# THE EFFECT OF FREEZE-DRIED WHOLE RASPBERRY POWDER ON GAIT PERFORMANCE, MOBILITY, AND SERUM BIOMARKERS OF CARTILAGE METABOLISM IN SYMPTOMATIC KNEE OSTEOARTHRITIS

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ABSTRACT

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THE EFFECT OF FREEZE-DRIED WHOLE RASPBERRY POWDER ON GAIT PERFORMANCE, MOBILITY, AND SERUM BIOMARKERS OF CARTILAGEMETABOLISM IN SYMPTOMATIC KNEE OSTEOARTHRITIS

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***Objective:*** The purpose of the study was to investigate the effects of freeze-dried raspberry consumption on gait performance, mobility assessed by physical activity, and serum biomarkers of cartilage metabolism (YKL-40, insulin growth factor-1, insulin growth factor binding protein-3, and hyaluronic acid) in individuals with current symptomatic knee osteoarthritis.

***Methods:*** A double-blinded, randomized placebo-controlled study design was used with evaluations at baseline (before treatment intervention), midpoint (8 weeks), and after intervention (16 weeks). Sixty-three participants (both men and women) with self-reported mild to moderate degree of pain in the knee due to symptomatic OA were recruited. Participants were randomized into two groups, either placebo *(n = 29)* or treatment group *(n = 34*). The treatment group consumed 35 grams of freeze-dried raspberry powder mixed with 10-12 ounces of water, consumed daily. The placebo group consumed 35 grams of a control powder similar in color, fiber, carbohydrates, and calories. Anthropometric measurement of height, weight, blood pressure, and leg length were obtained at baseline, midpoint, and final visit. Additionally, participants filled out the International Physical Activity Questionnaire (IPAQ) at each visit. Finally, gait analysis was performed using the GAITRite® system at each visit. Overnight fasting

venous blood was collected at each visit to assess markers of cartilage metabolism (YKL-40, IGF-1, IGFBP-3, and hyaluronic acid). Treatment effects were analyzed using repeated measures ANOVA.

***Results:*** A total of 44 participants completed the study with an attrition rate of 30%. Average sitting time, measured by minutes per day, showed a progressive decrease from baseline to end of the study in the raspberry group, where the placebo group showed a consistent level for the duration of the study. The raspberry group showed an increase in house-related physical activity, measured by metabolic equivalents (METs), whereas no significant changes were observed in the placebo group. For cartilage metabolism markers: hyaluronic acid, IGF-1, IGFBP-3, and YKL-40, the raspberry group showed consistent biomarkers throughout the study without significant changes. The placebo group showed a progressive increase in IGFBP-3 and YKL-40 throughout the duration of the study, markers that are associated with advancing degradation of cartilage. IGF-1 and hyaluronic acid showed no significant changes over time in the placebo group. At a normal walking cadence, the placebo group showed a significant increase in cadence, velocity, right and left leg single support percentage, and right and left leg step length; additionally, with a decrease in right and left leg single support percentage and right and left leg cycle time, suggesting an improvement based on the placebo effect. The raspberry group showed improvement with an increase in cadence and a decrease in right leg cycle time at a normal walking cadence. However, at a fast walking cadence, only the raspberry group showed significant improvements, whereas the placebo showed no significant improvements. The raspberry group increased in cadence, velocity, and left leg single

support, while decreasing in left and right leg double support percentage and left and right cycle time.

***Conclusions:*** The findings of this study suggest that incorporation of whole raspberries may increase physical activity, improve gait performance, and prevent further cartilage degradation; therefore improving quality of life in individuals with symptomatic knee OA.

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# CHAPTER I

# INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis, a degenerative joint disease that affects the cartilage and surrounding tissues of generally the hands, hips, and knees (Center for Disease Control and Prevention [CDC], 2018a). Early onset OA symptoms includes pain with mobility, especially weight bearing activity, where rest may result in temporary relief from pain (Mahan & Raymond, 2017). The causation of OA is due to injury, loss of cartilage, or imbalance of inflammatory and anti-inflammatory processes. Due to the complexity of the disease, diagnosis can be based on medical history, physical exam, magnetic resonance imaging (MRI), x-ray, or joint aspiration (Arthritis Foundation, n.d.). OA can result in decreased physical mobility, increased pain and stiffness, and reduction in range of motion which could negatively impact activities of daily living (ADL).

OA is the most common degenerative disease among the elderly (Shen et al., 2012). According to the CDC, OA affects over 30 million individuals in the United States alone (CDC, 2018a). OA is the major cause of disability and loss of function amongst the elderly population (Shen et al., 2012). According to the 2010 World Health Organization (WHO) Global Burden of Disease Study, OA ranks 11th worldwide for cause of living with disability (Palazzo, Nguyen, Lefevre-Colau, Rannou, & Poiraudeau, 2016). OA also increases individuals' risk of mortality due to comorbidities, including cardiovascular disease, which may be related to decreased physical activity levels (Palazzo et al., 2016).

In severe cases, total joint replacement is required to improve mobility and reduce pain levels. With over 600,000 total knee replacements in the US annually, it has been estimated to cost approximately $12 billion (Palazzo et al., 2016).

Currently, since there is no known treatment or cure for reversing the condition of OA, it is most feasible to find therapeutic modalities that relieve symptoms such as pain and improve mobility (Shen et al., 2012). Typical treatments may include medication such as nonsteroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase-2 (COX-2) inhibitors, physical activity, muscle strengthening exercises, weight loss, and surgery (CDC, 2018a). The American Academy of Orthopaedic Surgeons (AAOS) recommends the use of strengthening exercises, knee arthroplasty, NSAIDs, and weight loss to a normal body mass index (BMI). Yet based on these recommendations, a study found orthopedic surgeons are increasing the usage of intra-articular corticosteroids, even though they are currently not recommended by the AAOS, due to limited research supporting beneficial effects (Carlson et al., 2018). Research has shown prolonged use of NSAIDs can result in gastrointestinal complications with the most severe cases involving peptic and duodenal ulcers which are susceptible to perforation and bleeding (Tramèr, Moore, Reynolds, & McQuay, 2000). Additionally, medications such as COX-2 inhibitors function as vasodilators and inhibit platelet aggregation. Prolonged use of COX-2 inhibitors increases rates of cardiovascular events (Mukherjee, Nissen, & Topol, 2001). Therefore, an alternative, more natural approach is necessary in order to reduce side effects, act as a preventative measure, and treat by increasing cartilage repair, reducing pain levels, and improving mobility.

Research has shown that dietary polyphenols pose beneficial effects when incorporated into human diets by acting as an antioxidant. Polyphenols are anti-inflammatory, anti-carcinogenic, and provide cardiovascular protection (Han, Shen, & Lou, 2007). Free radicals are compounds with one or more unpaired electrons that may cause alteration of proteins, lipids, and nucleic acids by attacking these components in order to seek electrons (Lobo, Patil, Phatak, & Chandra, 2010). Polyphenols reduce risk of these diseases through their ability to accept an electron from free radicals, thus helping to stabilize the free radicals and ultimately inhibiting a chain oxidation reaction (Pandey & Rizvi, 2009). Cell model studies, animal studies, and clinical trials have suggested polyphenols can reduce inflammatory markers and provide chondroprotective effects in OA (Jean-Gilles et al., 2012; Shakibaei et al., 2007; Dave et al., 2008; Suh et al., 2011; Figueira et al., 2014; Du et al., 2019; Ghoochani et al., 2016).

Raspberries are a commonly consumed berry with a rich source of polyphenols, especially anthocyanins and ellagitannins. Studies have shown raspberry consumption to be beneficial in metabolic, oxidative, and inflammatory diseases (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Red raspberries are one of many berries that act as COX-1 and COX-2 inhibitors in order to reduce inflammation, but has only been shown to be effective *in vitro* (Seeram, Momin, Nair, & Bourquin, 2001). Two rat model studies investigated the effects of red raspberry extract on OA, both showing promising results on reduction of cartilage degradation and inflammation (Suh et al., 2011). However, no clinical trial has investigated the effects of whole raspberries on mobility (physical activity), gait performance, and cartilage metabolism markers in individuals with OA.

## Hypothesis and Specific Aims

### Hypothesis

The main hypothesis of the study was that there would be a significant improvement in gait performance, mobility as assessed by physical activity, and serum biomarkers of cartilage metabolism (YKL-40, IGF-1, IGFBP-3, and hyaluronic acid) in individuals with symptomatic knee OA by consuming 35 grams of whole freeze dried raspberry powder for 16 weeks.

**Specific Aim 1**. Determine the effects of freeze-dried whole raspberry powder when compared to a placebo powder intervention on gait performance before, during (midpoint), and after intervention.

**Specific Aim 2**. Determine the effects of freeze-dried whole raspberry powder when compared to a placebo powder intervention on mobility assessed by physical activity before, during (midpoint), and after intervention.

**Specific Aim 3**. Determine the effects of freeze-dried whole raspberry powder when compared to a placebo powder intervention on cartilage metabolism serum biomarkers (YKL-40, IGF-1, IGFBP-3, and hyaluronic acid) before, during (midpoint), and after intervention.

# CHAPTER II

# REVIEW OF LITERATURE

## Osteoarthritis

OA is the most common form of arthritis, a degenerative joint disease that effects the cartilage and surrounding tissues of any joint but more commonly the hands, hips, and knees (CDC, 2018a). OA is not an autoimmune or systemic condition, but rather involves destruction of cartilage resulting in inflammation (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Common symptoms are stiffness, pain, swelling, and decreased range of motion that worsens slowly over a period of time (CDC, 2018a). OA can result in decreased physical mobility, increased pain and stiffness, and reduction in range of motion which could negatively impact activities of daily living (ADL). Currently, there is no known treatment or cure for OA, it is most feasible to find therapeutic modalities that relieve symptoms (Shen et al., 2012).

### Incidence and Prevalence

OA is the most common degenerative disease among elderly (Shen et al., 2012). According to the CDC, OA affects over 30 million individuals in the United States (CDC, 2018a). The National Arthritis Data Workgroup identified a 30% increase in OA cases in the U.S. between 1995-2005, and these numbers continue to rise with the expanding aging population (Umlauf, Frank, Pap, & Bertrand, 2010). By 2040, it is estimated that in the U.S. alone, diagnosed cases of OA will increase by 49% to 78.4 million (Arthritis Foundation, 2018). This issue expands past the U.S. to a global concern as well. OA is ranked as the fifth most prominent cause for disability globally. The prevalence of OA in

individuals over the age of 65 living in high income countries is estimated up to 80% (Arthritis Foundation, 2018).

## Impacts and Economic Burden

OA is the major cause of disability and loss of function amongst the elderly population (Shen et al., 2012). According to the 2010 WHO Global Burden of Disease Study, OA ranks 11th worldwide as a cause of living with disability (Palazzo et al, 2016) . Additionally, individuals with OA have an increased rate of work disability when compared to controls (Bitton, 2009). The Framingham studies suggest individuals with OA require some level of assistance with activities such as stair climbing, walking longer distance, housekeeping, and carrying items. Additionally, OA was ranked equally debilitating alongside congestive heart failure, chronic obstructive pulmonary disease, and heart disease (Breedveld, 2004). OA increases individuals' risk of mortality due to comorbidities, such as cardiovascular disease and hypertension, which may also be associated with decreased physical activity levels. These comorbidities lead to greater medical cost (Palazzo et al., 2016). One study estimated 41% of individuals with OA are also taking medication for hypertension (Breedveld, 2004).

In severe cases, total joint arthroplasty (TKA) is required in order to improve mobility and reduce pain levels. Approximately, half of a million Americans undergo joint replacements each year (Krasnokutsky, Attur, Palmer, Samuels, & Abramson, 2008). With over 600,000 TKA, it has been estimated to cost approximately $12 billion annually, not including the cost of therapy or rehab post-operatively (Palazzo et al., 2016). A study matched OA individuals compared to individuals without OA based on age, gender, geographic region, health plan type, and Medicare eligibility, in order to

draw a comparison of medical bill cost between the two groups. The study concluded, OA individuals had two times the amount of direct medical cost ($18,435 versus $7,494) over a 12 month period, with a significant increased inpatient, outpatient, and pharmaceutical cost in comparison to the non-OA individuals (Le, Montejano, Cao, Zhao, & Ang, 2012). OA can also induce indirect costs such as work-related losses, child-care cost, home-care cost, and remodeling of home due to inability to access related to disability. One study suggested these cost averaging $4,603 per person annually (Bitton, 2009). OA can decrease quality of life (QOL) as well as create emotional and financial burden in individuals undergoing the treatment process.

**Risk Factors**

Risk factors for developing OA include age, gender, obesity, genetics/family history, and race (CDC, 2018a). The exact pathogenesis of OA continues to remain unknown, but a few intrinsic and extrinsic factors based on trends have been identified qualifying as risk factors for the development of this condition. OA risk increases with every decade of age, especially over age 50, mainly due to "wear and tear" of the joints (Deshpande et al., 2016). Additionally, age affects the composition of the extracellular matrix (ECM) and the organization of the cartilage cells. The number of chondrocytes remain unchanged, but as we age, chondrocytes become more concentrated at the deeper levels of cartilage. This leaves less chondrocytes at superficial levels, resulting in a decrease in hydration of the ECM and an increase in stiffness and cartilage loss (Sophia Fox, Bedi, & Rodeo, 2009). Data analyzed from the National Health Interview 2007-2008 concluded that 57% of individuals suffering from OA were non-Hispanic white. Furthermore, OA occurrence in younger adults was found at increased rates in ethnic

minorities. In older adults with OA, increased rates are reported in non-Hispanic white population (Deshpande et al., 2016).

Woman have an increased risk of developing OA in comparison to men (CDC, 2018a). This may be multifactorial due to anatomical and hormone differences between the sexes. One study found that men have a greater volume of tibial and patellar cartilage compared to women in a non-arthritic knee joint, giving men more knee joint protection compared to women (Hame & Alexander, 2013). Women experience an increased risk of developing OA once reaching menopause, due to sex hormones potential role in the development of OA (Boyan et al., 2013). One study identified estrogen receptors on articular cartilage, showing a relationship between estrogen levels and cartilage health (Hame & Alexander, 2013). A study by Kinney, Schwartz, Week, Lotz, and Boyan (2005) suspects that female chondrocytes respond to the use of sex specific steroid hormones (estrogen) and can increase DNA synthesis in chondrocytes (Kinney et al., 2005).

The questions of nature versus nurture indicates that genetics is tied to OA development through twin studies, adoption studies, family clustering, and obtaining family history (Tim D. Spector & MacGregor, 2004). OA has not been tied to one particular gene, but it is suspected to be caused by an interaction of multiple genes (Litwic, Edwards, Dennison, & Cooper, 2013). One family study concluded that siblings of individuals who had a TKA from idiopathic OA had five times the risk for needing a TKA compared to their spouses who served as controls (Chitnavis et al., 1997). Another study that compared female identical twins to female fraternal twins with risk of developing OA in the hands or knee, demonstrated an increased correlation with evidence

of joint space narrowing and knee pain in the identical versus fraternal twins (T. D. Spector, Cicuttini, Baker, Loughlin, & Hart, 1996). It has been concluded that genetics play a role in the development of OA, raising potential for specific gene involvement. Studies have yet to identify those genes or whether OA may be a result of single nucleotide polymorphism (SNP). From a review of literature, genetic contenders under investigation associated with cartilage synthesis/breakdown include VDR, Col2A, AGC1, IGF-1, ER-α, TGF-β, CRTM (cartilage matrix protein), CRTL (cartilage link protein), A1ACT, COL9A1, COL11A1, COl1A1; there is a lack of strong evidence (Tim D. Spector & MacGregor, 2004).

Post-traumatic osteoarthritis is the "wear and tear" of weight bearing joints due to physical injury. Repetitive injury to cartilage can also be one of the causations of OA, such as in athletes or obese individuals, which could initiate and worsen the condition of cartilage destruction (CDC, 2018b). Certain types of labor intensive professions, such as coal miners or construction workers have been shown to have an increased risk for knee osteoarthritis due to the repetitive kneeling or squatting (Litwic et al., 2013). Athletes, who engage in high-impact exercise, commonly develop OA earlier in life due to joint overuse. A systemic review found soccer, long distance running, weight lifting, and wrestling to have a three times higher prevalence of developing knee OA compared to other elite sports (Driban, Hootman, Sitler, Harris, & Cattano, 2017). Repetitive injury can also result from inactivity, such as increased weight on knees from obesity. According to the 2018 data from Behavioral Risk Factor Surveillance Survey (BRFSS), obesity rate exceed 35% in nine states, 30% in 31 states, and 25% in 48 states (Behavioral Risk for Surveillance Systems, 2019). One causation of obesity is due to

America's inactivity, where less than 5% of adults participate in 30 minutes of physical activity each day and only one in three adults receive the recommended amount of physical activity each week (President's Council on Sports, 2017).

## Anatomy of the Knee Joint

In a normal healthy knee joint, there are five main components: bone, cartilage, ligaments, tendons, and synovial fluid/membrane. Three bones intersect the knee joint, the femur, tibia, and patella; where the femur and tibia meet, the bones are covered in articular cartilage, where the patella is covered on the posterior side (AAOS, 2014). Articular cartilage is approximately 2-4 mm in thickness surrounding the joint in order to protect the subchondral bone, allowing for smooth gliding of the two bones during movement (Mahan & Raymond, 2017). Articular cartilage is composed of extracellular matrix (ECM) with specialized cells called chondrocytes. Articular cartilage has two layers: thin layer, exposed to synovial fluid, and a deeper impermeable layer. Chondrocytes are responsible for the development, maintenance, and turnover of the ECM, with chondrocytes having a limited capacity for healing post injury (Sophia Fox et al., 2009). The two meniscus (lateral and medial) act as an important "shock absorber" in the knee, located between the femur and tibia. Meniscus is a type of cartilage different from articular cartilage in the fact that it is much tougher and resilient. Ligaments are the components that connect bone to bone, the knee has two collateral ligaments (medial and lateral) and two cruciate ligaments (anterior and posterior), acting to hold the knee in place. Finally, the knee has tendons, which connects muscle to bones via ligaments in order to facilitate movement (AAOS, 2014). The synovial membrane surrounds the non-cartilage areas of the knee joint; the membrane produces synovial fluid, a viscous fluid

responsible for lubricating the knee and circulating nutrients, also containing macrophages and fibroblasts (van der Kraan & van den Berg, 2007). During development, matrix components are developed due to stimulation by a variety of anabolic cytokines and growth factors, such as insulin growth factor-1 (IGF-1), a process which is slowed in healthy adults (Sandell & Aigner, 2001). All of these components work in a state of homeostasis in order to provide mobility of the knee joint and maintain the health of the cartilage.

## OA Pathophysiology

In an arthritic joint, there are two phases, biosynthetic phase, where chondrocytes attempt to repair the damaged matrix, and degradative phase, where synthesis of the matrix is inhibited and the enzymes continue to break down the matrix. Inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) are produced by the synovium and chondrocytes. TNF- α and IL-1β act by increasing synthesis of matrix metalloproteinases (MMPs), decreasing MMPs enzyme inhibitors, and decreasing synthesis of cartilage matrix (Sandell & Aigner, 2001). Anabolic cytokines, such as IGF-1, act to stimulate the synthesis of cartilage matrix (Sandell & Aigner, 2001). OA is a disease process that not only affects the articular cartilage, but it also affects the subchondral bone, synovial fluid, synovial membrane, ligaments, and the nerves and muscles around the joint (Nelms, Sucher, & Lacey, 2016). In an arthritic joint, as the disease progresses, cartilage can begin to thin, erode, and become soft, eventually losing both structure and function. With diminished cartilage, the subchondral bone becomes exposed and unprotected; to compensate, growth of cartilage and bone in the joint margins can result in osteophytes (bone spurs), which can exacerbate the symptoms

and limit range of motion of the knee (Nelms et al., 2016). Osteophytes occur due to the narrowing space of the joint and increased friction, a localized response for increasing surface area to better distribute the impact of force, which commonly occurs in weight bearing joints at an increased rate, but can also occur in fingers due to overuse (van der Kraan & van den Berg, 2007). Recent studies have shown a cycle of weak muscle tone, in relation to the surrounding knee muscles and worsening OA. One study found muscle impairments in atrophy and activation deficit to the quadriceps, hamstrings, and hip muscles in individuals with OA (Alnahdi, Zeni, & Snyder-Mackler, 2012). Another study was able to show similar results in a rat model, suggesting OA causes inflammation around the joint, increasing atrophy and decreased muscle regeneration (Silva et al., 2018). Additionally, levels of physical activity typically decline in individuals with OA due to increased levels of pain, resulting in straying from physical activity and increased muscle loss and impairments.

**Diagnosis**

Early onset of OA includes pain with mobility, especially weight bearing activity, where rest may result in temporary relief from pain (Mahan & Raymond, 2017). Due to the complexity of the disease, diagnosis may include medical history, physical exam, and other diagnostic tools. Medical history can help to detect risk factors for developing OA, whereas a physical exam can be used to determine pain levels, range of motion, balance, and gait. Additionally, a diagnostic test may include a) joint aspiration to detect broken down cartilage in the synovial fluid or b) magnetic resonance imaging (MRI) or x-ray in order to detect any damage, structural abnormalities, and narrowing of the joint space (Arthritis Foundaton, n.d.). The disease condition OA can be staged into four categories:

stage 0 no abnormalities, stage 1 beginning of OA and osteophyte formation, stage 2 moderate joint space narrowing and subchondral sclerosis, stage 3 >50% joint space narrowing and extensive subchondral sclerosis and osteophyte formation, and stage 4 joint destruction and little to no joint space (Litwic et al., 2013). Diagnosis of OA is usually based on disease progression and physiological and anatomical changes, but there are serum biomarkers associated with disease progression that cannot be utilized alone. These markers include glycoprotein-39 (YKL-40), insulin-growth factor-1 (IGF-1), insulin growth factor binding protein-3 (IGFBP-3), hyaluronic acid, along with inflammation markers such as c-reactive protein (CRP) and interleukin 1-beta (IL-1β).

**Biomarkers Associated with Cartilage Metabolism**

Hyaluronic acid (HA), also known as hyaluronan or hyalurnate, is a large linear glycosaminogycan (GAG) that is a component of cartilage and synovium (Sophia Fox et al., 2009). The articular cartilage is dependent on the synovial fluid for nutrition and waste removal. When cartilage is broken down, HA enters the synovial fluid and is removed into the blood stream through the synovial membrane (Moreland, 2003). HA is a component of synovial fluid, giving the fluid the viscoelastic properties; studies have shown that both concentration and molecular weight of HA are decreased in individuals with OA versus a healthy joint. With a decrease in HA, there is a decrease in synovial fluid, ultimately increasing the joint friction and pain (Moreland, 2003). In a healthy middle age adult, normal HA levels vary from 10-100mcg/L, but average 30-40mcg/L. Hence, these biomarkers can be useful indicators of cartilage health as well as progression and staging of OA.

IGF-1 is the predominant growth factor that contributes to cartilage matrix (De Ceuninck, Caliez, Dassencourt, Anract, & Renard, 2004). In children and adolescences, IGF-1 is important for linear growth of bones; in adults, IGF-1 plays a vital role in maintaining the homeostasis of the cartilage by stimulating production of proteins for cartilage matrix (De Ceuninck et al., 2004). IGF-1 increased in individuals with OA due to pro-inflammatory cytokines stimulating it's release, but chondrocytes have been less responsive to the anabolic effect in the arthritic joint (Hooshmand, Juma, Khalil, Shamloufard, & Arjmandi, 2015). A study found significant results for a relationship between IGF-1 serum concentration levels with osteophyte size and OA disease progression (Schouten, Van Den Ouweland, Valkenburg, & Lamberts, 1993). IGF-1 is responsible for the homeostasis of the cartilage while IGFBP-3 regulates the accessibility of IGF-1 to its receptor (De Ceuninck et al., 2004). IGFBP-3 is a protein in the class of IGFBPs, a class of six distinct binding proteins responsible for circulation of insulin growth factors (IGFs) to target tissues, each playing distinct roles (Allard & Duan, 2018). IGFBP-3 is responsible for regulation and transporting IGF-1 in the blood stream to target tissue, specific to ECM and chondrocytes (Hooshmand et al., 2015). Normal IGFBP-3 serum levels average around 100nM/L and are increased in individuals suffering from OA (Allard & Duan, 2018). In order to regulate the amount of free IGF-1, IGF-1 can stimulate the release of IGFBP-3 from chondrocytes to down regulate concentrations. Up regulation of IGF-1 concentration in the free form can be a result of the action of proteases, which release IGF-1 from IGFBP-3. IGFBP-3 has been three times higher in OA individuals compared to healthy cartilage and has been directly related with OA disease progression (De Ceuninck et al., 2004).

Human cartilage glycoprotein-39, commonly known as YKL-40, has been shown *in vitro* to be one of the proteins secreted by chondrocytes of an arthritic joint, and is relatively absent or at very low concentrations in individuals with non-osteoarthritic joints (Volck et al., 2001). YKL-40 is a significant growth factor for connective tissue cells, stimulating DNA synthesis and initiating a cascade of reactions leading to cell proliferation (Recklies, White, & Ling, 2002). YKL-40, classified as a marker of synovial inflammation and joint destruction, will be significantly increased in both serum and synovium fluid in patients suffering from OA (Conrozier et al., 2000). A cross-sectional study found a 36% increase in YKL-40 levels in individuals suffering from hip OA compared to a control group (Conrozier et al., 2000). Additionally, a study by Johansen et. al (1996), concluded the normal serum levels of YKL-40 in healthy adults averages 102mcg/L, where individuals with knee OA had 1.5 fold increase in YKL-40 levels.

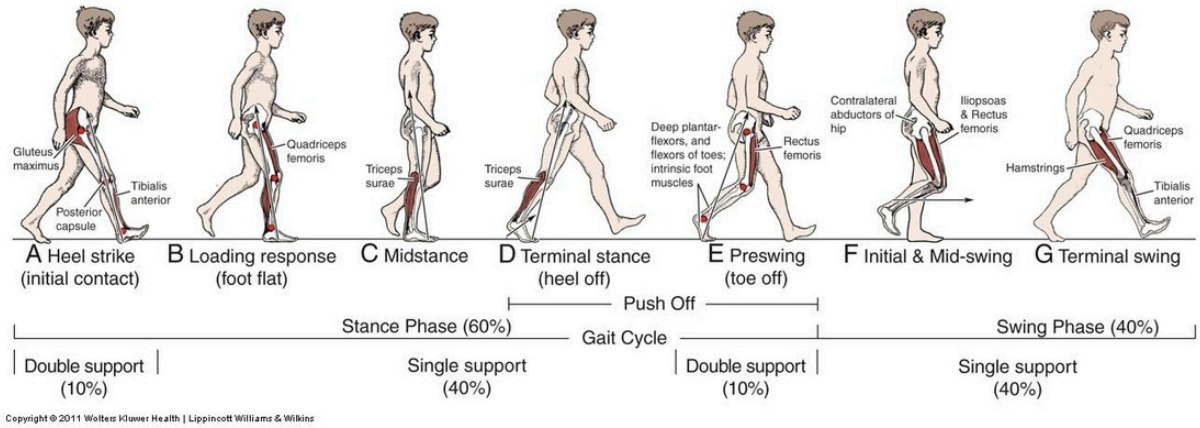
## Osteoarthritis and Mobility/Physical Activity

According to the WHO, the recommended physical activity for older adults includes engaging in 150 minutes of moderately intense aerobic physical activity or 75 minutes of vigorously intense aerobic physical activity weekly (WHO, n.d.). Vigorous activity is defined as activities requiring hard physical effort and resulting in substantially increased heart rate and breathing pattern. Moderate activity is defined as activities that require moderate physical effort and result in a slightly increased heart rate and breathing pattern (Craig et al., 2003). Additionally, recommendations also include muscle strengthening exercises that involve activation of all major muscle groups at least two days per week (WHO, n.d.).

Meeting these guidelines can be difficult for individuals suffering from OA due to disability and pain. Individuals suffering from mild to moderate OA, typically complain of pain when movement is initiated, such as walking or bending at the knee (Litwic et al., 2013). This pain and stiffness results in avoidance of physical activity and consequently continues the progression of the disease and decline in functional status (Pisters, Veenhof, de Bakker, Schellevis, & Dekker, 2010). Misconceptions exist regarding engaging in physical activity in individuals suffering from knee pain, with the idea that physical activity worsens the condition rather than improving it. A systemic review investigating the safety of physical activity and OA confirmed long-term therapeutic exercise poses a beneficial effect rather than harm. Physical activity can actually decrease risk of disease progression and the need for TKA (Quicke, Foster, Thomas, & Holden, 2015). Physical activity is important for individuals suffering from OA and can actually help improve pain, function, and QOL while delaying disability and other comorbidities. The physical activity guidelines should be followed, with adjustments made to focus more on joint friendly activities such as walking and swimming (CDC, 2018b). Many studies have confirmed the benefit of physical activity and its delay in the progression of OA especially in the hip and knee (Pisters et al., 2010).

## Osteoarthritis and Gait

The walk is a vital movement for human activities of daily living, requiring movement on both flat and rough terrain. This movement involves both the muscles and the skeletal system in a motion requiring stretching, compressing, and rotating with the goal of smooth movement without expending excess energy (Azahari, Siswanto, Ngali, Salleh, & Yusup, 2017). A human walk is defined as 'method of locomotion involving the use of two legs to provide both support and propulsion; with at least one foot in contact with the ground at all times' (Whittle, 2007, p.48). Gait refers to the pattern of walking, where walking is the action itself (Whittle, 2007). An upright and balanced posture is important for proper alignment to minimize stress on the bones and joints, while minimizing energy expended (Azahari et al., 2017). Gait cycle is composed of all phases of walking and is the interval between two successful occurrences of one repetitive event of walking; Figure 1, shown below, shows a complete gait cycle of the




right leg (Whittle, 2007).

*Figure 1.* Complete Gait Cycle of the Right Leg

The time duration in which the gait cycle last is called the cycle time (Whittle, 2007). A gait cycle is divided into two main phases: the stance phase and the swing

phase. The stance phase is classified as the cycle foot in contact with the ground, making up 60% of the cycle, whereas swing phase is when the cycle foot is the foot causing propulsion, making up 40% of the cycle (Physiopedia contributors, 2015). The stance phase, also known as the support/contact phase, is subdivided into loading response (flat foot), mid-stance, terminal stance (heel off), and pre-swing (toe off). The swing phase, initiated when the toe leaves the surface (termed toe off) until the next initial contact with the surface is subdivided into initial-swing, mid-swing, and terminal-swing. Gait also has two support components: single support (one leg) and double support (both legs). For example, using the right leg, the walk starts in double support, the right stance phase begins with right heel contact and ends with right toe off; the right swing phase is initiated with right toe off and ends with right heel contact (Whittle, 2007).

## Variables with Gait Analysis

Variables used for gait analysis include spatial (distance) and temporal (time). Spatial variables include stride length, step length, walking base (also known as stride width), and foot angle (angle of toe out). Stride length is the distance between two successful placements of the same foot; for example, the distance of the initial left foot placement to new left foot placement. One stride length is made up of two step lengths, distance between steps both left and right. In a normal gait, one stride length should be double a one-step length; in an abnormal gait one step length may be longer in distance than the opposite leg step length. Walking base or stride width is the side-to-side distance occupied in a walk. Foot angle or toe out, referring to the foot placement, is the degree of the toe out (or in) from the reference line of the heel (Whittle, 2007).

Temporal variables include step time, stride time, stance time, swing time, single support time, double support time, cadence, and velocity. Step time is the amount of time spent during a single step, measured in seconds. Whereas, stride time or cycle time, is the time it takes to complete one stride, measured in seconds. Stance time is the time spent in the stance phase on one limb, where swing time is the time spent in the swing phase for one limb, measured in seconds. Cadence is the number of steps in a given time, measure in steps per min. The normal range for cadence of female adults age 50-80 is 96-137 steps/min while for males it is 81-126 steps/min. Velocity, or speed, is the distance covered in a given period of time, measure in meters per second (Whittle, 2007).

## Gait Characteristics in Individuals with Knee Osteoarthritis

Gait pattern can show abnormalities when using normal human walk movements as the standard. Kaufman, Hughes, Morrey, Morrey & An (2001) examined the effects of stage 2 knee OA on walking, stair ascent, and stair descent in 139 individuals in comparison to 20 healthy individuals acting as controls. The study concluded velocity was significantly slower in individuals with OA. There was a significant decrease in internal knee extensor compared to controls, reflecting that participants with OA compensated in order to reduce knee joint loading associated with increased levels of pain (Kaufman et al., 2001). In a systemic review of 30 published research studies, it was concluded that there is a significant decrease in gait speed, stride length, and flexion of the knee in individuals with OA when compared to healthy controls (Ornetti et al., 2010). A large clinical trial including 2,911 individuals investigated the correlation of OA stage and gait parameters: cadence and stride length and OA severity stages. The study concluded that with each increased stage of OA, there was a decrease in cadence and

stride length (Elbaz et al., 2014). Gait change has shown in numerous research studies to be related to the severity and disease progression of OA. GAITRite is a valid, precise tool used to measure spatial (distance) and temporal (time) in walk patterns (Webster, Wittwer, & Feller, 2005a).

## Treatment and Therapies for OA

Treatments for OA depend on a patient's medical history and severity of symptoms with a combination of treatments typically used in order to improve mobility and decrease pain, while delaying the progression of the disease. Treatments can be surgical, pharmacological, and non-pharmacological. Typical treatments may include medications, such as NSAIDs or COX-2 inhibitors, supplement use, intra-articular injections, physical activity, muscle strengthening exercises, weight loss, and surgery (CDC, 2018a).

### Surgical

In 2008, the AAOS published evidence based best practices for non-surgical options in the treatment of OA; it was updated in 2015 to include surgical recommendations (Carlson et al., 2018). According to a recent study published by the AAOS, investigating the compliance of surgeons with evidence based recommendations, the most common intervention surgeons used for stage 4 OA was TKA, which according to the AAOS does have strong supporting evidence as well as highly recommended successful outcomes (Carlson et al., 2018). TKA has improved pain, disability, function, physical activity and overall QOL (Litwic et al., 2013). Surgery comes with the risk of infection. A meta-analysis including 20 published studies, showed an increase rate of infection, debridement, and knee revisions in obese individuals post-operatively when

compared to non-obese individuals (Kerkhoffs et al., 2012). Prior to a TKA, less invasive surgical options include arthroscopy with debridement of knee compartment or meniscectomy in the case of a torn meniscus. It has been suggested that only 50-75% of patients have relief post arthroscopy and proceed with a TKA within one year (Manen, Nace, & Mont, 2012).

**Pharmacological**

Ibuprofen, an NSAID, is a common first prescribed treatment for OA, due to being readily available over the counter and inexpensive. According to the AAOS 2015 guidelines, strong evidence supports the use of NSAIDs in treatment of OA (Carlson et al., 2018). Over the counter NSAIDS, are lower in dosage and will only provide pain relief. In order to receive pain relief and reduction in inflammation, a prescribed dosage is required (Arthritis Foundation, 2019). Pain reduction begins within 2 hours of oral administration of NSAID, but for a reduction in inflammation, consistent use is necessary for up to two weeks (Arthritis Foundation, 2019). NSAIDs are used to reduce inflammation by blocking the action of the cyclooxygenases (COX), enzymes that synthesizes prostaglandins which facilitate the inflammatory process. COX-1 plays a critical role in protecting the lining of the stomach and general housekeeping processes, whereas COX-2 is associated with inflammation. Due to the non-specific action of the NSAIDs on the COX enzymes, both COX-1 and COX-2 will be inhibited, resulting in reduced protection of the stomach lining (Arthritis Foundation, 2019). COX-2 inhibitors are NSAIDs that specifically target the COX-2 enzyme, therefore, impacting the stomach lining. A common COX-2 medication prescribed for OA patients is Celebrex (Celecoxib). Research indicates that prolonged use of NSAIDs can result in

gastrointestinal complications with the most severe cases involving peptic and duodenal ulcers, which make an individual susceptible to gastrointestinal perforation and bleeding. When left untreated, it can result in protein loss, blood loss, and potentially death (Tramèr, Moore, Reynolds, & McQuay, 2000). Side effects of NSAIDS also include bruising and heart and kidney complications (Arthritis Foundation, 2019). In addition to reducing inflammation, research has shown that COX-2 inhibitors result in enhanced vasodilation and inhibition of platelet aggregation. One study suggested that these mechanisms associated with prolonged use of COX-2 inhibitors can result in an increase in cardiovascular events (Mukherjee, Nissen, & Topol, 2001). Pharmacological treatments can pose adverse effects on health when used on a regular basis for a prolonged period of time.

There is increased interest in using intra-articular (IA) knee injections of hyaluronic acid or corticosteroids as a pharmacological treatment for knee OA. Hyaluronic acid injections are suspected to replace the hyaluronic acid in the synovial fluid, which is lost in the progression of OA, a method approved by the Food and Drug Administration (FDA; Gower, n.d.). Corticosteroid injections are suspected to be beneficial for reduction of local joint inflammation. A meta-analysis of 10 published studies concluded that, six of the 10 studies showed possible benefit from IA corticosteroid injection, providing pain relief up to two weeks (Arroll & Goodyear-Smith, 2004). Randomized-controlled trials have shown an increase length of time of relief with hyaluronic acid injections compared to corticosteroid injections, one month versus one week respectively (Manen et al., 2012). A recent study published by the AAOS, investigating the compliance of surgeons with AAOS evidence-based recommendations,

found that the most common intervention used by surgeons for knee OA stages 2 and 3 was IA hyaluronic acid and IA corticosteroids injections. The AAOS strongly recommends against the use of IA hyaluronic acid as some studies suggest little to no improvement in OA symptoms. The AAOS cannot either recommend nor discourage the use of IA corticosteroids and feel additional research is necessary (Carlson et al., 2018). Aside from a lack of research, IA injections increase the risk of infection especially in those who receive an IA injection within seven months following total knee arthroplasty (Carlson et al., 2018). Such pharmacological treatments only manage the symptoms of pain without reversing, treating, or altering the biological causation of the condition. Therefore, it is necessary to identify an alternative, a more natural approach to reverse OA condition, to act as a preventative measure, and treat by increasing cartilage repair and reducing pain levels.

**Nonpharmacological**

The AAOS 2008 recommendations are based on strong evidence that supports the use of strengthening exercises with neuromuscular education or through physical therapy; as well as moderate evidence supporting weight loss to lower body mass index (BMI) to less than 25 $kg/m^2$ through diet and exercise (Carlson et al., 2018). The Framingham study showed a 50% reduction in knee OA pain with a decrease in BMI by two points or more (Manen et al., 2012). OA is clinically defined by the loss of articular cartilage, but OA affects the surrounding muscle and can lead to decrease muscular strength in the lower extremities. One study estimated quadricep deficits of 10-56% in OA individuals compared to healthy controls (Alnahdi et al., 2012). A systemic review suggests that quadricep muscle strength is a major component of performance and physical function in

individuals with OA and can benefit from resistance training of the lower extremities in order to reduce pain and improve overall mobility (Alnahdi et al., 2012). Individuals often have difficulty maintaining an exercise regimen, where if not maintained, benefits can start to regress after six months (Manen et al., 2012).

Glucosamine and chondroitin are two common dietary supplements used in the treatment of OA. Glucosamine is an aminosaccharide found in two forms, glucosamine sulfate and glucosamine hydrochloride. Chondroitin is commonly found as chondroitin sulfate (Bruyere & Reginster, 2007). Glucosamine and chondroitin are two structural components that make up cartilage, naturally produced in the body, and pose benefit by assisting the ECM to retain water and prevent breakdown (Arthritis Foundation, n.d.). Controversial studies show whether glucosamine poses benefit or not where one study compared the effects of glucosamine to the use of NSAIDs on OA symptoms. It was concluded NSAIDs worked faster when compared to glucosamine, but both had similar long-term effects with a 48% improvement with glucosamine and 52% improvement with NSAIDs (Muller-Fabender, Bach, Haas, Rovati, & Setnikar, 1994). Other studies have shown no benefit with the use of glucosamine, chondroitin, or a combination of the two in their use for pain reduction with OA (Clegg, Reda, Harris, Klein, & O'Dell, 2006). According to the AAOS 2008 guidelines, the use of supplemental glucosamine and chondroitin are currently not recommended due to evidence suggesting no significant beneficial effect (Carlson et al., 2018).

## Diet, Inflammation, and OA

Inflammation is the body's response to stimuli that cause harm to the body and includes the presence of toxic components or damaged cells, in order to reduce the

stimuli and initiate the healing process in an attempt to regain homeostasis. The inflammation process can be broken down into four steps: 1) recognition of harmful stimuli; 2) inflammatory pathway activation; 3) inflammatory mediator release; and 4) inflammatory cell are recruitment (Chen et al., 2017). Cytokines are proteins secreted by cells for the purpose of communication and signaling between cells and include both pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines are produced as a response of activated macrophages. Examples of pro-inflammatory cytokines include interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α), two of the most common cytokines associated with pathological pain (Zhang & An, 2007). Anti-inflammatory cytokines are regulatory molecules that help to control and regulate the pro-inflammatory cytokine response and include IL-10, a common cytokine that suppresses IL-6 and TNF-α inflammatory responses (Zhang & An, 2007) .

A major risk factor for the development of OA is age. *Inflamm-aging*, a term coined by Franceschi et al (2000), correlates the direct relationship between progressive inflammation and aging. Age-related inflammation can be both systemic and local. Systemic inflammation can be driven by age-related changes such as a decrease in muscle mass and an increase in adipose tissue due to a progressive decline in metabolism and decrease in physical activity (Greene & Loeser, 2015). Adipose tissue is considered to be an endocrine tissue due to its ability to secrete adipokines and alter the shift to pro-inflammatory cytokines. Adipose tissue is capable of directly secreting pro-inflammatory cytokines, especially IL-6 and TNF- α (Nishimura, Manabe, & Nagai, 2009). With an increase in adipose tissue there is a direct increase in the production of pro-inflammatory cytokines specifically IL-6 and TNF- α (Greene & Loeser, 2015).

A diet rich in fruits and vegetables has benefits in the prevention and management of many chronic diseases. The dietary guidelines for Americans suggest eating a variety of nutrient dense foods while focusing on portion sizes as a key component of a healthy eating pattern. Recommended serving sizes include 2 and a half cups of vegetable equivalents per day and 2 and a half cups of fruit equivalents per day with a focus on variety and fresh produce to meet adequate fiber intake, which are a recommended 25 grams per day for women and 38 grams per day for men. Currently, the majority of Americans eat a diet pattern that is low in fruits, vegetables, and fiber, while being high in refined grains, added sugar, saturated fat, and sodium. It is estimated that three-fourths of Americans do not meet their daily recommendations for fruits, vegetables, and fiber (*HHS, 2015*).

Diet patterns have shown to both negatively and positively impact the rate age-related illness and chronic diseases, such as cardiovascular disease, diabetes mellitus, kidney disease, osteoarthritis, etcetera (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Many studies have revealed an inverse relationship between the consumption of fruits and vegetables and inflammatory markers. One study examined fruit and vegetable intake amongst cigarette smokers and the effect on their high-sensitivity c-reactive protein (HS-CRP) serum levels, a marker of inflammation. Participants were fed four servings of fruits and vegetables for four weeks, then the servings were increased to eight servings of fruits and vegetables for an additional four weeks. The study results identified a significant decline in HS-CRP serum levels with the increased fruit and vegetable intake (Galland, 2010). One study by Messier, Mihalko, Legault, and Miller (2013) investigated the effects of diet and exercise on knee OA in 450 obese individuals. The study had three

intervention groups: a) intensive diet-induce weight loss; b) exercise-induced weight loss; and c) both diet and exercise induced weight loss. The study found a greater weight loss and decrease in IL-6 levels in the diet and the combined diet and exercise groups in comparison to the exercise only group (Messier et al., 2013). More recently, the role of dietary antioxidants in arthritis management has been examined by researchers in a number of studies.

Vitamin E, a fat-soluble vitamin, has shown to be a powerful plant-source antioxidant. Free radicals are unstable atoms that can cause damage to tissue by seeking electrons from other compounds. Vitamin E has a phenolic hydroxyl group that donates a hydrogen in order to reduce free radicals, preventing free radicals from damaging tissues. A double-blind, placebo-controlled trial using OA induced in dogs, investigated the effects of a 400IU vitamin E supplement. This study found a reduction in inflammation markers, histological expression, and pain levels in the vitamin E treatment group versus no changes in the control group (Rhouma, de Oliveira El Warrak, Troncy, Beaudry, & Chorfi, 2013).

Vitamin C is a water-soluble vitamin found predominantly in fruits and vegetables. Vitamin C functions in cartilage biosynthesis as well as being an antioxidant vitamin. Research has shown that vitamin C contributes to the regeneration of other antioxidants in the human body such as vitamin E (National Institute of Health, 2018). In the longitudinal Framingham Studies, it was concluded that participants with moderate to high intakes of vitamin C experienced a reduction in the progression of their OA (McAlindon et al., 1996). More recently, a study by Chiu et al. (2017) investigated both *in vivo* and *in vitro* effects of vitamin C on OA. This study indicated that vitamin C has a

preventative role in OA progression including a decrease in apoptosis and reduction in pro-inflammatory cytokines (Chiu et al., 2017).

Vitamin D, a fat-soluble vitamin, plays a vital role in bone health through the formation, homeostasis, and turnover of bones. Vitamin D in the activated form (calcitriol) involved in the formation of hydroxyapatite, a calcium and phosphorous complex that makes up the major component of structural bone matrix (Cakar et al., 2018). Recent research suggests that there is a connection between vitamin D levels and the homeostasis and turnover of articular cartilage health, with indications that there is a significant increase in vitamin D receptors (VDRs) on damaged cartilage (Garfinkel, Dilisio, & Agrawal, 2017). Vitamin D has also been connected to inflammation with VDRs present on immune cells. When binding of vitamin D to VDRs is increased, there is an increase in activation of vitamin D receptor elements (VDREs). Activation of VDREs promotes blocking of gene transcription of inflammation markers that are associated with diseases including inflammatory bowel disease and systemic lupus erythematous (Garfinkel et al., 2017). Research has been unable to draw a strong correlation between vitamin D and the progression or inflammation associated with OA. A study that investigated the findings from two large longitudinal studies, the Framingham and Boston OA studies, found no significant relationship between vitamin D serum levels and progression of OA (Felson et al., 2007). Additionally, there does not appear to be a strong correlation between vitamin D deficiency and an increase in inflammatory cytokines associated with OA (Barker et al., 2014). Alternatively, a double-blind placebo-controlled study by Sanghi et al. (2013), suggested a small, but statistically significant improvement in function of knee and reduction in pain with supplementation

of vitamin D in individuals with OA and vitamin D deficiency. Vitamin D deficiency may act as a risk factor for developing OA, but stronger evidence is needed in order to draw a conclusion that vitamin D supplementation can be beneficial in the treatment of osteoarthritis.

Dietary omega ratio of omega-6 to omega-3 fatty acids play a role in inflammation. Omega-6 fatty acids are essential for normal development but should be maintained in a healthy ratio with omega-3s as excess omega-6s have the potential of being pro-inflammatory when in excess. The recommended ratio of omega-6 to omega-3 fats, in order to decrease disease related risks, ranges from 2:1-5:1. The typical western diet average ratio of omega-6 to omega-3 is between 15:1-16.7:1, and may be associated with an increase in diseases such as cardiovascular disease, cancer, and other inflammatory diseases (Simopoulos, 2002). Many published studies investigating the effects of omega-3 fatty acids on dogs with OA have shown beneficial results with one study finding an improvement in ability to walk and play in about six weeks in dogs consuming food with a 24-fold increase in omega-3s compared to the control (Roush, Dodd, et al., 2010). Additionally, consumption of food containing 3.5% fish oil omega-3s showed an improvement in weight bearing of 82% in dogs in the treatment group compared to a control group (Roush, Cross, et al., 2010). One randomized-controlled clinical trial investigated the effects of a high dose (4.5g) and low dose (.45g) of fish oil with both groups showing an improvement in pain levels when compared at two years (Hill et al., 2016). There is a need for additional research as a clinical trial on OA disease progression before distinct recommendations can be made.

The Mediterranean diet is a diet pattern that focuses on the consumption of fruits, vegetables, whole grains, and olive oil. Additionally, it also recommends a moderate intake of fish and poultry while limiting amounts of dairy and beef consumption. This dietary pattern follows a lower ratio of omega-6 to omega-3. This dietary pattern has shown beneficiary effects on inflammatory diseases such cardiovascular disease and cancer through its ability to normalize inflammatory markers such as HS-CRP and IL-6 (Galland, 2010). A large cohort study by Veronese et al. (2016), investigated the QOL as assessed by changes in stiffness, pain, disability, and depression in 4,470 individuals with OA and their adherence to the Mediterranean diet. Adherence was confirmed by using a Mediterranean diet score in order to put numerical data to common foods in the Mediterranean diet then grouping based on a score of 1 to > 18. The study identified a greater QOL, lower BMI, and healthier aging in individuals with a higher Mediterranean diet adherence score (Veronese et al., 2016). Another study looked at the changes in range of motion of knee and hip, as well as biomarkers associated with OA with the adaption of the Mediterranean diet for 16 weeks. Food frequency records were monitored by a Registered Dietitian Nutritionist to ensure compliance with a traditional Mediterranean diet. The study found a decrease in cartilage degradation markers and IL-1α, pro-inflammatory marker, from pre-diet and post-diet serum collection with no significant changes in the markers in the control group who remained on their normal diet (Dyer, Davison, Marcora, & Mauger, 2017).

## Polyphenols

Phytochemicals are a family of non-nutrient, non-essential compounds that are biologically active in the body with polyphenols being one of the largest classes of these

compounds (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Dietary polyphenols are the most abundant source of antioxidant compounds in the human diet; which is a component of a variety of foods such as fruits especially berries, vegetables, chocolate, and tea. Polyphenols are metabolites that contribute to the taste and color of plants and are used to protect the plant from ultraviolet radiation and pathogens (Pandey & Rizvi, 2009). Research has shown that dietary polyphenols impart beneficial effects when incorporated into human diets by acting as an antioxidant; and having properties of being anti-inflammatory, anti-carcinogenic, and providing cardiovascular protection (Han, Shen, & Lou, 2007). fPolyphenols are classified into sub categories based on their simple or complex structures on the number of phenolic rings in their structure and include phenolic acid, flavonoids, stilbenes, tannins, and lignans (Han et al., 2007). Color of fruits and vegetables are strongly associated with their polyphenol component. Berries with their bright red and blue color are high in the polyphenol anthocyanins (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Red raspberries, specifically, are a great source of the polyphenols anthocyanins (a subclass of flavonoids) and ellagitannins (hydrolysable tannins; Jean-Gilles et al., 2012).

Oxidative stress plays a vital role in degenerative diseases including OA due to its role in alteration of proteins, lipids, and nucleic acids (Han et al., 2007). Free radicals are compounds with one or more unpaired electron that can alter structures when seeking electrons (Lobo, Patil, Phatak, & Chandra, 2010). Polyphenols act as antioxidants and can help with oxidative stress through their ability to accept an electron, thus stabilizing the free radicals and ultimately inhibiting the chain oxidation reactions (Pandey & Rizvi, 2009). Free radicals that contain oxygen are called reactive oxygen species (ROS), and

when overproduced, can lead to an imbalance of ROS and antioxidant defenses. This imbalance results in a state called “oxidative stress” which can result in impaired cell function (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Increased ROS levels have been associated with a higher presence of degenerative aging diseases such as OA (Lobo et al., 2010). Chondrocytes, cells that play a vital role in maintaining cartilage matrix, can be negatively affected by increased levels of ROS. This results in increased apoptosis of chondrocytes, causing degradation of cartilage, inflammation, and the progression of OA (Krasnokutsky et al., 2008).

Oxidative stress and inflammation are two key players in the development of cardiovascular disease induced through endothelial dysfunction and its role in blood pressure and atherosclerosis development. Endothelial tissue is one of the most susceptible tissues to oxidative stress (Burton-Freeman, Sandhu, & Edirisinghe, 2016). A study by Suh et al (2011), found that when hamsters were fed raspberry juice, it resulted in lower LDL cholesterol while increasing HDL cholesterol when compared to placebo. In a study using hypertensive rats showed raspberry extract increased nitric oxide activation, decreased vasoconstriction, and resulted in an overall improvement in endothelial function (Jia et al., 2011). Anthocyanins have reduced LDL oxidation, lipid peroxidation, DNA damage, inflammation markers, and ROS generation, while increasing antioxidant enzyme activity associated with cardiovascular disease (Burton-Freeman, Sandhu, & Edirisinghe, 2016).

Fruit and vegetable intake have been proved to be inversely related to cancer incidence, with a focus on fruits and vegetables high in polyphenols. Studies have investigated different sources of polyphenols such as pomegranates, green tea, and

berries and their effect on a variety of cancers. In a study by Pan et al. (2015), it was concluded that consumption of freeze-dried black raspberries was beneficial in individuals with colorectal cancer through its effect on apoptosis, proliferation, and angiogenesis. A 15 year study investigated a variety of berries and their effect on esophageal and colon cancer in rats. The study findings suggested that berries are beneficial at inhibiting chemically-induced esophageal cancer in rats by 30-60% and 80% for colon cancer (Stoner et al., 2007).

## Polyphenols and OA *In Vitro* Studies

Polyphenols from a variety of food sources have been studied using cell models in order to show their beneficial effects on inflammation, OA, and cartilage health. A study by Jean-Gilles et al. (2012), used IL-1β to stimulate proteoglycan and type II collagen release from bovine nasal explant cell culture. When treated with red raspberry extract enriched with polyphenols, there was a significant decrease in the degradation of the matrix structure in treated cells (Jean-Gilles et al., 2012). Curcumin, the main component of the spice turmeric, is classified as a curcuminoid polyphenol. A study by Shakibaei Shakibaei, John, Schulze-Tanzil, Lehmann, and Mobasheri (2007) showed that curcumin has a positive effect in human chondrocytes by reducing inflammatory markers IL-1β and tumor TNF-α, as well as showing properties of being a COX-2 inhibitor. Similar results have been shown with resveratrol in human chondrocytes, by protecting against IL-1β inflammatory effects and preventing chondrocyte apoptosis (Dave et al., 2008).

## Polyphenols and OA *In Vivo* Studies

### Animals

A collagen induced rat model of arthritis, investigated the effects of red raspberry extract enriched with polyphenols. The findings demonstrated a significant improvement in cartilage protection and inflammation in ankle joints of rats that received a high dose of the raspberry extract (Suh et al., 2011). In a rat model with collagen-induced OA, raspberry extract was shown to improve swelling, osteophyte formation, and cartilage destruction when compared to a control group (Figueira et al., 2014). A research study investigating surgically induced OA in rabbits with four treatment groups, a control and three dose levels of resveratrol (10, 20, 50nmol/kg), demonstrated a cartilage protective effect with increasing raspberry extract dose, with all doses showing a significant difference from the control group. The benefits to the cartilage included decreased cartilage destruction, chondrocyte apoptosis, and nitric oxide levels in the synovial fluid (Wang, Gao, Chen, Li, & Tian, 2012). Another study using the rabbit model of OA investigate pomegranate extract on inflammatory serum markers with a pre- and post-treatment comparison. The rabbits were fed 10mL of polyphenolic rich pomegranate extract. Blood was collected for comparison pre-treatment and two hours post-treatment. The study identified a significant decrease in COX-1 and COX-2 activity and inhibition of IL-β induced nitric oxide production (Shukla, Gupta, Rasheed, Khan, & Haqqi, 2008).

### Humans

A clinical study by Schrager, Hilton, Gould, and Kelly (2015) investigated the effects blueberry juice consumption on gait performance. The authors found a significant improvement in gait speed in the blueberry juice group when compared to placebo group

(Schrager et al., 2015). A recent study by Du et. al (2019), investigating whole freeze-dried blueberry powder consumption, found an improvement in gait performance and a reduction in inflammation and pain levels in the blueberry group when compared to the placebo group. A study evaluating 200mg of curcumin supplementation in individuals suffering from OA for a 3-month period demonstrated a significant increase in walking distance on a treadmill and a decrease in serum C-reactive protein (CRP) levels when compared to the control group (Belcaro et al., 2010). A 6-week placebo-controlled randomized clinical trial by Ghoochani, Karandish, Mowla, Haghighizadeh, and Jalali (2016) investigated the effects of pomegranate juice on individuals suffering from OA. The study's findings indicate an improvement in physical function and a reduction in pain levels, stiffness, and serum enzymes responsible for cartilage degradation in the treatment group when compared to the control group (Ghoochani et al., 2016).

## Raspberries

Studies have demonstrated that the bioactive component present in raspberries affect metabolic, oxidative, and inflammatory conditions (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Red Raspberries (*Rubus idaeus* L.) are a rich source of micronutrients (vitamin C, magnesium, and vitamin K), fiber (6.5g fiber/100g fresh berries), and polyphenolic compounds, specifically anthocyanins (92.1+- 19.7 mg anthocyanins/100g fresh) and ellagitannins (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Pomegranate, blueberries, and raspberries are all rich sources of antioxidant compounds, especially anthocyanins. Research investigating the benefits of red raspberry polyphenols include inflammatory diseases such as Crohn's and other gastrointestinal inflammatory conditions. A study concluded that red raspberry supplementation facilitated epithelial

tissue repair and reduced risk of cancer development in mice with induced colorectal inflammation (Bibi, Du, & Zhu, 2018). Red Raspberries also have shown benefits with cardiovascular disease with various cell model studies showing a reduction in LDL oxidation, lipid peroxidation, DNA damage, and higher antioxidant enzyme activity (Burton-Freeman, Sandhu, & Edirisinghe, 2016). Red raspberries facilitate an anti-inflammatory action through inhibition of COX-2, but this has only been shown *in vitro* (Seeram et al., 2001). Dietary polyphenols are known for their antioxidant properties and have been commonly studied for their anti-inflammatory role in varies conditions. Existing data implicates a benefit of polyphenolic rich foods, with promising affects on symptoms associated with inflammation of OA, but no clinical trial has investigated the effects of whole raspberries on physical activity levels, gait performance, and cartilage damage in individuals with OA.

# CHAPTER III

# METHODOLOGY

## Study Design

A double-blinded, randomized placebo-controlled pre-test and post-test design procedure was used with an evaluation at baseline (before treatment intervention), during (midpoint), and after intervention. A total of 63 participants (both men and women) with a self-reported mild to moderate degree of pain in the knee due to symptomatic OA were recruited. Participants were recruited from Texas Woman's University (TWU) via email, through local Denton organizations/living facilities associated with active independent living older adults via email/newspaper advertisement, and through social media platforms such as Facebook (see Appendix A for recruitment flyer). All participants agreed to restrain from use of COX-2 inhibitors, chondroitin sulfate, glucosamine sulfate, or glucosamine hydrochloride during the study due to their potential effect on reducing symptoms of knee pain. Additionally, participants agreed to not consume other berries or berry products throughout the duration of the study.

Eligible participants were systematically randomized to either the raspberry treatment *(n = 34)* or control group *(n = 29)*. The treatment group consumed 35 grams of freeze-dried raspberry powder, while the control group consumed 35 grams of placebo powder equivalent in color, fiber, carbohydrates, and calories compared to the freeze-dried raspberry powder, consumed once per day for the duration of the 16-week study. The participants were instructed to mix two scoops (leveled off) of assigned treatment with 10-12 ounces of water to consume immediately at any desired time throughout the day. Participants remained on their respective treatment for the duration of the study. The

study protocols were approved by the Institutional Review Board at Texas Woman's University before any clinical work was initiated (see Appendix B).

**Inclusion/Exclusion Criteria**

Qualification for inclusion or exclusion was assessed through phone screening (see Appendix C) with an extensive medical history questionnaire including demographic information, medical history, smoking history, medication/supplement list, special diet, and food allergies. Inclusion criteria includes individuals between the age of 45 and 79 with symptomatic OA. Exclusion criteria included individuals that were medicating with drugs/supplements that influence OA; drugs that are classified as COX-2 inhibitors, supplemental chondroitin sulfate, glucosamine sulfate, glucosamine hydrochloride, and berry products. Additionally, a history of liver, kidney, heart, or any other acute or chronic disease that could affect their conditions, and heavy smokers defined as smoking more than 20 cigarettes per day and those who have undergone knee replacements were excluded from participation.

**Baseline, Midpoint, and Final Measurements**

Qualified participants were invited to participate in the 16-week study with 3 study visits (baseline, midpoint (8 weeks), and final). At baseline, participants were randomly assigned to the raspberry powder treatment *(n = 34)* or control group *(n = 29).* At baseline visit, participants were provided with a written consent form (see Appendix C), which informed participants of every aspect of the study; any questions or concerns were answered prior to starting the study. At each visit, anthropometric measurements were obtained including height, weight, leg length, and blood pressure with all measurements obtained three times and the average recorded. Furthermore, a fasting

blood draw, gait performance (walk pattern test), and physical activity questionnaire were collected at each visit.

## Treatment Compliance

Treatment compliance was tracked using a calendar for daily consumption of the designated treatment powder as well as a section to document over the counter pain medication use. Calendars were provided to participants at baseline and midpoint visits; participants were instructed to bring the calendar back to following visit. Participants were eliminated from study with < 80% compliance. Follow-up phone calls and emails were made to ensure compliance and answer any concerns with treatment consumption.

## Blood Collection and Inflammatory Marker Analysis

Participants were instructed to fast overnight (at least 8 hours) prior to blood isolation at which time, venous blood was obtained by a certified phlebotomist at baseline, midpoint, and final visits. The blood was centrifuged at 1500x g for 15 minutes and serum aliquoted within 2 hours of collection; then stored at -70°C until analysis. YKL-40 was assessed as a marker of cartilage degradation and analyzed with enzyme-linked immunosorbent assay (ELISA) kits from Quidel Corporation (San Diego, California). The MicroVue YKL-40 assay was performed in three steps. First, serum samples were mixed in YKL-40 antibody coated plates. Second, samples were exposed to alkaline phosphate conjugated rabbit anti-YKL-40, binding to the immobilized biomarker. A substrate solution then reacts with the conjugated enzyme causing a color change. Using a standard curve generated using a linear regression analysis, the color intensity was measured at an optical density of 405nm with color development proportional to YKL-40 concentration in the samples.

Human Free IGF-1 and Human IGFBP-3 were analyzed using quantitative sandwich enzyme immunoassay technique from R&D Systems Inc. (Minneapolis, Minnesota). Samples for both kits were diluted 100-fold in duplicate in assay buffer as concentrations were determined to be higher than assay standards. IGF-1 served as the biomarker for assessment of anabolic cartilage homeostasis and IGFBP-3 served as an assessment for IGF-1 availability. Kits for both markers followed the same procedure. The microplate is pre-coated with IGF-1 or IGFBP-3 specific antibody, which causes the samples and standards to be bound by the immobilized antibody adherence to the plate. Through a series of washes, additional unbound substances are washed away. Secondly, an enzyme-linked monoclonal antibody specific to IGF-1 or IGFBP-3 is added to the well; a series of washes removes unbound antibody-enzyme reagent. Third, a substrate is added in order to react with the enzyme causing a color change. Using a standard curve generated using a linear regression analysis; the color intensity was measured at an optical density of 450nm, with color level proportionate to the concentration of IGF-1or IGFBP-3 levels in the samples.

Hyaluronic acid, a biomarker that serves as an assessment for anabolism of the cartilage matrices. Hyaluronic acid was analyzed with ELISA kits from TECOmedical Group (Sissach, Switzerland) using a hyaluronic acid binding protein (HABP) coated microtiter plate. Samples were diluted 50-fold in assay buffer as concentrations were determined to be higher than assay standards. The ELISA was performed in three steps. First, serum samples were mixed in HABP coated microtiter plates. Second, samples were exposed to HABP-hyaluronic acid receptor protein (HRP) conjugate, binding to the immobilized biomarker. A substrate solution then reacts with the conjugated enzyme

causing a color change. Using a standard curve generated using a four-parameter curve, the color intensity was measured at an optical density of 450nm, with color intensity proportionate to the concentration of HA levels in the samples.

These four biomarkers served as an assessment of cartilage metabolism, cartilage degradation markers and anabolic growth factors associated with the cartilage matrix.

### Assessment of Gait and Mobility Analysis

Gait was analyzed by a trained research at baseline, midpoint, and final visits using a 10-meter GAITRite® system, a portable electronic pressure sensitive walkway used to measure gait abnormalities. Participants were instructed to walk at a normal walking cadence for a total of three trials which was then repeated with their fastest walking cadence, while not running. A 20 second break was inserted between each test run. The average of the three trials from each cadence was recorded for analysis. The GAITRite® system has been validated and used as a reliable measurement for multiple spatial (distance) and temporal (time) gait parameters (Webster, Wittwer, & Feller, 2005b).

Mobility analysis was assessed by the International Physical Activity Questionnaire (IPAQ; see Appendix E) administered to participants at baseline, midpoint, and final visits. IPAQ addresses vigorous and moderate activities of daily living, specifically with reference to their previous seven days. The questionnaire is split into five domains: job-related physical activity, transportation-related physical activity, housework related physical activity, recreational related physical activity, and average time spent sitting. A reference list of examples of vigorous and moderate activities were given to participants for assistance. Vigorous activity was defined as activities that

require hard physical effort that result in harder to breathe than normal. Moderate activity was defined as activities that require moderate physical effort and result in slightly elevated breathing. Due to attributes of the target population, three out of five domains were assessed, omitting job-related physical activity and transportation related-physical activity. Total walking time, and total moderate and vigorous activity were also assessed. IPAQ has been validated as a reliable questionnaire to assess diverse physical activity and inactivity (Craig et al., 2003).

## Statistical Analysis

A minimum total sample size of 60 participants was required for analysis with alpha = 0.5, power = 0.87, and a moderate effect size in mobility, gait, and markers of cartilage metabolism. Descriptive statistics were calculated for all variables, comprising means, and standard deviations for all variables. Statistical distribution was determined for all data to determine if normally distributed and parametric testing is appropriate, extreme outliers were removed as appropriate. Repeated measures ANOVAs were performed to determine significant outcomes between groups at each point in time as well as changes within the group over time for anthropometric measurements, serum test hyaluronic acid, YKL-40, IGF-1, and IGFBP-3, physical activity parameters and gait. All data was analyzed in SPSS 25.0.0.

# CHAPTER IV

# RESULTS

## Demographics

A total of 113 individuals were screened for the study with 95 meeting the qualification requirements. Of the 95 individuals, 63 agreed to start the study and were scheduled for an initial visit. Of the 63 participants, 17 were male and 46 were female. Over the duration of the study, 19 participants withdrew from the study due to lack of interest, lack of compliance, or experiencing gastrointestinal issues with powder consumption. A total of 44 participants completed the study while maintaining compliance. Data on recruitment of participants, compliance, and drop out is shown in Table 1.1 , with demographics highlighted in Table 1.2.

There was no significant difference through the duration of the study for body weight (see Figure 2). The raspberry treatment group had a starting body mass index (BMI) of 29.7 $kg/m^2$ and the placebo group with a BMI of 30.1$kg/m^2$. There was no statistically significant difference between participants' BMI through the duration of the study (see Figure 3). There was no significant difference in systolic *(p* = .234) or diastolic *(p* = .755) blood pressure between groups at baseline visit; however, the raspberry group showed a significant decrease in both systolic *(p* = .022) and diastolic *(p* = .021) blood pressure from baseline to end of study (see Figure 4 and Figure 5).

## International Physical Activity Questionnaire (IPAQ)

There was no significant difference between treatment groups at baseline for any of the domains being assessed. For average sitting time, measured minutes per day, there was a significant decrease in average sitting time per day in the raspberry treatment group

from baseline to end of the study *(p* = .018); as well as from 8 weeks to 16 weeks *(p* = .04). The placebo group did not show a significant change from baseline to end of the study in average sitting time (see Figure 6). For housework-related physical activity measured in metabolic equivalents (METs), the raspberry group over time showed a significant increase from 8 weeks to end of the study *(p* = .05). The placebo group showed no significant changes over the duration of the study in home-related physical activity over time (see Figure 7). There were no significant changes over time for either treatment group for recreational-related physical activity, total walking, total moderate physical activity, and total vigorous physical activity (see Figures 8-11).

## Cartilage Biomarkers

The two markers for cartilage formation, serum hyaluronic acid and serum IGF-1, showed no statistical significance between treatment groups. Concentrations of hyaluronic acid stayed steady throughout the study for both treatment groups with no statistically significant changes (see Figure 12-13).

Serum levels of IGFBP-3, were measured as indicators of bioavailability of IGF-1 being bound and unavailable for cartilage tissue. There was a statistically significant increase in the placebo group IGFBP-3 serum levels from baseline to 8-week period *(p* = .001) as well as from baseline to 16-week period *(p* = .005). The raspberry group showed consistent concentrations of IGFBP-3 serum levels throughout the study without any statistically significant changes between time points (see Figure 14).

Serum levels of YKL-40 were used as a marker to assess cartilage degradation. There was a significant increase in YKL-40 serum levels over time from baseline to 16

weeks in the placebo group *(p* = .004). The raspberry group showed consistent concentrations of YKL-40 serum levels throughout the duration of the study without significant changes (see Figure 15).

## Gait Performance

Gait performance was analyzed using the GAITRite® system, a portable 10-meter electronic walkway. The parameters that indicate an improvement in gait includes a decrease in velocity, cadence, single leg support percentage, and cycle time; while increasing in double leg support. Baseline differences between groups were observed only in fast walking cadence and fast walking right leg step length, all other parameters showed no statistical difference at baseline. At a normal walking cadence, the raspberry treatment group showed improvements in cadence, left leg double support percentage, and both legs cycle time. However, the placebo group showed significant changes at a normal walking cadence, in the parameters cadence, velocity, both legs single support percentage, both legs double support percentage, step length, and cycle time.

At a fast walking cadence, the raspberry treatment group showed a significant increase from baseline to 8 weeks *(p* = .004); as well as from baseline to end of the study *(p* = .011). The placebo group showed no statistical significance in fast walking cadence over time (see Figure 16). For velocity, there was a significant increase in fast walking velocity in the raspberry group over time from baseline to 8 weeks *(p* = .012) as well as baseline to the end of the study *(p* = 0.04). The placebo group showed no significant changes in fast walking velocity over time (see Figure 17).

For single support of cycle percentage and double support of cycle percentage at fast walking cadence, the placebo group showed no significant changes over time for either leg at either cadence. However, the raspberry group showed a significant increase in left leg single support cycle percentage at fast walking cadence between baseline to 8-weeks *(p* = 0.004), as well as baseline to 16-week period (*p* = 0.029; see Figure 18). Additionally, the raspberry group showed a significant decrease in left leg double support cycle percentage between baseline and 8-week period *(p* = 0.02) and from baseline to end of the study *(p* = 0.025). The raspberry group also showed a significant decrease in right leg double support cycle percentage between baseline to 8-week period *(p* = 0.026) and from baseline to end of the study (*p* = 0.029; see Figure 19). At a fast cadence, neither group showed statistically significant changes in left or right step length over time (see Table 1.3).

No significant changes were evident in the placebo group for both left and right leg cycle time at a fast walking cadence. The raspberry treatment group showed a significant decrease in left leg cycle time at fast walking cadence between baseline and 8-week period *(p* = .002) and from baseline to the end of the study (*p* = .011). Additionally, the raspberry group showed a significant decrease in right leg cycle time at fast walking cadence between baseline and 8-week period (*p* = .002) and from baseline to end of the study (*p* = .005; see Figure 20).

# CHAPTER V

# DISCUSSION

OA is the major cause of disability and loss of function amongst the elderly population (Shen et al., 2012). Individuals suffering from OA usually experience a decrease in physical activity due to exacerbated pain with movement. This study determined changes in levels of physical activity as assessed by the IPAQ questionnaire. Raspberry treatment resulted in a decrease in total average sitting time while increasing in home-related physical activities from baseline to final point with a 21% decrease and a 51% increase, respectively. A study by Ghoochani et. al (2016) suggested an improvement in physical function in individuals with OA, when consuming pomegranate juice, a fruit also rich in polyphenols. Belief that physical activity worsens the condition of OA rather than improving is a misconception. A systematic review investigating the safety of physical activity and OA confirmed long-term therapeutic exercise poses a beneficial effect rather than harm (Quicke et al., 2015). Physical activity is important for individuals suffering from OA and can actually improve pain, function, and QOL while delaying disability and other comorbidities. Our findings suggest raspberry consumption may help individuals with OA improve their physical activity and functional abilities, therefore improving QOL.

In our study, serum HA for both the raspberry and placebo treatment groups were maintained throughout the study. However, the raspberry group showed an incline trend and the placebo group a decline trend. When cartilage is broken down HA enters the synovial fluid, which is removed into the blood stream. (Moreland, 2003). One study by Pavelka et. al (2004), showed a direct relationship between radiological progression of

OA and HA serum levels. Since the stage of OA and joint space was not addressed in this study, HA levels may not have changed due to OA not showing substantial progression in disease to impact serum levels.

IGF-1 is the predominant growth factor that contributes to the anabolic process associated with cartilage matrix, with a vital role in adults for maintenance of cartilage homeostasis (De Ceuninck et al., 2004). Our study did not show a significant change in IGF-1 in the raspberry group. IGF-1 is responsible for the homeostasis of the cartilage while IGFBP-3 regulates the accessibility of IGF-1 to its receptor, showing an inverse relationship in an arthritic joint (De Ceuninck et al., 2004). IGF-1 has shown to increase in individuals with OA due to pro-inflammatory cytokines stimulating release; but chondrocytes have been less responsive to the anabolic effect in the arthritic joint partially due to the increased presence of IGFBP-3 (Hooshmand et al., 2015). IGFBP-3 levels have shown to increase in individuals with OA, therefore binding to IGF-1 inhibiting the use of the anabolic mechanism of cartilage (De Ceuninck et al., 2004). Our study showed no statistical changes in serum IGFBP-3 levels in the raspberry treatment group. However, the placebo group did experience a significant rise in IGFBP-3 levels as early as the 8 weeks period and continued to increase until the end of the study in comparison to baseline measurements. These results suggest that incorporating raspberries into the diet can act against increasing IGFBP-3 serum levels therefore allowing more IGF-1 to be available for cartilage synthesis.

YKL-40 is a biomarker associated with synovial inflammation and joint destruction. YKL-40 levels have been shown to be significantly increased in both serum and synovial fluid in patients suffering from OA (Conrozier et al., 2000). Our data

suggests that including raspberries in the diet helps to maintain YKL-40 serum levels, while the placebo group showed a progressive increase in YKL-40 serum levels through the duration of the study. When comparing the 16-week point to baseline, the placebo group had 21% increase in YKL-40 serum levels, where the raspberry treatment group actually had a 2.5% decrease in YKL-40. A cross-sectional study found similar results with a 36% increase in YKL-40 serum levels in individuals suffering from hip OA when compared to a control group (Conrozier et al., 2000). A cell model study by Lucero, Vijayagopal, and Juma (2014) found similar results with whole grape polyphenols suggesting a significant decrease in YKL-40 levels in chondrosarcoma cells that were treated in comparison to the control group. Our data suggests that raspberry consumption can prevent further cartilage degradation and progression of OA.

Gait performance was measured using indicators of step length of left and right leg, single support percentage of cycle of left and right leg, double support percentage of cycle of left and right leg, cycle time, velocity, and cadence. All of these gait parameters were assessed at both a normal and fast walking cadence. At a normal walking cadence, the raspberry treatment group showed improvement in cadence, double support percentage of both legs, and cycle time of both legs. However, the placebo group showed significant changes at a normal walking cadence in cadence, velocity, single support percentage of both legs, double support percentage of both legs, step length, and cycle time. Due to both groups showing significant difference at normal cadence it can be concluded the placebo group experienced the placebo effect at a normal walking cadence due to perceived decrease in pain levels. However, when observing at a fast walking cadence, the placebo group showed no significant improvement through the duration of

the study. The raspberry treatment group, on the other hand, showed gait improvements in cadence, velocity, single support percentage of left leg, double support percentage of both legs, and cycle time of both legs through the duration of the study. Improvement was shown as early as the 8-week period and continued to show improvement to end of the study. Spatiotemporal gait analysis has correlated with stage of OA. Improvement in gait performance was evident based on a decreased double leg support percentage, and cycle time; whereas, an increase in velocity, cadence, single leg support. Where double leg and single support cycle make up one complete cycle, as one increases, the other decreases. Increased single leg support percentage and decreased double leg support percentage is associated with decreased pain levels and improvements of overall function of the knee (Elbaz et al., 2014). A study investigating the effects of blueberry consumption and gait found improvements in the normal walking cadence of the blueberry group, but with no significant results at a fast walking cadence (Du et al., 2019). Raspberry consumption suggests improvements in gait performance at both a normal and fast walking cadence.

The study has some limitations which could have impacted the outcomes. First, the study did not determine stage of OA, and therefore, it was harder to distribute participants based on disease progression. Additionally, sex differences were not assessed and thus the effects of hormonal effects on OA progression were not assessed. Testing through MRI or x-ray and joint aspiration to assess the stage of OA was not determined; this would have allowed for investigation of joint space narrowing and synovial fluid contents and cartilage residues. Additional biomarkers such as inflammatory markers and matrix metalloproteinases (MMPs) could have better helped to assess if raspberry

consumption could slow down the degradation of the cartilage matrix. The study would have benefitted from a higher number of participants in order to increase those completing while still recognizing the attrition rate. Palatability of the product was undesirable by some participants who reported mixing the powder with other juices or making into a smoothie; which could have affected the bioavailability of the polyphenols within the powder. Compliance was based on participants self-reporting by documenting on a provided calendar. Compliance could have better determined if participants were given pre-mixed daily bottles for consumption rather than being responsible for proper mixing and consumption. Participants were asked to incorporate raspberry/placebo powder with the only diet instruction, to avoid raspberry products and to not make significant changes within their diet. Further studies would benefit from either a cross-over study or further investigation into the composition of the participants diet. Additionally, further studies in the change of physical activity need to be conducted using a more reliable tool due to possible over reporting of physical activity. The IPAQ is a self-administered questionnaire used for the age population of 15-65 years old. Our population included an older age group up to the age of 79, which may have affected the results. Over reporting was suspected with the questionnaire. Additionally, the IPAQ may have shown more significant results in a larger population as to the questionnaire would have shown greater reliability and validity in larger populations.

In summary, our study investigated the effects of whole freeze-dried raspberry powder consumption for preventing cartilage degradation and improving quality of life through increased physical activity and gait performance in individuals with OA. Findings suggest that raspberries have a positive effect on cartilage degradation, gait

performance, and physical activity levels; therefore, improving quality of life in individuals with symptomatic OA. Future research is needed with a larger population size, more compliance control, and dietary control in order to strengthen the findings on the beneficial effects of raspberries on cartilage health and improvement of physical activity levels including gait performance in individuals with symptomatic OA.

Table 1.1
*Participant Screening and Drop Out Rate*

| Participants Screened | Qualified and Initiated Study | Completed Study | Participant Drop Out | Drop Out Rate |
|---|---|---|---|---|
| 113 | 63 | 44 | 19 | 30% |

Table 1.2
*Demographics of Study Participants*

| | | Baseline | Midpoint | Final |
|---|---|---|---|---|
| Raspberry Group | Male (n) | 9 | 7 | 6 |
| | Female (n) | 25 | 17 | 14 |
| | Total (n) | 34 | 24 | 20 |
| | Avg. Age | 62.76 | 62.00 | 62.14 |
| Placebo Group | Male (n) | 8 | 8 | 8 |
| | Female (n) | 21 | 16 | 16 |
| | Total (n) | 29 | 24 | 24 |
| | Avg. Age | 58.10 | 58.54 | 58.54 |
| Total | Male (n) | 17 | 15 | 14 |
| | Female (n) | 46 | 33 | 30 |
| | Total (n) | 63 | 48 | 44 |
| | Avg. Age | 60.43 | 60.27 | 60.34 |
| Drop Rate (%) | | N/A | 23% | 30% |

Table 1.3
*Effect of Raspberry and Placebo on Step Length*

| | | Raspberry Group | | | Placebo Group | | |
|---|---|---|---|---|---|---|---|
| Cadence | Parameters | Baseline | Midpoint | Final | Baseline | Midpoint | Final |
| Normal | Left Step Length (cm) | 56.03±1.44 | 58.59±1.78 | 58.47±1.34 | 58.59±1.51 | 62.10±1.84* | 62.92±1.90* |
| | Right Step Length (cm) | 55.84±1.37 | 57.99±1.78 | 58.04±1.37 | 59.47±1.43 | 62.69±1.74* | 63.47±1.89*,^ |
| Fast | Left Step Length (cm) | 67.52±1.40 | 68.62±1.82 | 68.33±1.95 | 73.23±2.03^ | 72.40±2.30 | 73.25±2.12 |
| | Right Step Length (cm) | 66.98±1.54 | 68.52±1.90 | 67.25±1.97 | 73.36±1.83 | 72.73±2.14 | 73.31±2.01 |

Table 3 Step Length. Mean ± SEM. $N = 20$ for Raspberry group, $N = 24$ for Placebo group.
*, significance as compared to baseline $(p < 0.05)$
^, significance as compared to Raspberry group $(p < 0.05)$

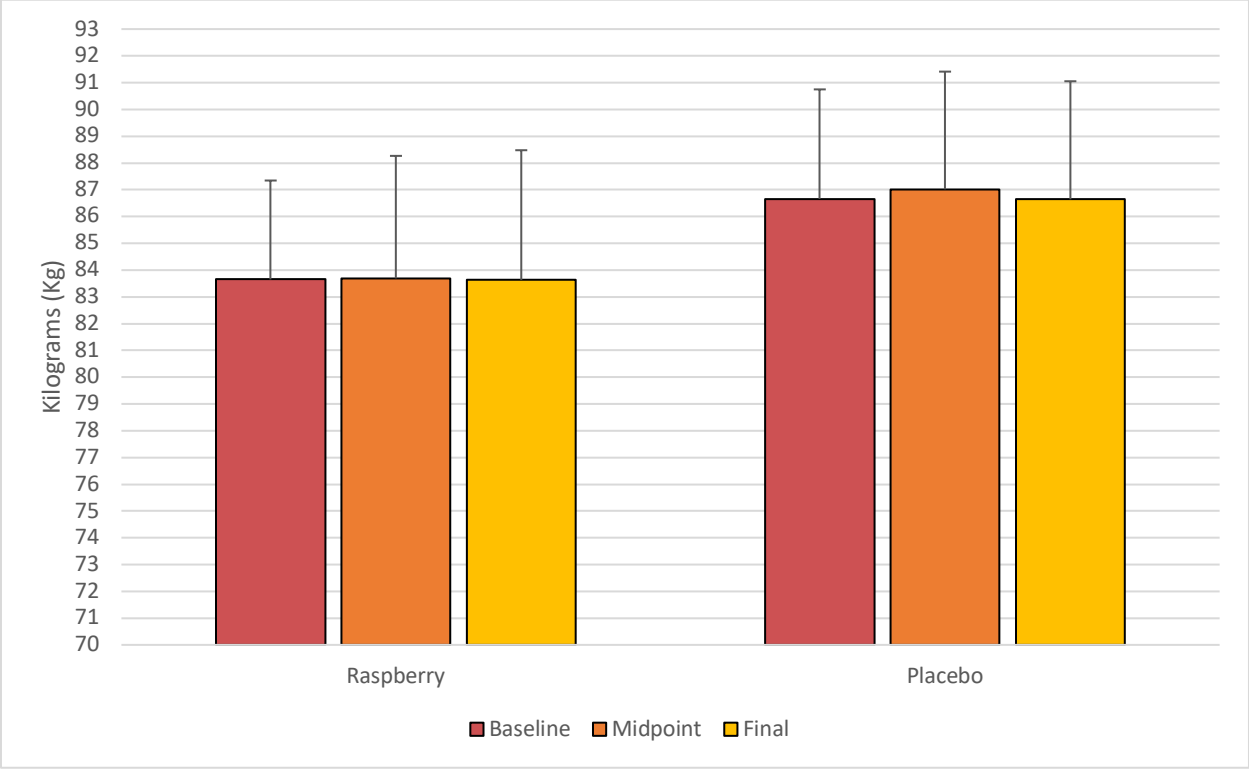



*Figure 2.* Effect of Raspberry and Placebo on Weight. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
No significant changes over time or between groups.

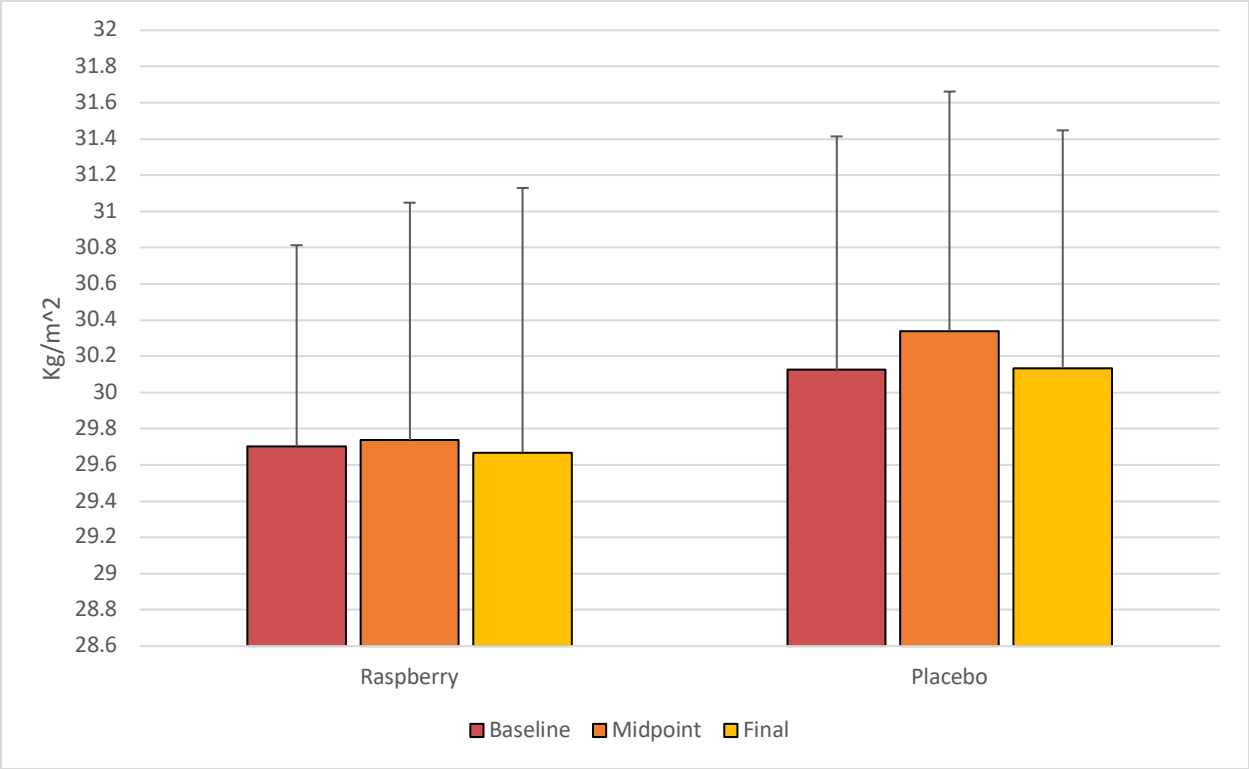



*Figure 3.* Effect of Raspberry and Placebo on BMI. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
No significant changes over time or between treatment groups.

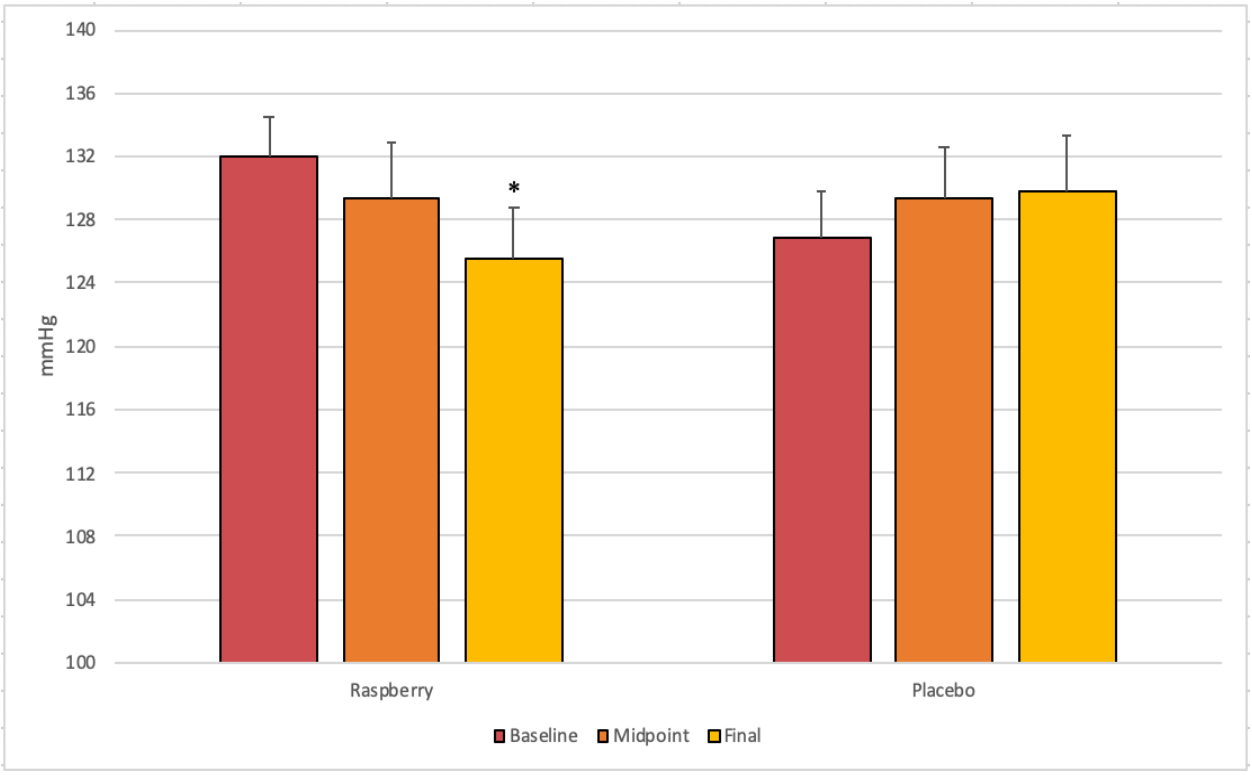



*Figure 4.* Effect of Raspberry and Placebo on Systolic Blood Pressure. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

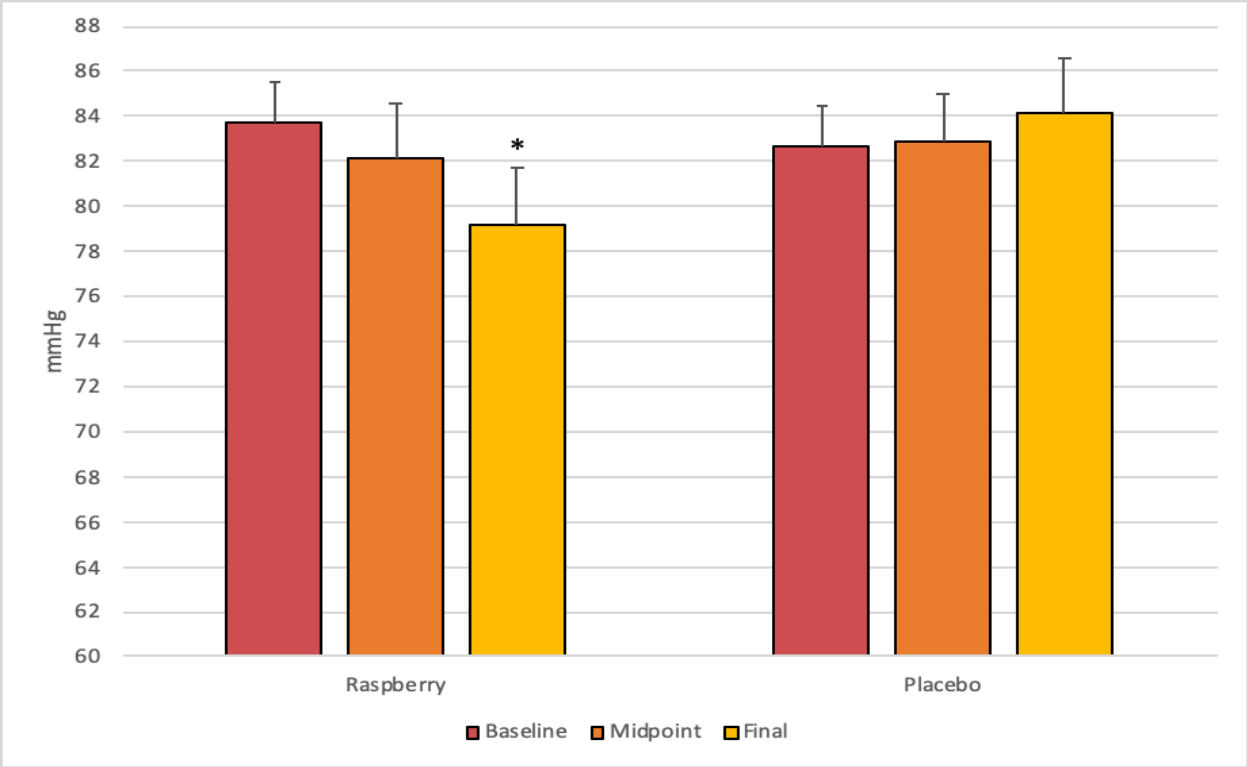



*Figure 5.* Effect of Raspberry and Placebo on Diastolic Blood Pressure. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

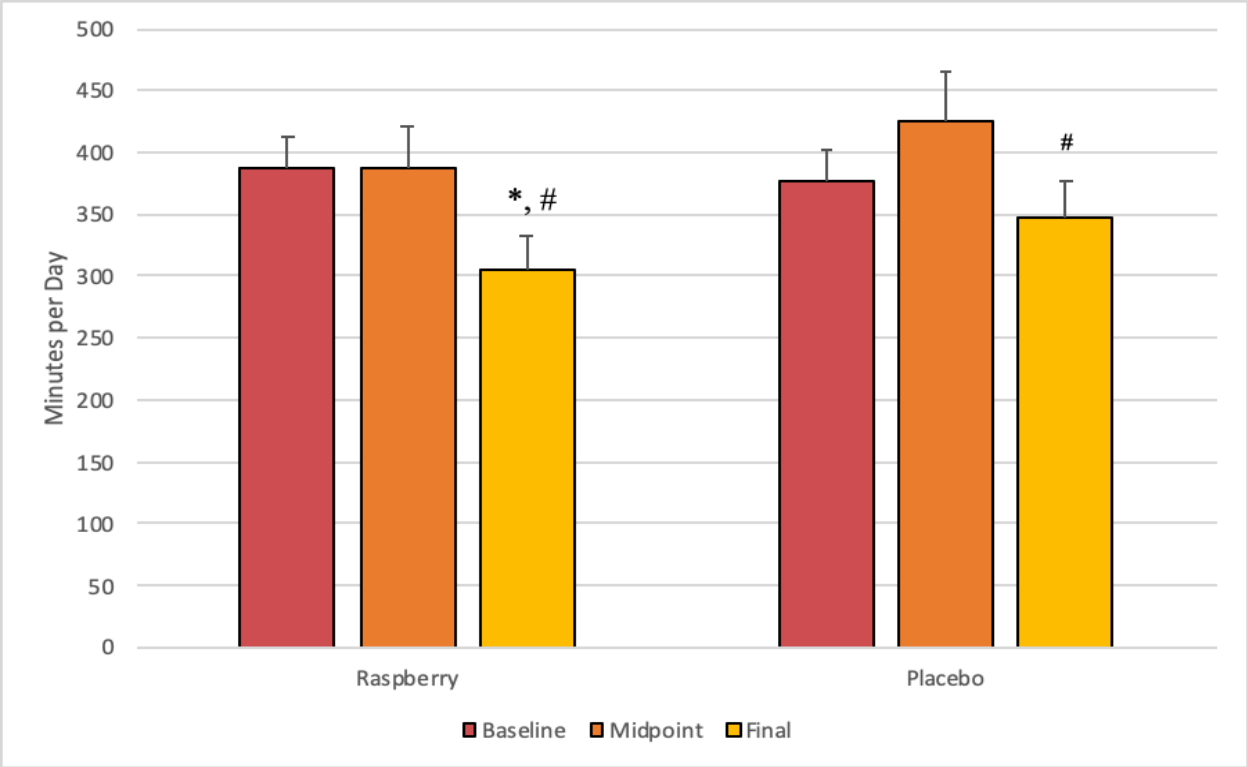



*Figure 6.* Effect of Raspberry and Placebo on Average Sitting Time. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*
#, significance as compared to midpoint *(p < 0.05)*

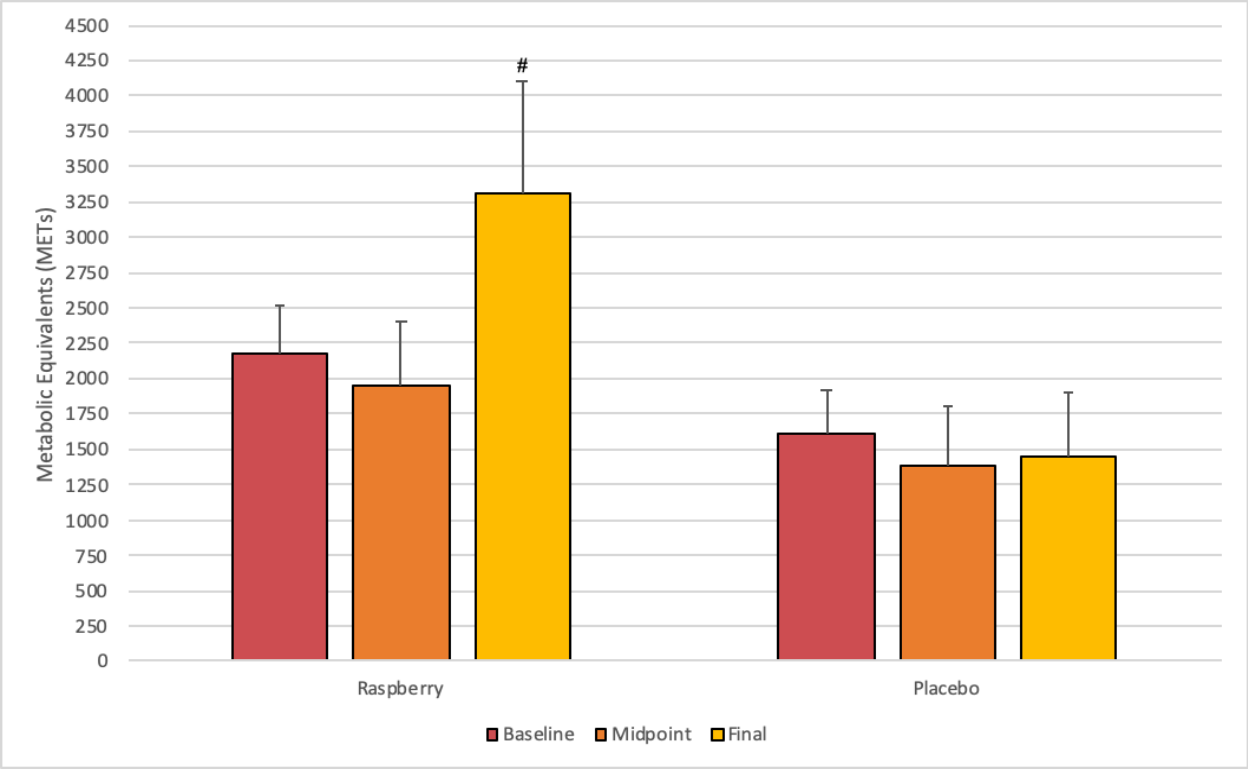



*Figure 7.* Effect of Raspberry and Placebo on Housework Related Physical Activity. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed.
#, significance as compared to midpoint *($p < 0.05$)*

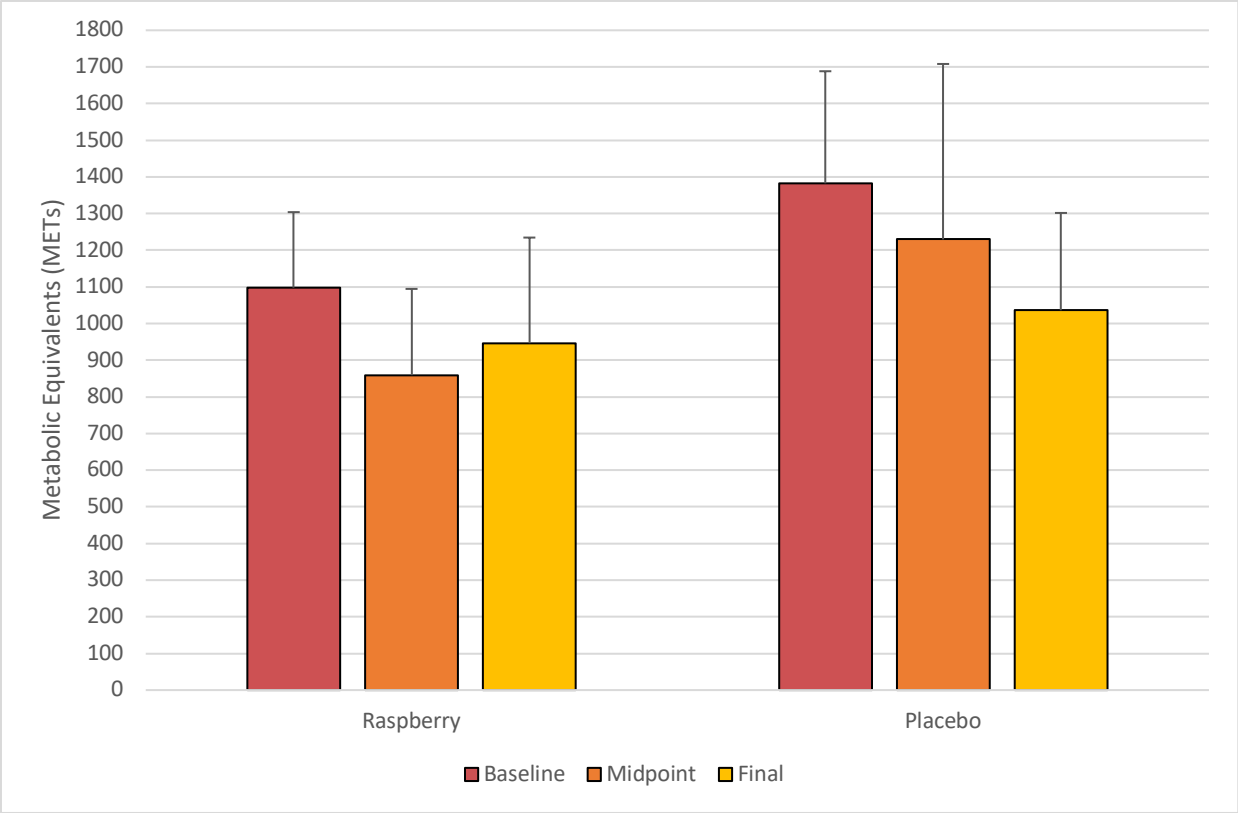



*Figure 8.* Effect of Raspberry and Placebo on Recreational Physical Activity. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed. No significant difference over time or between groups.

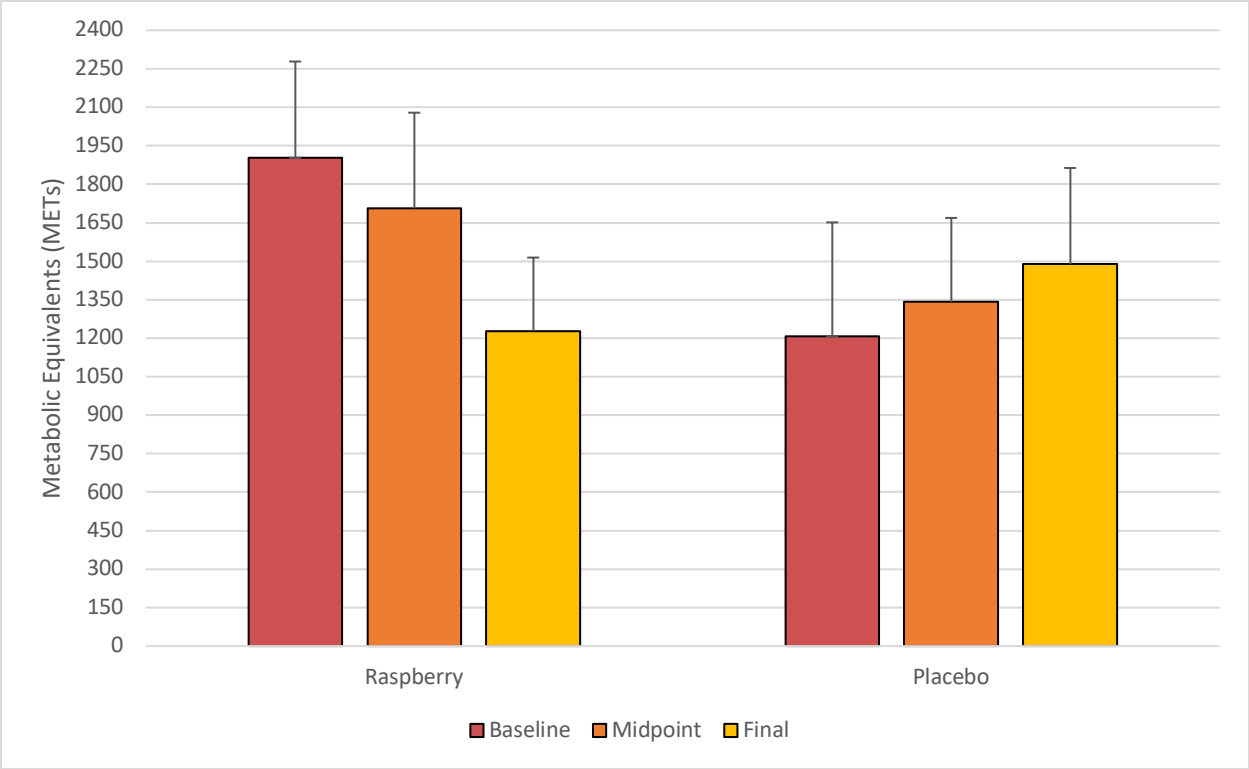



*Figure 9.* Effect of Raspberry and Placebo on Total Walking. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed.
No significant difference over time or between groups.

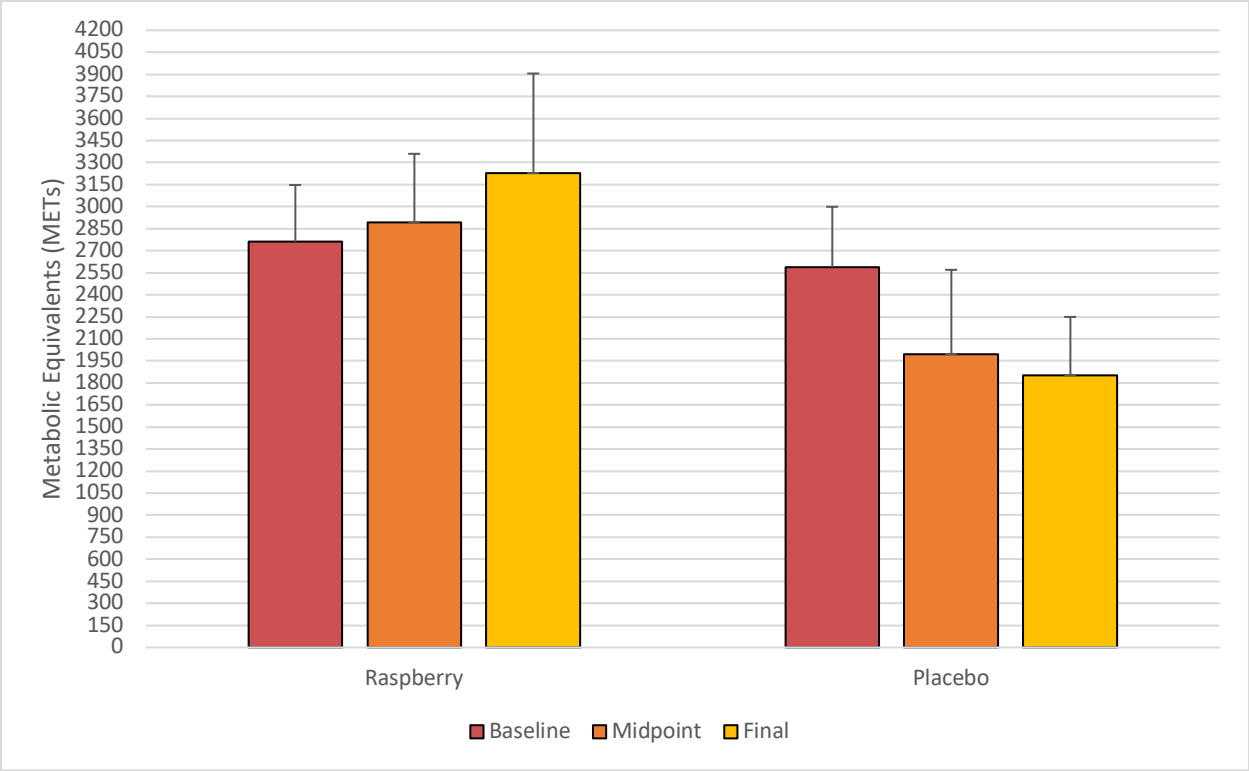



*Figure 10.* Effect of Raspberry and Placebo on Moderate Activity. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed.
No significant difference over time or between groups.

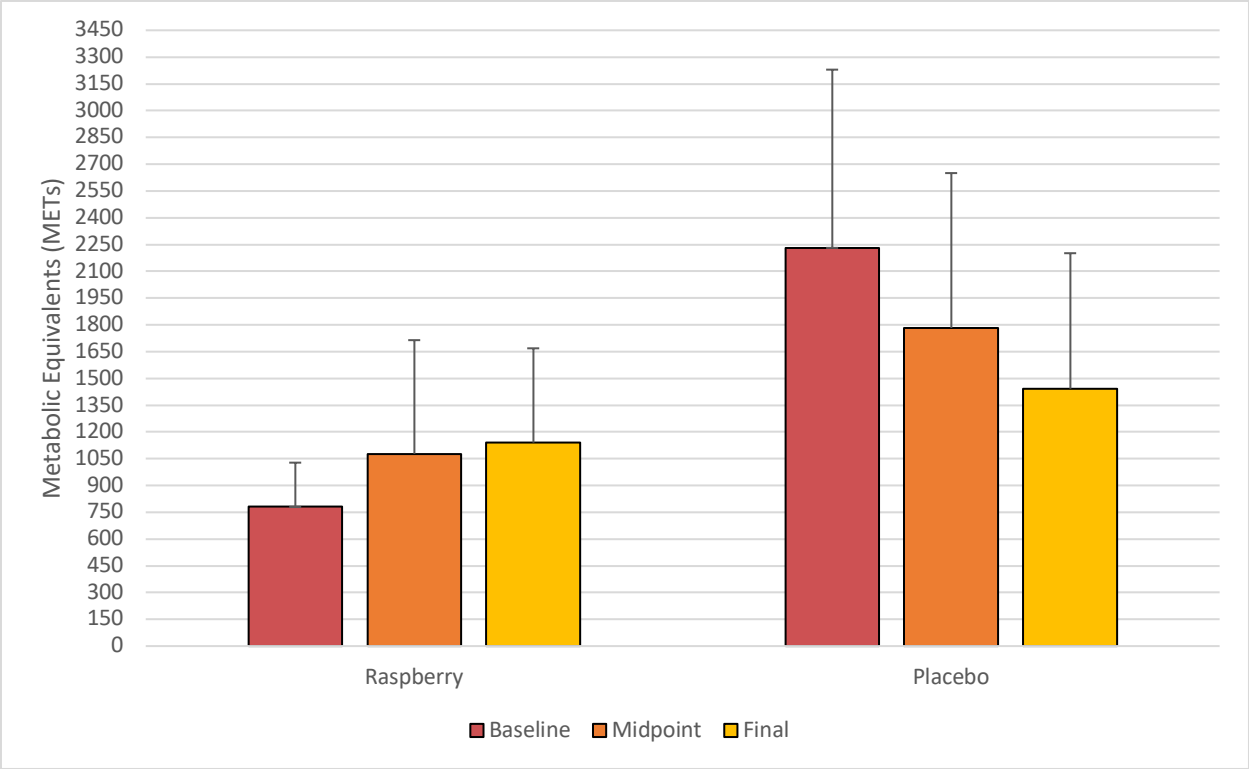



*Figure 11.* Effect of Raspberry and Placebo on Total Vigorous Activity. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed.
No significant difference over time or between groups.

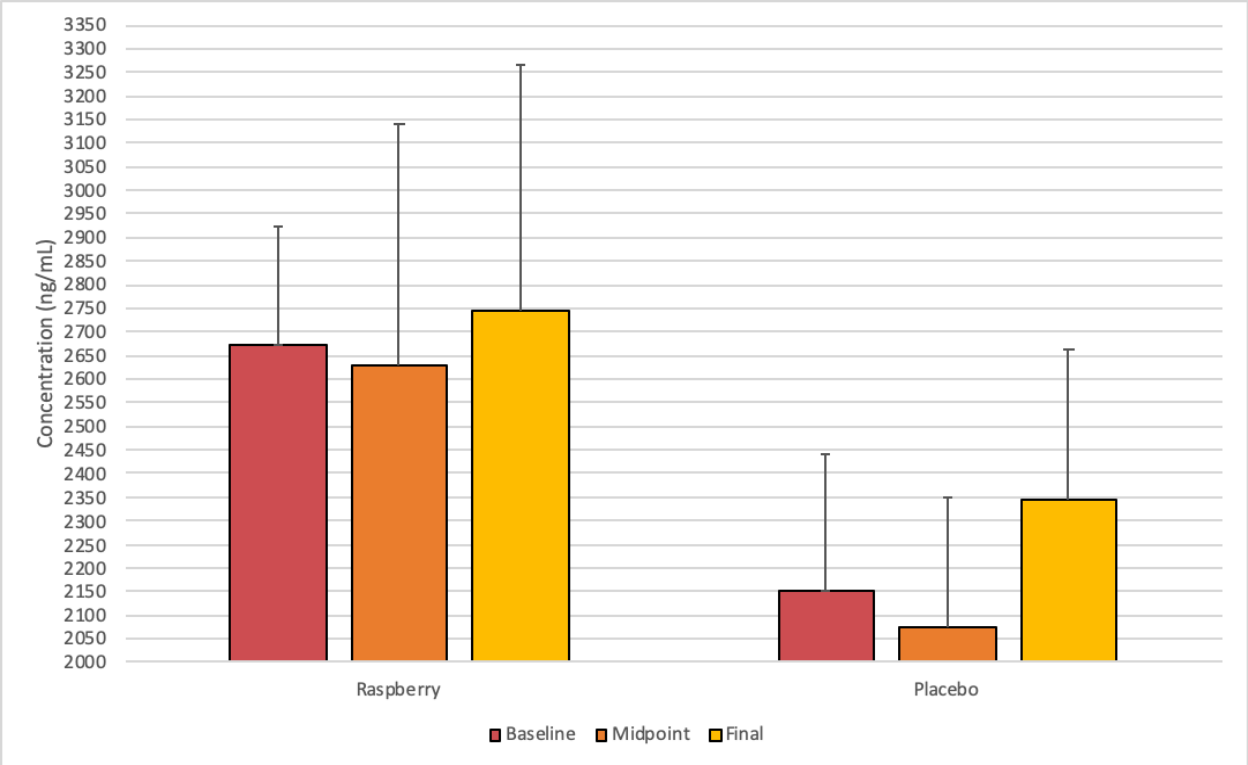



*Figure 12.* Effect of Raspberry and Placebo on Plasma Hyaluronic Acid Levels. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
No significant difference over time or between groups.

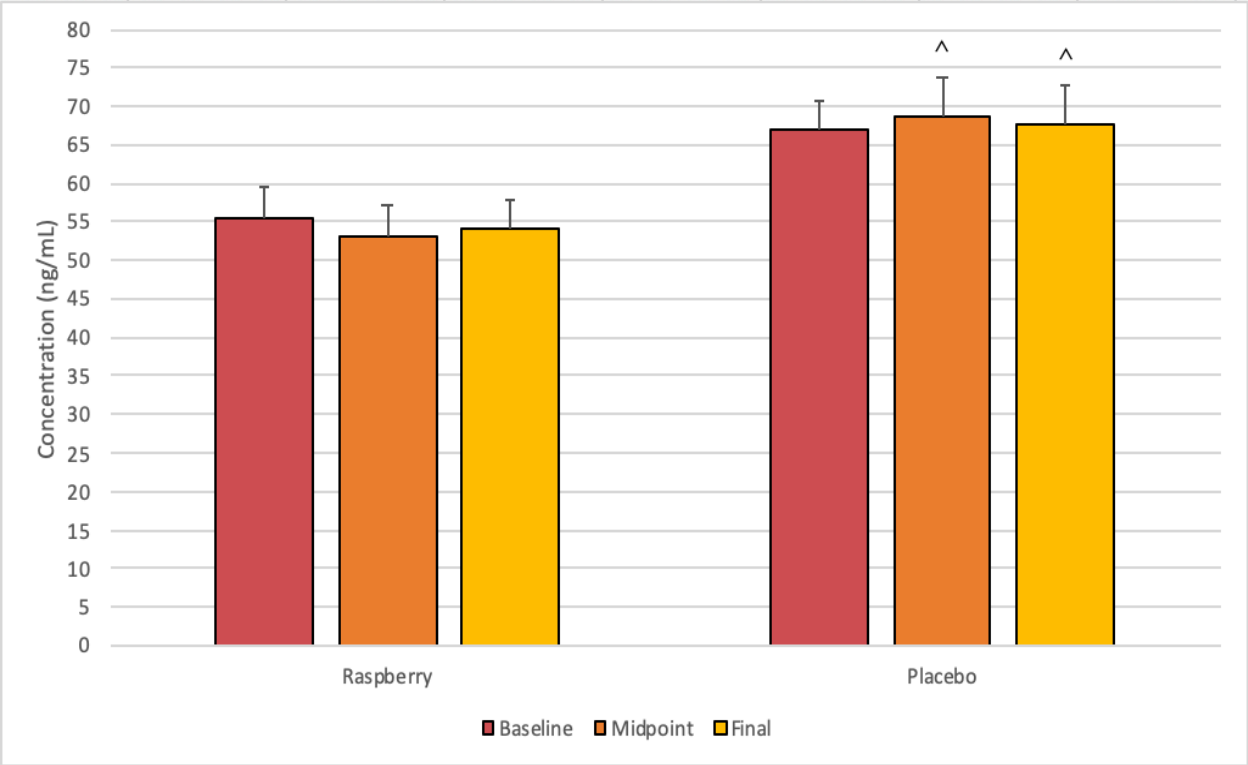



*Figure 13.* Effect of Raspberry and Placebo on Plasma Insulin-Like Growth Factor-1 Levels. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. ^, significance as compared to Raspberry group *($p < 0.05$)*

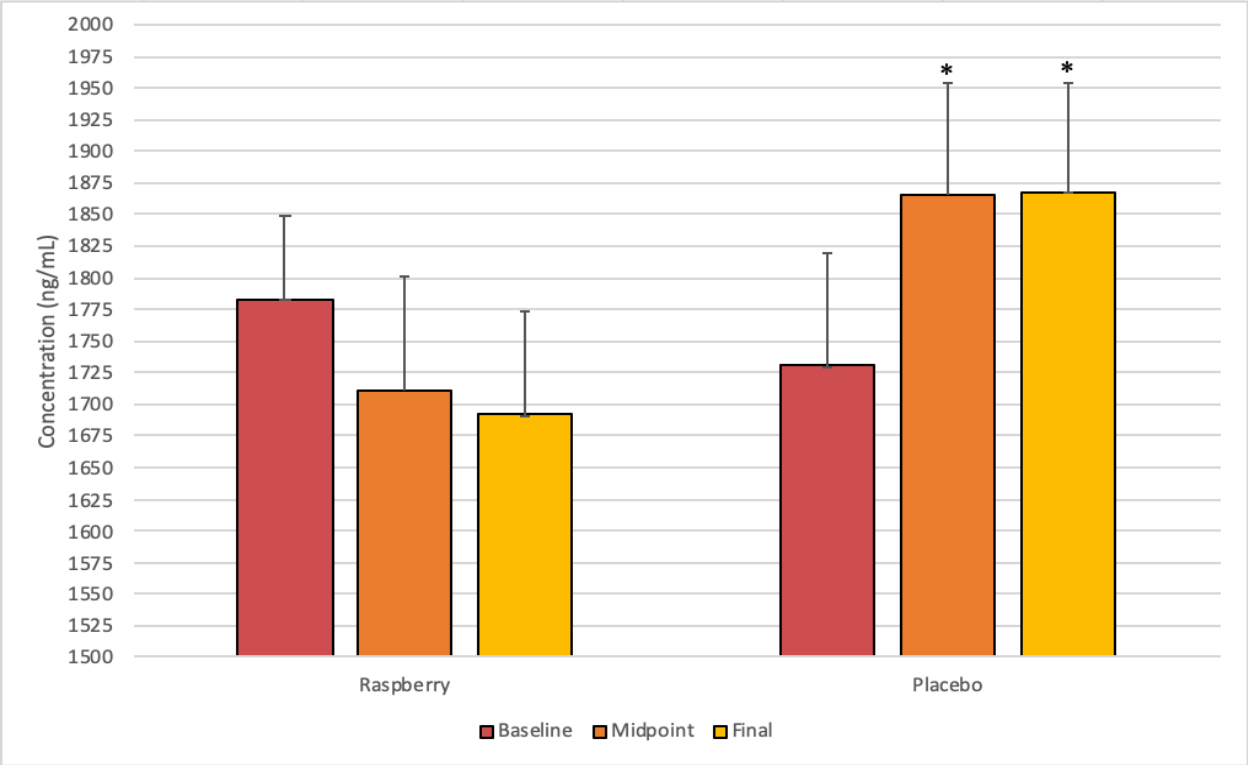



*Figure 14.* Effect of Raspberry and Placebo on Plasma Insulin-Like Growth Factor Binding Protein 3 Levels. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *($p < 0.05$)*

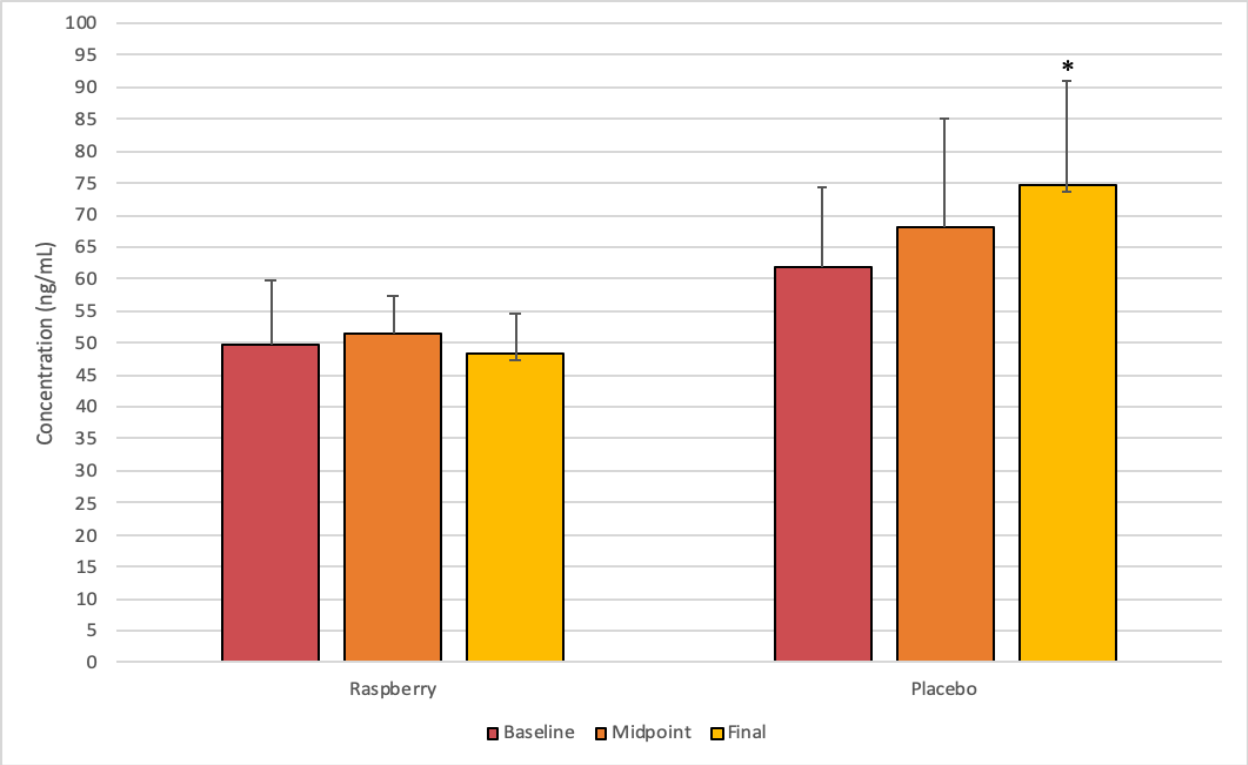



*Figure 15.* Effect of Raspberry and Placebo on Plasma Glycoprotein-39 Levels. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *($p < 0.05$)*

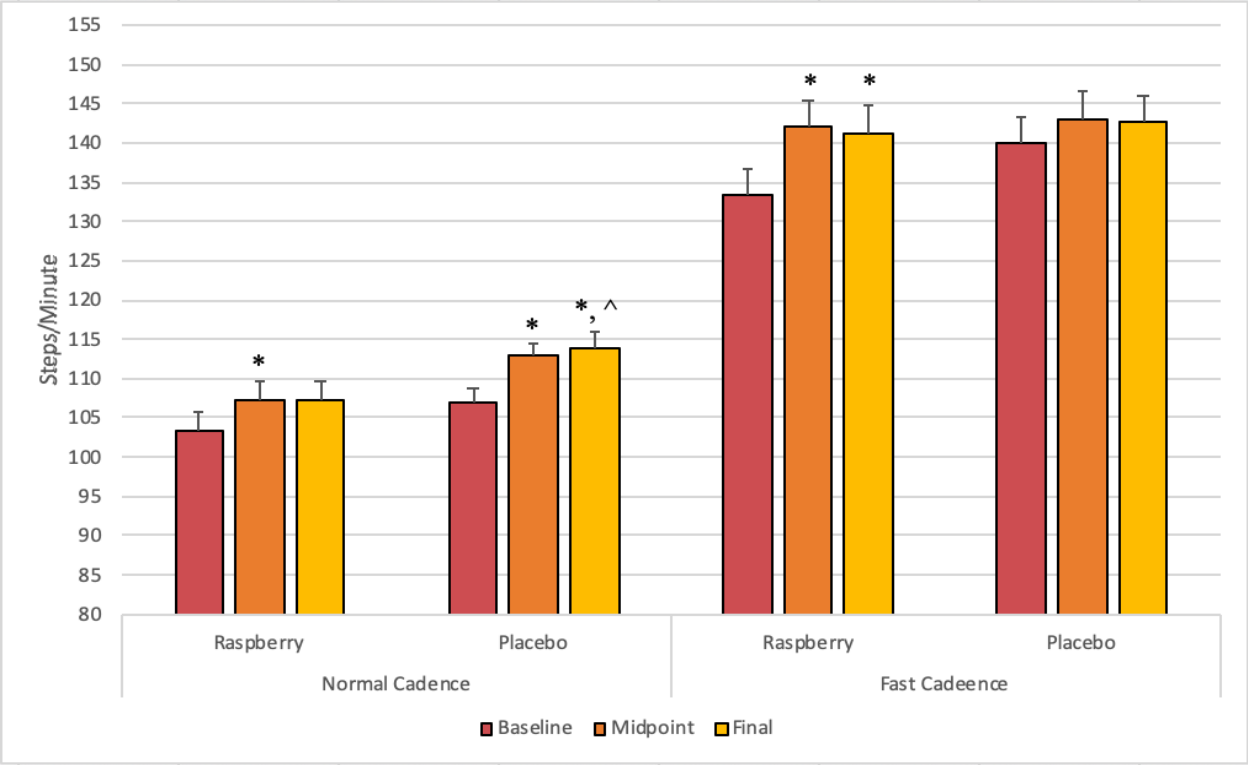



*Figure 16.* Effect of Raspberry and Placebo on Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were removed.
*, significance as compared to baseline *(p < 0.05)*
^, significance as compared Raspberry group *(p < 0.05)*

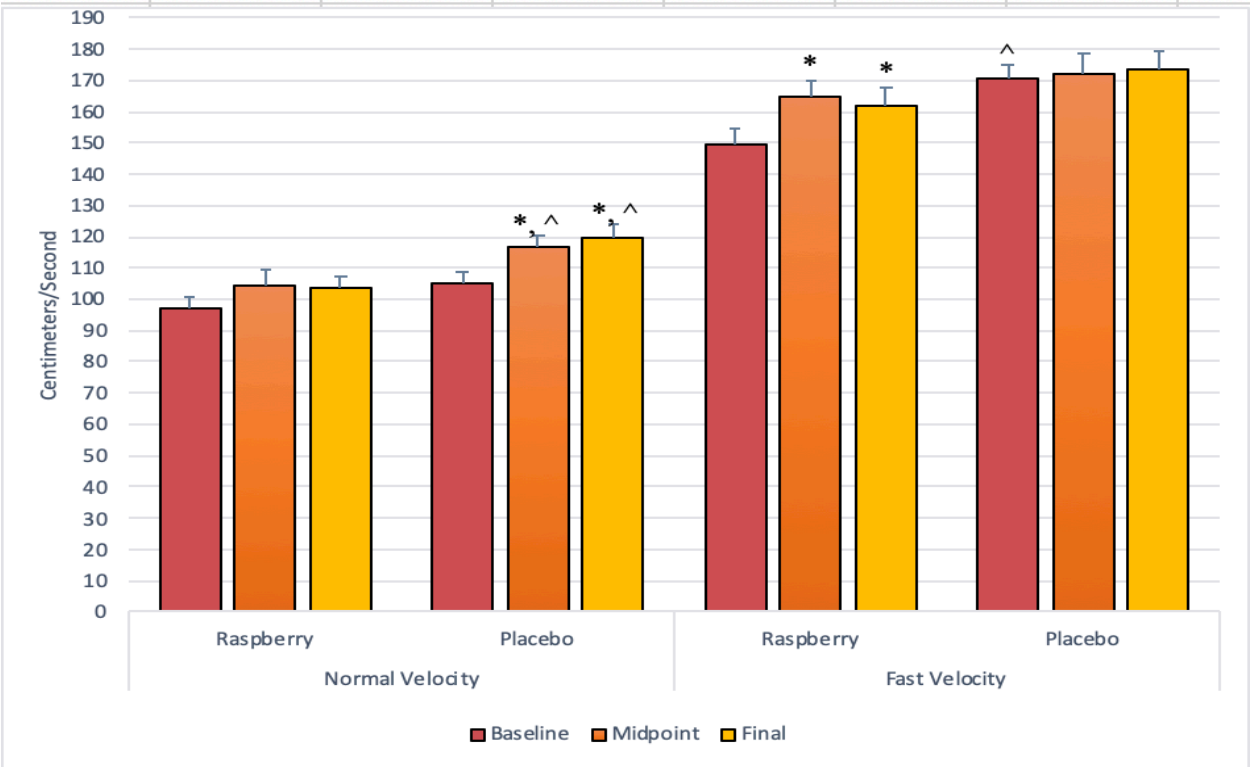



*Figure 17.* Effect of Raspberry and Placebo on Velocity. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*
^, significance as compared to Raspberry group *(p < 0.05)*

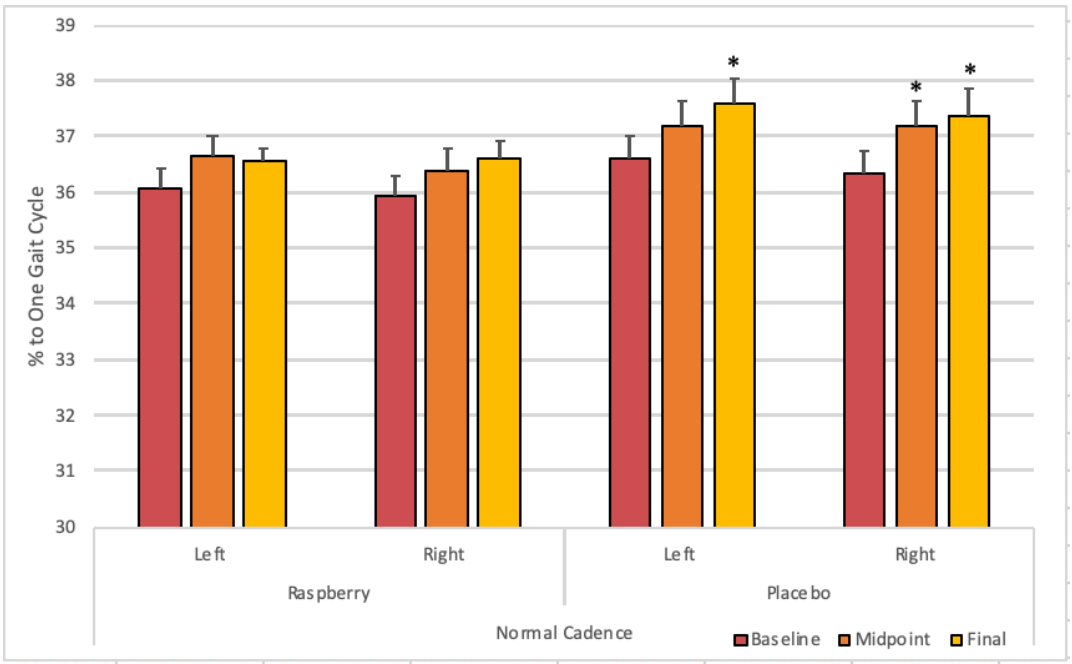



*Figure 18a.* Effect of Raspberry and Placebo on Single Support Percentage to One Gait Cycle at Normal Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

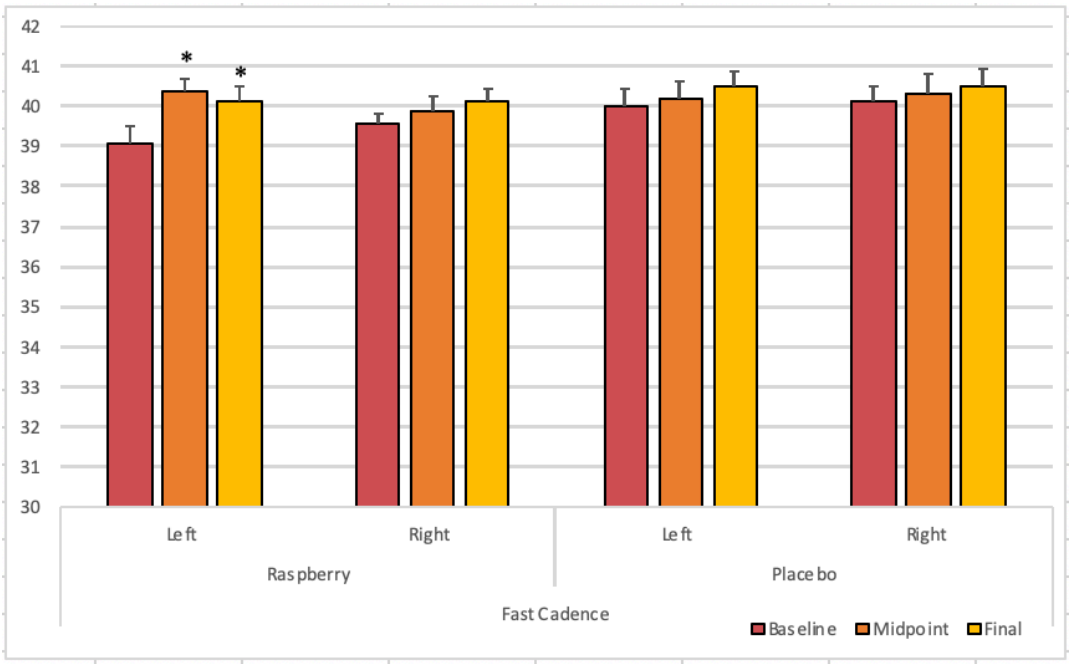



*Figure 18b.* Effect of Raspberry and Placebo on Single Support Percentage to One Gait Cycle at Fast Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

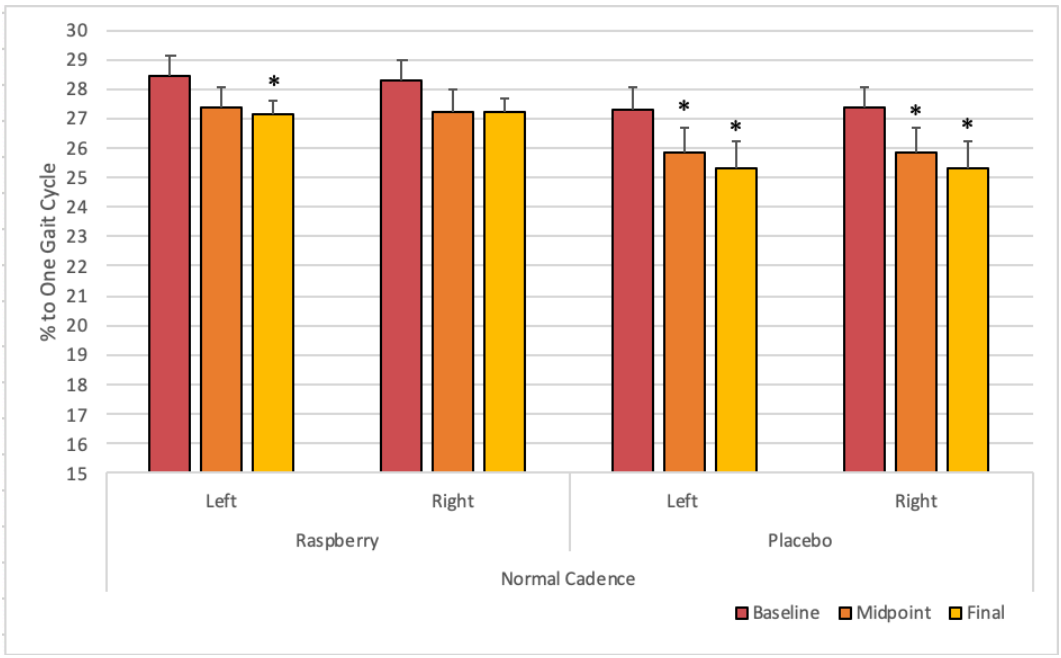



*Figure 19a.* Effect of Raspberry and Placebo on Double Support Percentage to One Gait Cycle at Normal Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

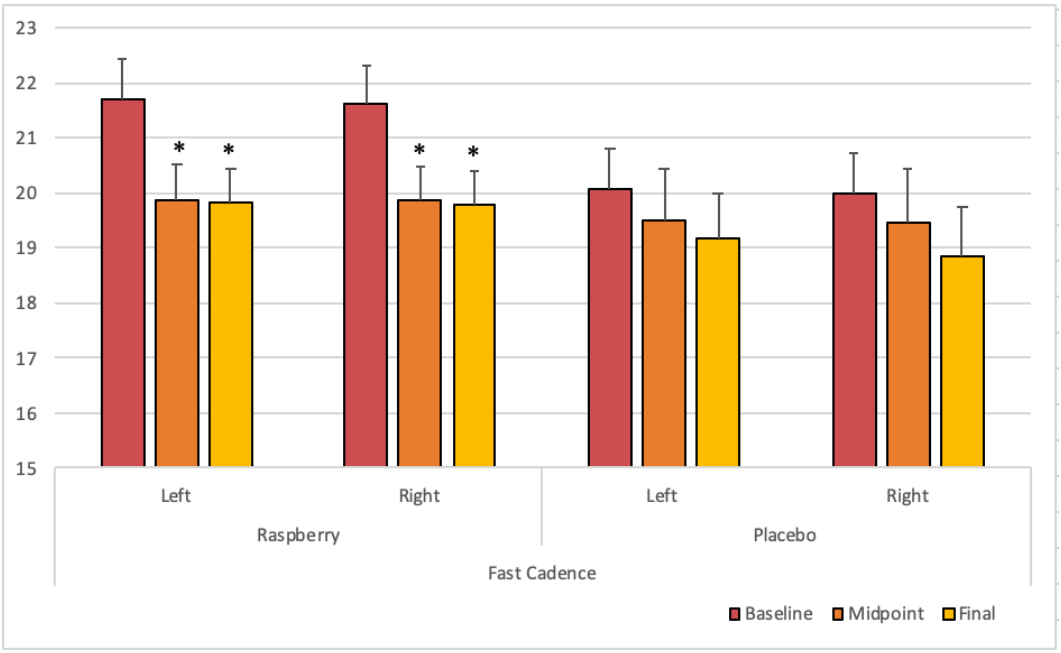



*Figure 19b.* Effect of Raspberry and Placebo on Double Support Percentage to One Gait Cycle at Fast Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group.
*, significance as compared to baseline *(p < 0.05)*

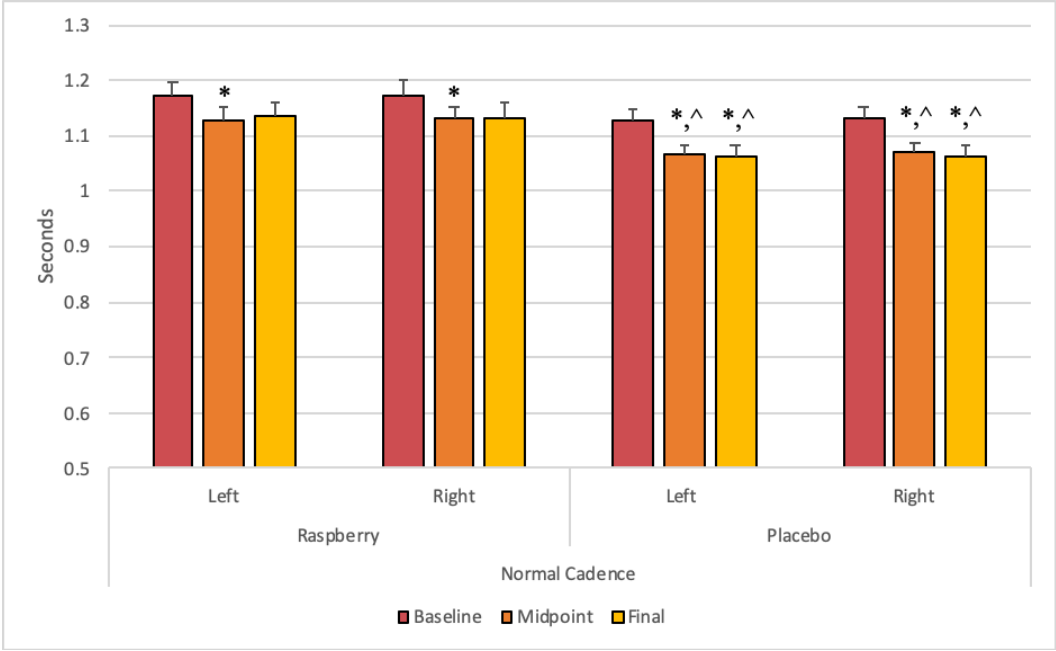



*Figure 20a*. Effect of Raspberry and Placebo on Cycle Time at Normal Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were omitted.
*, significance as compared to baseline *(p < 0.05)*
^, significance as compared to Placebo group *(p < 0.05)*

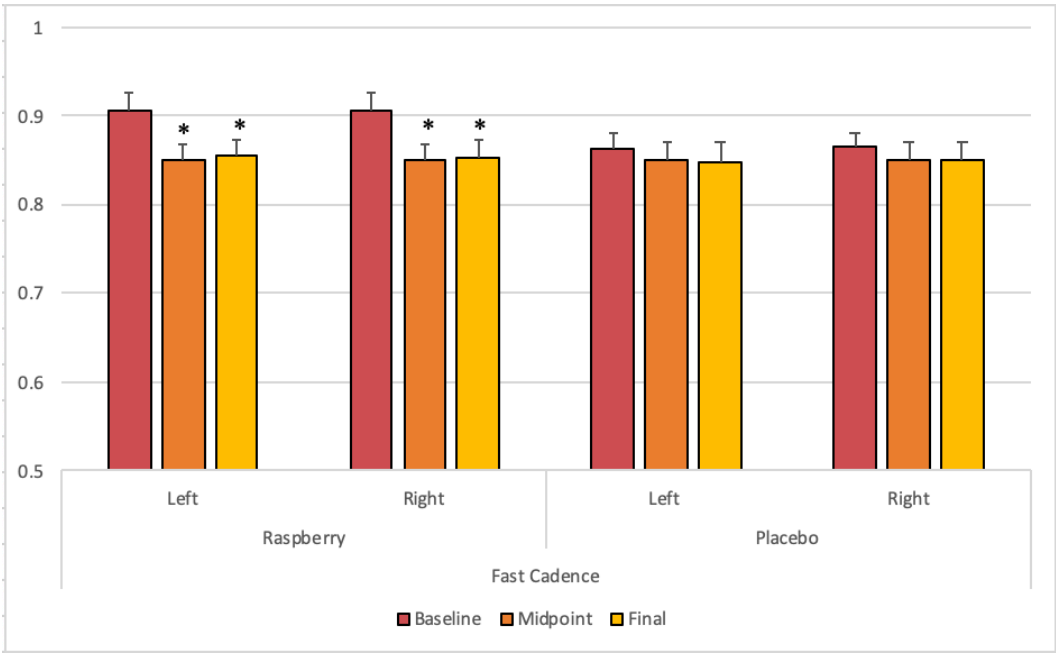



*Figure 20b.* Effect of Raspberry and Placebo on Cycle Time at Fast Walking Cadence. Mean ± SEM. *N = 20* for Raspberry group, *N = 24* for Placebo group. Outliers were omitted.
*, significance as compared to baseline *(p < 0.05)*
^, significance as compared to Placebo group *(p < 0.05)*

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# APPENDIX A

# IRB APPROVAL LETTER

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

DATE: September 7, 2018

TO: Dr. Shanil Juma
Nutrition & Food Sciences

FROM: Institutional Review Board (IRB) - Denton

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

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

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. Shane Broughton, Nutrition & Food Sciences

# APPENDIX B

# RECRUITMENT FLYER


# Who?

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

# Yes?

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

# Contact

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

# Additional Criteria

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

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

# Benefits

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

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

# APPENDIX C

# INFORMED CONSENT

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

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


Investigators: Shanil Juma, PhD 940-898-2704(sjuma@twu.edu)
Young-Hoo Kwon, PhD 940-898-2598(ykwon@twu.edu)
Parakat Vijayagopal, PhD 940-898-2709(pvijayagopal@twu.edu)


Explanation and Purpose of Research

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

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

Research Procedures

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

**First Visit**

During the first visit you will be asked to arrive at the study site fasted (not to eat any food overnight or at least 10 hours). A phlebotomist (person taking the blood) will draw 15 milliliters (approximately 1 tablespoon) of your blood from one of the veins of your arms. You will be provided with a snack and drink (cookies, crackers, and orange juice). This will be followed with a spot urine collection. You will be asked to provide a urine specimen after the first morning void. A trained personnel of the same gender will take your height and weight measurements. Filtered water and a light snack will be available for you at the study site. You will be asked to complete a physical activity questionnaire regarding your activity habits over the past week. You will complete a questionnaire regarding pain and stiffness. A measurement of knee motion (flexibility) will be done in a lying down position on a patient table and repeated three times during this visit by a trained personnel of the same gender associated with the study. A gait analysis to evaluate walking parameters will be done by trained personnel with instructions to walk short distance (30 feet) at usual speed and fastest speed. Each walking speed will be repeated three times with a 30 second rest between each walk. At the end of the baseline visit, you will be randomly assigned to a treatment based on chance, like a flip



Participant Initials _____

of a coin. Neither you nor the researcher chooses your assigned treatment group. You will have an equal chance of being in either group. You will be provided a 60 day supply of either the study treatment (freeze dried red raspberry powder packaged in pouches) or a control (comparative placebo freeze-dried powder without red raspberry). The daily dosage of placebo and raspberry powder (30 grams) will be reconstituted by you using 12 ounces of cold water and mixed in a blender bottle that will be provided to you as part of the study.

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

**Final Visit**
At the end of the study (4 months), you will be asked to come in for your last visit and not to eat any food overnight (10 hours) for a blood draw (1 tablespoons of blood will be obtained). You will be provided with snacks and filtered water. You will be asked to provide a spot urine specimen. A trained personnel of the same gender will measure height and weight. We will also ask you to complete a physical activity questionnaire regarding activity habits over the past week. You will complete a questionnaire regarding pain and stiffness. A measurement of knee motion (flexibility) will be done in a lying down position on a patient table and repeated three times during this visit by a trained personnel of the same gender associated with the study. A gait analysis to evaluate walking parameters will be done by trained personnel

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

Potential Risks

A potential risk to you as a participant in this study is release of confidential information. Confidentiality will be protected to the extent that is allowed by law. To protect confidentiality, you will be given a code number which will be used in all records. Only the principal investigator will know your identity. All records will be stored in a locked filing cabinet in the principal investigator's office. The records will be shredded within 5 years of completion of the study. Your name or any other identifying information will not be included in any publication that may result from the study. There is a potential risk of loss of confidentiality in email, downloading, and internet transactions.

A second possible risk is that you may not like the freeze-dried red raspberry powder or the comparative placebo powder without red raspberry. If you do not like the randomized treatment, there is no penalty for not consuming it. You are free to quit the study at any time. Freeze dried red raspberry powder or the placebo powder without red raspberry is from a whole fruit source or equivalent to the sugar content of the whole fruit that has been custom prepared and packaged for our study. It has been previously used in other human clinical studies and is deemed safe for consumption and not harmful in any way.

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

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

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

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

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

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

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

Participation Benefits

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

Questions Regarding the Study

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

_____ _____

Signature of Participant Date

This page will be detached and filled separately.

* If you would like to receive a summary of the results of this study, please provide an address to which this summary should be sent:

_____
_____
_____



Participant Initials _____

# APPENDIX D

# SCREENING QUESTIONNAIRE

Screening Tool

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

# APPENDIX E

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (IPAQ)

# 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.**