DIET, PAIN, AND INFLAMMATION IN PARTICIPANTS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

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OSTEOARTHRITIS

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Abstract: Osteoarthritis (OA) is a degenerative joint disease that affects the cartilage, subchondral bone, and synovial fluid. Risk factors for knee OA include increase adiposity, where excess adipose tissue release pro-inflammatory cytokines and adipokines, creating a state of chronic inflammation, and leading to the activation of matrix metalloproteinases, which facilitate cartilage breakdown. Individuals with knee OA experience pain upon walking or standing, decreased range of motion, and loss of function in the affected joint. The purpose of this study is to: (1) evaluate the relationship of dietary nutrient intakes with quality of life indicators, pain scores, and serum biomarkers of inflammation and cartilage degradation in participants with symptomatic knee OA (observational) and (2) evaluate the effects of freeze-dried strawberries on serum biomarkers of cartilage degradation in participants with symptomatic knee OA (intervention). Study participants (n=17) with abdominal obesity and symptomatic knee OA completed baseline self-reported dietary records, VAS and ICOAP pain score evaluations, HAQ-DI quality of life indicator assessments, and serum blood draws to assess biomarkers of inflammation and cartilage degradation. Subjects were then randomly assigned to consume 50g of freeze-dried strawberry powder each day or 50g of placebo powder in this 26-week, randomized, double-blind, placebo controlled, cross-over intervention trial. Results of the observational data showed significant inverse correlations of soluble fiber with serum MMP-3, total sugar with MMP-8, saturated fat with MMP-3, vitamin C with MMP-8, and copper with IL-6. Results from our 26-week cross-over intervention showed freeze-dried strawberry supplementation significantly reduced serum concentrations of MMP-3 compared to the placebo. Our study identifies intriguing relationships between dietary nutrient intakes and biomarkers of inflammation and cartilage degradation by suggesting that a well-balanced diet, with adequate intakes of saturated fat, sugar, vitamin C, soluble fiber, and copper, may have a protective role against the activation of cartilage degrading enzymes in knee OA. Additionally, strawberry supplementation decreased serum MMP-3 concentrations suggesting that well-balanced eating habits should also incorporate an array of antioxidant rich foods, including the bioactive strawberry, as an adjunct therapy in knee OA.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease that affects the articular cartilage, subchondral bone, and synovial fluid of weight bearing joints such as the hips, knee, spine, or feet. OA causes pain, stiffness in joints, loss of motion, and is classified by the loss of articular cartilage.[1,2,5] OA affects more than 27 million individuals in the United States.[93] Recently, it has been estimated that over 14 million Americans have symptomatic knee OA.[94] Over the past several decades, epidemiological data has associated knee OA with older age, obesity, the female population, metabolic syndrome, inflammation, and elevated serum levels of cartilage degrading enzymes.[13,63,69-74,88,89] The pathological mechanism by which OA develops is both mechanical and metabolic. Metabolically, excess adipose tissue releases adipokines and pro-inflammatory cytokines that lead to the onset and progression of the disease by creating a state of chronic inflammation and activating matrix metalloproteinases (MMP's) that facilitate cartilage breakdown.[14,15,27,56] For this reason, one of the biggest contributing factors to knee OA is increased adiposity and metabolic syndrome.[9,16] Mechanically, excess weight increases force and loading of joints which results in a breakdown of articular cartilage, ligaments, and other supporting structures.[12] As a result, individuals with knee OA experience pain upon walking or standing, as well as loss of motion and function in the affected joint.

Although previous observational studies have attempted to identify the relationship between food and pain, little is known about the correlations that exists between dietary nutrient

intakes with pain scores, quality of life assessments, and biological parameters, such as inflammation and cartilage degradation, in diagnosed knee OA.[112] Epidemiological data from the Framingham Study and the Osteoarthritis Initiative suggest that both prevalence of knee OA and the pain associated with the condition, are influenced by diet; specifically the intake of total fiber, omega-3 polyunsaturated fatty acids (PUFA), and vitamin C.[114-116] However, it is unclear as to what relationships exist between dietary nutrient intakes (macronutrients, micronutrients, antioxidants, phytochemicals) and both symptoms and metabolic dysregulation associated with this condition.

Currently, there is no cure for osteoarthritis. Many of the treatment strategies focus on alleviating pain with the use of pharmaceuticals or non-steroidal anti-inflammatory drugs (NSAIDS). In addition, weight loss is also commonly recommended as a treatment strategy to relieve pain and improve joint function.[28] Many studies have evaluated the role of dietary polyphenols and their ability to improve endogenous enzyme systems and reduce pain, inflammation, and oxidative stress damage in individuals with symptomatic knee OA.[75-80] In addition, other nutraceuticals such as ginger powder, avocado/ soybean extracts, and omega-3 fatty acids have also been shown to reduce pain and pro-inflammatory biomarkers associated with knee OA.[67,82,83]

Strawberries, a popularly consumed berry fruit, are a rich source of soluble fiber, vitamin C, folate, tocopherols, carotenoids, and polyphenols.[98] The strawberry polyphenolic composition has shown to have anti-inflammatory and antioxidant capacities in individuals with diabetes, cardiovascular disease, and hypertension. In modulating cardiovascular disease (CVD) risks, strawberries reduced low-density lipoprotein (LDL) cholesterol, circulating adhesion molecules, and lipid peroxidation in the form of malondialdehyde (MDA). [48,49,100,101,110] However, to date, there has not been a preclinical or a clinical study to evaluate the effects of strawberry polyphenols on inflammation and cartilage degradation associated with symptomatic knee OA,

especially in individuals with characteristic of metabolic syndrome. Thus, this study aims to examine the hypothesis that strawberry supplementation will lower serum biomarkers of cartilage degradation in participants with knee OA.

The goal of this study is to identify the relationship of dietary nutrient intakes with pain scores, quality of life indicators, and biochemical parameters in participants with symptomatic knee OA using a cross-sectional approach with baseline data. Additionally, this study will also investigate the relationship of pain scores and quality of life indicators with biochemical parameters in our participants. Finally, this study will attempt to identify strawberries as a potential alternative treatment method for decreasing serum biomarkers of cartilage degradation in participants with symptomatic knee OA.

The specific aims are to:

Observational

1. To examine whether dietary nutrient intakes at baseline are in any way correlated with biomarkers of inflammation, biomarkers of cartilage degradation, pain scores, and quality of life indicators in participants with symptomatic knee OA
2. To examine whether biomarkers of inflammation and cartilage degradation at baseline are in any way correlated with pain scores and quality of life indicators in participants with symptomatic knee OA

Intervention

3. To examine the effects of strawberry intervention on biomarkers of cartilage degradation by assessing serum levels of nitrite, MMP-3, and MMP-8 in participants with symptomatic knee OA

For the present study, we hypothesize that:

1. Total fat and total carbohydrate intakes will be positively correlated with pain scores, circulating biomarkers of inflammation, and circulating biomarkers of cartilage degradation in subjects with symptomatic knee OA
2. Total fat and total carbohydrate intakes will be inversely correlated with quality of life indicators in subjects with symptomatic knee OA
3. Micronutrient and antioxidant intakes will be inversely correlated with pain scores, circulating biomarkers of inflammation, and circulating biomarkers of cartilage degradation in subjects with symptomatic knee OA
4. Micronutrient and antioxidant intakes will be positively correlated with quality of life indicators in subjects with symptomatic knee OA
5. Pain scores will be positively correlated with circulating biomarkers of inflammation and cartilage degradation in subjects with symptomatic knee OA
6. Quality of life indicators will be inversely correlated with circulating biomarkers of inflammation and cartilage degradation in subjects with symptomatic knee OA
7. Strawberry supplementation will significantly decrease serum concentrations of nitrite, MMP-3, and MMP-8 in subjects with symptomatic knee OA

CHAPTER II

REVIEW OF LITERATURE

OA is a degenerative joint disease that affects the cartilage, subchondral bone, and synovial fluid. OA generally involves weight bearing joints such as the hips, knee, spine, and feet.[1] OA causes pain, stiffness in joints, and loss of motion which can be debilitating to those who suffer from this chronic condition.[2] The pathological mechanism in which OA develops focuses on the relationship between cartilage, the synovium, and subchondral bone. OA is classified by the degradation of articular cartilage found in joints. Previous findings indicated that OA results from injury or excessive loading of weight to a joint as the main cause of cartilage degradation. However, new research suggests that systemic inflammation and the release of cytokines and matrix metalloproteinases may be equally responsible for cartilage degradation.[4] In addition, evidence has shown that inflammation of the synovium (synovitis) may also contribute to the progression of OA.[3]

Among the more than 200 arthritic conditions, OA is the most commonly treated.[5] OA affects more than 27 million individuals in the United States.[93] Recently, it has been estimated that over 14 million Americans have symptomatic knee OA.[94] Over the last several decades, epidemiological data has associated knee OA with older age, obesity, the female population, metabolic syndrome, inflammation, and elevated serum levels of cartilage degrading enzymes (Table 1). In addition, family predisposition, potential for injury, and lifestyle also contribute to the incidence of OA.[11] Likely one of the most significant contributing factors in knee OA is

excess weight.[13] The mechanism that relates obesity to OA is both mechanical and metabolic. Mechanically, excess weight increases the forces and loading of joints which results in a breakdown of articular cartilage, ligaments, and other supporting structures. In addition, decreased muscle strength can also contribute to the degradation of articular cartilage by altering the load placed on the joint. Therefore, weak muscles associated with joints can put increased stressed on an already compromised joint.[12] Metabolically, adipose tissue or fat stores, release a number of different molecules such as adipokines (leptin, adiponectin, visfatin, resistin) and cytokines interleukin-1 (IL-1β) and tumor necrosis factor-α (TNF-α). In OA, these proteins appear to have pro-inflammatory effects and may contribute to both inflammation and the progression of the disease. [14,15] Age is attributed as a contributory factor in the development of OA due to the decrease in cartilage homeostasis. Over time, chondrocytes do not respond to growth factor stimulation which results in a pathological change to the cartilage.[10] However, given the obesity epidemic in the United States, more individuals under the age of 65 are presenting with symptomatic knee OA due to the metabolic contribution.[94] For this reason, OA warrants future research efforts to identify strategies for prevention and treatment of this chronic condition.

I. Obesity, Metabolic Syndrome, and OA

When assessing the prevalence of OA, it is important to consider two conditions that contribute greatly to the onset and progression of this debilitating disease; obesity and metabolic syndrome. Overweight and obesity are identified by calculating body mass index (BMI) where a person's weight in kilograms is divided by height in meters squared. A BMI between 25.0 and 29.9 kg/m^2 is classified as overweight and a BMI greater than 30.0 kg/m^2 is considered obese.[4] As

stated, obesity relates to OA not only due to increased mechanical loading on the knee, but also excess adipose tissue and the release of adipokines.

According to the National Institutes of Health (NIH), metabolic syndrome is a group of biochemical and physiological conditions that significantly increase an individual's risk for heart disease and other chronic illnesses such as diabetes, stroke, and cancer.[6] To obtain a diagnosis of metabolic syndrome, one must present with at least three of five following conditions: (1) waistline measuring greater than 35 inches for women and greater than 40 inches for men, (2) blood triglycerides greater than or equal to 150 mg/dL, (3) high-density lipoprotein (HDL) cholesterol lower than 40 mg/dL for men and lower than 50 mg/dL for women, (4) blood pressure measuring equal to or greater than 130/85 mmHg, (5) insulin resistance measured by a fasting plasma glucose of greater than or equal to 100 mg/dL.[4, 6,7] Of these factors, insulin resistance or an impaired response to insulin in cells, appears to be the most significant cause of metabolic syndrome.[8] Insulin is important not only in glucose homeostasis, but also in the regulation of pro-inflammatory cytokines. In a study by Jeschke, et al., insulin showed to reduce the plasma concentrations of pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-1β, macrophage migration inhibition factor (MIF), and TNF-α.[9]

The main concept linking metabolic syndrome to OA is insulin resistance, increased adiposity, and the presence of inflammation or pro-inflammatory cytokines which upregulate inflammation.[9,16] In a review conducted by Wang et al., the researchers propose a cluster of metabolic abnormalities that relate metabolic syndrome and inflammation to OA: (1) dysregulated metabolites, (2) abnormal body composition, (3) presence of inflammatory cytokines and acute-phase proteins, and (4) presence of inflammatory adipokines.[17]

II. Inflammation and OA

Dysregulated nutrient metabolism, such as altered lipid profiles, have been associated with cartilage degradation in OA patients.[18,19] As lipid and glucose metabolism become dysregulated, an individual's body composition changes, which may result in an increased amount of adipose tissue. As stated previously, adipose tissue acts as an endocrine organ by releasing adipokines. Therefore, when more adipose tissue is present, adipocytes release an excess of adipokines such as leptin, resistin, visfatin, and adiponectin.[15] These adipokines are strongly correlated with inflammation in knee OA by activating pro-inflammatory cytokines.[20,21]

Likely the most significant relation between metabolic syndrome and OA is the presence of pro-inflammatory cytokines. The three pro-inflammatory cytokines associated with the pathogenesis of OA appear to be IL-6, IL-1β, and TNF-α.[15] IL-6 is a cytokine produced by cells such as T cells, B cells, monocytes, fibroblasts, osteoblasts, keratinocytes, endothelial cells, mesangial cells, and tumor cells.[22] The inflammatory response and the release of IL-6 is not only seen in low grade inflammation, but also in the development of atherosclerosis.[26] In addition, there is also a strong correlation between inflammatory markers such as highly sensitive C-reactive protein (hs-CRP), an acute phase protein produced by the liver in response to inflammation, and IL-6 in individuals with metabolic syndrome.[25] In terms of knee OA, IL-6, has been shown to be elevated in both the synovial tissue and chondrocytes, contributing to the onset and progression of the disease.[23,24]

IL-1β and TNF-α are also two important pro-inflammatory cytokines associated with OA. Both IL-1β and TNF-α are produced within the synovium, including the synovial fluid and membrane; as well as in chondrocytes, osteoblasts, and mononuclear cells. IL-1β and TNF-α can stimulate the production of IL-6, thus contributing to the pro-inflammatory cascade associated with these cytokines in OA.[24] In addition, IL-1β and TNF-α appear to have a major impact on cartilage degradation by stimulating the release a matrix metalloproteinases, enzymes responsible for the breakdown of the cartilage matrix.[27]

MMP's are a family of proteolytic enzymes found throughout the human body and facilitate the breakdown of extracellular matricies.[56] In knee OA, several MMP's, such as MMP-1, MMP-3, MMP-8, and MMP-13, have been identified as having a significant influence in the degradation of articular cartilage in the knee. In particular, MMP-3 or stromelysin-1, is produced by chondrocytes and appears to be directly correlated to the disruption of cartilage homeostasis in knee OA. MMP-3 facilitates the breakdown of articular cartilage in the knee, which leads to joint-space narrowing and pain in the affected joint.[56] However, MMP's are not solely responsible for the progression of OA, and are greatly influenced by inflammatory cytokines.[27,56] There is also evidence which suggests that the adipokine leptin may upregulate the expression and amplify the production of MMP-3 in chondrocytes.[68] Clinical studies have also shown a significant increase in IL-1β, MMP-1 and MMP-3 in the synovial fluid of osteoarthritic knees compared to healthy controls.[57] Therefore, in the presence of adipokines and inflammatory cytokines, there is an increased risk for the activation of these cartilage degrading enzymes.

Similarly, the combination of leptin and pro-inflammatory cytokines, such as IL-1β, is capable of producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) which further contributes to the progression of OA.[20,27] Nitric oxide (NOx) is a RNS produced via inducible nitric oxide synthase (iNOS) from the amino acid L-arginine.[58] When converted into its active form, NOx can trigger inflammation, tissue destruction, and apoptosis.[59] NOx has the ability to enhance the activity and synthesis of MMP's as well as inhibit the synthesis of collagen and proteoglycans.[60,61] In addition, the relationship between NOx, iNOS, and certain pro-inflammatory cytokines appears to be synergistic. NOx appears to be activated by IL-1β and TNF-α via iNOS. In the presence of these inflammatory cytokines, the enzyme iNOS is significantly upregulated leading to the increased production of NOx.[62,65] Moreover, NOx appears to significantly increased in both serum and synovial fluid in individuals with primary knee OA compared to healthy controls.[64]

III. Pain and Knee OA

Pain is a commonly assessed and monitored clinical outcome in the pathogenesis and management of OA. Pain is the most common symptom associated with knee OA and the focus of many treatment remedies.[2,28] There are a number of ways to assess pain level associated with knee OA: (1) Visual Analog Scale (VAS) for pain, (2) Short-form McGill Pain Questionnaire (SF-MPQ), (3) Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), and (4) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC).[102,103] Additionally, to understand disease state, the Health Assessment Questionnaire Disability Index (HAQ-DI) is often used combination with pain score to evaluate the overall functional status of each patient.[104] Epidemiological research studies have suggest a positive correlation of inflammatory biomarkers, such as hs-CRP, IL-1β, and TNF-α, with increased pain.[105,106] However, to our knowledge, there have not been an observational study which evaluates the relationships of pain with biomarkers of cartilage degradation, such as serum levels of MMP-3 and MMP-8 in knee OA.

ICOAP is a series of questions which are divided into two sections and assess intermittent pain or pain that comes and goes, and constant pain or pain that is always present. The scale quantifies the patient response to each question providing a score from 0 to 4; (0) 'not at all' and (4) 'extremely'.[107] The VAS pain scale is used to quantify a visual and verbal representation of pain by the use of a horizontal or vertical line ruler which is 100mm in length. This self-reported pain method allows a participants to rank their pain from 0 (no pain) to 10 (worst pain imaginable) and to place a marker perpendicular to the VAS indicator. The pain score is quantified by measuring the distance from 0 (no pain) to the marker indicated by the participant.[103] In addition to assessing pain, it is also important to understand how this condition impacts an individual's quality of life. HAQ-DI is a questionnaire which aims to understand the burden of a chronic condition, such as knee OA, on daily activities such as dressing, eating, and

walking.[104] Utilization of ICOAP, VAS, and HAQ-DI allows a more in-depth understanding into how knee OA impacts the individuals suffering from this condition.

IV. Diet and Knee OA

According to the Arthritis Foundation, some of the most common treatments for knee OA involve physical activity, weight loss, pharmacological treatment, surgery, and the use of knee braces or sleeves.[28] One factor that must be considered in both onset and progression of OA is nutrient intake. Although a well-balanced, nutrient rich diet is a staple for maintaining a healthy lifestyle, it is important to consider the role of dietary nutrient composition and dietary intakes of naturally occurring phytochemicals, in the prevention and management of chronic conditions such as OA.

A. Dietary Risk Factors

Over the past several decades, there have been a number of epidemiological studies conducted to identify dietary risk factors for the development of knee OA. Nutrient risk factors for knee OA were identified in the early 1990's with data from the Framingham Study. The results from this large cohort study suggests that lower dietary intake of certain micronutrients, such as vitamin C, vitamin E, and β-carotene, are positively associated with risk of developing knee OA.[95,116] Lower intakes of vitamin C and vitamin D have been shown to be more common among individuals with knee OA compared to health controls, and associated with increased risk of knee OA.[117] Similarly, Wang et. al suggest that high consumption of vitamin C-rich fruits is associated with a reduction of bone marrow lesions (BML) development in healthy individuals, suggesting a potential protective role in the development of knee OA.[113] In addition, previous research has also found associations between vitamin K intake and knee OA. Lower dietary

intakes and serum levels of phylloquinone (vitamin K) have shown a positive association with both the development and progression of knee OA.[53, 119]

Macronutrient intakes, specifically fat and carbohydrate intake, have also been studied for their role in the risk of knee OA. Higher intakes of total energy, carbohydrates, and sugar have shown a positive association with the development of BML's in knee OA.[97] Data from the Osteoarthritis Initiative identified that an increase in total fat intake resulted in a decrease in joint space narrowing after 48 month follow-up. This study also showed an inverse association between PUFA intake and joint space narrowing and concluded that there was a significant risk of knee OA progression with an increased intake of saturated fats, and a reduced risk among those with higher intakes of PUFA and MUFA.[118] Additional data analyzed from the Osteoarthritis Initiative suggest the prevalence of knee OA is lower among individuals with dietary patterns consistent with a Mediterranean diet. The findings of this study suggests that diets higher in whole grains, legumes, fruits, vegetables, olive oils, and lean proteins may have a protective role against knee OA.[114] These studies suggest that dietary nutrient quality may have a strong influence on the pathogenesis of knee OA and may have a role in the prevention of this chronic condition.

B. Dietary Nutrient Intakes on Knee OA Symptoms

Although dietary risk factors have been widely studied in knee OA, very little is known about the relationship of normal dietary intakes with symptoms of knee OA, such as pain, mobility, and quality of life. Choi et al. conducted a cross-sectional observational study to identify the relationship between momentary knee pain and dietary intakes. The results of this study indicated that as participants pain increased, so did their energy consumption, specifically in total fat and total sugar. This study suggests that as calorie intake increases, pain increases, thus contributing to obesity and the progression of knee OA symptoms.[112] In a clinical trial conducted by Clinton et. al, a whole-food, plant-based diet rich in phytochemicals and low in

animal products revealed an improvement in VAS pain scores among individuals with OA compared to participants that continued a normal Western diet. The researchers suspect the decrease in pain may be due to the nutrient quality of the (whole-food, plant-based) diet which is rich in omega-3 fatty acids and fiber.[96] Further findings from the Osteoarthritis Initiative, suggest that higher fiber intake (approximately 25g each day), was associated with a decrease in WOMAC pain score over an 8 year period.[115] Although these studies provide some insight to the relationship between diet and pain, a gap in the research still exists when evaluating the relationship of common dietary nutrient intakes with pain and quality of life indicators.

C. Polyphenols

Polyphenols are naturally occurring compounds or phytochemicals found in an array of foods such as fruits, vegetables, spices, nuts, tea and cocoa. As a non-pharmacologic therapy for knee OA, a number of polyphenolic compounds have been studied in clinical trials (Table 2). Curcumin, the polyphenol derived from the spice turmeric, has been studied in clinical trials, *in-vitro*, and *in-vivo*. For decades, curcumin has been studied for its anti-inflammatory and antioxidant capabilities related to prevention and treatment of chronic diseases.[42,43] In OA, curcumin has shown to reduce expression of inflammatory cytokines, MMP's, and NOx *in-vitro* and *in-vivo*.[44,45,90] Additionally, curcumin has also been shown to reduce NSAID use by approximately 55%, as well as improve VAS pain score in human subjects.[76]

In overweight and obese humans with clinically diagnosed OA, polyphenols found in pomegranate juice, passion fruit extract, tart cherry juice, and rosmarinic spearmint tea have shown to significantly reduce WOMAC pain scores by an average of 30%, as well as reduce inflammation by 15% (measured by hs-CRP) and MMP-13 by 19%.[75,78-80] *In-vitro* studies have concluded that polyphenolic compounds such as resveratrol have the ability to inhibit the production of inflammatory cytokines and have also been shown to decrease the production of

ROS by more than 75%.[46] Similarly, green tea catechins decrease the expression of cyclooxygenase-2 (COX-2) and iNOS in IL-1β stimulated chondrocytes by 64% and 94%, respectively.[87] *In-vivo*, epigallocatechin gallate (EGCG) has demonstrated the ability to reduce the expression of the cartilage degrading enzymes MMP-1, MMP-3, MMP-8, and MMP-13, as well as the proinflammatory cytokines IL-1β and TNF-α in osteoarthritic mice.[91] (Table 4)

D. Nutraceuticals

An emerging area of research in the field of nutrition and the fight against chronic disease is the use of nutraceuticals. In 1989, Dr. Stephen DeFelice coined the term "Nutraceutical" and defined it as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease.[92] Many clinical and *in-vitro* studies have been conducted to show the efficacy of nutraceuticals as a treatment option for knee OA (Table 3 and Table 4). Ginger has been widely studied for its ability to act as an anti-inflammatory agent by suppressing nuclear factor kappa B (NF-*K*B) and inhibiting iNOS, TNF-α, and IL-1β.[66] In overweight and obese humans, ginger and burdock root tea have had promising outcomes in treating inflammation associated with knee OA by significantly reducing serum levels of CRP, NOx, and IL-6.[67,81] Other nutraceuticals such as avocado/soybean unsaponifiables (ASU) and omega-3 PUFA have also shown to significantly improve VAS and WOMAC pain scores, as well as reduce NSAID use associated with knee OA.[82,83]

ASU's are vegetables extracts that do not form into soaps after saponification. The remaining oil is highly concentrated containing one part avocado oil and two parts soybean oil. ASU consists of phytosterols, fatty acids, fat-soluble vitamins, and alcohols.[39] *In-vitro* studies have demonstrated an average decrease of 65% in the IL-6 and prostaglandin E_2 (PGE_2) production among chondrocytes treated with an ASU ratio of 1 part avocado and 2 parts soybean (A1S2) (Table 4).[40,41] Next, omega-3 fatty acids are linked to inflammation because of their

ability to produce eicosanoids, molecules that regulate inflammation. For example, it is thought that high levels of arachidonic acid, an omega-6 PUFA, may translate to cells within the body and increase the production of inflammatory eicosanoids, thus increasing inflammation.[35] Conversely, omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), produce eicosanoids with anti-inflammatory properties and may act to inhibit the metabolism of arachidonic acid, thus lowering inflammation.[35] Studies have shown how supplementation of omega-3 fatty acids reduces both inflammation and pain in knee OA.[36,37,83]

E. Weight Loss

Diet and weight loss may be an effective approach in reducing both pain and inflammation associated with OA. In obese individuals with clinically diagnosed knee OA, a low calorie diet, approximately 800-1,000 kcal/day, comprising of 15-20% protein, 45-60% carbohydrate, and less than 30% fat, showed a significant improvement in WOMAC pain and function scores. When combined with 1 hour of exercise, 3 days a week, results showed a 45% improvement in WOMAC pain and a 15% reduction in serum IL-6 levels.[30] (Table3)

F. Supplements and NSAID's

In order to treat pain associated with knee OA, the most common initial treatment option is the use of pharmaceuticals such as Paracetamol (Tylenol) and NSAIDS.[29] However, pharmacological therapy only temporarily reduces inflammation associated with pain and does not address the underline cause of OA. Two of the most well-known supplements associated with the treatment of arthritis are glucosamine and chondroitin. Both glucosamine and chondroitin sulfate are naturally formed elements of cartilage and synovial fluid.[31] Because these elements can be consumed via the diet, they have received much attention as a treatment option for OA.[32-34, 84-86] However, supplementation with these compounds has only been evaluated for pain and

joint narrowing. There is insufficient evidence to suggest that glucosamine and chondroitin treat the inflammation cascade associated with knee OA.

V. Berries: Strawberries

A. Composition and Active Compounds

Berries, such as strawberries (*Fragaria X Ananassa*), are a commercially grown fruits that contains an array of micronutrients and phytochemicals making them highly bioactive.[47] Every 100g of fresh weight strawberries provide a rich source of dietary fiber, vitamin C, and folate, approximately 2g, 59mg, and 24µg, respectively. Strawberries also contain an adequate supply of carotenoids, in the form of lutein and zeaxanthin (26mg/ 100g), tocopherols (1mg/ 100g), and approximately 40 phenolic compounds such as anthocyanins (27mg/ 100g) and pelargonidin (25mg/ 100g), providing strawberries with both antioxidant and anti-inflammatory capacities.[98,99,111]

B. Effects on Oxidative Stress and Inflammation

Consumption of berry polyphenols have been evaluated in large-scale epidemiological studies and shown to have a role in reducing cardiovascular disease risk and incidence of cardiovascular events.[108,109] In humans, strawberries have been studied for their ability to reduce oxidative stress and improve lipid profiles. Clinical research has shown that strawberries, in the form of freeze-dried strawberry powder reduces the risk of cardiovascular disease by decreasing serum levels of total cholesterol including LDL cholesterol and small LDL particles, as well as circulating vascular adhesion molecules (VCAM). [48,49,100] Moreover, strawberries have the ability to significantly reduce lipid peroxidation, increase endogenous antioxidant enzymes, and reduce oxidative byproducts such as MDA and hydroxnonenol (HNE).[49,100,101] In addition, strawberries

have displayed anti-inflammatory properties by reducing pro-inflammatory cytokines such as IL-1β and IL-6, and increasing the anti-inflammatory cytokine interleukin-10 (IL-10) in peritoneal macrophages of mice.[50,51] The available clinical and *in-vivo* research shows promising effects of strawberries as a measure to prevent or manage chronic disease. However, to our knowledge, there has not been a clinical trial to evaluate the effects of strawberries on markers of inflammation and cartilage degradation associated with knee OA suggesting a window for novel research in this area.

VI: Summary

This section provides an overview of the pathophysiology and disease state associated with knee OA. Given the metabolic and mechanical attributes of knee OA, successful management of OA symptoms, such as pain, should aim to address the metabolic dysregulation, such as chronic inflammation and activation of cartilage degrading enzymes. This review outlines the dietary risk factors associated with the onset and progression of knee OA, and clearly identifies a lack of evidence on the relationships of normal dietary intakes with biomarkers of inflammation, biomarkers of cartilage degradation, pain scores, and quality of life indicators. This review also highlights the previous research efforts, which focus on potential dietary and nutrient approaches to treating knee OA. Polyphenolic compounds from sources such as pomegranate, passion fruit, cherry, and rosmarinic spearmint tea have shown promising results in clinical studies at reducing pain and inflammation associated with knee OA. However, to our knowledge, there has not been a clinical study to evaluate the effects of strawberries on serum biomarkers of cartilage degradation in individuals with symptomatic knee OA. The high polyphenolic composition of strawberries have promising effects on lowering inflammation, adhesion

molecules, and oxidative stress, and deserves further attention as for other chronic conditions such as OA.

Table 1. Summary of Epidemiological Studies Relating Obesity, Metabolic Syndrome, and Inflammation to Knee OA				
Study Design	**Sample Size**	**Population Characteristics**	**Significant Findings**	**Ref**
Retrospective Cohort	n=1420	Men and women, with or without elevated weight, and high or low physical activity	Risk of developing knee OA associated with overweight and obesity	[13]
Cross-Sectional	n=1808	Men and women over 18 years of age with knee and/or hip OA BMI = 28.3 ± 6.0 kg/m^2	Knee OA associated with old age, the female population, and obesity	[69]
Prospective Cohort	n=142	Women without clinically diagnosed knee OA or knee injury BMI (Baseline) = 25.0 ± 5.0 kg/m^2	Knee OA associated with increased weight results in elevated bone marrow lesions, and tibiofemoral cartilage defects	[70]
Prospective Cohort	n=1,764,061	Men and women ≥ 40 years of age without history of diagnosed OA	Knee OA associated with overweight and obesity	[71]
Prospective Cohort	n=908	Caucasian women aged between the ages of 45 and 64	Knee OA associated with increased BMI and higher levels of IL-6[1]	[74]
Prospective Cohort	n=403	Men and women aged 45 to 60 years old with asymptomatic diagnosed knee OA, who had not previously had surgery BMI = 28.5 ± 4.9 kg/m^2	Cartilage degradation in knee OA associated with metabolic risk factors such as diabetes mellitus, high blood pressure, and high abdominal	[72]
Prospective Cohort	n=1202	Participants age 27 to 75 with diagnosed knee OA, known anthropometric measurements (blood pressure and serum lipid levels), and did not have diabetes or known elevated fasting plasma glucose BMI = 29.7 ± 5.3 kg/m^2	Knee OA associated with multiple metabolic syndrome risk factors including central obesity and hypertension	[89]
Prospective Cohort	n=1384	Men and women with or without diagnosed knee OA Average BMI (Baseline) = 23.1 ± 3.4 kg/m^2	Incidence of knee OA is influenced by glucose intolerance and hypertension Progression of knee OA is associated with overweight/obese and hypertension	[73]
Cross-Sectional	n=105	Men and women age 45 to 70 with symptomatic knee OA	Knee OA associated with increased levels of MMP-3[2], MMP-9[3], and NO[4]	[63]
Prospective Cohort	n=3465	Men and women with clinically diagnosed knee OA and available serum biomarkers of atherosclerosis BMI = 26.8 ± 4.0 kg/m^2	Knee OA associated with elevated levels of VCAM-1[5] and CD40L[6] among women	[88]

[1] IL-6 = Interleukin-6, [2] MMP-3 = Matrix Metalloproteinase-3 (Stromlysin-1), [3] MMP-9 = Matrix Metalloproteinase-9 (gelatinase-B), [4] NO = Nitric Oxide (RNS), [5] VCAM-1 = Vascular Cell Adhesion Molecule-1 , [6] CD40L = CD40 Ligand

Table 2. Summary of Clinical Studies with Polyphenols and Clinically Diagnosed Knee OA				
Intervention & Daily Dose	**Study Design & Sample Size**	**Duration**	**Effects of polyphenol vs. control**	**Ref**
Pomegranate Juice Pomegranate Juice (200mL) vs. no intervention	Randomized control trial (n = 38)	6 weeks	Decrease in WOMAC[1] pain score and serum MMP-13[3] Increase in serum GPx[4]	[75]
Passion Fruit Extract Extract (150mg) vs. Placebo (150mg)	Randomized, double-blind control trial (n = 33)	60 days	Decrease in WOMAC pain and physical function scores	[80]
Tart Cherry Juice Cherry Juice (16oz.) vs. Control (16oz.)	Randomized, double-blind cross-over trial (n = 46)	6 weeks	Decrease in WOMAC pain and physical function scores, decrease in serum hs-CRP[5] levels	[79]
Curcumin (Theracurmin ©) Theracumin (180mg) vs. Placebo (180mg)	Randomized, double-blind control trial (n = 50)	8 weeks	Decrease in VAS[6] pain score and overall NSAID[7] use	[76]
Curcumin (C3 Complex®) C3 Complex (1500mg) vs. Placebo (1500mg)	Randomized, double-blind control trial (n = 40)	6 weeks	Decrease serum MDA[8], Increase in serum SOD[9] and GSH[10]	[77]
Rosmarinic Acid Tea Rosmarinic Spearmint Tea (280mg/ 600mL) vs. Control (26mg/ 600mL)	Randomized, double-blind control trial (n = 46)	16 weeks	Increase in QoL[11] score, decrease in WOMAC pain score	[78]

[1] WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index, [2] MMP-1 = Matrix Metalloproteinase-1, [3] MMP-13 = Matrix Metalloproteinase-13, [4] GPx = Glutathione Peroxidase, [5] hs-CRP = highly sensitive C-Reactive Protein, [6] VAS = Knee Pain Visual Analog Scale, [7] Non-Steroidal Anti-Inflammatory Drugs, [8] MDA = Malonedialdehyde, [9] SOD = Superoxide Dismutase, [10] GSH = Glutathione, [11] QoL = Quality of Life

Table 3. Summary of Clinical Studies with Nutraceuticals and Dietary Approaches in Clinically Diagnosed Knee OA

Intervention & Daily Dose	Study Design & Sample Size	Duration	Effects of Active Agent vs. Placebo/Control	Ref
Ginger Powder Ginger Powder (1,000mg) vs. Placebo (1,000mg)	Randomized, double-blind control trial (n=100)	3 months	Decrease in serum hs-CRP[1] and NO[2]	[67]
Burdock root tea Burdock Tea (6g/ 450mL) vs. Control (150 cc Boiled Water)	Randomized, control trial (n=36)	6 weeks	Decrease in serum MDA[3], hs-CRP, and IL-6[4], increases in TAC[5] and SOD[6]	[81]
Avocado/ soybean unsaponifiables (ASU) ASU (300mg or 600mg) vs. Placebo	Randomized, multicenter, double-blind randomized control trial (n=206)	90 days	Decrease in NSAID[7] intake and VAS[8] pain score	[82]
Omega-3 Supplement (Phytalgic ©) Phytalgic vs. Placebo	Randomized, double-blind, parallel groups control trial (n=81)	3 months	Decrease in WOMAC[9] pain score and NSAID use	[83]
Glucosamine Sulfate Glucosamine Sulfate (1500mg) vs. Placebo (1500mg)	Randomized, double-blind, control trial (n=139)	3 years	No changes in joint-space width Decrease in WOMAC pain scores	[84]
Chondroitin Sulfate (Condrosulf®) Condrosulf (800mg) vs. Placebo (800mg)	Randomized, multicenter, double-blind control trial (n=110)	1 year	No changes in joint-space width Decrease in AFI[10] and VAS scores	[85]
Glucosamine and/or Chondroitin Sulfate Glucosamine (1500mg) vs. Chondroitin (1200mg) vs. Combination (2700mg) vs. Celexicob (200mg) vs. Placebo	Randomized, double-blind control trial (n=357)	24 months	Glucosamine alone had lowest loss in joint-space width compared to chondroitin alone	[86]
Diet and Exercise Diet Only (800 to 1,000kcal/day) vs. Diet & Exercise (800 to 1000 kcal/day and 3 hours/week of physical activity) vs. Exercise Only (3 hours/week of physical activity)	Randomized, single center, double-blind control trial (n=399)	18 months	Decrease in weight and serum IL-6 among diet only and diet & exercise group Decrease in WOMAC pain score among diet & exercise group	[30]

[1]hs-CRP = highly sensitive C-Reactive Protein, [2] NO = Nitric Oxide, [3] MDA = Malonedialdehyde, [4] IL-6 = Interleukin-6, [5] TAC = Total Antioxidant Capacity, [6] SOD = Superoxide Dismutase, [7] NSAID = Non-Steroidal Anti-Inflammatory Drugs, [8] VAS = Knee Pain Visual Analog Scale, [9] WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index, [10] AFI = Lequesne's Algo-function Index

Table 4. Summary of *in-vitro* and Animal Studies with Nutraceuticals and Knee OA

Nutraceutical & Dose	Cell/ Animal Model	Effects of Active Agent vs. Control/ Vehicle	Ref
Curcumin 5µM vs. 10µM vs. 15µM vs. 20µM	Human chondrocytes	Reduced production of NO[1], IL-6[2], and MMP-3[3] in IL-1β[4] induced chondrocytes	[44]
Resveratrol 100mg/mL	Human chondrocytes	Reduced IL-1β expression and IL-1β produced ROS[5]	[46]
Omega-3 Fatty Acids 10 to 100µg/ mL of Omega-3 and fatty acid blend	Bovine chondrocytes	Reduced mRNA expression of Agg-1[6], Agg-2[7], IL-1α[8], and TNF-α[9] in bovine chondrocytes	[36]
Avocado/Soybean Unsaponifiables (ASU) Avocado Only-(3.3 and 10µg/ mL) vs. Soybean Only-(6.6 and 10µg/ mL) vs. 1 part Avocado, 2 part Soybean-(10µg/ mL)	Human chondrocytes	Reduction in IL-6, NO, and MMP-3 production (with and without IL-1β stimulation)	[41]
Green Tea IL-1β (5ng/mL) and 100 µg/ mL of EGCG[10] vs. IL-1β (5ng/mL) and 200 µg/ mL of EGCG vs. 200 µg/ mL of EGCG Only	Human chondrocytes	Reduction in the activity of iNOS[11] and COX-2[12], reduction in the production of NO and PGE_2[13]	[87]
Curcumin Oral Curcumin (50mg/kg) vs. Topical Curcumin Nanoparticles (0.07mg of 10µg curcumin/ 1mg nanoparticles) vs. Vehicle Control (Coconut Oil)	C57BL/6 Mice	Suppression of mRNA expression of IL-1β, TNF-α, MMP-1[14], MMP-3, and MMP-13[15] Oral administration decreased OA disease progression Topical nanoparticles decreased synovitis and subchondral plate thickness, decreased expression of adipokines and pro-inflammatory mediators in IPFP, and decreased pain	[90]
Green Tea Intraperitoneal injection of EGCG (25mg/kg) vs. control vehicle	C57BL/6 Mice	Reduced levels of MMP-1, MMP-3, MMP-13, IL-1β, and TNF-α in articular cartilage	[91]

[1] NO = Nitric Oxide, [2] IL-6 = Interleukin-6, [3] MMP-3 = Matrix Metalloproteinase-3 (Stromylsin-1), [4] IL-1β = Interleukin-1-beta, [5] ROS = Reactive Oxygen Species, [6] Agg-1 = Aggrecanase-1 , [7] Agg-2 = Aggrecanase-2, [8] IL-1α = Interleukin-1-alpha, [9] TNF-α = Tumor Necrosis Factor-alpha, [10] EGCG = Epigallocatechin-3-gallate, [11] iNOS = inducible Nitric Oxide Synthase, [12] COX-2 = Cyclooxygenase-2, [13] PGE_2 = Prostaglandin-E2, [14] MMP-1 = Matrix Metalloproteinase-1, [15] MMP-13

CHAPTER III

METHODOLOGY

Participants

Approval from Oklahoma State University's International Review Board (IRB) was obtained prior to recruitment for this study. Investigators and research assistants involved in this study completed training through the Collaborative Institutional Training Initiative (CITI). Additionally, all participants provided written consent to the study prior to being enrolled.

Participants were recruited from the Oklahoma Medical Research Foundation (OMRF) patient registry through recruitment flyers/emails, as well as referrals from the Oklahoma University Health Science Center (OUHSC). Subjects included in the study were ambulatory men and women (over the age of 18) with an enlarged waist circumference (women >35 inches, men >40 inches) and knee pain due to age-related degenerative knee OA (based on medical history and baseline questionnaire). Participants stable on hypolipidemic, hyperglycemic, and/or anti-hypertensive medications were also included in the study. Individuals were excluded from the study if they had any type of pre-existing disease such as cancer, coronary heart disease, liver failure, or renal disorders. Additionally, participants were also excluded if they were pregnant or lactating, had knee surgery, a traumatic knee injury, allergic to strawberries, taking mega doses (greater than 1,000mg) or antioxidants or fish oil supplements, recent participation in a weight loss program, or those who smoke. After recruitement, 17 subjects were enrolled and completed the entire 26-week, cross-over trial as shown in Figure 1.

Study Design

In a 26-week randomized placebo-controlled crossover trial, participants were randomized to either the intervention or placebo group. The intervention group consumed 50 gram of freeze-dried strawberry powder reconstituted in water each day divided into two doses. The placebo group followed an identical protocol consuming 50 grams of placebo powder each day divided into two doses. Participants spent 12 weeks in the intervention or placebo arm of the study, completed a 2-week washout period, and then crossed over to the opposite arm. The freeze-dried strawberry and placebo powder were provided to study participants in vacuum-sealed packs with storage instructions.

Blood draws and laboratory tests

Fasting blood samples were collected at baseline, end of week 12 (end of phase 1), week 14 (end of wash-out), and week 26 (end of phase 2). All patients were seen at the Oklahoma Clinical and Translational Science Institute (OCTSI) or at OUHSC, and blood samples were sent to OU Medical Center for analyses of glucose, HbA1c, lipid profiles, and hs-CRP. Serum collected was separated and stored at -80°C until analyzed. Serum nitrite levels were assessed using Promega Griess Reagent System (Promega Madison, WI) and serum levels of MMP-3, MMP-8, IL-6, and IL-1β were assessed using the R&D Human Quantkine ELISA (R&D Systems, Minneapolis, MN) immunoassay system.

Dietary Intakes

All study participants were asked to maintain their usual dietary habits throughout the duration of the study. Participants were required to complete a three-day food record to assess

normal or common dietary intakes. Dietary intakes were provided by the subjects at baseline, end of week 12 (end of phase 1), week 14 (end of wash-out), and week 26 (end of phase 2). Nutrient composition was analyzed using Nutritionist Pro Software (Axxya Systems, Redmond, WA).

Pain and Quality of Life Assessments

Pain scores and quality of life assessments were obtained at baseline, end of week 12 (end of phase 1), week 14 (end of wash-out), and week 26 (end of phase 2). Knee pain analysis was assessed using ICOAP and VAS pain scales. ICOAP is a series of questions which are divided into two sections and assess intermittent pain or pain that comes and goes, and constant pain or pain that is always present. The scale quantifies the patient response to each question providing a score from 0 to 4; (0) 'not at all' and (4) 'extremely'.[107] Scores were then normalized between 0 (no pain) and 100 (extreme pain). The scores for constant pain (five questions) were scored out of 20, while scores for intermittent pain (six questions) were scored out of 24. Percentages were then calculated for each participant.

The VAS pain scale is used to quantify a visual and verbal representation of pain by the use of a horizontal or vertical line ruler which is 100mm in length. This self-reported pain method allows a participants to rank their pain from 0 (no pain) to 10 (worst pain imaginable) and to place a marker perpendicular to the VAS indicator. The pain score is quantified by measuring the distance from 0 (no pain) to the marker indicated by the participant.[103] Scores were categorized as follows: 0-4 (no pain), 5-44 (mild pain), 45-75 (moderate pain), 76-100 (extreme pain). Scores were then translated to represent a score between 0 and 3, where 0 indicated no pain and increasing scores indicated worsening pain.

In combination with ICOAP and VAS, HAQ-DI was also utilized to evaluate the health state of the participants. HAQ-DI is a questionnaire which aims to understand the burden of a

chronic condition, such as knee OA, on daily activities such as dressing, eating, and walking.[104] HAQ-DI questionnaires assess eight domains, allowing a score of 0 (indicating ease with performing the task) and 3 (indicating difficulty or inability to perform the task). Total scores are calculated into a percentage out of a total possible score of 24. Scores were then normalized between 0 (indicating no difficulty with a task) and 100 (indicating severe difficulty or inability to perform a task). All non-invasive indicators will contribute to pain score evaluations and the association to dietary intakes at baseline.

Statistical analyses

Observational Study – Baseline Data

Demographic data, disease characteristics, dietary intakes, serum biochemical markers, pain scores, and quality of life assessments were summarized for all study participants at baseline and expressed in mean and standard error of the mean. Frequencies were identified from baseline data and correlations were expressed using Pearson correlation coefficient. A second model was generated to adjusted for total energy intakes with the highest and lowest calories dropped from the correlation analysis.

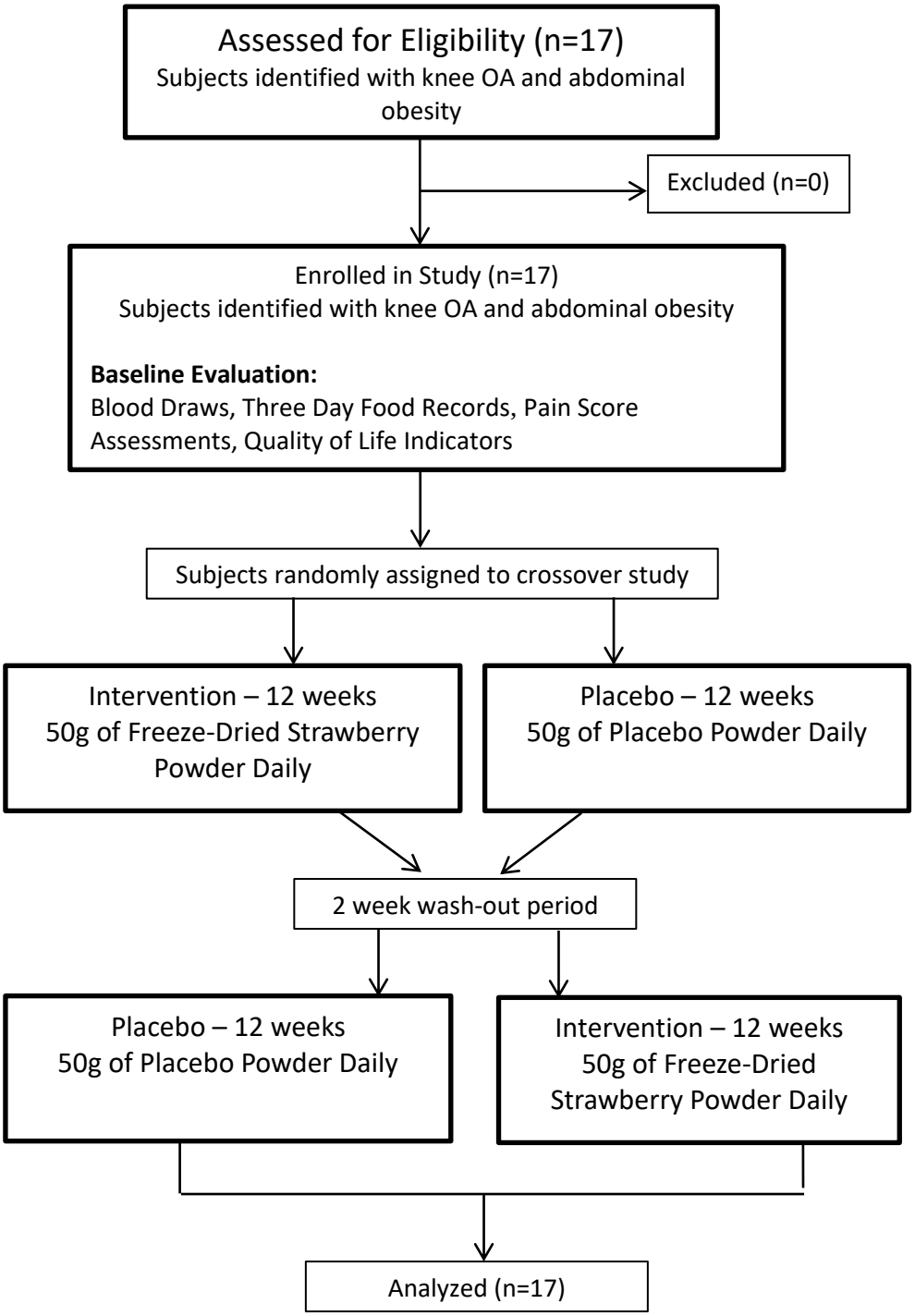
1. Correlations of dietary nutrient intakes (macronutrient, micronutrient) vs. serum markers of inflammation and cartilage degradation (hs-CRP, IL-1β, IL-6, NOx, MMP-3, MMP-8), pain scores (ICOAP, VAS), and quality of life indicators (HAQ-DI)

2. Correlation of serum biomarkers of inflammation and cartilage degradation (hs-CRP, IL-1β, IL-6, NOx, MMP-3, MMP-8) vs. pain scores (ICOAP, VAS) and quality of life indicators (HAQ-DI)

Intervention

Demographic data, disease characteristics, dietary intakes, serum inflammatory markers, pain scores, and quality of life assessments were summarized for all study participants at baseline and expressed in mean and standard error of the mean. Change in serum biomarkers of cartilage degradation were compared at different time points between the strawberry intervention and placebo groups using a PROC MIXED analysis adjusting for baseline measure as a covariate. Data was collected at the end of the washout phase; however, since no carryover effects were noted, baseline data was used to adjust in the final model. All analyses were conducted using Statistical Analysis Software version 9.2 (SAS Institute, Cary, NC). A two sided p-value of less than 0.05 was considered statistically significant.

Figure 1. Participant Allocation



CHAPTER IV

FINDINGS

Nutrient Composition of Freeze-Dried Strawberry Powder

A nutrient analysis was completed by the California Strawberry Commission to determine the macronutrient composition of the freeze-dried strawberry powder and the placebo (Table 5). Freeze-dried strawberry powder (50g) used in our study is equivalent to approximately 500 grams of fresh weight strawberries (1.10 lb). The analysis indicated that 50 grams of freeze-dried strawberry powder or placebo powder provided approximately 150 and 180 calories, respectively, to each participant's daily dietary intakes. The overall calorie contribution for each macronutrient of the freeze-dried strawberry powder was 88% carbohydrate, 9.3% protein, and 6% fat. The freeze-dried strawberry powder provided significant amounts of polyphenols and anthocyanins, while these phytochemicals were not detectable in the placebo powder.

Baseline Characteristics

Anthropometrics, Blood Pressure, Lipid Profiles, Medications

The baseline characteristics are summarized in Table 6. Among the 17 participants recruited for this pilot study, 4 were male and 13 were female. The age of the participants ranged from 45 to 75 years of age, with the mean age being 56.5 years (± 1.7). Additionally, 16 of the 17 participants were classified as obese, having a BMI greater than 30.0 kg/m^2. Each participant

presented with an enlarged waist circumference (greater than 35 inches of females and 40 inches for males) with the mean size being 46.9 inches (± 1.1). The average blood pressure for all participants can be considered normal (<130/85 mm/Hg), however, of our 17 participants, 11 were taking blood pressure medications at baseline. Other metabolic factors indicated that on average, participants had an elevated fasting blood glucose (> 100mg/dL), with 2 participants diagnosed with diabetes, and 6 participants being on lipid lowering medications.

Pain Scores, Quality of Life Indicators, Biochemical Parameters

At baseline, study participants, on average, reported mild to moderate pain based on ICOAP and VAS scores. ICOAP scores for intermittent pain ranged from 2 to 15 out of a possible score of 24, with an average score of 9.2 out of 24 or 38.5%. Constant pain scores ranged from 0 to 11 out of a possible 20, with an average score of 6.4 or 31.8%. Overall, total ICOAP pain ranged from 4 to 26 out of a possible 44, with an average score of 15.6 or 35.4%. VAS pain scores varied from 0.3 (indicating no or little pain) to 2.8 (indicating extreme pain), with an average of 1.4 (indicating moderate pain). Finally, HAQ-DI scores reported by the participants indicated mild to moderate difficulty performing daily tasks. Scores ranged from 0 to 1.0 with an average score of 0.62.

Biochemical data also varied among participants. At baseline CRP ranged from 1.5 to 20.6 mg/L. In addition, a large variation was seen among IL-1β, ranging from 3.1 to 52.4 pg/mL. Less variation was seen among IL-6, nitrite, MMP-3, and MMP-8 with serum levels ranging from 5.3 to 11.8 pg/mL, 3.7 to 11.4 μM, 2.1 to 10.4 ng/mL, and 0.8 to 5.3 ng/mL, respectively.

Dietary Intakes

All dietary data was summarized based on three-day food records provided by the participants at baseline. Total calorie intake varied dramatically ranging from 767 kcals per day to 4,316 kcals per day. Based on calorie contribution for each macronutrient, diets consisted of

approximately 42% carbohydrate, 40% fat, and 18% protein, with saturated fat intake accounting for 13% of total calories. Participants were consuming a daily average of 67 grams or 5.5 tablespoons sugar. The average daily fiber intake was 17.7 grams which falls well below the daily recommendation of 25 grams per day for women, and 38 grams per day for men.[52] In addition, dietary intakes among study participants fell short of the Recommended Daily Allowance (RDA) for vitamin C (44.7 mg/day compared to the RDA of 75 mg/day), vitamin D (55 IU/day compared to the RDA 600 IU/day), vitamin E (4.6 mg/day compared to the RDA 15 mg/day). Finally, although there is no set RDA for omega-3 PUFA intake, the designated Adequate Intake (AI) was also not met on average by study participants.[52]

Observational Findings: Correlation Analyses Among Dietary Nutrient Intakes and Biomarkers of Pain, Inflammation, and Cartilage Degradation

Correlations between variables were identified using baseline data (Table 8, 9, 10) and expressed in r-values. Assessment of macronutrient intakes revealed a statistically significant moderate inverse relationship of soluble fiber with serum MMP-3 concentrations, total sugar with MMP-8, and saturated fat with MMP-3. Upon assessment of micronutrient intakes, a significant moderate inverse relationship was identified between vitamin C and MMP-8, as well as between copper and IL-6 ($p<0.05$).

Although not statistically significant, there were several trends identified in our data worth mentioning. First, VAS pain trended toward a positive relationship with serum IL-1β concentrations ($p=0.086$). Additionally, there was a trend toward an inverse relationship of total carotenoids (RE), β-carotene, and vitamin C with serum MMP-3 concentrations ($p=0.055$, 0.097, 0.093, respectively).

Due to the variation in participant calorie intake, correlations were also analyzed with all nutrients adjusted to 1,000 calories with the highest and lowest total calories excluded from the analyzed data (n=15). The results showed a statistically significant inverse correlation between HAQ-DI scores and total cholesterol ($p=0.024$). Additionally, there was a significant positive correlation between ICOAP constant pain and total carbohydrate intake ($p=0.022$). Adjusted correlations between nutrients and serum biomarkers revealed inverse relationship of soluble fiber with MMP-8 ($p=0.030$), vitamin C with MMP-8 ($p=0.034$), and copper with IL-6 ($p=0.040$).

Intervention Findings: Effects of Freeze-Dried Strawberries on Biomarkers of Cartilage Degradation

Three parameters were used to assess cartilage degradation and inflammation in the study participants (Table 11). After the 12 week strawberry intervention, serum concentrations of the cartilage degrading enzyme, MMP-3, was significantly reduced by 18.8% ($p<0.05$). MMP-8 and serum nitrite levels showed no significant changes after strawberry intervention.

Table 5. Strawberry Beverage Composition		
	Strawberry	Placebo
Serving Size (g)	50.0	50.0
Calories (kcal)	150.0	180.0
Fat (g)	1.0	1.0
Protein (g)	3.5	0.2
Carbohydrates (g)	33	43.9
Total Polyphenols (mg)	2006.0	n.d
Total Anthocyanin (mg)	154.0	n.d
Phytosterols (mg)	50.0	n.d
Total dietary fiber (g)	4.0	5.2

Strawberry powder was obtained through the California Strawberry Commission. (g) = grams; (mg) = milligrams; (kcal) = kilocalories

Table 6. Participant Baseline Characteristics[1] (n=17)	
Age (yrs)	56.5 ± 1.7
Gender (m/f)	4/13
Height (cm)	169.4 ± 2.8
Weight (lbs)	244.9 ± 7.2
BMI (kg/m^2)	39.3 ± 1.6
Waist Circumference (inches)	46.9 ± 1.1
Systolic BP (mm/Hg)	126.8 ± 2.0
Diastolic BP (mm/Hg)	81.7 ± 1.3
Triglycerides (mg/dL)	130.3 ± 13.4
Total Cholesterol (mg/dL)	187.6 ± 8.9
HDL (mg/dL)	54.3 ± 3.5
LDL (mg/dL)	105.5 ± 7.9
Fasting Glucose (mg/dL)	112.3 ± 5.3
HbA_{1C} (%)	6.1 ± 0.2
Blood Pressure Medication (n)	11/17
Lipid Medication (n)	6/17
Diagnosed w/Diabetes (n)	2/17
Vitamin Supplement Use (n)	11/17
HAQ-DI	0.6 ± 0.1
VAS : Pain	1.4 ± 0.2
VAS : Health	0.7 ± 0.1
ICOAP : Constant Pain Subscale %	31.8 ± 3.5
ICOAP : Intermittent Pain Subscale %	38.5 ± 3.4
ICOAP : Total Pain Score %	35.4 ± 3.1
C-Reactive Protein (mg/L)	5.75 ± 1.2
Interleukin-6 (pg/mL)	8.85 ± 0.4
Interleukin-1β (pg/mL)	18.65 ± 4.0
Serum Nitrite (μM)	6.45 ± 0.8
MMP-3 (ng/mL)	7.0 ± 0.6
MMP-8 (ng/mL)	1.85 ± 0.3
[1] values expressed in mean ± standard error	

(BMI) = Body Mass Index; (m) = Male; (f) = Female; (cm) = Centimeter; (lbs) = Pounds; (kg) = Kilogram; (m^2) = Meters squared; (yrs) = Years; (mm/Hg) = Millimeters of Mercury; (mg/dL) = milligrams per deciliter; (n) = numerical value; (HAQ-DI) = Health assessment questionnaire disability index; (ICOAP) = Measure of intermittent and constant osteoarthritis pain; (VAS) = Visual analog scale; (mg/L) = milligrams per liter; (pg/mL) = pictograms per milliliter; (ng/mL) = nanograms per milliliter; (μM) = micromolar; (MMP) = Matrix Metalloproteinase

Table 7. Baseline Total Dietary Nutrient Intakes[1] (n=17)	
Calories (kcal)	2,026.5 ± 182.7
Fat Calories (kcal)	807.2 ± 107.4
Saturated Calories (kcal)	269.8 ± 34.2
Protein (g)	92.3 ± 11.2
Carbohydrate (g)	214.6 ± 19.5
Fiber (g)	17.7 ± 1.9
Soluble Fiber (g)	0.9 ± 0.2
Sugar (g)	66.74 ± 5.8
Fat (g)	89.8 ± 12.0
Saturated Fat (g)	30.0 ± 3.8
Monounsaturated Fat (g)	16.9 ± 3.8
Polyunsaturated Fat (g)	7.9 ± 1.9
Trans-Fat (g)	1.1 ± 0.4
Cholesterol (mg)	304.2 ± 53.9
Vitamin A (IU)	4,130.8 ± 1,134.2
Carotenoids (RE)	127.4 ± 50.8
β-Carotene (mcg)	584.7 ± 304.1
Vitamin C (mg)	44.7 ± 9.0
Vitamin D (IU)	55.3 ± 14.5
Vitamin E (mg)	4.6 ± 1.7
Copper (mg)	0.6 ± 0.1
Iron (mg)	9.9 ± 1.2
Zinc (mg)	6.7 ± 1.0
Omega-3 (g)	0.8 ± 0.3
[1] values expressed in mean ± standard error	

(kcal) = kilocalories; (g) = grams; (mg) = milligrams; (IU) = International Units; (RE) = Retinol Equivalents; (mcg) = micrograms

Table 8. Pearson's Correlation[1] Dietary Nutrient Intakes vs. Pain Scores and Quality of Life Assessments (n=17)						
	HAQ-DI	VAS: Pain	VAS: Health	ICOAP: Constant	ICOAP: Intermittent	ICOAP: Total
Total Caloies (kcal)	-0.030	0.373	-0.008	0.025	0.049	0.043
Protein (g)	-0.132	0.275	-0.103	-0.193	-0.102	-0.157
Total Carbohydrate (g)	-0.193	0.385	-0.071	-0.141	-0.005	-0.074
Total Fiber (g)	-0.114	0.342	0.118	-0.138	0.000	-0.069
Soluble Fiber (g)	0.181	0.332	0.068	**0.463**	0.276	0.400
Total Sugar (g)	0.138	0.316	-0.099	0.169	-0.212	-0.038
Total Fat (g)	-0.262	0.176	0.269	-0.244	-0.303	-0.304
Saturated Fat (g)	0.130	-0.098	0.305	0.178	0.367	0.308
Monounsaturated Fat (g)	-0.132	0.273	-0.102	-0.195	-0.103	-0.159
Polyunsaturated Fat (g)	-0.193	0.385	-0.071	-0.141	-0.005	-0.074
Trans Fat (g)	-0.290	-0.018	0.033	-0.340	-0.110	-0.238
Cholesterol (mg)	-0.307	0.203	-0.062	-0.159	-0.077	-0.126
Vitamin A (IU)	0.058	0.122	0.200	0.359	0.147	0.270
Carotenoids (RE)	-0.216	0.120	0.386	0.349	0.357	0.389
B-carotene (mcg)	-0.206	0.082	0.404	0.342	0.369	0.392
Vitamin C (mg)	-0.255	0.164	0.408	0.128	0.100	0.125
Vitamin D (IU)	0.069	0.055	-0.100	0.028	-0.124	-0.058
Vitamin E (mg)	0.095	-0.095	-0.135	-0.213	-0.306	-0.288
Copper (mg)	0.107	0.078	-0.122	-0.091	-0.268	-0.215
Iron (mg)	0.169	0.387	-0.036	-0.070	-0.166	-0.133
Zinc (mg)	-0.235	0.168	0.109	-0.267	-0.221	-0.239
Omega-3 PUFA (g)	-0.205	-0.155	0.048	-0.377	-0.080	-0.239
Omega-6 PUFA (g)	-0.339	0.188	-0.118	-0.252	-0.133	-0.207
[1] Values expressed in r-values						

***p<0.05**

Bold values indicate possible trends

(kcal) = kilocalories; (g) = grams; (mg) = milligrams; (IU) = International Units; (RE) = Retinol Equivalents; (mcg) = micrograms; (IL-6) = Interleukin-6, (IL-1β) = Interleukin-1 beta, (MMP-3) = Matrix Metalloproteinase-3, (MMP-8) = Matrix Metalloproteinase-8

Table 9. Pearson's Correlation[1] Dietary Nutrient Intakes vs. Biochemical Data (n=17)						
	CRP	IL-6	IL-1β	NOx	MMP-3	MMP-8
Total Calories (kcal)	-0.127	0.201	0.155	0.299	-0.326	-0.041
Protein (g)	-0.196	0.237	0.021	0.284	-0.197	0.093
Total Carbohydrate (g)	-0.277	0.071	0.043	0.133	-0.228	0.181
Total Fiber (g)	-0.201	-0.056	0.107	-0.055	0.061	0.104
Soluble Fiber (g)	0.108	0.148	0.296	-0.055	**-0.541***	-0.316
Total Sugar (g)	0.150	-0.253	0.160	0.284	-0.141	**-0.531***
Total Fat (g)	-0.140	0.089	0.093	-0.112	-0.298	**-0.264**
Saturated Fat (g)	0.279	0.143	0.152	-0.105	**-0.509***	-0.325
Monounsaturated Fat (g)	-0.195	0.237	0.020	0.284	-0.195	0.092
Polyunsaturated Fat (g)	-0.278	0.071	0.043	0.133	-0.228	0.181
Trans Fat (g)	-0.179	0.004	-0.165	0.155	0.225	-0.112
Cholesterol (mg)	-0.272	0.032	0.072	0.050	-0.043	-0.053
Vitamin A (IU)	-0.070	-0.130	-0.107	-0.177	0.058	-0.042
Carotenoids (RE)	0.137	0.071	-0.164	-0.177	**-0.473**	-0.300
B-carotene (mcg)	0.086	0.122	-0.178	-0.235	**-0.416**	-0.224
Vitamin C (mg)	0.063	0.089	0.004	-0.060	**-0.420**	**-0.490***
Vitamin D (IU)	-0.197	0.183	**0.455**	0.282	0.119	-0.181
Vitamin E (mg)	0.023	-0.357	-0.156	0.003	0.367	-0.285
Copper (mg)	0.203	**-0.492***	-0.082	-0.215	0.185	-0.335
Iron (mg)	0.153	-0.167	0.152	0.227	-0.175	-0.274
Zinc (mg)	-0.085	-0.163	0.108	-0.021	0.123	-0.309
Omega-3 PUFA (g)	-0.184	0.115	-0.178	0.199	0.346	0.004
Omega-6 PUFA (g)	-0.063	-0.058	-0.100	0.127	0.294	0.019
[1] Values expressed in r-values						

***p<0.05**

Bold values indicate possible trend

(kcal) = kilocalories; (g) = grams; (mg) = milligrams; (IU) = International Units; (RE) = Retinol Equivalents; (mcg) = micrograms; (IL-6) = Interleukin-6, (IL-1β) = Interleukin-1 beta, (MMP-3) = Matrix Metalloproteinase-3, (MMP-8) = Matrix Metalloproteinase-8

Table 10. Pearson's Correlation[1] Biochemical Data vs. Pain Scores and Quality of Life Assessments (n=17)						
	HAQ-DI	VAS: Pain	VAS: Health	ICOAP: Constant	ICOAP: Intermittent	ICOAP: Total
CRP (mg/L)	0.316	0.055	-0.315	0.227	0.211	0.240
IL-6 (pg/mL)	-0.064	-0.109	0.193	-0.234	-0.096	-0.176
IL-1β (pg/mL)	0.099	**0.428**	-0.016	0.135	-0.140	-0.013
Nitrite (μM)	0.202	0.176	-0.161	-0.108	-0.364	-0.269
MMP-3 (ng/mL)	0.050	-0.319	-0.140	-0.299	-0.337	-0.352
MMP-8 (ng/mL)	0.129	0.027	0.018	-0.160	0.128	-0.006
[1] Values expressed in r-values						

***p<0.05**

Bold values indicate possible trends

(HAQ-DI) = Health assessment questionnaire disability index; (ICOAP) = Measure of intermittent and constant osteoarthritis pain; (VAS) = Visual analog scale; (mg/L) = milligrams per liter; (pg/mL) = pictograms per milliliter; (ng/mL) = nanograms per milliliter; (μM) = micromolar; (MMP) = Matrix Metalloproteinase; (IL-6) = Intereukin-6; (IL-1β) = Interleukin-1 beta; (CRP) = C-reactive protein

Table 11. Serum Biomarkers of Cartilage Degradation Following Strawberry and Control Intervention (n=17)				
	Baseline	Strawberry 12wk	Washout 2wk	Control 12wk
MMP-3 (ng/mL)	6.9±0.6	**5.3±0.5***	7.1±0.6	6.8±0.5
MMP-8 (ng/mL)	1.8±0.3	2.2±0.3	2.4±0.2	2.1±0.2
Nitrite (μM)	6.4±0.7	9.9±2.2	6.4±0.8	7.5±0.8

***p<0.05 vs. control**

(MMP) = Matrix Metalloproteinase; (ng/mL) = nanogram per milliliter; (μM) = micromolar

CHAPTER V

CONCLUSIONS

Overall, our study identified several important relationships of dietary nutrient intakes with serum markers of inflammation and cartilage degradation in participants with symptomatic knee OA. Using baseline data, we identified inverse relationships of macro and micronutrients, such as fiber, saturated fat, total sugar, vitamin C, and copper with biomarkers of inflammation and cartilage degradation such as MMP-3, MMP-8, and IL-6. As part of our strawberry intervention, we found that daily consumption of 50g of freeze-dried strawberries significantly decreased serum concentration of MMP-3 in participants with symptomatic knee OA.

Observational: Correlation Analyses Among Dietary Nutrient Intakes and Biomarkers of Pain, Inflammation, and Cartilage Degradation

To our knowledge, this is the first study to identify correlations between normal dietary intakes and serum concentrations of the cartilage degrading enzymes, MMP-3 and MMP-8. MMP's are a family of cartilage degrading enzymes and can be detected at the systemic level. In knee OA, MMP's, specifically MMP-3, facilitates the breakdown of articular cartilage, which contributes to joint space narrowing in the affected joint.[56] Our study revealed that higher intakes of soluble fiber and saturated fat were correlated with lower serum concentrations of MMP-3. Additionally, our data also showed higher total sugar and vitamin C intakes were correlated with lower serum concentrations of MMP-8.

The relationship of dietary macronutrient intakes with pain and loss of articular cartilage has been previously studied in both the development and progression of knee OA.[112,118] However, studies to evaluate relationships between dietary macronutrient intakes and serum biomarkers of inflammation and cartilage degradation in knee OA is lacking. Our findings suggest that lower serum concentrations of MMP-3 were correlated with higher saturated fat intake. However, these findings are not consistent with previous research efforts. Lu et al. identified a positive relationship between saturated fat intake and joint space narrowing.[118] These findings suggest that the degradation of articular cartilage, which is facilitated by MMP's, is seen among individuals with higher saturated fat intake. However, this particular study did not directly evaluate serum markers of cartilage degradation, specifically MMP's, nor did it identify the sources of saturated fat. In our study, participants consumed approximately 13% of calories from saturated fat which is slightly greater than the recommended daily intake set by *2015-2020 Dietary Guidelines for Americans* (recommending less than 10% of total calories).[121] Although most of the research on saturated fat intake has been largely related to risk for cardiovascular disease, there appears to be no clear consensus on how different sources of saturated fat contribute to disease risk. Otto et al. revealed that saturated fat coming from dairy products was associated with lower CVD risk, whereas saturated fat coming from animal meat was positively associated with CVD risk.[129] In contrast, the most recent review from the American Heart Association Presidential Advisory, identifies dairy saturated fat as a contributing factor to CVD.[130]

Since our study focuses on the relationships of nutrients with pain scores and biological parameters as they relate to OA and not CVD, we must consider how each source of saturated fat (i.e. milk products, animal meat) provide other micro and phytonutrients. For example, along with saturated fat, 1% milk provides other nutrients such as protein, calcium, phosphorus, potassium, folate, vitamin A, and vitamin D.[98] Animal meat, such as top sirloin, provides potassium, zinc, niacin, folate, and mono/polyunsaturated fatty acids.[98] Our study did not evaluate food group

composition, therefore we are unable to identify the sources of saturated fat. However, it is possible the inverse correlation seen between saturated fat and MMP-3 is related to the added nutrients found dairy and animal meats versus processed foods which provide little or no nutritional value. We must also consider the fact that dietary nutrient intakes and serum concentrations of MMP's has not been previously researched; therefore, this relationship may be a new finding in metabolic knee OA.

We also identified a moderate inverse relationship of total sugar intake with serum concentrations of MMP-8. This finding suggests that higher total sugar intake is correlated with lower MMP-8 concentrations. This finding of total sugar intake, which includes both added sugars and natural sugars found in products such as fruits, vegetables, and grains. Among study participants, total sugar accounted for approximately 31% of total carbohydrates and 13% of total calories. Much of the literature suggest that adverse health conditions such as obesity, diabetes, and heart disease are associated with excessive intakes of added sugars.[131] As discussed with saturated fat, it is important to understand the food source in which the sugar is coming from. For example, sugar sweetened beverages, such as soda, provide absolutely no nutrients beneficial to human health. Fruit, such as strawberries, provide fiber, vitamin C, folate, carotenoids, and phytochemicals, such as polyphenols.[98,99,111] Given that individual food items were not specifically analyzed for each participant, we are unable to speculate as to whether total sugars accounted for added or natural sugar. It is possible that the inverse correlation seen between total sugar and serum concentrations of MMP-8 is related to the added nutrients found in fruits, vegetables, and whole grains versus added sugars. Finally, as stated with saturated fat intake, we must consider the fact that since these relationships have not been previously researched; this relationship may be a new finding in metabolic knee OA.

In addition to macronutrient consumption, micronutrient consumption has also been well studied in knee OA. One of the earliest epidemiological studies in knee OA identified the relationship between knee OA risk and dietary intakes of vitamin C, vitamin E, and β-carotene, suggesting lower consumption of these vitamins was associated with an increased risk of developing knee OA.[95,116] In our study, there was a statistically significant moderate inverse correlation of vitamin C intake with MMP-8. In addition, our adjusted model (n=15) also showed statistical trends which suggest higher intakes of vitamin C, total carotenoids, and β-carotene are associated with lower MMP-3 concentrations. Vitamin C and carotenoids (including β-carotene) are potent antioxidants found naturally in fruits and vegetables. Vitamin C is necessary for the homeostatic mechanism in the glutathione peroxidase cycle, which is responsible for scavenging lipid radicals. In addition, carotenoids, such as β-carotene, are also necessary for scavenging free radicals and maintaining the redox balance in the body. The disruption in the redox balance results from over-production of ROS and RNS and can occur in the serum and synovial fluid in knee OA. ROS have the ability to independently upregulate the gene expression and production of MMP's.[122] Therefore, it is very possible that higher consumption of these vitamins may have a possible mechanism which involves reducing oxidative stress to decrease the activation of both MMP-3 and MMP-8.

Next, the moderate inverse relationship seen between soluble fiber intake and serum MMP-3 suggest that MMP-3 concentrations tend to be lower individuals with higher soluble fiber intake. However, it is important to understand this correlation was only seen among soluble fiber, not total fiber. Additionally, in our study, soluble fiber accounted for approximately 5% of the total fiber among the participants, with the average total fiber intake being approximately 17 grams per day. Human studies have identified the beneficial role of soluble fiber and its ability to improve lipid profiles, reduce risk of CVD, improve glycemic control, and aid in weight loss.[55] In addition, Ma et al. conducted a long-term epidemiological study and identified an inverse

relationship between soluble fiber and serum CRP.[120] These associations were not seen in our study which may be due to the difference in baseline data for both total soluble fiber intake and serum CRP. It is possible that if soluble fiber intake had been higher among our study participants, we may have not only seen an inverse relationship among CRP, but also with other inflammatory biomarkers. It is possible soluble fiber, with many benefits to human health, may have the ability to reduce the overall inflammatory burden, thus resulting in lower production of MMP's.

Finally, we identified an inverse correlation between dietary copper intake and serum concentrations of IL-6. Copper is an essential co-factor needed for many important enzymes systems such as ceruloplasmin, superoxide dismutase (SOD), cytochrome oxidase.[132] However, evidence to support the relationship of dietary copper intakes with biomarkers of inflammation and oxidative stress is lacking. Much of the literature identifies the impact of serum copper concentrations and the clinical implications associated with deficiency and toxicity. It has been speculated that copper has both antioxidant and pro-oxidant capabilities which are largely related to the production of ROS due to decreased SOD activity (copper deficiency) and increased Fenton reactions (copper toxicity).[132,133] Bo et al. identified higher serum hs-CRP concentrations among participants with higher dietary copper intakes. Although this study did not directly evaluate serum IL-6 concentrations, they hypothesize that higher inflammation may be a result of higher dietary copper intakes, higher serum copper concentrations, and increased oxidative stress.[134] In our study, average dietary intake of copper among participants fell well short of the RDA and that of the previously mentioned study (1.5mg/day, Bo et al.).[52,134] Since our study did not evaluate serum copper concentrations, it would be difficult to speculate the specific mechanism behind the inverse relationship of copper with IL-6. It is possible this is a new finding in metabolic knee OA. However, future studies would need to be conducted to determine the

influence of dietary copper intakes with both serum copper concentrations and IL-6 concentrations in participants with knee OA.

It is important to address why there were no statistically significant correlations identified among dietary nutrient intakes with pain scores and quality of life indicators; as well as among all biochemical parameters with pain scores and quality of life indicators. In 2014, Choi et al. conducted an ecological momentary study to evaluate the relationship between pain and eating among individuals with symptomatic knee OA. The researchers concluded the relationship between dietary intakes and pain is cyclic; where higher pain yields higher energy intakes (specifically in the form of total fat and total sugar), and higher energy intakes, further perpetuate pain in knee OA.[112] Additionally, other studies have identified that higher energy intakes, total carbohydrate intakes, and total sugar intakes are positively correlated with the development of BML's in knee OA.[97] The development of BML's have also been associated with higher pain levels among individuals with symptomatic knee OA.[135] The findings from these previous studies lead us to hypothesize that higher dietary intakes of total fat and total carbohydrate would be positively correlated with pain scores and biochemical parameters, and inversely correlated with quality of life indicators. These findings were not identified in our unadjusted analysis (n=17). However, we did identify several statistically significant correlations in our adjusted model (n=15), suggesting an inverse correlation of total cholesterol intake with HAQ-DI and total carbohydrate intake with ICOAP: constant pain. At baseline, participants reported having only mild to moderate pain, as indicated in VAS and ICOAP questionnaires; as well as only mild to moderate difficulty performing daily tasked, indicated by HAQ-DI questionnaires. Had our participants reported moderate to severe pain, moderate to severe difficulty with daily tasks, or had different dietary patterns, we may have identified statistically significant correlations among these variables in our unadjusted model. The fact that we did identify two statistically significant correlations in our adjusted model, leads us to suspect that there is an important relationship that

exists between dietary nutrient intakes and pain scores or quality of life indicators, which should be confirmed in a fully powered study.

Although not statistically significant, we did identify one trend in our unadjusted model suggesting a possible positive correlation of soluble fiber intake with ICOAP: constant pain percent. This suggests that higher soluble fiber intakes trended toward being positively correlation with higher pain scores for this assessment. This trend is contrary to previous research conducted by Dai et al. who identified that higher total fiber intakes (amounts equal to 25g per day), was associated with lower WOMAC pain scores among participants with symptomatic knee OA.[115] Based on these findings we anticipated an inverse correlation of total fiber intake with pain scores and a positive correlation with quality of life assessments. However, these expectations were not met. As stated previously, participant's total fiber intake fell well short of the recommended 25g for women and 38g for men.[52] Therefore, it is possible we did not identify the anticipated correlations due to low fiber intake among our study participants, or due to our participants reporting having only mild to moderate pain, and mild to moderate difficulty with daily tasks.

Lastly, our findings did not reveal any statistically significant correlations of biochemical parameters (serum concentrations of CRP, IL-6, IL-1β, nitrite, MMP-3, and MMP-8) with pain scores or quality of life assessments among study participants at baseline in our unadjusted model. We hypothesized that higher serum concentrations of these biomarkers would be positively correlated with pain scores, and inversely correlated with quality of life indicators. Two studies have identified a positive relationship between serum concentrations of inflammatory biomarkers and OA pain. Strurmer et al. found that participants with symptomatic hip or knee OA, had a 5.7% increase in serum hs-CRP concentrations for every 10mm increase in VAS pain scores.[105] Similarly, Stannus et al. also identified positive associations of hs-CRP, TNF-α, and IL-6 with WOMAC pain scores at baseline or after a 2.7 year follow-up.[106] The

findings from these previous studies were not confirmed in our study participants. Comparatively, our participants reported having less pain and presented with much higher baseline concentrations of hs-CRP and IL-6. However, although not statistically significant, we did identify a positive trend among IL-1β and VAS pain scores in our unadjusted model. This trend suggested that higher IL-1β concentrations may be positively correlated with higher VAS pain scores. Although the overall findings among these particular variables was not as expected, this trend proposes there may be some sort of positive relationship between inflammatory biomarkers and pain scores and should be confirmed in a full powered study.

Intervention: Effects of Freeze-Dried Strawberries on Biomarkers of Cartilage Degradation

To our knowledge, this is the first human study to report the impact of the polyphenol-rich strawberry supplementation on biomarkers of cartilage degradation in knee OA. Most of the current research investigating the impact of phytochemicals on MMP-3 concentrations have been conducted in cell and animal studies. Curcumin has been widely studied in OA and has shown to decrease in IL-1β stimulated human chondrocytes, and reduce the mRNA expression of MMP-3 in C57BL/6 mice.[44,90] In addition, *in-vitro* studies evaluating ASU have also seen a decrease in MMP-3 with and without IL-1β stimulation.[41] Lastly, green tea catechins have also shown to reduce MMP-3 in the articular cartilage of C57BL/6 mice.[91] However, very few studies have evaluated the effects of polyphenols and nutraceuticals on serum levels of MMP's in humans. Mirtaheri et al. evaluated the effects of alpha-lipoic acid on serum concentrations of MMP-3 in participants with rheumatoid arthritis, but found no significant changes after an 8 week intervention.[54] Additionally, Goochani et al. showed that the polyphenolic rich pomegranate was capable of reducing serum levels of MMP-13, another cartilage degrading enzyme studied in knee OA.[75]

One important mechanism to consider when evaluating the results of our study is the impact the strawberry intervention had on biomarkers of inflammation. MMP-3 is greatly influenced by inflammatory cytokines such as IL-1β.[27,56]Although beyond the scope of this paper, our study also measured serum levels of IL-6 and IL-1β, which significantly decreased after strawberry intervention.[123] It is important to consider that this decrease in MMP-3 may also be a result of the reduction in these pro-inflammatory cytokines. Other clinical studies examining the effects of strawberries have identified their ability to significantly reduce lipid peroxidation, increase endogenous antioxidant enzymes, and reduce oxidative bi-products such as MDA and hydroxnonenol (HNE).[49,100,101] Therefore, one proposed mechanism of action, could be that strawberries reduce overall oxidative stress and decreasing the inflammatory burden, thus reducing the activation of cartilage degrading enzymes, such as MMP-3.

It is also important to address why similar effects were not seen for serum nitrite and MMP-8. Nitrite is a byproduct of the effector molecule NOx which spontaneously breakdowns down into nitrate and nitrite.[124] The production of NOx is facilitated by three forms on nitric oxide synthase; eNOS, which is important in endothelial vasodilation, iNOS, which can trigger inflammation and tissue destruction, and nNOS, which is important in cell signaling.[59,125] Our study showed a small but insignificant increase in serum nitrite levels after strawberry supplementation. Although nitric oxide synthase gene expression was not assessed in this study, it is possible that the polyphenol rich berry had an effect on increasing gene expression of eNOS resulting in an increase in NOx production. This proposed mechanism has been addressed *in-vitro* by Lazzé et al. and Xu et al. who have shown the ability of anthocynains to upregulate gene expression of eNOS in human and bovine endothelial cells.[126,127] In addition to serum nitrite, the null effects of strawberry intervention on serum concentrations of MMP-8 were also observed in our study. Although MMP-8 has been identified as a culprit in human articular cartilage degradation *in-vitro*, to date, no study has been reported on the effects of polyphenol intervention

on serum concentrations of this enzyme in humans with OA.[128] It is possible that in metabolic OA, serum concentrations of MMP-8 may not be modulated by polyphenols. It is also possible the dose of strawberries was not large enough to modulate a response, or that our sample size was simply too small to identify changes in MMP-8 after strawberry intervention. Future research should be conducted in larger clinical trials to identify if polyphenol intervention has an effect on serum concentration of MMP-8 in participants with knee OA..

Strengths, Limitations, and Future Research

The most significant limitation to our study is the small sample size of our pilot study (n=17). Therefore, it may be difficult to generalize the findings presented in this study to a larger population. Future research efforts should attempt to perform a fully powered study with a large sample size to confirm and strengthen our findings. Another limitation to this study is the cross-sectional design, which only evaluates participants with a previously diagnosed knee OA. Although the study provided us with valuable insight into the relationship between dietary habits, pain, and inflammation, future research should include longitudinal epidemiological data to assess how dietary intake impact the development of this chronic condition. Additionally, future observational studies may want to assess food group composition to better understand sources of nutrients. In doing so, this may provide further insight into protective dietary patterns in knee OA. Another potential limitation is the study lacks a dose-response design to evaluate the optimal serving of strawberries that have an effect on serum biomarkers of inflammation and cartilage degradation. Our participants consumed 50 grams per day of freeze-dried strawberries and placebo powder each for 12 weeks. It is possible that alternative dosages, whether smaller or larger, may modulate a more desirable outcome and better reflect common eating patterns.

Our study does have several strengths as well. First, by using three-day food records collected at baseline, we were able to get a better understanding of each participant's common

dietary patterns. Compared to 24-hour recalls, three-day food records allowed us to take an average of macro and micronutrients over several days which is more likely to reflect their normal diet. Next, by collecting three-day food records at each visit (baseline, end of phase 1, end of washout, and end of phase 2), we were able to confirm that participants did not change dietary habits throughout the duration of the study. Therefore, we believe the changes in these biochemical parameters were a result of the strawberry intervention rather than habitual dietary changes. Finally, the cross-over design of our intervention phase allowed each participant to serve as his or her own control, eliminating confounding variables. Additionally, each arm of the study accounted for a substantial amount of time in either the intervention or treatment group (12 weeks in each, 24 weeks total), allowing ample time for the bioactive components to facilitate a desired effect, with the two-week wash out period minimizing the potential for a carry-over effect.

Conclusion

Not only are the number of individuals with symptomatic knee OA increasing in the U.S., the condition is becoming more prevalent in a younger population given the obesity epidemic.[94] The majority of treatment strategies focus on pain management with pharmaceuticals and weight loss to decrease adiposity and the mechanical loading of the knee joint. The overall goal of any treatment strategy in knee OA should focus on reducing the chronic inflammatory burden associated with metabolic knee OA. In doing so, this may aim to not only reduce pain, but suppress the cartilage degrading enzymes that further contribute to the progression of this condition.

In our study, lower serum concentrations of cartilage degrading enzymes were seen among higher intakes of soluble fiber, total sugar, saturated fat, and higher vitamin C. Although our study does not identify cause and effect, it provides insight into the relationship between food intake and disease characteristics. We can conclude that a well-balance diet which includes

adequate intakes of saturated fat, total sugar, soluble fiber, vitamin C, carotenoids, and copper may be protective against the activation of cartilage degrading enzymes in symptomatic knee OA.

We also showed that freeze-dried strawberry intervention significantly decreased serum concentrations of MMP-3 compared to the placebo. Based on the findings from our pilot study, strawberry supplementation as a therapeutic intervention is not recommended. However, as an adjunct therapy, strawberry supplementation may have an implication in knee OA. Dietary habits in the management of knee OA symptoms and biological parameters should focus on incorporating an array of antioxidant-rich fruits and vegetables, including the bioactive strawberry fruits.

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APPENDICES

Appendix A: IRB Approval

Appendix B: Informed Consent

Appendix C: ICOAP Questionnaire

Appendix D: HAQ-DI Questionnaire

Appendix E: Food Diary Form

Appendix A: IRB Approval

Oklahoma State University Institutional Review Board

Date:	Thursday, March 19, 2015
IRB Application No:	HE1517
Proposal Title:	Effects of freeze-dried strawberries on systemic markers of inflammation and knee function in participants with osteoarthritis (OA)
Reviewed and Processed as:	Expedited

Status Recommended by Reviewer(s): Approved Protocol Expires: 3/18/2016

Principal Investigator(s):

Arpita Basu
301 HES
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

- The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1.Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval. Protocol modifications requiring approval may include changes to the title, PI advisor, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2.Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3.Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of the research; and
4.Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Dawnett Watkins 219 Cordell North (phone: 405-744-5700, dawnett.watkins@okstate.edu).

Sincerely,

Hugh Crethar, Chair
Institutional Review Board

Appendix B: Informed Consent

Consent Form

University of Oklahoma Health Sciences Center (OUHSC)
Oklahoma State University (OSU)

Title: Effects of Freeze-dried Strawberries on Systemic Markers of Inflammation and Knee Function in Participants with Osteoarthritis (OA).

Sponsor: Oklahoma State University, Department of Nutritional Sciences
California Strawberry Growers Association (providing strawberry powder)
Principal Investigator: Robert H. "Hal" Scofield, MD
Co-Principal investigator: Arpita Basu, PhD, RD

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part in them. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this study because you have documented osteoarthritis (OA) in the knee.

Why Is This Study Being Done?
The purpose of this study is to find out about the effects of strawberry intake on blood markers of inflammation and to see whether strawberries can lower these markers and improve function in the knee.

What is the Status of the Drugs (Devices or Procedures) involved in this study?
This study involves the use of strawberry powder. The strawberry powder is not approved by the FDA as a "drug".

How Many People Will Take Part In The Study?
We anticipate recruiting about 25 people with the hope that 20 will go on to complete the entire study.

What Is Involved In The Study?
This is a 26- week study that will be conducted at Oklahoma Clinical and Translational Science Institute (OCTSI), located in the Harold Hamm Diabetes Center.

You will be randomized to receive either strawberry powder or a control "placebo" powder (made of dietary fiber and sugar). Randomization means that you are put in a group by chance, like a flip of a coin. A computer program will make this random assignment; you will not know which group you will be in. About halfway through the study, you will stop taking the first assigned powder (either the strawberry or the control powder) and you will not take any powder for a couple of weeks. Then, you will start taking the powder again (either the placebo or the strawberry powder).

During the study, please do not consume any other berries other than the strawberry powder that will be provided to you.

If you take part in this study, you will have the following tests and procedures:

Screening visit:
During your first visit we will do some tests and measurements to determine if you qualify for the study. This will involve:

- Reading and signing the consent form;
- Measuring your height, weight, blood pressure, and waist size; and,
- Drawing about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and blood counts to find out how well your cells, liver, and kidneys are working.

If you qualify, we will let you know over the telephone and ask you to come back to begin the study. The study visits will be approximately every four (4) weeks for a total of eight (8) visits. The table below explains what study events will occur:

Study Interventions	Screening	Visit 1 (within 4 weeks of screening)	Visit 2 (end of 4 weeks +/-2 days)	Visit 3 (end of 8 weeks +/-2 days)	Visit 4 (end of 12 weeks +/-2 days)	Washout (no powder for ~2 weeks)
Informed Consent & HIPAA	X					
Bloodwork	X				X	
24-hour urine collection		X			X	
Serum for biomarkers		X			X	
Vitals*	X				X	
Food Diary			X	X	X	
Surveys		X		X	X	
Dispense powder/ placebo		X	X	X		

*Vitals = Blood pressure, height, weight, waist circumference

3/9/15
3/8/16
HE1517

Study Interventions	Visit 5 (end of 14 weeks +/-2 days)	Visit 6 (end of 18 weeks +/-2 days)	Visit 7 (end of 22 weeks +/-2 days)	Visit 8 (end of 26 weeks +/-2 days)
CMP, HgA1c, Lipids, HS CRP	X			X
24-hour urine collection	X			X
Serum for biomarkers	X			X
Vitals*	X			X
Food Diary		X	X	X
Surveys	X		X	X
Dispense powder/placebo	X	X	X	

*Vitals = Blood pressure, height, weight, waist circumference

You will mix the strawberry and control powders with water for consuming. The powders will come in 25g packets and you will take one packet in the morning and one in the evening. You will do this for about 12 weeks and then you will have a period of time where you will not take any powder. This is called a washout period. After the two-week washout period, your powders will be switched and you will begin taking the other powder for an additional 12 weeks.

We will ask you to keep a food log for at least three days a week for each week that you are on the study. You will bring these food diaries with you to each study visit.

There will be health surveys to complete at some of the study visits. These should take about 15-25 minutes to complete.

Depending on the study events, the study visits may be as short as 30 minutes, or as long as an hour.

How Long Will I Be In The Study?

You will be in the study for 26 weeks. There may be circumstances under which your participation may be stopped by the investigator without regard to your consent. This may occur if you do not follow the study requirements, such as keeping the food diaries and keeping your study visit appointments.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

3/9/15
3/18/16
HE1517

What Are The Risks of The Study?
While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict.

Likely: the risks involved with drinking the strawberry or control drink may include stomach ache, gas, or headache.

Less likely: the color of your stools may change.

There is also the risk of pain, bruising and infection with the blood draw. There is a slight possibility you could develop an allergy to strawberries. An allergic reaction may include things like: rash, itching, or diarrhea. Typically, if these symptoms develop, they will go away once you stop taking the strawberry powder. You will remain allergic to strawberries in the future though.

In a severe allergic reaction, you may experience difficulty breathing or throat swelling. If either of these symptoms develops, these are medical emergencies and you should go to your nearest emergency room or call 911 for immediate assistance.

Are There Benefits to Taking Part in The Study?
If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope that the information learned from this study will benefit other people in the future.

What Other Options Are There?
You may choose not to participate in the study. You may also obtain strawberries outside of the study if you choose not to participate.

What About Confidentiality?
Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. All personal information will be coded and all files will be kept in locked cabinets in the offices of the study researchers at OUHSC and OSU. Stored data in the computer will be protected by passwords known only to the study researchers. All information linked to specific names will be coded and names will be deleted after data collection is complete. After that, only numerical codes will be used to identify the study participants. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration and the OUHSC & OSU Institutional Review Boards. However, all data will be coded and no personally identifiable information will be shared.

What Are the Costs?
The study sponsor will pay for the laboratory specimens and the powders related to your participation in this study.

Will I Be Paid For Participating in This Study?
You will be reimbursed for your time and travel during the study. You will be reimbursed $10 for the screening visit. At study visits 1, 2, 3, 5, 6, and 7, you will be reimbursed $20 for your time and travel. At study visits 4 and 8, you will be reimbursed $30 for your time and travel. If you complete the entire study, your total reimbursement would equal $190.

What if I am Injured or Become Ill While Participating in this Study?
In the case of injury or illness resulting from this study, emergency medical treatment is available. No funds have been set aside by the University of Oklahoma Health Sciences Center or Oklahoma State University to compensate you in the event of injury.

What Are My Rights As a Participant?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?
If you have questions about the study or have a research-related injury, contact Dr. Hal Scofield, MD at 405-271-7061 (anytime) or Dr. Arpita Basu, PhD at 405-744-4437 (9AM-5PM, Monday-Friday) or at 405-612-2434 (anytime), or Janice Gales, RN at (405) 271-8000, extension 34881 (8:00AM-5:00PM).

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at (405) 271-2045 or email IRB@OUHSC.edu. Or, contact Dr. Sheila Kennison, IRB Chair at Oklahoma State University, at 405-744-3377 or irb@okstate.edu.

Signature:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study.

PARTICIPANT SIGNATURE (age ≥18) *(Or Legally Authorized Representative)*	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

Volunteers Needed

For a Study related to:

The Effects of Strawberries on Knee pain.

You may qualify for this study if you are a male or female with the following:

- A waist circumference of greater than 40 inches in men or 35 inches in women
- Have been diagnosed with knee pain related to osteoarthritis
- Have a knee x-ray
- Visit a physical therapist

Following an initial telephone questionnaire, eligible participants will be scheduled for a screening visit and 8 follow-up visits.

There is no charge to participate in the study. Study participants will receive compensation for each follow-up visit. Visits will take place locally at the Department of Nutritional Sciences, 307 Human Sciences at Oklahoma State University, Stillwater, OK

For more information, please contact Dr. Arpita Basu at arpita.basu@okstate.edu or (405) 744-4437

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Appendix C: ICOAP Questionnaire

{merge ID here}

A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP: KNEE Version

People have told us that they experience different kinds of pain (including aching or discomfort) in their knee. To get a better sense of the different types of knee pain you may experience, we would like to ask you about any "constant pain" (pain you have all the time) separately from any pain that you may experience less often, that is, "pain that comes and goes". The following questions will ask you about the pain that you have experienced in your knee in the PAST WEEK. Please answer ALL questions.

A) CONSTANT PAIN

For each of the following questions, please select the response that best describes, on average, your *constant knee pain* in the PAST WEEK.

1. **In the past week, how intense has your *constant knee pain* been?**

$\square_0$	$\square_1$	$\square_2$	$\square_3$	$\square_4$
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

2. **In the past week, how much has your *constant knee pain* affected your sleep?**

$\square_0$	$\square_1$	$\square_2$	$\square_3$	$\square_4$
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

3. **In the past week, how much has your *constant knee pain* affected your overall quality of life?**

$\square_0$	$\square_1$	$\square_2$	$\square_3$	$\square_4$
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

4. **In the past week, how frustrated or annoyed have you been by your *constant knee pain*?**

$\square_0$	$\square_1$	$\square_2$	$\square_3$	$\square_4$
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

5. **In the past week, how upset or worried have you been by your *constant knee pain*?**

$\square_0$	$\square_1$	$\square_2$	$\square_3$	$\square_4$
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

{merge ID here}

B) PAIN THAT COMES AND GOES

For each of the following questions, please select the response that best describes your *knee pain that comes and goes*, *on average*, in the PAST WEEK.

6. In the past week, how intense has your most severe *knee pain that comes and goes* been?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

7. In the past week, how frequently has this *knee pain that comes and goes* occurred?

☐0	☐1	☐2	☐3	☐4
Never/ No knee pain that comes and goes	Rarely	Sometimes	Often	Very Often

8. In the past week, how much has your *knee pain that comes and goes* affected your sleep?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

9. In the past week, how much has your *knee pain that comes and goes* affected your overall quality of life?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

10. In the past week, how frustrated or annoyed have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

11. In the past week, how upset or worried have you been by your *knee pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

THANK YOU!

{merge ID here}

A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP: HIP Version

People have told us that they experience different kinds of pain (including aching or discomfort) in their hip. To get a better sense of the different types of hip pain you may experience, we would like to ask you about any "constant pain" (pain you have all the time) separately from any pain that you may experience less often, that is, "pain that comes and goes". The following questions will ask you about the pain that you have experienced in your hip in the PAST WEEK. Please answer ALL questions.

A) CONSTANT PAIN

For each of the following questions, please select the response that best describes, on average, your *constant hip pain* in the PAST WEEK.

1. In the past week, how intense has your *constant hip pain* been?

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant hip pain	Mildly	Moderately	Severely	Extremely

2. In the past week, how much has your *constant hip pain* affected your sleep?

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant hip pain	Mildly	Moderately	Severely	Extremely

3. In the past week, how much has your *constant hip pain* affected your overall quality of life?

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant hip pain	Mildly	Moderately	Severely	Extremely

4. In the past week, how frustrated or annoyed have you been by your *constant hip pain?*

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant hip pain	Mildly	Moderately	Severely	Extremely

5. In the past week, how upset or worried have you been by your *constant hip pain*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant hip pain	Mildly	Moderately	Severely	Extremely

{merge ID here}

B) PAIN THAT COMES AND GOES

For each of the following questions, please select the response that best describes your *hip pain that comes and goes*, *on average*, in the PAST WEEK.

6. In the past week, how intense has your most severe *hip pain that comes and goes* been?

☐0	☐1	☐2	☐3	☐4
Not at all/ No hip pain that comes and goes	Mildly	Moderately	Severely	Extremely

7. In the past week, how frequently has this *hip pain that comes and goes* occurred?

☐0	☐1	☐2	☐3	☐4
Never/ No hip pain that comes and goes	Rarely	Sometimes	Often	Very Often

8. In the past week, how much has your *hip pain that comes and goes* affected your sleep?

☐0	☐1	☐2	☐3	☐4
Not at all/ No hip pain that comes and goes	Mildly	Moderately	Severely	Extremely

9. In the past week, how much has your *hip pain that comes and goes* affected your overall quality of life?

☐0	☐1	☐2	☐3	☐4
Not at all/ No hip pain that comes and goes	Mildly	Moderately	Severely	Extremely

10. In the past week, how frustrated or annoyed have you been by your *hip pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No hip pain that comes and goes	Mildly	Moderately	Severely	Extremely

11. In the past week, how upset or worried have you been by your *hip pain that comes and goes*?

☐0	☐1	☐2	☐3	☐4
Not at all/ No hip pain that comes and goes	Mildly	Moderately	Severely	Extremely

THANK YOU!

Appendix D: HAQ-DI Questionnaire

HEALTH ASSESSMENT QUESTIONNAIRE

Patient ID _____ Date _____

In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add any comments on the back of this page.

Please check the response which best describes your usual abilities OVER THE PAST WEEK:

	Without ANY difficulty[0]	With SOME difficulty[1]	With MUCH difficulty[2]	UNABLE to do[3]
DRESSING & GROOMING				
Are you able to:				
-Dress yourself, including tying shoelaces and doing buttons?	☐	☐	☐	☐
-Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
-Stand up from a straight chair?	☐	☐	☐	☐
-Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
-Cut your meat?	☐	☐	☐	☐
-Lift a full cup or glass to your mouth?	☐	☐	☐	☐
-Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
-Walk outdoors on flat ground?	☐	☐	☐	☐
-Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of these activities:

- ☐ Cane
- ☐ Walker
- ☐ Crutches
- ☐ Wheelchair
- ☐ Devices used for dressing (button hook, zip, long-handled shoe horn, etc.)
- ☐ Built up or special utensils
- ☐ Special or built up chair
- ☐ Other (Specify:_____)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ☐ Dressing and Grooming
- ☐ Arising
- ☐ Eating
- ☐ Walking

Please check the response which best describes your usual abilities **OVER THE PAST WEEK:**

	Without ANY difficulty [0]	With SOME difficulty [1]	With MUCH difficulty [2]	UNABLE to do [3]
HYGIENE				
Are you able to:				
-Wash and dry your body?	☐	☐	☐	☐
-Take a tub bath?	☐	☐	☐	☐
-Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
-Reach and get down a 5-pound object (such as a bag of sugar) from just above your head?	☐	☐	☐	☐
-Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
-Open car doors?	☐	☐	☐	☐
-Open jars which have been previously opened?	☐	☐	☐	☐
-Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
-Run errands and shop?	☐	☐	☐	☐
-Get in and out of a car?	☐	☐	☐	☐
-Do chores such as vacuuming or yardwork	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of these activities:

- ☐ Raised toilet seat
- ☐ Bathtub seat
- ☐ Jar opener (for jars previously opened)
- ☐ Bathtub bar
- ☐ Long-handled appliances for reach
- ☐ Long-handled appliances in bathroom
- ☐ Other (Specify:_____)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ☐ Hygiene
- ☐ Reach
- ☐ Gripping and opening things
- ☐ Errands and chores

We are also interested in learning whether or not you are affected by pain because of your illness.

How much pain have you had because of your illness IN THE PAST WEEK:

PLACE A VERTICAL (|) MARK ON THE LINE TO INDICATE THE SEVERITY OF THE PAIN

NO PAIN 0 ——— SEVERE PAIN 100

We are interested in understanding how your overall health state is today.

To help people say how good or bad a health state is, we have drawn a scale on which the best state you can imagine is marked by 0, and the worst state you can imagine is marked by 100. Please indicate on the scale how good or bad your health is today by drawing a line across the scale on the point that matches how good or bad your health is currently.

Best health state |----------| Worst health state
0 100

Appendix E: Food Diary Form

STRAWBERRY & KNEE OA Study

As a part of this study, you will be asked to keep a Diary of *everything* you eat and drink for 3 days. These 3 days should include 2 weekdays and 1 weekend day, *example*: Thursday, Friday and Saturday.
Begin with the first food or beverage in the morning and write down what you eat as you go through the day.
The Nutritionist will review your completed Food diary.

When you come back, please bring in any bottles/packages of dietary or nutritional supplements you have taken within the past week.
This would include any pills, powders, capsules, oils, tablets, or liquid vitamin/mineral supplements, herbal supplements, herbal teas or tinctures or any other type of dietary supplement you have taken.

GENERAL INSTRUCTIONS FOR RECORDING FOOD INTAKE

1. Please record on the Food Diary Form the place (home, home of a friend, restaurant) of each meal and snack.

2. Record one food item per line on the Food Diary Form. Space is provided on both sides of the form. Be sure to include gum, candy and beverages.

3. Record the amount and food item on the Food Diary Form using common household measurements, for example: Tablespoons, cups, package size etc.

4. Remember to record everything you possibly can about a food. The more detail you include the better.

5. When you record an item, please note if it was baked, boiled, broiled, fried, or roasted. This is extremely important, especially for meats.

6. Record any additions to a food item. This would include sugar, relish, margarine, butter, catsup, pickles, mayonnaise, mustard, gravies, cream, etc., which were served with the food.

7. When eating out, record the menu item and amount eaten. Refer to Hints for Eating Out.

8. List the method of mixing a package mix if it is different form the directions given on the package. You may record this on a Recipe Form.

9. Use the Recipe Form to record any homemade items you have prepared. Measure each ingredient and record the method of preparation on the bottom of that form.

10. If you have any questions, please call Janice Gales at 405-271-3480 (x34881) or e-mail Janice-gales@ouhsc.edu, or Arpita Basu at 405-744-4437 or e-mail arpita.basu@okstate.edu

HINTS FOR EATING OUT

1. Record the name of the restaurant.

2. Quiz the wait staff regarding portion sizes.

3. Record amounts in standard household measurements, ie: teaspoons (ts), tablespoons (Tb), ounces, cups, etc.

4. For items such as bacon, rolls, and cucumbers, record the number of each item eaten.
   For example: 3 small white rolls
   4 cucumber slices
   2 medium bacon slices

5. For meats, record the dimensions of the cooked meat. Do not include the bone.
   For example: 2 slices of roast beef 4" x 3" x ¼". State the weight of the meat if it is mentioned on the menu.

6. Refer to the Food Description Flow Charts to describe your food.

7. For national fast food restaurants, (i.e. McDonald's, Arby's, Burger King), record the name of the sandwich/item you ate (i.e. Big Mac, Whopper).

Strawberry & Knee OA Study

Food Diary

Name: _____ ID# _____ Protocol No: _____

Date of Record: _____ Day of Week: _____

Please record everything you eat today. Please include descriptions, brand names, and weighed and measured amounts (Please save labels). In the first column under meal and place, please put what meal you ate and where you ate it. You may use the codes at the bottom of the page for convenience. Thank you.

Meal* Place*	Amount	Food & Beverage Description	Office Use Only

*Meal Codes: Breakfast - BR
Morning Snack – MS
Lunch – LU
Afternoon Snack –AS
Supper – SU
Evening Snack – ES

*Place Codes: Home – HO
Restaurant – RE (Please Specify name of Restaurant)
Friends –FR
Work- W

VITA

Danielle Christiansen

Candidate for the Degree of

Master of Science

Thesis: DIET, PAIN, AND INFLAMMATION IN PARTICIPANTS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

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