/2017. 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 E

PHYSICAL ACTIVITY QUESTIONAIRE

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

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

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

Background on IPAQ

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

Using IPAQ

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

Translation from English and Cultural Adaptation

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

Further Developments of IPAQ

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

More Information

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

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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

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

PART 1: JOB-RELATED PHYSICAL ACTIVITY

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

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

   ☐ Yes

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

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

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

   _____ **days per week**

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

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

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

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

   _____ **days per week**

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

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

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

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

_____ **days per week**

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

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

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

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

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

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

_____ **days per week**

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

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

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

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

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

_____ **days per week**

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

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

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

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

_____ **days per week**

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

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

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

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

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

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

_____ **days per week**

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

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

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

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

_____ **days per week**

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

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

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

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

_____ **days per week**

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

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

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

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

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

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

_____ **days per week**

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

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

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

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

_____ **days per week**

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

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

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

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

_____ **days per week**

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

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

PART 5: TIME SPENT SITTING

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

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

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

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

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

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

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)