EFFECTS OF FREEZE-DRIED STRAWBERRIES ON BIOCHEMICAL VARIABLES, MARKERS OF INFLAMMATION AND KNEE FUNCTION IN OBESE ADULTS WITH RADIOGRAPHIC EVIDENCE OF KNEE OSTEOARTHRITIS

By

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Bachelor of Science in Nutritional Sciences

Oklahoma State University

Stillwater, Oklahoma

2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2017

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ACKNOWLEDGEMENTS

Here I want to acknowledge all the people who have helped guide me through my graduate program and my entire college career. Including friends, family members, and colleagues. I want to thank Dr. Arpita Basu for being both my mentor, and a fellow collaborator in the laboratory. I admire her for helping introduce me to the field of research, and further for helping me grow as an individual in both competence and autonomy. I would also like to thank my fellow committee members for both their input and assistance on this graduate thesis. I also want to acknowledge my mother Laurie Robarts who financially supported these six years of academic development and enlightenment, here at Oklahoma State University. Who also loved me and fought to keep my dreams alive. Reminding me that the purpose to life is to find your purpose. I also want to acknowledge the memory of my brother Daris Schell who has always pushed me to continue with my education. Lastly, to all the additional family members and friends, who helped me through this milestone, thank you for your support.

Name: JACE DEAN SCHELL

Date of Degree: MAY, 2017

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Major Field: NUTRITIONAL SCIENCES

Abstract: Osteoarthritis (OA) is characterized by inflammation, joint pain and cartilage degeneration. OA remains without any effective pharmacological cure. Persons at risk of developing OA include obese individuals. We have previously reported the effects of dietary berries in improving cardio- metabolic outcomes in obese adults. To our knowledge no previous reports have examined the effects of berries on OA. Thus, in the present study we examined the hypothesis that strawberries will improve metabolic profiles and symptoms of pain in obese participants with radiographic evidence of knee OA.

In a randomized placebo-controlled crossover trial, obese participants [n=17 (Females 13, Males: 4); age: 56±7y; BMI: 39±6 kg/m^2(mean±SD)] were randomized to one of two groups: strawberry (50g freeze-dried) or placebo, each for 12 weeks. Blood draws, anthropometric measurements, surveys for pain scores [visual analog scale of pain (VAS), Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP)] and quality of life indices Health assessment questionnaire disability index (HAQ-DI)] were administered at screen, 12, 14 (washout) and 26 weeks of the study. We found significant decreases in pain scores (VAS) in the strawberry vs. placebo group at 12 weeks (0.71±0.31 and 0.90±0.47, respectively, $p<0.05$). IL-6 levels were significantly different in strawberry versus placebo group at 12 weeks (2.56±0.53 and 7.38±1.14, respectively, $p<0.05$). No differences were noted in biochemical profiles, especially, glucose, HbA1c, total- and LDL cholesterol and C-reactive protein. HDL-cholesterol was significantly lower in the strawberry vs. placebo group at 12 weeks (50±16 and 55±6.5mg/dL, respectively, $p<0.05$). Thus, strawberry bioactive compounds may help alleviate pain symptoms in knee OA. These findings deserve further investigation in larger clinical trials.

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CHAPTER I

INTRODUCTION

Like most degenerative diseases of the musculoskeletal system, arthritic conditions such as knee osteoarthritis (OA) have a high prevalence in aged populations.[1] OA is a condition caused in part by injury; loss of cartilaginous structure and function, and an imbalance in proinflammatory and anti-inflammatory pathways.[1] Knee pain is often a prominent and disabling symptom of OA of the knee. [2] Knee pain is associated with high body mass index (BMI) (30.0 kg/m^2) and knee structural changes.[2-4] One in every two elderly adults are diagnosed with symptomatic knee OA.[4] The primary factors that cause knee OA include aging and obesity.[3] Secondary factors include increased proinflammatory cytokines, including tumor necrosis factor-alpha (TNFα) and interleukin (IL)-6 .[2-3] Research suggests that increases in TNFα and IL-6 are associated with radiographic evidenced knee OA and knee cartilage loss.[2] Moreover, inflammation may be associated with knee pain genesis and severity.

Strawberries are well known for their role in combating inflammation through putative anti-inflammatory mechanisms, and having antioxidant properties.[6] Strawberries are reported to be the largest contributors of cellular antioxidant activity among all fruits consumed in the United States (US), and have been shown to

significantly inhibit free radicals, reduce oxidative LDLs, and oxidative stress.[6] We investigated strawberries as a therapeutic intervention on knee OA. To our knowledge this is the first study to investigate the role of strawberries, and their effect on knee OA. Our central hypothesis is that strawberry intervention (50g freeze-dried) vs. placebo will improve OA-related biomarkers of inflammation, especially knee pain scores, and lower serum cytokine levels in obese adults with radiographic evidence of knee OA.

Purpose: The purpose for our study is to reveal dietary strawberries as a means of reducing biomarkers and symptoms associated with knee OA. This study explores the prospect of strawberry intervention in the management of inflammation and pain symptoms, as related to knee OA. Specifically, our study investigates strawberry's therapeutic role in lowering inflammatory biomarkers and reducing knee pain scores.

Specific Aim: This study aimed to assess the effects of strawberries as a therapeutic strategy on selected inflammatory biomarkers and pain scores in obese adults with radiographic evidence of knee OA.

Null Hypotheses:

1. Freeze-dried strawberries will have no effects on biochemical variables (Glucose, Lipids, HbA1c, Interleukin-6, Interleukin-1β and C-reactive protein) vs. placebo in obese participants with radiographic evidence of knee OA

2. Freeze-dried strawberries will have no effects on pain scores and health assessment indices vs. placebo in obese participants with radiographic evidence of knee OA.

CHAPTER II

LITERATURE REVEIW

I. Biomarkers Contributing to Knee OA

Osteoarthritis: OA is a major age-related health condition in the US and its prevalence continues to grow worldwide.[1] OA affects nearly 27 million Americans or roughly 12 % of the adult US population.[7-9] By the year 2030 the prevalence of OA is predicted to increase to 72 million or nearly 20 % of the adult US population.[7] OA causes pain and dysfunction in 20% of elderly individuals, and affects one third of the population aged between 63 and 94 years.[10] OA is the most common form of disability in women, and OA-related symptoms are the most common reason for visits to health-care providers.[4]

OA is the leading cause of impairment in physical function in the aging US population, with the knee joint being a primary site for disease.[3] Alongside the prevalence of aging, obesity is another major risk factor of OA.[3] Further, metabolic disturbances such as metabolic syndrome (MetS) can increase an individual's risk of

OA.[11] Current treatments for OA include drug therapy, exercise, weight control, weight loss, and surgical intervention.[7] These treatment strategies focus on reducing symptoms [10, 12] and in preventing further functional deterioration, since there is no known cure for OA.[8]

OA Pathophysiology: Research suggests that OA is predominately driven by both physical wear and tear, and by inflammatory mediators released by the articular cartilage, bone and synovium.[13, 14] The articular cartilage is the smooth muscle that covers the ends of bones.[1] The articular cartilage surrounding bone allows for proper joint mobility and decreases mechanical stress. Degeneration of articular cartilage of the joints, leads to additional forces pressing against the bones responsible for weight bearing movements. Such bones include the hips and knees.[14] This results in pain and joint space narrowing amongst those with OA.[1, 14] Articular cartilage, once damaged cannot partake in a normal inflammatory response due to the tissue being non-vascularized, and having to rely solely on joint movement to dissipate the necessary inflammatory molecules.[13] As a result, joint swelling or synovitis may form, which is the local inflammation of the synovium as a result of increased inflammatory mediators associated with OA.[13]

Other factors that have a role in contributing to OA include metabolic intermediate abnormalities such as those seen in MetS, which can result in impaired glucose utilization and hormonal imbalance.[11] Further, the over-expression of reactive

oxygen species (ROS) is another major contributor to the catabolism of the articular cartilage, and is recognized as an intermediate involved in OA pathophysiology.[15] ROS are produced naturally in the body to maintain normal redox reactions.[8] Pathological conditions such as inflammation can overexpress ROS, which results in oxidative stress.

Proinflammatory Mediators of OA: Inflammation is a common defense mechanism driven by the normal immune response.[13] The innate immune response is induced by an increase in both local and systemic soluble mediators, such as proinflammatory cytokines.[13-14] OA is associated with increases in specific cytokines, such as IL-1β, IL-6 and TNFα.[13-14] IL-6 is regularly produced in low amounts within the body, though under pathological circumstances such as OA, IL-6 concentrations increase upon stimulation by IL-1β.[14] TNFα has also shown to increase IL-6 activity, as seen in animal models.[14, 16] These animal models reported higher levels of IL-6 concentrations in individuals with OA when compared to control groups.[14, 16-19]

Both IL-1β and TNFα have been investigated in a number of studies as a potential early indicator for OA. Mouse models have shown that IL-1β plays an important role in pain sensitivity.[20] Levels of IL-1β were increased in the early stages of disease, but were reduced with regression of synovitis.[14, 21] Likewise, TNFα has been positively correlated with pain, joint stiffness, and higher radiographic severity of the disease.[14, 22] Both of these inflammatory mediators require further study to clarify their efficacy as potential biomarkers of OA.

Another potential inflammatory mediator of OA is C-reactive protein (CRP), which is produced by the liver in response to increased IL-6 activity.[23-24] CRP is a marker of low-grade systemic inflammation, and high serum levels are present in early OA.[1] One study concluded that OA and pain severity were both associated with low-grade systemic inflammation, which was assessed by increased CRP concentrations.[25] These findings suggest that proinflammatory mediators are important biomarkers of OA.

***Knee OA & Pain Scores**:* The knee is the most common joint associated with the clinical symptoms and cause of disability due to OA.[26] Knee OA develops due to genetic inheritance, aging, obesity, trauma and other systemic diseases.[27] OA of the knee is more common amongst women, and its prevalence increases with age from 7% of those aged between 65-70 to 11.2% of those aged 80 and older.[26, 28] Studies have shown that serum levels of CRP, TNFα IL-1β and IL-6 contribute to symptomatic knee pain.[13] These biomarkers along with many other quantitative methods are available to assess for knee pain caused by knee OA.

One measure of knee pain is the visual analog scale for pain (VAS) (0 to 100). [26] The VAS is a unidimensional measure that is widely used to assess pain severity.[29] VAS is a continuous scale, anchored by 2 symptom descriptors rating no pain (0) to a severe pain (100) score. Study participants place a mark in between these 2 descriptors, marking a place on the horizontal line that is most closely representative of their level of pain. Scores representative of no pain (0-4), mild pain (5-44), moderate pain (45-75) and

severe pain (75-100) are then assessed for analysis. Scores of 30 and over are associated with needing medical care.[26-28] VAS has been used as a method of recruitment in clinical trials assessing knee OA to help identify those with specific pain severity (40-100). [30] Further, clinical studies have used VAS to assess treatment outcomes and effectiveness from baseline to end of study.[31] Meta-analysis of randomized clinical trials using analgesic pharmaceuticals, including non-steroidal anti-inflammatory drugs (NSAIDS), concluded that VAS is a highly predictive measure of pain intensity from baseline, 2, 6 and 12 weeks of study duration.[32]

Another evaluation tool is the Measure of Intermittent and Constant Pain (ICOAP). [29] This multidimensional OA-specific measure was designed to evaluate pain experience including the following: pain intensity, pain frequency and impact on quality of life, alongside being a measure of physical disability.[29] ICOAP evaluates an individual's level of both constant pain and intermittent pain. Scores (0-4) are normalized from 0 (no pain) to 100 (extreme pain). Higher scores indicate a worse experience with OA. Versions of ICOAP exist for OA of both the knee and hip. In a longitudinal cohort study utilizing ICOAP as a measure of knee pain in both men and women, found that constant and intermittent pain scores were a predictor for physical dysfunction and future limitation.[33] Both ICOAP and VAS can help determine levels of pain severity, treatment outcomes and physical disability associated with knee OA.

The Health Assessment Questionnaire Disability Index (HAQ-DI) has also been used to assess OA.[34] HAQ-DI is widely utilized as an assessment of both disease-specific disability and quality of life.[35] Both observational and clinical studies have used HAQ-DI for a number of chronic diseases and found its scores to be an important predictor of work disability[36], morbidity[36, 37], and mortality.[38] One study assessing the measurement properties of HAQ-DI generalized OA and concluded that the HAQ-DI showed good construct validity and reliability.[34] Based on this information, HAQ-DI, ICOAP and VAS may be regarded as appropriate tools for evaluation and analysis of knee pain in OA studies

Metabolic Syndrome and Obesity on knee OA: MetS is a cluster of metabolic risk factors including obesity, hypertension, dyslipidemia, diabetes, and insulin resistance, that are commonly present in patients with OA.[39-41] MetS is defined as having any three out of five components, which include: large waist circumference (102/88 cm for men/women), low HDL (<40-50mg/dL), hypertension (130/85 mmHg), hyperglycemia (fasting plasma glucose ≥5.6 mmol/L) and hypertriglyceridemia (≥1.7 mmol/L). [39,-40] Research has also shown that obese individuals and individuals with elevated BMI with progressive knee OA or hip OA have increased circulating levels of CRP, which places them at risk of developing low-grade systemic inflammation, as a result of inflammatory mediators released from adipose tissue.[42-47] Obesity and MetS both linked by low-grade systemic inflammation, have been shown to subsequently increase risks of OA.[39]

Biomarkers of Inflammation and OA-Related Progression in Epidemiological Studies: As shown in Table 1, epidemiological studies have reported associations between the selected biomarkers of inflammation related to knee OA. These studies report that serum levels of CRP are a marker for systemic inflammation and of OA progression.[23, 25, 43-44] Specifically, Sturmer *et al.* reported that CRP was a major predictor of OA-related pain severity and pain level indices with reports of a high median VAS score (73/100).[5, 25, 44] Sowers *et al.* associated CRP levels with BMI, obesity and radiographically defined knee OA, further reporting that obesity was a modifier of CRP concentrations.[23] The study concluded that CRP is a measure of the acute phase response in the inflammatory process associated with OA.

Studies also report that serum cytokine levels of IL-6 were associated with matrix cartilage loss, found in elderly adults with knee OA, suggesting that low-level inflammation was associated with the pathogenesis of knee OA.[48] Pearle *et al.* reported no significance in serum IL-6 and IL-1β from synovial fluid, in individuals with synovial inflammation and knee OA vs. those without OA.[43] Moreover, the incidence of MetS was highly correlated with risk of knee OA.[39] Further, high BMI and women with high hip-waist circumference were shown to have an increased prevalence of MetS and knee OA.[49]

Table 1- Epidemiological Studies and OA

Author, Year	Study Design	Population Characteristics	Outcomes
Sharif *et al.* 2000	Cohort/ Longitudinal	Patients w/ Knee OA n=90	Elevated Serum CRP was Predictive of Later Forms of OA Progression (3 Years)
Sowers *et al.* 2002	Cohort/ Longitudinal	Pre-Menopausal Women n=1025	CRP is Associated w/ Knee OA Obesity was a Modifier of CRP Concentrations
Sturmer *et al.* 2004	Retrospective Design	Patients w/ Knee or Hip OA n=770	Pain Severity was Highly Associated w/ Low Level Increase of CRP Indicating Systemic Inflammation Median VAS: 73/100
Pearle *et al.* 2007	Cross-Sectional Study	Patients w/OA Undergoing Knee Arthroplasty n=54	Elevated CRP Levels Reflect Synovial Inflammation IL-6 and IL-1β were not Significantly Different from Controls
Engstrom *et al.* 2009	Cohort Study	Healthy Adult Population n=5171	MetS was Associated w/ Having Increased Risk of Knee OA
Gandhi *et al.* 2010	Longitudinal Study	Patients Undergoing Knee Replacement Surgery n=200	Among Patients w/ Knee OA, There is Greater Risk of MetS in Those With High BMI and in Women w/High WHR
Stannus *et al.* 2010	Cross Sectional/ Cohort/	Elderly Adults w/knee OA n=172	Serum Levels of IL-6 are Associated w/ Knee Cartilage Loss

OA-Osteoarthritis; VAS-Visual Analog Scale; CRP-C-Reactive Protein; BMI-Body Mass Index; WHR-Waist Height Ratio; IL-6- Interleukin 6

II Diet, Polyphenols and Knee OA

Fruit polyphenols: Polyphenols are the natural components found in fruits, teas, spices, wine and vegetables.[8] Polyphenols are known for having anti-inflammatory and antioxidant activity, and have shown to increase innate antioxidant defenses. [8, 50] Dietary polyphenols include resveratrol, currcuminoids, quercetin, and anthocyanins.[51] Moreover, polyphenols can be broadly categorized into four different groups as follows: flavonoids, stillbenes, lignans, and phenolic acids. [52] It is estimated that polyphenol consumption ranges within 20-25 mg/day in the typical American diet. It is suggested that polyphenols are able to reduce inflammatory pathways.[8] These pathways are inhibited in individuals who consume both adequate amounts of polyphenols and consume a diverse array of polyphenol sources. Further, polyphenols are advantageous in reducing cytokine expression and other cardiometabolic risk factors.[8, 50] Health benefits of polyphenols include: increasing antioxidant capacity, anti-inflammatory properties and cardiometabolic protection.[8] However, such effects of polyphenols are mainly based on cell or animal-based studies, and further clinical studies are needed to support these findings in human health and disease.

Strawberries as a functional food: Strawberries are considered a functional fruit due to having a diverse array of nutrients, including phytochemicals and fiber.[53] Strawberries are known for their antioxidant properties, which are attributed to their polyphenol and vitamin content.[54] Approximately 40 phenolic compounds have been

identified and these include: glycosides of quercetin, kaempferol, anthocyanin, pelargonidin, ellagic acid and ellagitannins. The most significant polyphenols in strawberries that contribute to their antioxidant capacity include ellagic acid, ellagitannins, and anthocyanin.[53] Investigators have reported more than 25 different anthocyanin pigments. Strawberries also contain vitamins such as folate, vitamin C and vitamin E, along with potassium, folic acid, carotenoids, and specific flavonoids[53-54]. Thus, strawberries as a functional food may have some potential of therapeutic intervention in knee OA by reducing biomarkers of inflammation, cardiometabolic markers, and knee pain.

Clinical studies on Strawberry Intervention on Inflammatory Biomarkers: As shown in Table 2, clinical studies report varying levels of knee-OA related biomarkers, which include CRP IL-6, and IL-1β, shown to be altered after strawberry intervention. Participants in these studies were both overweight and obese individuals, and individuals with MetS and type-2 diabetes .[6, 55-59] Few studies reported significant decreases in CRP levels, and/or marginal postprandial changes in CRP after strawberry drink intervention. [57-59] However, other studies assessing CRP levels after strawberry intervention found no decreases in serum CRP at 4 and 12 weeks.[6, 56]One clinical trial reports postprandial changes in IL-6, with significant decreases in serum levels after 6 hours of consumption of strawberry drink, with lower but not significant changes in IL-1β. Marginal postprandial differences were found in IL-1β levels in another study, which

conducted a 6 week intervention.[58] Another study found that subjects given 50 g/day of strawberry intervention had significantly increased serum antioxidant levels, though no decreases in inflammatory status.[59] Based on these clinical studies, further investigation on the effects of strawberries on inflammatory biomarkers in human subjects is needed in larger clinical trials.

Table 2- Clinical Studies on Strawberry Interventions Measuring Inflammation

Author, Year	Study Design and Duration	Population Characteristics	Intervention	Outcomes
Basu *et al.*2009	Randomized Controlled Trial 4 Weeks	Females w/MetS n=16	Consumed 2 Cups of FDS 25 g/Daily	No Effects on Levels of CRP
Edirisinghe *et al.* 2011	Randomized Controlled Trial Assessed Within 6 Hours	Overweight Adult Subjects n=24 10 Men, 14 Women	FDS Beverage 10 g /Serving/ Daily	Significantly Decreased CRP Significant Decrease in IL-6 and Lower but not Significant Decrease IL-1β Levels
Ellis *et al.* 2011	Randomized Controlled Trial 6 Weeks	Overweight and Obese Subjects n=24 10 Men, 14 Women	FDS Beverage 10 g/Serving/ Daily	No Significant Differences in IL-6 Levels, Elevated IL-6 Levels in Placebo Group Marginal Postprandial Differences in IL-1β and CRP at 6 weeks
Moazen *et al.* 2013	Randomized Controlled Trial 6 Weeks	Obese Subjects w/T2D n=36 13 Men, 23 Women	FDS Beverage 50g/Daily	Significantly Decreased Levels of CRP Increases in Serum Antioxidants
Basu *et al.* 2014	Randomized Controlled Trial 12 Weeks	Adult Subjects w/Abdominal Obesity n=60 5 Men, 55 Women	Low Dose FDS: 25g/Daily High Dose FDS: 50g/Daily	No Significant changes in CRP

OA-Osteoarthritis; VAS-Visual Analog Scale; CRP-C-Reactive Protein; BMI-Body Mass Index; WHR-Waist Height Ratio; IL-6- Interleukin 6; FDS- Freeze-Dried Strawberries

Strawberries on cardiometabolic biomarkers: As shown in Table 3, individuals with hypertension and symptoms associated with cardiovascular disease (CVD), strawberry and anthocyanin interventions decreased hypertension, improved endothelial function, and decreased diastolic blood pressure.[62-64] Following strawberry intervention (10-50g/day) in both MetS and hyperlipidemic sample populations, study participants showed significant decreases in oxidized low-density lipoprotein (LDL) levels, decreases in total cholesterol, decreases in lipid peroxidation, improved lipid profile and increased LDL particle size.[6, 55, 61, 65-66] Decreased LDL size is positively correlated with risk of developing CVD, due to their increased susceptibility for oxidation.[55] Small LDL particles contribute to oxidative LDL production, due to their ability to more easily pass through the endothelium.

Clinical studies have shown that strawberry intervention further improved antioxidant status in healthy individuals, and improved platelet function.[67] Platelet function is a major component in the risk of CVD, since increased platelet aggregation can cause thrombotic events such as myocardial infarction (MI). Some studies found no significant changes in the study subjects' high-density lipoprotein (HDL) cholesterol levels and blood pressure after strawberry intervention.[68] More research investigating cardiometabolic risk factors is essential to better understand the factors that contribute to cardiovascular health and for the management of chronic diseases.

Table 3- Strawberries on Cardiometabolic Biomarkers

Author, Year	Study Design and Duration	Population Characteristics	Intervention	Outcomes
Basu *et al.* 2009	Randomized Controlled Trial 4 Weeks	Females w/ MetS n=16	2 Cups Strawberry Drink/Day 25 g/Daily	Significantly Decreased in TC and LDL Significantly Decreased Lipid Peroxidation
Basu *et al.*2010	Randomized Controlled Trial 8 Weeks	Subjects w/ MetS n=27	4 cups FDS Beverage 50 g/Daily	Decreases in Atherosclerotic Risk Factors Significant Decreases in TC, and Small LDL Particle Concentration
Burton-Freeman *et al.*2010	Randomized Controlled Trial 12 Weeks	Hyperlipidemic Subjects n=24	Strawberry Beverage 10/g /Daily FDS	Reduction in Risk of CVD Reduced Risk of LDL Oxidation
Zunino *et al.* 2012	Randomized, Clinical Trial 7 Weeks	Obese Adults n= 20	Strawberry Drink 4 Servings 2 serving/day 80g/serving	Significantly Increased in LDL Particle Size Significant Reduction in Plasma Concentrations of Cholesterol
Basu *et al.* 2014	Randomized Controlled Trial 12 Weeks	Adults w/Abdominal Obesity n=60	Low Dose FDS: 25g/Daily High Dose FDS: 50g/Daily	Blocks Meal-Induced Increase in Oxidative LDLs Improves Lipid Profile by Decreasing Lipid Peroxidation

Amani *et al.* 2014	Randomized Controlled Trial 6 Weeks	Adults w/T2D n=36	Two Cups of Flavonoid Beverage 25 g /Serving/Daily	Significant Reduction in TC Diastolic Blood Pressure was Significantly Reduced Post-Intervention in Experimental Group

Type 2 Diabetes: T2D; Freeze-Dried Strawberry: FDS; C-reactive protein: CRP; Interleukin: IL

Strawberry Intervention in Animal Models: As shown in Table 4, strawberry interventions in animal studies have shown significant reductions in cardiometabolic and inflammatory markers. [69-72] These animal models have shown significant reductions in levels of CRP, blood glucose, proinflammatory mediators, triglycerides and increases in antioxidant defense systems following strawberry intervention. Though no changes in circulating CRP were found in an induced MetS rat model after treatment with strawberry pomace extract in one study.[69 -70] Regardless there was a significant decrease in total cholesterol and triglycerides in this study.[69] Additional studies found significant reductions in blood glucose after strawberry intervention, and found that greater dosages of strawberry intervention lead to greater reductions in blood glucose levels.[70, 72] Strawberry phytochemicals were also shown to reduce TNFα and IL-6 levels.[70-72] Researchers noted that IL-6 levels were elevated in animals fed a high fat diet.[70] Moreover, animals showed decreased expression of mRNAs for TNFα, IL-6, and IL-1β.[71]

Strawberry intervention was shown to decrease ROS production, and increase antioxidant defense enzymes that prevent oxidative stress.[72] More specifically strawberry intervention increased superoxide dismutase and catalase defense enzymes, which protect against superoxide anion and hydroxyl radicals, that cause oxidative damage. Thus, strawberry intervention showed increases in antioxidant capacity in animals and lowered oxidative stress.[72] More investigation on these inflammatory and

antioxidant biomarkers are needed in human studies, especially with conditions such as knee OA characterized by these pathological changes.

Table 4- Strawberry Intervention in Animal Studies

Author, Year	Animal model	Intervention	Outcomes
Jaroslawska *et al.* 2011	Male Wistar Rats n= 48 4 Weeks	Polyphenol-Rich Strawberry Pomace on Induced MetS 16.8 mg/Pellet/Daily	No Significant Changes in CRP Lowered Triglycerides and TC
Parelman *et al.* 2012	C57BL/6J Mice n= 36 24 Weeks	Low Fat Diet + 2.6% SB Powder High Fat Diet + 2.6% SB Powder	Significant Decrease in CRP Significant Decrease in BG Lower levels of TNFα Increase in IL-6 after Fed High Fat Diet
Shi *et al.* 2015	Male Crj:CD-1 Mice n=50 20 Weeks	Strawberry Phytochemicals Diet Containing: 2.5%, 5.0% or 10.0% Strawberries	Down Regulated the Expression of mRNA of TNFα, IL-6, IL-1β and iNOS
Ibrahim *et al.* 2015	White Albino Rats n=30 30 Days	Strawberry leaf Extract 3 Dosages: 50, 100, 200 mg/kg	Decreased BG and Greater Decreases in Interventions w/Greater Dosages Increases in CAT and SOD Decreases in TNFα and IL-6

BMI-Body Mass Index; WHR-Waist Height Ratio; TC-Total Cholesterol Cardiovascular disease: CVD; Myocardial Infarction: MI; Freeze Dried Strawberry: FDS; Low Density Lipoprotein: LDL; Metabolic Syndrome: MetS

Effects of Other Dietary Supplements on OA: As shown in table 5, those with symptomatic knee OA are prone to seek alternative medications, specifically 47% of adults with symptomatic knee OA are seeking alternative treatments.[8] Use of curcumin, ginger, avocado-soybean oil, green tea, pomegranate and resveratrol on OA have been previously reported to decrease proinflammatory mediators and expressions of signaling pathways related to OA progression.[73-78]A meta-analysis of randomized clinical trials found that avocado-soybean oil supplementation suppresses TNFα and IL-1β, and decreases levels of inducible nitric oxide synthase(iNOS) in patients with OA.[73] Another meta-analysis analyzing clinical trials of curcumin intervention showed significant decreases in circulating IL-6 concentrations.[74]

Ginger powder intervention in patients with OA also noted significant reductions in nitric oxide, and in addition reported significant reductions in circulating levels of CRP.[75] In vitro studies utilizing green tea polyphenols, resveratrol and pomegranates also showed significant reductions in IL-1β, iNOS expression and nitric oxide production[76-77] as well as decreased levels of IL-1β.[78] These studies show potential as therapeutic strategies for the management of OA, although nutraceuticals need further investigation before they can be integrated into conventional management strategies for OA.

Table 5-Studies Utilizing Nutraceuticals in the Management of Knee OA

Author, Year	Study Design and Duration	Study Model	Intervention	Outcomes
Singh *et al.* 2002	In Vitro 12-24 Hours	Human Primary Chondrocytes	Green Tea-EGCG 1, 10, 50 or 100 µM	Significant Reduction in IL-1β, iNOS Expression and NO production
Ahmed *et al.* 2005	In Vitro 24 Hours	Human Primary Chondrocytes	Pomegranate 6.25, 12.5, 25 or 50 mg/L	Significant Reduction in IL-1β
Christensen *et al.*2008	Meta-Analysis 6 Months	Randomized Controlled Trials on OA Patients	Avocado-Soybean Oil Reports of Varying Dosages	Reduced Levels of iNOS Suppressed TNFα and IL-1β
Lei *et al.* 2012	In Vitro 8 Hours	Rat Primary Articular Chondrocytes	Resveratrol 5, 10 or 20 µM	Significant Reduction in IL-1β, iNOS Expression and NO production
Derosa *et al.* 2016	Meta-analysis 2009-2016	Randomized Clinical Controlled Trials	Curcuminoids Reports of Varying Dosages	Significant Reductions in Plasma IL-6 Levels
Naderi *et al.* 2016	Randomized Controlled Trial 12 Weeks	Patients w/ Knee OA n=100	Ginger Powder 2 Servings of 500 g/Daily	Significant Reductions in Nitric Oxide and CRP

MetS-Metabolic Syndrome; CRP-C-reactive Protein; BG-Blood Glucose; SB-Strawberry; TC-Total Cholesterol; TNFα-Tumor Necrosis Factor Alpha; IL-Interleukin; iNOS-Inducible Nitric Oxide Synthase

Conclusion: Based on the review of literature, strawberry fruit may provide a therapeutic role in the management of knee OA. Epidemiological studies support a role of proinflammatory biomarkers in the pathogenesis of OA and their contribution to knee pain severity in OA populations.[25, 44] Strawberry phytonutrients and polyphenols, being an excellent source of antioxidants and anti-inflammatory compounds, may help in the management of proinflammatory mediators of knee OA.[6] Clinical and animal studies have shown that strawberry intervention significantly decreases levels of CRP and IL-6.[57, 59,70,72]

Further, animal studies have noted that strawberry intervention suppresses signaling pathways that lead to oxidative damage, ROS production and OA progression.[71] Strawberries improved the action of antioxidant defense systems and defense enzymes.[72] Strawberries have also been shown to reduce cardiometabolic biomarkers associated with CVD and MetS.[6, 61, 66] Keeping in view the lack of clinical studies in berries and related sources of polyphenols we aim to address the role of strawberries in modulating cardiometabolic parameters, inflammation, pain and overall quality of life indicators related to mobility and function in obese adults with radiographic evidence of knee OA.

CHAPTER III

METHODOLOGY

Study design: In a 26-week randomized placebo-controlled crossover trial, 17 participants with abdominal obesity and radiographic evidence of knee OA were randomized to the following groups following a 2-week washout phase between each treatment: strawberry (50g/day) and placebo (50g/day). The freeze-dried strawberry and placebo powders were provided to the study participants in vacuum-sealed packs with storage instructions. The participants were required to consume 50g daily divided into two doses of strawberry and placebo powder reconstituted in water. As shown in Table 6, strawberry and placebo beverage composition are similar in caloric value, along with total fat, carbohydrate, protein and fiber intake. Strawberry powder provides additional polyphenols, anthocyanins, and phytosterols when compared to the placebo. Patients were recruited from the Oklahoma Medical Research Foundation (OMRF) patient registry, recruitment flyers/emails, and also referrals from the HSC community. Compliance was measured by unused strawberry and placebo packets of the powder. The study met the criteria for approval and was approved by both the Oklahoma State University Institutional Review Board and the Oklahoma University Center for Health Services Institutional Review Board (Appendix pg. 63).

TABLE 6. STRAWBERRY BEVERAGE COMPOSITION

Table 6. 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. Grams-g; Milligrams-mg; Kilocalories-kcal

Inclusion Criteria: Ambulatory adults (21y or older-no age limit) with enlarged waist circumference (women> 35 inches, men>40 inches) and knee pain due to age-related degenerative OA were included in the study. OA was diagnosed based on medical history (documented evidence and/or referrals), X-ray reports and baseline questionnaire. Participants stable on hypoglycemic, hypolipidemic and/or anti-hypertensive agents were included in the study. Additionally, the screening tests were within expected normal limits.

Exclusion criteria*:* Any form of pre-existing disease, e.g. cancer, coronary heart disease, liver, or renal disorders, pregnancy and lactation, knee surgery, traumatic injury, allergic to strawberries, taking mega doses of antioxidants/fish oil supplements (> 1g/day), participation in a recent weight loss program, above normal limits on the screening specimens, and participants with smoking habits were excluded from the study.

Blood draws and laboratory tests**:** Fasting blood samples were collected at screen, 12, 14, & 26 weeks of the study for each enrolled participants. All patients were seen at the Oklahoma Clinical and Translational Sciences Institute (OCTSI) at OUHSC and blood samples were sent to OU Medical Center for analyses of glucose, HbA1c, lipid profiles, and high-sensitivity CRP. Blood samples were collected at the OCTSI recruitment site by trained phlebotomists. Serum samples were also prepared and stored at these time points for ELISAs involving cytokines IL-6, and IL-1β (IL-6 and IL-1β

Human ELISA Kits, R&D Systems Minneapolis, MN). The CV% were within 5-7% for each assay.

Knee pain scores, Visual Analog Scale (VAS) and Health Assessment Questionnaire Disability Index (HAQ-DI):

Questionnaire Disability Index (HAQ-DI): Knee pain was assessed using the Intermittent and Constant Osteoarthritis Pain Measure (ICOAP) (As shown in Appendix pg.58). This measure is available for the evaluation of both knee OA and hip OA. ICOAP is a patient-reported pain measure to assess both intermittent pain (pain that comes and goes) and constant pain (pain that persists). Participant responses to 11 questions in the questionnaire were scored between 0-4, with 0 experiencing no pain and 4 experiencing extreme pain. These scores were normalized between 0-100. Scores were categorized from 0 (no pain) and 100 (extreme pain). Scores in the section for constant pain (five questions) were scored out of 20. While scores in the intermittent pain section (six questions) were scored out of 24. Percentage scores were then calculated for each participant. HAQ-DI (As shown in Appendix pg. 60) was also utilized to evaluate the health state of the participants. HAQ-DI further defines the difficulty participants with knee OA experience when performing daily-activities. HAQ-DI is a questionnaire that assesses eight domains as follows: dressing, arising, eating, walking, hygiene, reach, grip, and common activities. [37-38] HAQ-DI was originally formulated and validated at Stanford University in 1978 and has proved to be a reliable tool for the assessment of individuals with knee OA.[37] Evaluation of HAQ-DI includes scoring the eight domains

between 0 and 3. A score of 0 indicates ease with preforming tasks, while a score of 3 indicates difficulty and an inability to preform tasks. Scores obtained out of 24 are converted to percentages, which are then normalized between 0 and 100. Score of 0 indicates no difficulty with tasks, while scores up to 100 indicate increasing difficulty. VAS (As shown in Appendix pg. 62) was also assessed in order to determine the participant's relative pain score and provide a representative measure of pain levels. VAS is a visual portion of the HAQ-DI, that measures both pain and health state. VAS is a visual measure with scores between 0 and 100. Subjects were asked to place a score representative of their level of pain or health state along a 15 cm line by placing a mark on the scale. Scores are categorized as 0-4 (no pain), 5-44 (mild pain) 45-75 (moderate pain) and 75-100 (extreme pain). Scores are translated to a representative score between 0 and 3. A score of 0 indicates no pain and in good health, while increasing scores reflect increasing pain and poorer health.

Food record: Nutrient intake reports (As shown in Appendix pg. 55) were measured through dietary recall at baseline, 12, 14, and 26 weeks using 3-day food records. Participants were asked to recall all food and beverage consumed at least 3 days of the week and these records were collected at the above mentioned time points. Dietary records were analyzed using Nutritionist Pro software, and data were collected for calorie, fats, proteins and carbohydrate as well as fiber intake.

Statistical analyses: Demographic and disease characteristics (pain scores and duration of OA) at baseline were summarized prior to being randomized to one of the two treatment groups. Descriptive statistics were reported using means and standard error measures for each variable at the end of each 12-week strawberry and placebo phase. Changes in measures of glycaemia, lipid profiles, biomarkers of inflammation, and pain scores were compared among different time points using PROC MIXED adjusting for baseline measures as a covariate. Data were also collected at the end of washout phase (two weeks between each intervention) and since no carry-over effects were noted we used screen data to adjust our final model. All analyses were performed with level of significance at less than 0.05, and using SAS/STAT software version 9.2 of the SAS system for windows (SAS Institute Inc., Cary, NC).

CHAPTER IV

FINDINGS

Baseline characteristics: As shown in Table 7, seventeen subjects (13 females, 4 males) were enrolled in the crossover study, and all subjects completed the study. The study had no dropouts. Mean age of these subjects was 56.5 years, weight, 246.4 lbs, and BMI 39.3 kg/m^2. Eleven individuals were on blood pressure prescription medications, and six individuals were taking lipid prescription medications throughout the 26-week study. Two participants were diagnosed with diabetes prior to study. Eleven individuals were taking a multivitamin/mineral supplement on a regular basis and continued to do so throughout the study. On average all participants met the general criteria for the MetS. These prevalent features of MetS in our study participants were enlarged waist circumference (>35 inches for women and > 40 inches for men, on blood pressure medications and impaired fasting glucose >100mg/dL). All subjects maintained their usual diet and exercise patterns throughout the study duration. The strawberry powder reconstituted in water was well tolerated with no adverse effects, and participants showed active compliance (90%) with our study intervention.

TABLE 7. PARTICIPANT BASELINE CHARACTERISITICS

Table 7. Participant Baseline Characteristics (n=17)	
Age (yrs)	56.5 ± 1.7
Gender (m/f)	4/13
Height (cm)	169.7 ± 72.7
Weight (lbs)	246.4 ± 7.1
BMI (kg/m^2)	39.3± 1.4
Blood Pressure Medication (n)	11/17
Lipid Medication (n)	6/17
Diagnosed w/Diabetes (n)	2/17
Vitamin Supplement Use (n)	11/17

Body Mass Index-BMI; Male-m; Female-f; Centimeters-cm; Pounds-lbs; Kilogram-kg; Meters-m; Years-yrs; Numerical value-n

Biochemical variables: As shown in Table 8, we found no significant differences in glucose, HbA1c, triglycerides, total cholesterol and LDL-cholesterol between strawberry and placebo groups at 12 weeks of the intervention. HDL-cholesterol was significantly lower in the strawberry vs. placebo group at 12 weeks (52.3±3.0 and 54.3±3.4 mg/dL, respectively, $p<0.05$), but not at baseline. No significant changes in BMI and waist circumference measurements in strawberry versus placebo groups were noted. No significant changes were found in systolic and diastolic blood pressure between groups. The strawberry and placebo powder were well tolerated and did not affect safety variables, especially the liver and kidney function tests.

Inflammatory variables: As shown in table 8, among the inflammatory variables measured in the study, IL-6 levels were significantly lower in strawberry versus placebo group at 12 weeks (2.7±0.5 and 7.4±1.1, respectively, $p<0.05$) but not significantly different compared to baseline values. Further, the study found that IL-1β values were significantly lower in the strawberry vs. placebo group (7.5±0.7 and 16.2±1.2 pg/mL, respectively, $p<0.05$) at 12 weeks. Levels of CRP showed no significant change in strawberry or placebo groups from baseline.

TABLE 8. CARDIO-METABOLIC AND INFLAMMATORY BIOMARKERS AT 12 WEEKS

Table 8. Cardio-Metabolic & Inflammatory Biomarkers at 12 wks (means±SE)			
	Baseline	Strawberry	Placebo
BMI (kg/m^2)	39.3±1.4	39.1±1.5	39.3±1.5
Waistline (in)	46.4 ±1.1	46.5± 1.1	46.9±1.1
Systolic blood pressure (mm Hg)	124.7±2.5	124.5±2.2	126.8±1.9
Diastolic blood pressure (mm Hg)	79.9±1.6	81.7±1.3	81.7±1.2
Glucose (mg/dL)	113.4±5.6	118.9±8.7	112.3±5.1
HbA1c (%)	6.0±0.2	6.1±0.1	6.1±0.2
Total Cholesterol (mg/dL)	188.7±6.2	188.5±7.6	187.7±8.7
Triglycerides (mg/dL)	128.8±14.3	136.4±16.1	130.3±13.0
LDL Cholesterol (mg/dL)	109.2±6.3	107.8±7.0	105.5±7.7
HDL Cholesterol (mg/dL)	51.5±3.0	52.3±3.0*	54.3±3.4
C-Reactive Protein (mg/L)	5.8±1.2	4.6±0.9	4.8±0.9
Albumin (g/dL)	4.0±0.1	4.0±0.1	12.6±8.3
ALT (U/L)	40.5±2.8	38.7±2.3	40.7±3.0
AST (U/L)	30.1±2.6	28.9±2.3	31.4±2.1
Creatinine (mg/dL)	0.8±0.0	0.8±0.0	0.8±0.0
BUN (mg/dL)	15.5±1.0	14.5±0.7	14.5±1.0
Calcium (mg/dL)	9.3±0.1	9.3±0.1	9.2±0.1
IL-6 (pg/mL)	1.8±0.4	2.6±0.5*	7.4±1.1
IL-1β (pg/mL)	18.6±4.0	7.5±0.7**	16.2±1.2

* Significance found in strawberry versus placebo group at p<0.05; ** Significance found in strawberry versus placebo group and against baseline values at p<0.05;Body Mass Index-BMI; Hemoglobin A1c- HbA1c; Low Density Lipoprotein-LDL; High Density Lipoprotein; Aspartate

Transaminase- AST; Alanine Transaminase-ALT; Blood Urea Nitrogen-BUN; Interleukin-IL; Beta-β; Kilogram-kg; Meters-m; Inches-in; Millimeters-mm; Mercury-Hg; Milligram-mg; Deciliter-dL; Percentage-%; Grams-g; Units per Liter- U/L; Pictograms- pg; Milliliter-mL

Pain Scores: As shown in Table 9, we found significant decreases in pain scores (VAS) in the strawberry vs. placebo group at 12 weeks (0.7±0.3 and 0.9±0.5, respectively, $p<0.05$). No significance was found in HAQ-DI between strawberry and control groups. In general, the strawberry group exhibited numerically lower ICOAP intermittent, constant, and total pain scores, though these did not reach significance when compared to the placebo.

TABLE 9. PAIN SCORES AND QUALITY OF LIFE INDEX

Table 9. Pain Scores and Quality of Life index (means±SE)			
	Baseline	Strawberry	Placebo
HAQ-DI	0.6±0.1	0.4±0.1	0.6±0.1
VAS: Pain	1.4±0.2	0.8±0.1*	1.0±0.2
VAS: Health	0.7±0.1	1.0±0.2	0.7±0.4
ICOAP: Constant Pain %	31.8±3.5	13.8±3.6	24.2±4.1
ICOAP: Intermittent Pain %	38.5±3.4	24.3±4.7	34.6±3.0
ICOAP: Total Pain %	35.4±3.1	19.4±3.7	30.0±3.0

* Significance found in strawberry versus placebo group and against baseline values at $p<0.05$; Health Assessment Questionnaire Disability Index-HAQ-DI; Visual Analogue Scale-VAS; Measure of Intermittent Constant and Osteoarthritis Pain-ICOAP; Percentage-%

Dietary intakes: As shown in Table 10, dietary analyses from 3-day food record revealed no significant changes in total calories, and dietary macronutrients (fat, protein and carbohydrates) in strawberry and placebo groups. No changes in fiber intake were found between strawberry and placebo groups as well. On average macronutrient percentages of total kcal were: protein (13-19%), carbohydrate (38-58%) and fat (32-41%). In addition, food records revealed participants maintained their habitual food and beverage intakes throughout the study and refrained from other sources of dietary berries.

TABLE 10. DIETARY DATA

Table 10. Dietary Data			
	Baseline	Placebo	Strawberry
Caloric Intake (kcal)	2026.5±182.7	1882.8±207.8	2604.1±440.8
Protein (g)	92.3±11.2	75.7±7.2	106.9±18.9
Carbohydrates (g)	214.6±19.5	228.9±29.3	320.5±56.4
Fat (g)	89.8±12.0	77.6±9.5	100.6±21.9
Fiber (g)	17.7±1.9	20.2±2.5	23.5±3.2

Dietary data derived from 3-day food records; Kilocalories-kcal; Grams-g

CHAPTER V

CONCLUSIONS

To our knowledge this was the first study to examine the role of strawberries as a therapeutic strategy in reducing selected biomarkers of inflammation, cardiometabolic and biochemical markers and knee pain in obese adults with radiographic evidence of knee OA. Subjects consumed 50-g/day freeze-dried strawberry powder, which is equivalent to 500 g fresh strawberries (3.5 cups) or placebo for a duration of 12 weeks each. This dose of freeze-dried strawberries has been previously tested in our clinical trials and was shown to be effective in improving cardiometabolic risk factors.[6, 56,66] Strawberry supplementation in our participants with knee OA was shown to improve knee pain scores as assessed by VAS, and maintain lower serum levels of IL-6 and IL-1β vs. placebo at 12 weeks. We found numerically lower but not significant pain scores assessed by ICOAP. Similarly, strawberry consumption had no effects on total and LDL cholesterol, systolic and diastolic blood pressure, and BMI and waist circumference. Our findings address the limited published data on the role of functional foods, such as berry fruits in modulating inflammatory markers and pain scores as related to knee OA.[2]

Pain and Strawberries: We observed significant decreases in VAS-pain scores in the strawberry vs. placebo group at 12 weeks of the study. This may be explained by the significant decreases in serum IL-6 and serum IL-1β that were significantly lower in the strawberry vs. placebo group. Serum IL-6 has been previously correlated with knee pain in OA in other observational and clinical studies.[43, 48, 53] Epidemiological studies have also correlated biomarkers of inflammation, such as CRP and IL-1β with knee pain.[23, 25, 43-44] We also observed a generally lower ICOAP pain scores, though these were not statistically significant. The differences in survey structure, the VAS being based on visual measure of pain vs. ICOAP being a categorical questionnaire on pain level may explain differences in how participants are required to interpret their pain experience, and thus the observed differences in our study. Our study findings on lower VAS pain scores suggest that strawberries may decrease pain symptoms and may provide palliative effects for individuals with knee OA.

Other clinical studies using dietary bioactive compounds, such as curcumin and green tea polyphenols have also shown decreases in knee pain associated with OA. Nakagawa *et al.* showed that curcumin significantly lowered VAS pain scores in patients with knee OA in a randomized double-blind, placebo controlled study, following the use of Theracurmin containing 180 mg/day of curcumin.[79] Derosa *et al.* reported in a meta-analysis and systematic review of clinical trials that currcuminoids significantly lowered IL-6 levels[74] Furthermore, Hashempur *et al.* showed that green tea polyphenols can

significantly decrease knee pain.[80] Taking their findings into account, Singh *et al.* stated that green tea polyphenols significantly reduce IL-1β.[50] These results are similar to our study findings in which strawberry intervention lowered both IL-6 and IL-1β vs. placebo group.

Inflammation and strawberries: Several previous clinical trials have shown decreases in inflammatory markers, such as IL-6, IL-1β and CRP following strawberry intervention.[57-59] However, to our knowledge no such reports have been shown in knee OA. The present study showed that strawberries significantly suppress IL-6 levels compared to placebo, but did not lower serum levels against baseline values. IL-1β levels were also significantly lower vs. placebo and vs. baseline values. These findings may help postulate that strawberries may serve as a therapeutic strategy for lowering inflammatory markers, and a means for down regulating pro-inflammatory pathways related to chronic conditions such as OA.

Edirisinghe *et al.* previously reported that freeze dried strawberries at a dose of 10 g/day significantly decreased levels of CRP, IL-6, and IL-1β in overweight adults.[57] Their study utilized the same crossover design, which may account for some of our studies' similarities excluding significant changes in CRP. Though we have previously reported in other clinical studies a similar findings of no significant changes in levels of CRP following strawberry intake at 25 and 50 g/day.[6, 56] IL-1β has shown to up regulate

IL-6 levels[23-24] in previous clinical trials, which may explain the concomitant decreases in both IL-6 and IL-1β in our study.

Cardiometabolic effects of strawberries: In the present study strawberry intervention did not show any effects on cardiometabolic variables in obese participants with knee OA. In our previous trials and those by others[6, 55, 63, 66] strawberries were reported to decrease total and LDL cholesterol. Amani *et al.* previously reported decreased total cholesterol after supplemented flavonoid beverage at 25 g/day.[63] Further, Zunino *et al.* and Burton-Freeman *et al.* also found reductions in plasma cholesterol and reduced risk of LDL oxidation after strawberry supplementation.[55, 61] The differences found in our present study findings may be explained by the otherwise optimal glucose and lipid profiles in our participants with knee OA. We noticed a slight but significant increase in serum HDL in the placebo vs. strawberry group at 12 weeks. Though statistically significant, these values are not biologically meaningful and further studies are needed on the role of strawberries on cardiometabolic risk factors in OA.

Limitations: Our pilot study has some limitations, which include our small sample size (n=17). The small sample size limits our study's ability to generalize our findings to larger populations. Further, this study did not examine dose-response effects in our participants. Our participants were administered a single dose of 50g/day of strawberry powder. Varying dosages may reveal additional information on attenuating biomarkers of inflammation and/or reducing knee pain scores and thus must be

considered in future trials. Since we assessed biomarkers of inflammation in obese participants with symptomatic knee OA at a single time point, obesity itself may be a confounding factor in our analyses. Thus, a comparison group of non-obese participants with OA may help elucidate the effects of nutritional interventions on inflammation and other pathological symptoms associated with obesity in knee OA. Another limitation of this study was our assessment of the selected metabolic and inflammatory biomarkers. We did not measure other biomarkers, such as TNFα and matrix metalloproteinase, as well as biomarkers of oxidative damage that have also been correlated with knee OA. Thus, our pilot study findings warrant further investigation in larger trials in participants with knee OA.

Strengths: The main strength of our study is our crossover design and participant selection based on specific inclusion criteria to generalize our findings to obese participants with knee OA. Additionally, we took into consideration several excluding factors that can confound the effects of strawberries on knee OA, especially the exclusion of fish oil consumers and those on recent and ongoing weight loss programs. This allowed for greater accuracy in determining changes in biomarkers throughout the 26-week duration, and further added precision in determining pain scores and health indices. Our within-group design aimed to prevent confounding covariates since each individual served as their own control.

Conclusion: In the present study, strawberries decreased pain scores and associated inflammatory biomarkers in obese participants with knee OA. These findings add to the existing literature on the role of dietary bioactive compounds as a potential alternative therapy in the management of knee pain in OA. Our study has practical relevance based on the availability of strawberries in various forms to the general consumers, and thus the recommendation of incorporating strawberries as an effective dietary strategy in the management of knee OA.

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APPENDICES

Page

1. Food Records:

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 dairy.

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

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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

2. Measure of Intermittent and Constant Osteoarthritis Pain:

{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?**

☐0	☐1	☐2	☐3	☐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?**

☐0	☐1	☐2	☐3	☐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?**

☐0	☐1	☐2	☐3	☐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?***

☐0	☐1	☐2	☐3	☐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*?**

☐0	☐1	☐2	☐3	☐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!

3. Health Assessment and Disability Index 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 mo	☐	☐	☐	☐
-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, zipp 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

4. Visual Analog Scale:

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 — SEVERE PAIN

0 — 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

5. Institutional Review Board 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

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

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.

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 405-612-2414 (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. Shelia 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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VITA

Jace Dean Schell

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF FREEZE-DRIED STRAWBERRIES ON BIOCHEMICAL VARIABLES, MARKERS OF INFLAMMATION AND KNEE FUNCTION IN OBESE ADULTS WITH RADIOGRAPHIC EVIDENCE OF KNEE OSTEOARTHRITIS

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in May 2017.

Completed the requirements for the Bachelor of Science in Dietetics at Oklahoma State University, Stillwater, Oklahoma/United States in 2015.

Experience:

Server: Red Lobster

Club Coach: Oklahoma State Master's Swim Instructor

Swim Instructor: Swim America

BASIC Leadership Camp

Professional Memberships:

American Red Cross Certification

- Adult and Pediatric First Aid/CPR/AED

American Red Cross Certification

- Safety and Training Fast Course-Coaching

National Society of Collegiate Scholars