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KETOGENIC DIET AS A PREVENTATIVE MEASURE OR TREATMENT OPTION FOR OSTEOARTHRITISTARGETING NFL ATHLETES AS A HIGH-RISK GROUP

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KETOGENIC DIET AS A PREVENTATIVE MEASURE OR TREATMENT OPTION FOR OSTEOARTHRITIS: TARGETING NFL ATHLETES AS A HIGH-RISK GROUP

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Abstract

Osteoarthritis (OA) is a prevalent joint disease that results from the degradation of articular cartilage, leading to inflammation, pain, and eventual joint failure. At one time, it was thought that OA was only the result of the natural decline experienced in old age; however, various risk factors have now been identified that may contribute to an increased risk for developing OA. Risk factors may include joint loading, altered biomechanics, obesity, and joint injury, to which athletes are exposed at a high rate. NFL athletes are one group of athletes that have continued to show a high prevalence of arthritis in retirement, based on those risk factors. There is no current treatment outside of anti-inflammatory medications and joint replacements, both of which do not always lead to the most favorable outcomes. A high-fat, low carbohydrate diet, called the ketogenic diet, may be able to prevent the onset of OA or treat it once it has developed. The ketogenic diet produces ketone bodies which have been shown to decrease inflammation and reactive oxygen species, two leading factors of OA development or potentiation. While research is not conclusive on how this works, various studies have supported its claim. The following extended literature review aims to determine if current research supports using the ketogenic diet in preventing or treating OA in NFL athletes.

KEY WORDS: Osteoarthritis, ketogenic diet, NFL athletes, ketone bodies, joint loading

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List of Abbreviations

NFL	National Football League
OA	Osteoarthritis
KD	Ketogenic diet
ECM	Extra cellular matrix
PRR	Pattern recognition receptors
DAMPs	Damage-associated molecular patterns
TLR	Toll-like receptors
NLR	Nod-like receptors
NLRP3	NLR family pyrin domain containing 3
ROS	Reactive oxygen species
NOX	NADPH oxidases
NOS	Nitric Oxide synthases
ACL	Anterior cruciate ligament
CT	Computed tomography
MRI	Magnetic resonance imaging
ADL	Activities of daily living
KL	Kellgren-Lawrence
JSW	Joint space width
WOMAC	Western Ontario and McMaster Universities Arthritis index
NSAIDs	Non-steroidal anti-inflammatory drugs
CPT-1	Carnitine palmitoyl transferase
HMGCS2	3-hydroxymethylglutaryl-CoA synthase
AcAc-CoA	Acetoacetyl-CoA
HMG-CoA	3-hydroxymethylglutaryl-CoA
HMGCL	HMG-CoA lyase
AcAc	Acetoacetate
(D-βOHB)	D-β-hydroxybutyrate
BDH1	D-βOHB dehydrogenase
MCT	Monocarboxylate transporters
SCOT	Succinyl-CoA:3-oxoacid-CoA transferase
TCA	Citric acid cycle
PGD2	prostaglandin D2
AMPK	Adenosine monophosphate-activated protein kinase
NF-E2	Nuclear factor erythroid-derived 2
Nrf2	(NF-E2)-related factor 2
HDAC	Histone deacetylase

1. Introduction

Disease, in all its forms, continues to afflict millions of people, threatening death and decreasing quality of life. Researchers, physicians, scientists, and many more continue to fight and search for treatments that could potentially cure any of the plethora of diseases that plague this world. Some have prevailed while others have fallen short of this seemly impossible task. But what if the treatment in many cases need not be found but refocused to areas of daily life? According to the Centers for Disease Control, the four main factors for causing disease are tobacco use, poor nutrition, lack of physical activity, and excessive alcohol use—all of which can be managed with proper education and the initiative to make a positive change.[1] In the crisis of disease, there is a large emphasis on discovering new treatments or preventative measures to combat the development of a disease, yet there are known manageable factors that prove to play a significant role is disease pathology. Tobacco use, poor nutrition, lack of physical activity, and excessive alcohol have proven to cause some of the most prevalent diseases such as liver disease, obesity, diabetes, heart disease, and many others. And while it is easy for people to see how these factors contribute to disease pathology, many people fail to recognize that the converse of these factors can be used for good by preventing or treating disease. Eliminating tobacco use, limiting alcohol use, participating in physical activity, and maintaining a balanced diet can play a role in healing the body from disease or preventing it altogether. People have the power to influence their health in a positive way, but effective education is a must for real change to occur. The following literature review will attempt to do this by discussing osteoarthritis followed by proposing an alternative, diet-based treatment.

Osteoarthritis, simply defined by the Centers of Disease Control (CDC), is the morphological change and damage that occurs to the ends of bones as a result of the breakdown of cartilage between two bones of a joint.[2] This creates pain, stiffness, and swelling that affects the daily function of more than 32.5 million US adults, according to the CDC.[2] While the disease symptoms may be managed and some treatment may even stop further development of the disease, once damage is done to the joint, it cannot be reversed.[3] This places a greater emphasis on preventative measures for osteoarthritis. Individuals with painful osteoarthritis symptoms may experience a diminished quality of life through an inability to move in the same ways they once could. Those diagnosed may not be able to participate in activities of daily living or recreational activities, especially those that involve physical activity. In fact, some physical recreational activities can create an increased risk for the development of osteoarthritis, as will be discussed in the following review.

One group of individuals that have an increased risk for developing osteoarthritis are current or retired National Football League (NFL) athletes. The force and stress exerted on the joints of NFL athletes, a consequence of their sport, may damage the articular cartilage, thus increasing the risk for developing osteoarthritis. As an athlete myself, I imagine the frustration a former NFL athlete must experience when diagnosed with osteoarthritis, knowing their sport must have played a part. I can also understand the disappointment an NFL athlete may feel when discovering they have premature signs of osteoarthritis and must end their career for fear of further damage. Injuries, like most all diseases, threaten to limit people and trap them into a specific outcome. And while some treatments do exist for osteoarthritis, there are not many options that have shown positive results. Additionally, there is no way to prevent the disease outside of proper body mechanics, exercise,

and weight reduction. However, NFL athletes have teams of experts that monitor daily exercise, biomechanics, and even nutrition, yet they are still a high-risk group. There must be a better way.

In the following literature review, finding a better way was the primary goal, and this was found through the ketogenic diet. Through my research, I demonstrate that the ketogenic diet could provide a positive avenue to treat the disease and potentially prevent the disease. While the ketogenic diet has not been used in OA, it has been shown to benefit a plethora of other diseases. Through research completed in similar areas, I was able to hypothesize the potential benefits in osteoarthritis and provide another way for NFL athletes and all those diagnosed with osteoarthritis. In the following literature review, I will discuss the research results that has led to this conclusion followed by a hypothetical research proposal in this area of study.

2. Methodology

The following thesis is an extended literature review constructed through reviewing current and historical peer reviewed literature osteoarthritis, NFL athletes, and the ketogenic diet. The information gathered was used to explain the disease pathology and characteristics of osteoarthritis and provide a potential treatment alternative or preventative measure to high-risk populations. Literature was then gathered in order to design a murine model experiment to test the hypothesized treatment option and theorize what the experimental data would show from the hypothetical experiment. This experiment was designed following historical and present murine model experiments that involve osteoarthritis, ketogenic-like diets, and compression injuries. The data was primarily collected through databases such as PubMed, PMC, and EBSCO host using search terms such as ‘osteoarthritis,’ ‘osteoarthritis and compression injuries,’ ‘osteoarthritis and football,’ ‘ketogenic diet,’ ‘ketogenic diet as treatment,’ and ‘inflammation.’ The sources were found through journals such as *American Journal of Sports Medicine, Science, Nature Medicine*, and *Arthritis & Rheumatology*.

3. Osteoarthritis

Osteoarthritis (OA) is a debilitating musculoskeletal disease that affects many people across the globe. Statistically, it is the most prevalent joint disease, as well as the most prevalent type of arthritis, affecting an estimated 10% of men and 18% of women over the age of 60 with a total of 242 million people with symptomatic OA worldwide.[4,5] As a result of the various symptoms associated with OA, people experience movement limitations creating difficulty in activities of daily living (ADL) and regular exercise.[5] These limitations greatly affect the holistic well-being of the patient. For example, there is a 55% increase in mortality as a result of reduced physical activity, comorbid conditions, and adverse effects to medications.[5] Additionally, it has been determined that one-third of those diagnosed with OA that are over the age of 45 suffer from depression or anxiety.[6] Currently, the outlook is not grim as OA is the third most rapidly rising condition associated with disability, behind diabetes and dementia.[5] This creates a $136.8 billion industry for OA related costs each year which made it the second most costly health condition treated in hospitals in the United States in 2013.[7,8] Despite continued research in OA treatment, joint replacements still prevail as a leading way to inhibit the symptoms associated with OA. Approximately 54% of people diagnosed with knee OA will undergo a knee replacement in their lifetime.[9] This is one large contributor to the economic burden associated with OA as joint replacements can range from $30,000 to $112,000.[10] The physical, mental, and economic burdens associated with OA are supremely evident highlighting the need for research in this area.

OA is a disease of the joints that causes alterations in the composition, structure, and function of articular cartilage resulting in changes to bone morphology. This change in morphology was once considered to be the result of natural joint degeneration due to aging. This has since been disproven

and it is now known to be the result of an imbalance in the biochemical mechanisms within a joint.[11] These biochemical mechanisms that result in morphological changes include, but are not limited to, the following: degradation of articular cartilage, thickening of the subchondral bone, osteophyte formation, synovial inflammation, degeneration of ligaments, hypertrophy of the joint capsule, and changes in the muscles, nerves, and fat surrounding the joint.[12] Accompanying the morphological changes are physical symptoms such as stiffness of the joint, pain, and eventual joint failure.[13] Typically, symptoms do not arise until the degeneration has reached its final stage where it will be diagnosed as OA.[13] In order to prevent joint failure, treatment research must progress to find preventative measures and treatment options.

OA affects the joints of the body, but more specifically, it affects synovial joints.[14,15] Synovial joints are characterized by a joint cavity encapsulated by a fibrous connective tissue called the articular capsule, as shown in Figure 1.[16] This joint capsule is filled with synovium, a thick, fluid substance filled with synovial cells, in order to provide lubricant for bones to maintain healthy, gliding movement.[17] In addition to the synovium, the articular cartilage also aids in the smooth, healthy joint movement. Articular cartilage is a thin layer of hyaline cartilage that covers the ends of the articulating bones in order to protect and stabilize joints.[14,16] Articular cartilage is a key contributor to a healthy joint and is thus a key contributor to the development of OA when not functioning properly. The articular cartilage is composed of a single cell type, chondrocytes, which are then encased in the extracellular matrix (ECM) of collagen and proteoglycans.[18] Collagen is the main structural component of cartilage that supplies it with the tensile strength it needs.[17] Proteoglycans draw water into the joint to provide cushioning and support.[18] Chondrocytes produce and maintain the matrix by responding to changes in the joint in order to maintain

homeostasis.[18] These cells are usually quiescent and lack the ability to self-renew, making them a prime target for detrimental effects leading to OA.[19] Research has shown that chondrocyte death, as a result of varying factors, is the main factor of OA development.[19] This will be discussed further in the following section.

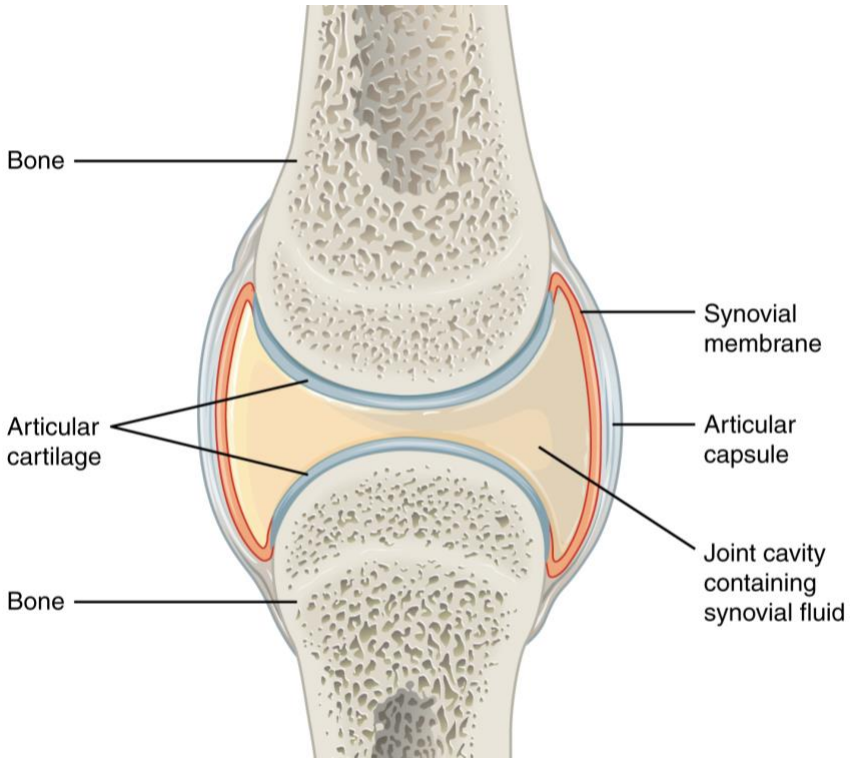

Figure 1. A normal synovial joint that allows for smooth bending. The articular capsule contains articular cartilage and synovial fluid for smooth movement while ligaments provide stability.[20]

3.1 Pathophysiology

There is no one singular cause currently known for the development of OA but a plethora of factors that contribute to the onset of joint destruction.[20] These could include genetic predispositions, aging, obesity, trauma, or other systemic diseases.[21] Although the etiologies from patient to patient may differ, the end-stage joint failure for each OA patient remains the same. The factors that result in end-stage failure are complex and involve inflammatory, mechanical, and metabolic factors that

prohibit the articular surface from properly functioning in distributing and absorbing mechanical load of a joint.[22] This ultimately leads to joint destruction and an OA diagnosis.

Chondrocytes are chiefly responsible for maintaining joint homeostasis through secretion of ECM components in low turnover conditions. ECM receptors reside on chondrocytes that that allow the cells to respond to stimulation, such as simple movement of the joint or weight placed on the joint.[18] This stress can be mostly inconsequential but act as a signal for the chondrocytes to maintain homeostasis. However, chondrocytes do not always respond in the most beneficial way. In the initial stages of OA development, chondrocytes respond harshly to various signals in a way that leads to a cascade of events, ultimately resulting in a dysfunctional joint. These signals vary depending on the patient and the predisposition at hand but some may include: high-magnitude mechanical stress, excessive or abnormal joint loading, introduction of inflammatory cytokines, reactive oxygen species, or injury to the surround cartilage or ligaments.[23] In response to these joint stimulators, chondrocytes and other cells in the synovium are activated and instead of responding in a way that maintains homeostasis, catabolic effects ensue. This response by chondrocytes may include upregulating synthetic activity, increasing production of inflammatory cytokines, or producing matrix-degrading proteinases.[18,23] As a result of this, proteoglycans and the collagen network become depleted and chondrocytes undergo apoptosis from the lack of their self-renewing capabilities.[18]

3.1.1 Inflammation

Inflammation is a cellular response to fight against infection in the body; however, in the case of OA and other chronic disease such as neurological disease, obesity, cancer, and autoimmune

disease, inflammation contributes to the development.[24] The role inflammation plays in the pathophysiology of OA is still unclear. It is still controversial whether inflammation is a primary factor that acts as a stressor to begin cartilage degradation or if inflammation occurs as a result of cartilage degradation.[18] Some studies have shown that proinflammatory factors drive the production of proteolytic enzymes that produce cartilage degradation seen in OA while other studies have shown that chondrocytes produce proinflammatory factors in the absence of any other inflammation.[18,25] In either case, inflammation is a key contributor to the disease that consistently appears in the synovial fluid of OA patients resulting in common OA symptoms such as pain, swelling, and stiffness.[18,26]

Chondrocytes and synovial cells express what are known as pattern recognition receptors (PRR).[27] PRR are part of the innate immune system and work as the first line of defense against invading pathogens.[27] They recognize damage-associated molecular patterns (DAMPs) and then respond with pathways leading to a wide range of host defenses and inflammatory factors. PRR include Toll-like receptors (TLR) and Nod-like receptors (NLR).[27] Recent evidence shows that a wide variety of endogenous products produced by cartilage matrix disruptions or cellular stress can bind to TLRs or NLRs and activate their response, furthering the controversy between primary or secondary inflammation.[15] Following PRR activation, chondrocytes or synoviocytes, begin to produce proinflammatory mediators, such as chemokines and cytokines.[18] These are also a part of the innate immune system and contribute to the development of OA by producing collagenases and aggrecanases leading to ECM degradation.[13] They also signal and arrange additional inflammatory cells, including macrophages, granulocytes, and lymphocytes.[15] Other downstream effects include the activation of NFκB, a protein complex that participates in cytokine production,

and upregulation of nitric oxide via inducible nitric oxide synthase.[18] A subset of Nod-like receptors are NLR family pyrin domain containing 3 (NLRP3) inflammasomes, protein complexes that activate inflammasomes leading to the activation of capase-1 and the cleavage of pro-IL-1β and pro-IL-18 to their active form.[27] NLRP3 inflammasomes have provided a central focus for OA research as will be discussed below. PRR initiate and potentiate inflammatory responses that induce catabolic action in chondrocytes, phenotypic shifts in the joint, and apoptosis of cells.[18] Repeated degradation of the articular cartilage coupled with constant inflammation results in narrowing joint space and osteophyte formation leading to pain, stiffness, and the potentiation of OA.[14] This OA progression process is outlined in Figure 2 below.

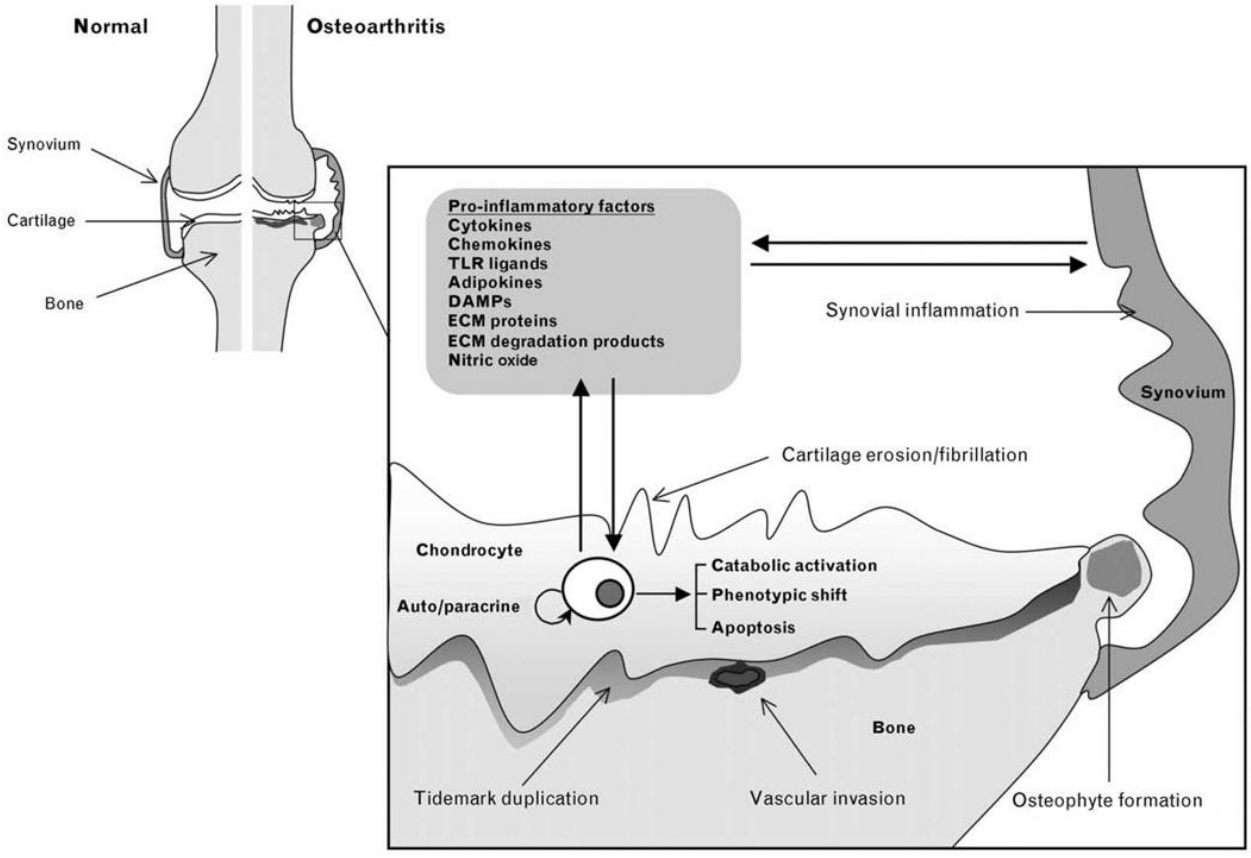

Figure 2. Alterations in the joint environment may lead to inflammation-induced and stress-induced signaling pathways resulting in the production of chemokines, cytokines, adipokines, Toll-like receptor ligands, and nitric oxide. The result is an upregulation of ECM degradation products leading to catabolic activity, phenotypic shifts, and apoptosis.[18]

3.1.2 Reactive Oxygen Species

Another influential factor that may contribute to the development of OA is oxidative stress as a result of reactive oxygen species. Reactive oxygen species (ROS) are free radicals that contain oxygen including hydrogen peroxide, hydroxy radical, superoxide anion, and nitric oxide.[13] They are compounds that contain an unpaired electron in their valence shell making them highly-reactive and unstable in search of stability.[28] The production of ROS are a part of normal physiological processes that are needed for survival, such as O_2- produced from electron transport chains in the mitochondria, and pose no threat to the body at very low concentrations.[19,28] However, when an imbalance occurs, deleterious effects may ensue.

Oxidative stress is the result of an imbalance between ROS and their clearance through antioxidant defense system. This imbalance has been shown to be a causative factor in many diseases, such as Alzheimer's, Parkinson's, cardiovascular disease, and cancer.[19,29,30] Oxidative stress has also been concluded to be a leading contributor to OA development. This has been proven by examining chondrocytes isolated from cartilage during end stage OA development and by finding lipid peroxidation or nitrosylation in synovial fluid, such as in studies completed by Nemirovskiy et al. in 2009 or Altay et al. in 2015.[31,32] The exact pathway that oxidative stress uses to induce OA development is still unclear.[33] However, evidence has shown that oxidative stress, more broadly, has a direct effect in chronic inflammation and redox signaling and control.[13,34] More specifically in OA, research has shown that oxidative stress can regulate intracellular signaling that effects chondrocyte apoptosis, extracellular matrix degradation, and disruption to the subchondral bone in an interdependent relationship with inflammation.[19]

ROS may be produced in the mitochondria or peroxisomes, as well as any structure that contains NADPH oxidases, Xanthine Oxidase, and Nitric Oxide synthases.[35] Within the aerobic articular cartilage, chondrocytes are host to numerous mitochondria where oxidative phosphorylation occurs with subsequent production of ROS.[36] One large contributor of ROS in OA development are NADPH oxidases (NOX) expressed by chondrocytes.[35] These are a family of seven enzymes that respond to stimuli to complete a variety of functions, including host defense, cellular signaling, and regulation of gene expression.[37] They do so by transporting electrons to create redox signaling and ROS generation.[38] In OA development, NOX catalyze electron transfers from NADPH to molecular oxygen, creating ROS.[39] In addition, Nitric Oxide synthases (NOS) are another family of enzymes that play a key role in ROS production in OA. NOS is available in three isoforms: neuronal NOS, endothelial NOS, and inducible NOS.[13] Inducible NOS are expressed by chondrocytes and have been shown to be upregulated in OA cartilage, producing nitric oxide.[13] Both NOX and inducible NOS contribute to an overproduction ROS making the antioxidant system incapable of responding adequately.

NOX and inducible NOS are pathways that occur in a healthy body, as well as in a developing OA patient. However, in OA patients, these pathways are upregulated to create a surplus of ROS leading to oxidative stress. These pathways have various activating signals throughout the body but in OA, their upregulation is dependent upon (1) inflammation and (2) mechanical stress. As already discussed, inflammation is a large contributing factor to OA development with inflammatory cytokines such as interleukin-1β, interleukin-17, tumor necrosis factor-α, interferon-γ present in OA joints.[40] In addition to their destructive effects on cartilage degradation, they also act as signaling molecules for NOX and inducible NOS. Additionally, mechanical stress is a large

factor for ROS development through these pathways. A study in 2007 on bovine articular cartilage completed by Tomiyama et al. showed that mechanical compression greatly increased ROS.[41] Another study in 2006 by Martin and Buckwalter showed that the introduction of antioxidant treatment decreased cell death in vitro of human articular cartilage.[42] The ability of both inflammation and mechanical stress to induce ROS in a joint is further facilitated by the cyclic compression naturally found in a joint during movement.[43] Free radical diffusion occurs throughout all joint compartments and allows for the transfer of ROS during walking, running, or daily activities.[43] This may contribute to increases cartilage exposure to ROS.

ROS largely affect joints by enhancing the inflammatory process as shown above, but there are also direct influences in OA progression. In general, research has shown that ROS directly influence OA development by inciting cartilage degradation through expression of matrix degrading proteases, inhibiting ECM synthesis, and inducing chondrocyte apoptosis, as outlined in Figure 3 below.[13] These is wide-ranging research currently available that shows the various disruptions ROS can cause in the joint capsule and how it may contribute to OA development. Altindag et al. in 2007 showed a positive correlation between oxidative stress and collagen degradation, a major component of the ECM.[44] Another study by Hashimoto et al. in 1998 showed that increased levels of apoptotic cells had a positive correlation with increased levels of nitrogen oxide production as OA progressed in rabbits.[45] In addition to chondrocyte and collagen degradation, ROS have also shown to have a role in preventing anabolic actions in the cartilage matrix. H_2O_2 has been proven to prevent proteoglycan synthesis by suppressing oxidative phosphorylation and adenosine triphosphate formation in chondrocytes.[46,19] Subsequently, this prevents the joint components from recovering following an event that induced cartilage injury.

Other research has also shown that ROS decrease chondrocytes' sensitivity to growth factors as well as prevent chondrogenic precursor cells to enter an injured area by reducing capacity.[47,48] Additionally, research has found a notable alteration in mitochondria function in chondrocytes and synovial cells in the presence of ROS which may be a leading cause of the factors outlined above.[18] All of these factors influence the healthy functioning of a joint and present a key target for OA treatment.

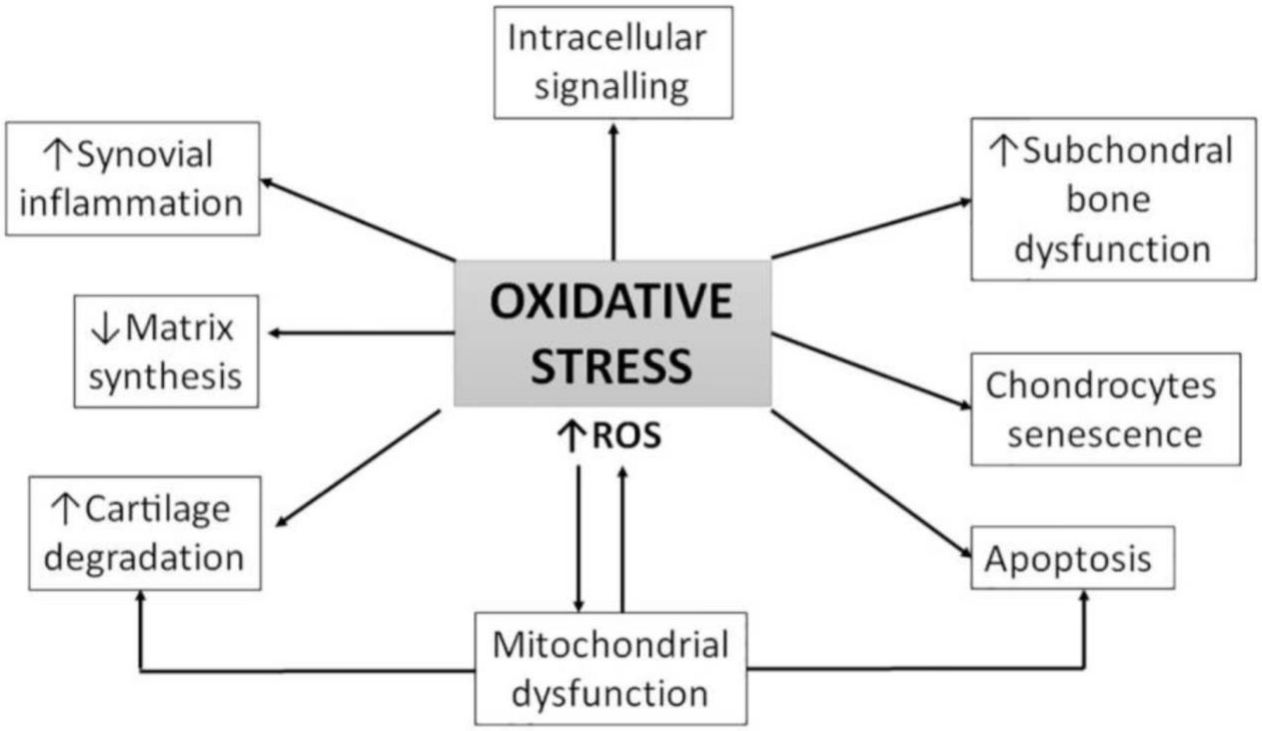

Figure 3. An increased level of reactive oxygen species leading to oxygen stress and its related pathogenesis in OA.[19]

3.2 Risk Factors

Although OA is a degenerative disease, age is not the only risk factor and certainly not the determining factor. The major risks can be divided into two main categories: natural predisposition and joint biomechanics.[14] Some individuals who develop OA exhibit natural predisposing factors

shown to increase the likelihood of contracting the disease, but do not necessarily cause it. Age is the strongest predisposing risk factor due to the body's inability to regenerate cartilage over time, as well as the accumulation of additional predisposing factors with time.[17] Genetics is another natural predisposing factor that has been researched, the results of which have identified that 11 loci can predispose OA, but it is not a strong enough factor to actually predict the onset of the disease.[17] Lastly, race can also be a natural predisposition with knee osteoarthritis occurring more frequently in African Americans compared to Caucasians.[14] None of these factors directly cause the disease, but rather put a person at a higher risk for development.

Along with natural predisposition, joint biomechanics expose people to a variety of risks for developing OA. Biomechanics that increase risk include injury, overload, and instability, which can be caused by a variety of factors.[14] Anatomical factors are a big risk to joint instability, as well as overload and injury issues. Anatomical factors can include uneven leg length, poor muscle strength, bone morphology, and many others.[17] These factors can be the result of an injury or naturally occurring. Although anatomical issues increase the likelihood of developing the disease, it is not an indicator or cause of the disease. Injury, overload, and instability leading to osteoarthritis is more prevalent in joints exhibiting anatomical issues compared to healthy joints, but osteoarthritis still occurs in otherwise healthy joints.[17,14] Furthermore, injury can cause structural issues in the joint that can lead to biomechanical risk factors, one instance of which is a ligament or meniscus tear. Direct injury to a joint that causes damage to the bone or articular cartilage can start the process of degradation and subsequent inflammation, pain, and stiffness that occurs with OA.[17] Additionally, obesity has been shown to be a large risk factor because it increases the load on weight-bearing joints and produces inflammatory adipokines.[17] Many of

these factors are presented in a wide variety of populations, but some are affected by multiple factors all at once.

3.2.1 Athletes at Risk

While the elderly population is at greatest risk for developing OA, young adults or even children are not free of risk. Recreational and occupational activities pose a great risk to developing osteoarthritis. In 2011, Cameron et al. found that active duty military personnel were shown to have a significantly higher risk for OA compared to a general group of people of the same age.[49] Another study in 2012 by Sulsky et al. found evidence to support job-related heavy lifting and standing as a hazard to developing hip OA.[50] Both of these provide evidence of physical load risk factors for developing OA. There is large indication throughout the literature to suggest that stressors applied to a joint through recreational or occupational activities often have detrimental long-term effects and place a person at a much higher risk for developing OA.

Athletes increase their risk of OA with the participation in athletic activities. A systematic review by Driban et al. in 2017 found that participants in long-distance running, wrestling, competitive weightlifting, and soccer have a greater prevalence of knee OA.[51] Another study in 1996 by Spector et al. saw a 2-3 fold increase in radiologic OA risks in the knees and hips of ex-elite female athletes of weight-bearing sports.[52] Research has even shown the effect of athletic activities regardless of age or presumed healthy individuals. Rall et al. in 1964 found that 80% of American football players who sustained a knee injury developed OA in the following 10 to 30 years.[53] Loading and injury, due to the nature of many athletic events, are the main factors that contribute to the increased risk of OA in athletes.

Loading of joints, especially on the knees and hips, applies stress to the joint that must be counteracted, by the work of the matrix, to sustain and protect the underlying bone. When force is applied to a joint, fluid in the articular cartilage shifts to sustain the impact of the weight and protect the bone from damage.[14] However, this process does not occur quickly. Athletes who load and unload joints in fast succession, do not give the fluid in the articular cartilage enough time to move to provide compression and stability.[14] The frequency at which most athletes participate in athletic activities, as well as the intensity and speed used in most sports, applies stress to the articular cartilage that is difficult for the joint to manage over long periods of time.

Direct, blunt injury to a joint can cause damage to the articular cartilage and eventually the whole joint. Athletes are more likely to sustain an injury than the average person, and this injury can lead to instability and deterioration of articular cartilage leading to the development of OA.[14] In addition to direct injury to a joint, injury to the surrounding muscles or ligaments puts the joint at risk. It has been estimated that 50% of people diagnosed with ligament injuries, such as a meniscus tear, will develop OA in the following 10 to 20 years.[54] Luc et al. in 2014 determined that one third of individuals that sustain an anterior cruciate ligament (ACL) rupture will develop OA in the following 10 years.[55] The stability and health of a joint depends on the whole musculoskeletal system, which is often compromised when athletes sustain injuries in joint areas during athletic participation.

3.2.2 NFL Athletes at Risk

The individual risk factors associated with OA development accumulate as a result of athletic participation leading to an overall higher risk for OA in athletes. This is true for athletes at any level, but the degree and exposure of the risk factors are greatly heightened with professional athletics. Athletes who earn their wage from participating in a sport dedicate years of their lives to the sport at an extremely high level. Many professional athletes participate in their sport for 10+ years before even entering into a professional field. This means there are years and years of running, lifting, kicking, hitting, throwing, etc. all on the same joints given at birth. As outlined above, these events greatly influence the development of OA. The risk of damage associated with professional athletics fluctuates depending on the sport itself, placing some professional athletes in more need of alternative treatment options. NFL athletes are one of the highest at-risk populations, making them a great target for continued research.

American football players continue to be a focus group for research in evaluating risk factors that may lead to any possible short-term or long-term effects. Some risk factors that have been associated with American football players may potentially lead to lasting effects on neurological, musculoskeletal, and cardiovascular systems, as well as behavioral and mental health.[56] Most notably for this research, studied have determined that NFL athletes are at a large risk for developing OA. Golightly et al. conducted a survey in 2009 with 2,538 retired NFL athletes and reported that 40.6% of retirees under the age of 60 had been diagnosed with some form of arthritis.[57] This stands in great comparison to the 11.7% of U.S. males diagnosed in same age group.[57] While these risks continue to be highlighted in research and news alike, football enthusiasts do not appear to be dissuaded from participation. In the 2017-2018 academic year,

football had the largest number of participants in the NCAA with 73,557 athletes.[58] While these are not professional athletes, many of them are working at a very high level for an extended period. For those possessing the athletic ability, football becomes a profession where their bodies are exposed to various risk factors for many more years to come.

The exact reason for this increased risk is something that is still being studied and one that is complex with many variables to consider. Some of the proposed factors have already been outlined above, which include: joint loading, increased weight, and injury. These factors are extremely prevalent in NFL athletes and often times lead to lasting and irreversible effects. One study by Davies et al. in 2019 surveyed 2,432 retired NFL athletes with an average of 15.2 years of participation in the NFL.[59] 11.4%, reported that they had a joint replacement with 7.7% reporting knee replacements and 4.6% reporting hip replacements.[59] Age, current weight, position, and each additional knee or hip injury was also shown to increase the risk of replacement. Linemen showed an 85% increase in hip replacement after retirement as compared to skill players.[59] Sustaining just one knee injury increased the prevalence of knee replacement by 78% with each additional knee or hip injury increasing the prevalence by almost twice that amount.[59] This research clearly shows the various and multi-dimensional factors that may contribute to the onset of OA in NFL athletes.

As outlined above, successive joint loading can cause an increased risk for developing OA. Athletes who participate in sports that require quick joint loading create a risk to articular cartilage by not providing enough time for joint fluids to counteract the blunt force being applied to a joint.[14] Joint loading in NFL athletes is very common, especially for linemen. Linemen play from a position known as the 3-point stance where they are squatting with one hand placed on the ground.

Athletes in these positions explode out of their stance and into the opposing players. This explosive position applies substantial force to the knee and hip joints.[60] Additionally, joint loading could also be a result of blunt trauma that occurs when helmets collide with joints, or minute trauma from repetitive loading applied to the joint in a way that it cannot counteract.[60,61]

Obesity is another risk factor that may contribute to the increased prevalence of OA in NFL athletes. It is known that obesity poses a risk for OA as it may produce inflammatory adipokines or create an even greater joint loading risk to the athlete.[17] In American football, weight gain is often encouraged for linemen in order to succeed at their highly physical position.[59] Maeda and Moll determined that there was an average BMI of 36.0 for line players in the American Football Conference in the NFL, indicating obesity, while other positions indicated a BMI between 27.9 and 37.1.[62] These statistics certainly indicate a potential risk for the increased OA prevalence. Additionally, research has shown a positive correlation between higher BMIs and prevalence of injuries to joints, such as the ankles or knees.[63] This leads to one of the greatest risk factors for OA in NFL athletes.

Injuries to lower extremities, especially to the knees, is very common in American football. In the same survey by Golightly et al., 52.8% of the retired NFL athletes reported knee injuries, 74.1% reported ligament/tendon injuries, and 14.2% reported anterior cruciate ligament tears from their time in the NFL.[57] This is an astounding percentage of athletes that have sustained injuries. These injuries are problematic for active players as these injuries may sideline athletes for an extended period of time. But not only are they problematic in the moment, research also suggests that they have lasting effects. Golightly et al. also determined that OA was more prevalent amongst those

that had sustained a knee injury or a ligament/tendon injury with a 95% prevalence ratio in both cases.[57] This study suggests a large correlation between injury and OA development. When joint areas are injured, the biomechanics of that joint are compromised. This could cause an altered and damaging joint loading process leading to articular cartilage breakdown. Injuries themselves can also start the degradation of the articular cartilage. In both these cases, over time, degradation of the cartilage creates harmful effects leading to inflammation, ROS, and OA development.

3.3 Diagnosis

Diagnosis of OA is typically a combination of patient history, physical exam, diagnostic imaging, and laboratory testing.[14,64] Pain is the leading symptom described by OA patients.[14] The pain may be described as deep or aching that is worsened by movement or activity.[64] At first, this pain can be relieved by analgesics, but one's body will eventually become unresponsive to medications.[14,64] This pain is what ultimately pushes people to seek help and a diagnosis from physicians. Joint stiffness, inflammation, crepitus, effusion, and decreased range of motion are other symptoms described by OA patients.[14,18,64] In majority of cases, symptoms only present once damage has already occurred which cannot be reversed.

Radiographic imaging, magnetic resonance imaging (MRI), computed tomography (CT), ultrasonography, and blood or urine tests are some ways physicians can confirm or deny an OA diagnosis.[64] Plain radiographic imaging is the most commonly used test to diagnose OA. Typical findings for OA patients include joint space narrowing, osteophytes, subchondral bony sclerosis, and cyst formation.[14,64] Figure 4 below shows a radiographic image with signs of OA. MRI or CT scans are used less often; but can still be used to determine if there are abnormalities. A CT scan

may determine if there is a misalignment, while a MRI scan may be used to look for chondral thinning, subchondral osseous changes, and osteophytes.[64] Additionally, MRIs may also be used to visualize articular cartilage or tissues supporting the joint, such as the meniscus or tendons, in order to determine if there is a tear, which is a common predisposition to OA.[64] Ultrasonography is another tool that is used less often, but may be used to monitor cartilage degeneration.[64] Urine and bloods tests are another way physicians can determine if there is a rise in effector molecules, such as cytokines, or extracellular matrix components, such as collagen, which can be a biochemical marker for OA.[17] These options all provide results once degradation has begun but there are not tests to show premature development, further emphasizing the importance of preventative measures.

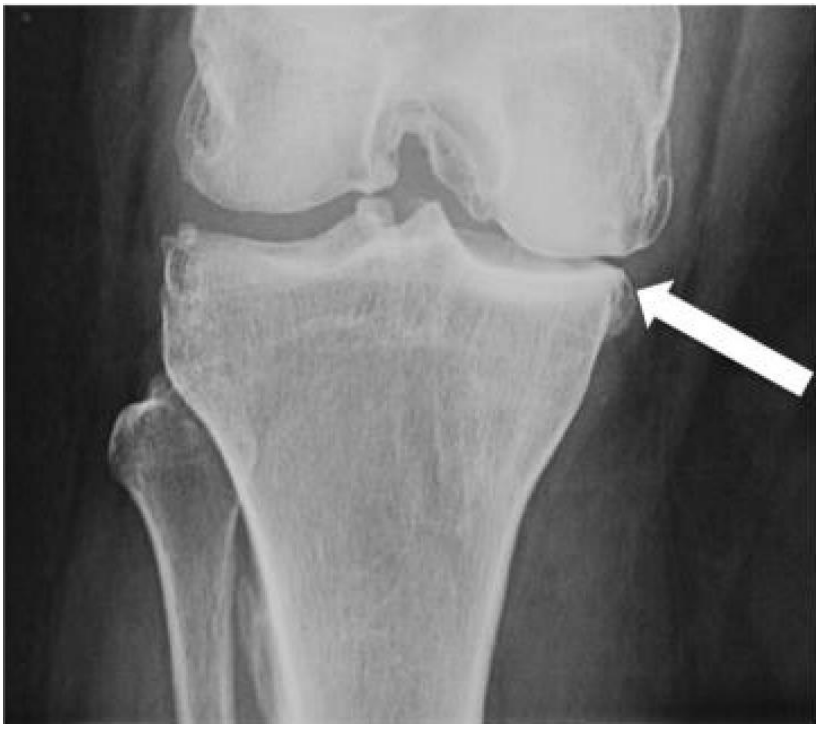

Figure 4. Radiographic image of an OA diagnosed knee. The arrow denotes medial joint space narrowing.[65]

As stated previously, pain is the leading symptom associated with OA that often times pushes patients to seek diagnostic help. People are usually not aware of the damage occurring in their joints without evidence through pain. However, pain develops late in the stages of OA.[17] By the time a person is experiencing joint pain, the disease is often times advanced and irreversible.[17] Pain is a subjective matter that varies across demographics with some individuals having higher pain tolerances than others. It has been shown that pain tolerance decreases with age, which might explain the prevalence of OA diagnoses in elderly populations.[14] Additionally, athletes might ignore pain by mistaking it for typical aches and pains associated with athletic competition. Admitting to subtle aches or pains may risk a player's opportunity to continue recreational or occupational athletics. Early diagnosis or recognition of OA is pivotal in successful treatment.

3.4 Current Treatment

Treatment for OA depends on the severity and duration of the disease as well as the age of the patients and desired goals after treatment. The main goal for any OA treatment is to alleviate symptoms and improve functional status, which is especially important in athletes.[64] Treatments for OA can be categorized by nonpharmacological, pharmacological, and surgical options. In any case, treatment interventions that have few or no adverse effects must also be of low-risk to the individual and proven to be effective for them to provide positive outcomes.[17] In the following sections, the current treatment options will be discussed.

Nonpharmacological options can include weight loss, physical activity, physical therapy, modifications of activities of daily living (ADL), joint protection techniques, use of assistive devices, bracing or taping joints, insoles, and much more.[14,64] However, these options vary

amongst demographics. Physical activity is a nonpharmacological treatment option that is still being investigated. Uthman et al. completed a study in 2013 with 8,128 OA patients and concluded that exercises that increase strength, flexibility, and aerobic capacity are likely to help treat OA.[66] Physical activity is something that can cause the disease, as seen in NFL athletes, but can also be used for benefit. In order for it be beneficial, specific regimens should be followed.

Another nonpharmacological form of treatment being investigated to slow the progression or prevent the onset of OA is dietary changes. A study was done by the Osteoarthritis Initiative on 2,757 patients with knee osteoarthritis with a mean age of 62.[67] The study compared what they called a traditional Western diet to what is known as a prudent diet.[67] The Western diet relied heavily on sugar-containing beverages, refined grains, desserts, processed meats, and French fries.[67] Conversely, the prudent diet consisted primarily of natural, unprocessed foods.[67] OA progression was monitored through radiographic imaging and measured by the Kellgren-Lawrence (KL) scale, loss in joint space width (JSW), and symptomatic progression by the Western Ontario and McMaster Universities Arthritis index (WOMAC).[67] The study found that the Western diet increased KL-worsening risk and increased odds of a higher WOMAC score.[67] In contrast, the prudent diet showed a decreased KL-worsening risk, decreased JSW loss, and decreased odds of higher WOMAC progression.[67] This is just one example of how diet can positively influence the progression of OA.

There has also been evidence of beneficial results for pharmacological options in OA patients. Pharmacological options primarily include the use of acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate pain and reduce inflammation.[64] Steroid injections, such

as corticosteroids, may also be used in the joint for its anti-inflammatory effect.[64] However, corticosteroids must be used with caution because of the cytotoxicity of steroids to chondrocytes.[17] These are primarily options to prevent pain and enhance one's quality of life. Other drug options have been found to show some disease treatment properties. For example, chondroitin and glucosamine have shown to be effective for its anti-inflammatory and anticatabolic effects on the joint cartilage.[17] Pharmacological options for pain and symptom relief are extremely prevalent but options for the prevention or treatment still require much additional research.

Surgery is another option for OA patients, and can include options such as articular cartilage transplant, regeneration stimulations, and joint replacement.[17] Surgery is typically not a preferred option for most patients, especially current athletes, due to the risks and recovery factors of surgery. Joint replacement has shown to provide poor functional outcomes, as well as short life span of the protheses.[61] In any case, the ultimate goal of treatment is to provide symptom relief and functional freedom with the lowest risk possible to the patient. This is a difficult task to achieve as many treatment options for any disease have at least some small risk. This is not necessarily the case for more natural options as will be discussed in the following section.

4. Ketogenic Diet

The ketogenic diet (KD) consists of eating high fats, moderate proteins, and low carbohydrates in order to put the body in a state of ketosis.[68] The ratio of fats to carbohydrates and protein of a classic KD consists of 3-4:1. There are many variations of the classic KD depending on the ratio which may include: the Modified Ketogenic Diet (2:1), the Modified Atkins Diet (1:1), and the Medium-Chain Triglyceride Oil Diet (1.9:1).[69] These diets work to limit carbohydrates, or sugars, which are the primary source of energy in the body and the easiest source for the body to access.[70] When the body is deprived of carbohydrates, the body enters a catabolic state where it will use its glycogen stores.[70] Once glycogen stores are depleted, the body enters gluconeogenesis, a state where the liver produces glucose primarily from lactic acid, glycerol, and the amino acids alanine and glutamine.[70] This, however, is not sustainable for long periods of time so the body must find another source of energy. This is when the body enters ketogenesis, a process where fatty acids stored in the body are metabolized to acetoacetate and beta-hydroxybutyrate.[70] Acetoacetate and β-hydroxybutyrate are the two main forms of ketone bodies in which the body uses for energy when glucose levels are depleted.[68] This process maintains homeostasis when the body is experiencing a caloric restriction but in the case of the ketogenic diet, glucose restriction activates it.[68] The production of ketone bodies and their effect are the hypothesized reasoning for the benefits seen when using the ketogenic diet.

The KD first emerged in the 20th century in the United States and France as a treatment form for epilepsy.[71] In 1920, Dr. Stanley Cobb and Dr. William Lennox determined that starvation had a positive effect on seizure control.[71] It was during this same period that Dr. RT Woodyatt at Rush Medical College identified the production of ketone bodies as a result of fasting.[71] He then

replicated these results with a high fat, low carbohydrate diet. It was through these findings that Dr. Russel Wilder at Mayo Clinic suggested a high fat, low carbohydrate diet be used for children suffering from epilepsy and termed it the ketogenic diet.[72] Since then, the KD has been shown to be effective in treating many other conditions. Research has shown that the KD can be effective in improving blood pressure, blood glucose, triglycerides, and HDL cholesterol levels.[70] Additionally, it may be effective in treating ischemic strokes, Parkinson's disease, Alzheimer's disease, sleep disorders, cancers, autism, amyotrophic lateral sclerosis, and many others.[71,73] Many of these improvements can be attributed to an enhanced neuro functioning as seen in research. One study in 2019 by Fortier et al. showed a 230% increase in brain ketone metabolism on a high fat diet and subsequently the subjects' episodic memory, language, executive function, and processing speed improved compared to baseline.[74] Another study by Appelberg et al. in 2009 reported that traumatic brain injury induced rats fed a KD improved more than those fed a regular diet.[75] Research using the KD continues to divulge into a variety of conditions as more is being discovered on the benefit of ketone bodies.

4.2 Ketone Bodies

The body is continuously producing small amounts of ketone bodies for energy, with each producing 22 ATP under normal circumstances.[76] These have provided mammalian energy for a variety of physiological states including starvation, the neonatal period, post-exercise, pregnancy, and low carbohydrate diets such as the KD.[73] In a typically healthy adult, there is approximately 100-250 μM ketone bodies in circulation.[73] This rises steadily in any of the physiological conditions listed above. After prolonged exercise or 24 hours of fasting, the circulation of ketone bodies may reach 1 mM.[73] In pathological states, such as diabetic ketoacidosis, this may rise as

high as 20 mM.[73] However, this pathological state seen in type 1 diabetics is not achievable by diet alone.[69] For the KD, optimal levels of 2 mM are consistently maintained when carbohydrates are avoided.[76] There is the possibility for minimal adverse events in this state such as gastrointestinal symptoms, weight loss, headaches, and fatigue but these symptoms typically resolve after a few weeks on the KD.[69]

Ketone bodies are water-soluble lipid molecules with two R-groups attached to a carbonyl group.[76] Ketogenesis is the process in which they are formed by the breakdown of fatty acids, the rate of which is proportional to total fat oxidation.[73] Approximately 80% of the stored energy in the human body is in the form of fatty acids contained in adipose tissue.[77] During periods of fasting or carbohydrate depletion, these fatty acids are mobilized from adipocytes to the liver where they are converted to ketone bodies.[77] Once formed, ketone bodies are transferred to other tissues where they are metabolized into acetyl-CoA and eventual ATP for energy.[77] Regulators of this metabolism is extensive and still not completely understood.

Ketone body production begins when fatty acids are brought into the mitochondria via carnitine palmitoyl transferase (CPT-1) and the acyl chains are transported through the mitochondrial membrane and undergo β-oxidation.[73] In the fate committing state, 3-hydroxymethylglutaryl-CoA synthase (HMGCS2) catalyzes acetoacetyl-CoA (AcAc-CoA) and acetyl-CoA to generate 3-hydroxymethylglutaryl-CoA (HMG-CoA), as shown in Figure 5 below.[73] HMG-CoA lyase (HMGCL) then cleaves HMG-CoA to release acetyl-CoA and acetoacetate (AcAc).[73] AcAc is then reduced in the final step to the ketone body, D-β-hydroxybutyrate (D-βOHB) by phosphatidylcholine-dependent mitochondrial D-βOHB dehydrogenase (BDH1) in a near-

equilibrium reaction with NAD+/NADH.[78] AcAc can also be converted to another ketone body, acetone, through a non-enzymatic decarboxylation.[76] Once production is complete, ketone bodies are transported out of the liver via monocarboxylate transporters (MCT1 and MCT2) into circulation for terminal oxidation in extrahepatic tissues.[73] The exact mechanism for transport is not currently known, but research has shown a higher concentration of circulating ketones as compared to extrahepatic tissue concentrations which suggests that ketone bodies move down a concentration gradient.[73] Acetone, however, is only excreted through urine or exhaled because it does not have the capabilities for terminal oxidation.[76] This has caused research to focus primarily on the work of β-hydroxybutyrate in the body.

Once transported outside of the liver, β-hydroxybutyrate must be catabolized in the mitochondria of extrahepatic tissues to acetyl-CoA to be made available for the citric acid cycle (TCA) for terminal oxidation.[73] This is then used for energy in the body during carbohydrate depletion. First, mitochondrial BDH1 catalyzes a reaction to oxidize βOHB in order to convert it back to AcAc.[73] AcAc is activated to AcAc-CoA when succinyl-CoA exchanges a CoA-moiety.[73] This reaction is catalyzed by succinyl-CoA:3-oxoacid-CoA transferase (SCOT) in a near-equilibrium reaction.[73] The energy released from hydrolysis of AcAc-CoA is greater than the release from succinyl-CoA which permits the reaction to favor AcAc formation.[73] Next, any of the four mitochondrial thiolases catalyzes a reaction to convert AcAc-CoA to two molecules of Acetyl-CoA.[73] These molecules then enter the TCA cycle where they are oxidized to later form ATP. It is also important to note that no ATP is used to activate ketone bodies, while glucose and direct fatty acids do require the investment of ATP.[73]

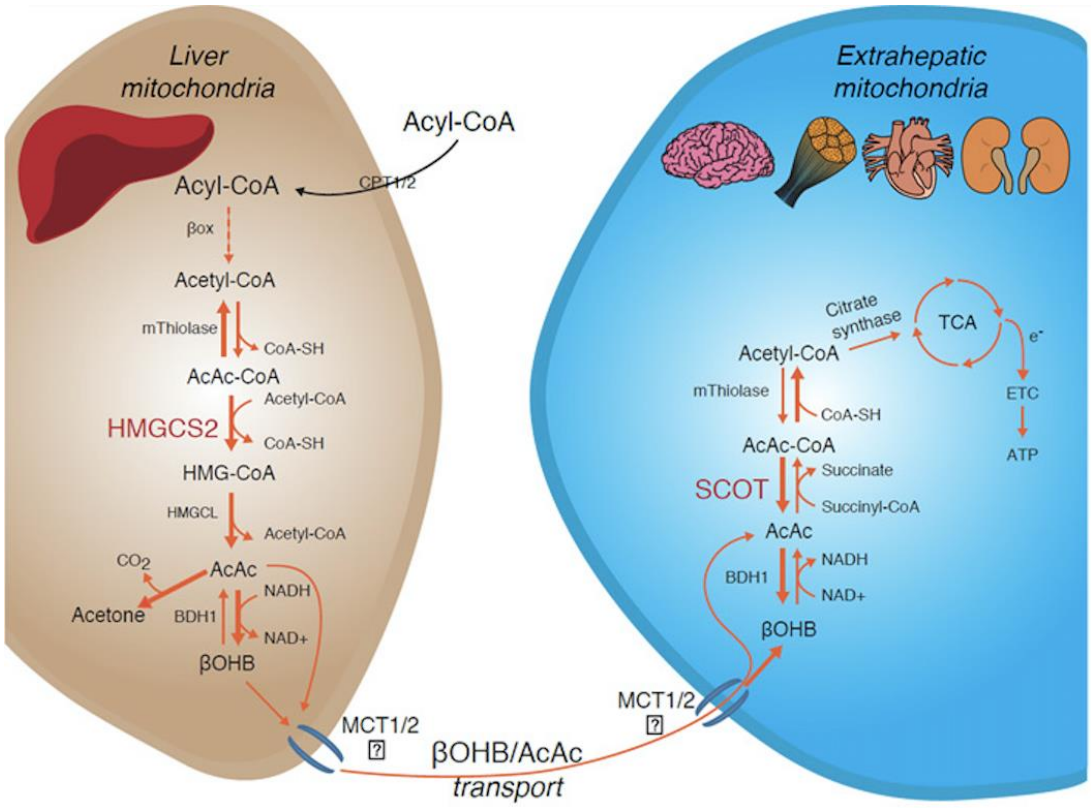

Figure 5. Ketogenesis occurs in the hepatic mitochondria starting from Acyl-CoA and ending with Acetone, Acetoacetate, or β-hydroxybutyrate. *HMGCS2 is the fate committing step that allows for ketone production after which they are transported to extrahepatic tissues. They then undergo reactions that require the fate committing step of SCOT and are terminally oxidized for energy use. There is still uncertainty for the mechanism of transfer, as indicated by the question marks.*[73]

Ketone body metabolism provides efficient and effective energy but that is certainly not its only role. Continued research in ketogenesis has shown that β-hydroxybutyrate is not only an energy carrier but plays a direct and indirect role is signaling that may affect gene expression, lipid metabolism, neuronal function, and metabolic rate.[77] β-hydroxybutyrate has been shown to have direct effects on the body through cell-surface receptors, competitive inhibition of enzymes, protein posttranslational modification, and modulation of ion channels.[77] These signaling functions' reduction effect on inflammation and oxidative stress has continued to be revealed

through research. This reduction provides a positive potential for an alternative treatment option in OA.

4.3 Anti-Inflammatory

The inhibition of glycolysis, or the breakdown of sugar by the body for energy, is known to dampen the body's pro-inflammatory responses, making ketone metabolism an interesting point of research for OA treatment.[27] Currently, research has suggested a role of ketone body metabolism in the regulation of the innate immune system.[79] This has made starvation states and the KD a prevalent area of research as this could be a way to treat multiple diseases that have a pro-inflammatory aspect, such as atherosclerosis, asthma, inflammatory bowel disease, arthritis, and many more.[73] In a study conducted in 2008 by Forsythe et al., 40 overweight subjects were fed either a very low in carbohydrate diet or an isocaloric diet for 12 weeks.[80] The results showed that both were associated with a decrease in several serum inflammatory markers; however, there was a greater anti-inflammatory effect with the KD, indicating a future research focus for ketone bodies and inflammation.[80] Despite growing research, the exact role of ketone bodies in the innate immune system is still not known. However, there are multiple proposed mechanisms for action, two of which will be discussed below.

4.3.1 GPR109A

Ketone bodies have been shown to have a signaling effect through G-protein coupled receptors through unclear mechanisms. One G-protein coupled receptor that ketone bodies have been shown to effect are GPR109A, also known as HCA2 or NIACR1.[73] These receptors are bound and activated by the ketone, β-hydroxybutyrate.[77] Currently, β-hydroxybutyrate is the only known

endogenous ligand for the receptor.[81] This receptor is expressed in many cell types, including immune cells such as macrophages and monocytes, microglia, and colonic epithelial cells.[73] In these cells, the receptor's activation leads to anti-inflammatory effects as a result of the activation of intracellular calcium release, prostaglandin alterations, and downstream inhibition of NF-κB.[77] NF-κB is a transcription factor that is considered to be a master regulator of the innate immune system, leading to pro-inflammatory responses of the body.[82] When GPR109A is activated, research suggests that it induces a macrophage phenotype related to prostaglandin D2 (PGD2) that works to resolve inflammation.[83] Additionally, research by Zandi-Nejad et al. in 2013, suggests that a metabolite of PGD2, known as IKB kinase, works as an inhibitor of NF-κB in macrophages leading to a decrease in the production of inflammation.[84] Another study by Fu et al. in 2014 similarly showed a decrease in proinflammatory enzymes and proinflammatory cytokines as a result of the inhibition of NF-κB from β-hydroxybutyrate.[85] The exact mechanism requires further research but these results provide substantial evidence for the work of β-hydroxybutyrate.

4.3.2 NLRP3 Inflammasome

The most prevalent area of research in regards to decreased inflammation as a result of β-hydroxybutyrate is the NLRP3 inflammasome. The NLRP3 inflammasome is a multi-protein complex sensor in the innate immune system that responds to damage in the body by activating capase-1 which promotes secretion of pro-inflammatory cytokines IL-1β and IL-18 in machrophages.[79,86] Removal of this inflammasome has shown to improve other diseases such as diabetes, bone loss, gout, Alzheimer's, age-related functional decline, atherosclerosis, and multiple sclerosis.[79] Research by Youm et al. in 2015 was the first to recognize the direct role that ketones played in limiting the NLRP3 inflammasome.[79] Prior to this, studies had shown that there was a

link between circulating ketones and the regulation of the inflammasome, but these studies suggested that it was a result of the reduced oxidative stress, increased adenosine monophosphate-activated protein kinase (AMPK), or autophagy.[79,87,88,89] Youm et al. determined that it was not the case.

In that same study by Youm et al. in 2015, macrophages derived from lipopolysaccharide-primed bone marrow of mice were treated with ATP, an NLRP3 activator, and two ketone bodies: β-hydroxybutyrate and acetoacetate.[79] β-hydroxybutyrate was shown to dose-dependently inhibit ATP-induced capase-1 cleavage and the processing of IL-1β, while AcAc was shown to be inconsequential.[79] The study also found that β-hydroxybutyrate acted on five other NLRP3 activators to prevent inflammation.[79] The results were determined to not be dependent on starvation regulated mechanisms like AMPK, ROS, or autophagy but on the ketones themselves.[79] The research concluded that β-hydroxybutyrate controls an unknown event that results in a reduction in K^+ efflux from macrophages, inhibition of ASC oligomerization and an inability for the assembly of the inflammasome. As a result of all of this, inflammation is decreased. This study provided substantial evidence to suggest that the ketogenic diet could positively affect the development of OA and a potential for furthered research with NLRP3 inflammasomes.

4.4 Reduced Oxidative Stress

Mitochondria are the powerhouse of the cell as this is where ATP is produced through oxidative phosphorylation. It is through this process that ROS are produced through the escape of electrons during transfer along the electron transport chain leading to oxidative stress, damaging the mitochondria in a positive feedback loop.[69] This increase in ROS and subsequent damage to the

mitochondria is decreased in ketone body metabolism as compared to glycolysis.[90] This reduction is due, in part, to the uncoupling proteins that are expressed during ketone metabolism.[91] These proteins lead to a reduced mitochondria potential, followed by a decrease in the ROS produced.[91] This alone provides a large benefit for one's general health, but it also has the potential to provide a large benefit for those at risk for developing OA or those already diagnosed with OA. Decreased ROS by this means is just one factor that has been shown to be affected by ketones, while many more theories of their benefit exist.

4.4.1 Nrf2

One of the primary ways that ketone bodies affect and reduce oxidative stress is through the activation of the nuclear factor erythroid-derived 2 (NF-E2)-related factor 2 (Nrf2).[69] This is a transcription factor that belongs to the cap "n" collar subfamily of basic-region leucine zipper transcription factor.[92,93] Nrf2 has been shown to be the central protein that interacts with a common DNA sequence, called the antioxidant response element, to activate gene transcription in response to rising oxidative stress.[93,94] This response mediates drug-metabolizing enzymes leading to elimination of exogenous and endogenous chemicals that lead to oxidative stress.[93] Research by Talalay et al. in 2003 showed that an increase in Nrf2 activity in mice through chemoprotective agents was associated with protection from oxidative stress.[95] Other research continues to support this claim.

Although research has continually supported the notion that β-hydroxybutyrate activates Nrf2, the mechanism by which it occurs is still not understood.[69] Milder et al. in 2010 showed that in the mitochondrion of the hippocampus of rats fed a KD, an initial increase in ROS occurred after only

1 day followed by a large reduction after 3 weeks of the diet.[96] Based on these results, Pinto et al. in 2018 hypothesized that the initial increase in H_2O_2 provided a redox signal for β-hydroxybutyrate to do its work.[69] Additionally, the initial increase in H_2O_2 may enhance the binding of Nrf2 to the antioxidant response element, as indicated in a study by Wilson et al. in 2005.[97]

4.4.2 HDAC

Another potential area of study that has shown to be influenced positivity by ketone bodies are histone deacetylases (HDAC). HDAC are nuclear proteins that consist of a deacetylase domain that typically work in large regulatory multiprotein complexes.[77] They function to suppress gene expression through deacetylating DNA, thus creating tightly condensed chromatin that cannot be transcribed.[98] β-hydroxybutyrate works to prevent this by competitively inhibiting HDACs. As a result, the transcription of genes that detoxify ROS can be upregulated. These include catalase, mitochondrial superoxide dismutase and metallothionein 2.[69] One study completed in 2020 by Solé et al. showed that β-hydroxybutyrate worked to slow the progress of OA in vitro and in vivo as a result of HDAC inhibition.[76] Another study by Shimazu et al. in 2013 showed similar results in mouse tissue treated with β-hydroxybutyrate.[87] The study showed that there was an increase in histone acetylation of the Foxo3a and Mt2 promoters and both of the genes were activated because of the selective depletion of HDACs.[87] This led to an increased protection from oxidative stress.[87] This research provides substantial evidence to suggest a potential for using the KD as a preventative measure or treatment option for OA.

5. Research Proposal

In light of the information gleaned from numerous completed studies, it is apparent that there is a need for alternative preventative measures or treatment options for those at risk for developing OA. Research shows that OA contributes to pain and decreased mobility, as well as mental and emotional struggles. For those that are diagnosed, it is a devastating reality to know that there is no way to stop continued bone breakdown. They have two options, anti-inflammatory medications once symptoms present or joint replacement once the joint has failed. Anti-inflammatory medications seem to only treat the pain but do little to stop the progression of the disease. Joint replacement is often very costly and is typically only an option at the end of disease development. There needs to be additional therapies.

There are many at risk for developing OA. These may include individuals with occupational hazards, increased age, certain genes, musculoskeletal trauma, obesity, and increased physical activity. While there are many populations of people that fit into these categories, NFL athletes continue to be a population that fit into many of these categories, not just one. That is why this research aims to focus on providing a preventative measure or treatment option for OA in NFL athletes. While there are currently no ways of perfectly predicting who will develop OA, there are a large number of factors that can be identified for those that are at higher risk, NFL athletes being one of those. With this in mind, a preventative measure would allow the significant number of NFL athletes who are diagnosed with OA to decrease. For those that choose not to partake in a preventative option, there needs to be a way to treat those that become diagnosed with OA later in life.

The ketogenic diet has existed since 1920; however, there are still a lot of unanswered questions for how it produces the effects it does on the body. While this research study does not aim to answer those questions, it does aim to determine how it may help to treat or prevent OA in high-risk groups. Though there are many unanswered questions, research does continually support the claim that it reduces oxidative stress and limits inflammation, two things that are key to OA development. Other studies conducted have shown a decrease in OA biomarkers using the ketogenic diet, but there are no current studies aimed at targeting NFL athletes specifically. Additionally, research has not been able to determine if OA begins by oxidative stress and inflammation or if they are only potentiators of the disease. Because of this, a research study would need to be conducted that tests the potential for the KD being used as a preventative measure and a treatment option in NFL athletes. In order to fully examine the ketogenic diet's effect on joint health, a model would need to be used to mimic what is seen in NFL athletes.

5.1 Research Model

The objective of this proposed research is to determine the potential benefits of the KD in preventing or treating OA in NFL athletes. Because the joint will need to be examined for specific markers of degeneration and inflammation, the research is designed to be carried out in an osteoarthritic mouse model. While this model will not perfectly represent the various factors that contribute to OA in NFL athletes, this study would provide preliminary testing to determine if further research should be conducted specifically with NFL athletes and the KD. This research study will also provide results for areas outside of NFL athletes. There are many OA high-risk groups that may benefit from the unique properties of the KD. This research specifically aims to

determine if the KD can prevent or treat osteoarthritis in C57B/6J mice that will resemble the joint loading representative of that seen in NFL athletes.

5.1.1 Mouse Model

The mouse model will consist of forty-eight C57B/6J mice that are at least 10 weeks old. At that age, the mice have developed enough that they are able to manage joint damage that would be representative of an adult human's ability.[99] Male gender is also important in the representative model as research has shown that OA is more prevalent in females and hormonal causes may contribute to their decreased ability to maintain joint health.[99] It will also need to be ensured that the mouse model does not have any genetic predispositions for developing OA. This will allow for a proper analysis of the effects of the KD on OA in NFL athletes without variance from genetic dispositions.

All of the mice will be treated appropriately, according to the Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Each of the mice will be held in separate, ventilated cages where they will have 12-hour cycles of light and dark. Food will be provided to them either in the form of the KD or the regular chow diet. The mice fed a KD will be fed at specific intervals in order to monitor the daily macronutrient levels. The control groups will be fed a regular chow ad libitum. The regular chow diet will be composed of agricultural byproducts, such as corn, oats, wheat, alfalfa, and a protein source, as well as supplemented with vitamins and minerals.[100] The ketogenic diet will consist of casein, cornstarch, sucrose, soybean oil or lard, and supplemented with vitamins and minerals.[100] This will be given to the test group animals in ratios of 8:1:1 for fat, protein, and carbohydrates, respectively. This allows the mice to enter a state of ketogenesis

where the body is relying on fatty acid breakdown and subsequent ketone bodies for energy, rather than the typical glucose stores.

5.1.2 Ketosis Monitoring

During the research conduction, it will be critical that the ketone body levels are monitored consistently. In order to do this, the Precision Xceed Pro Point of Care System will be used. This is a machine that is designed to determine blood ketone levels.[101] This requires puncturing the surface of the animal to draw blood out which will then be placed on the test strips. The reading will determine if the animal has entered ketosis or not. Ketosis is defined as having a steady ketone body composition of at least 2 millimole/L.[76] This will be used as the standard for determining ketosis in the mouse model.

5.1.3 Osteoarthritis Induction

In order for this study to adequately represent OA development that is seen in NFL athletes, it must be induced in a similar manner. However, there are many factors that contribute to their high-risk identification in NFL athletes with no research definitively pointing to a direct cause in every instance. Additionally, research has found that different positions on the team take on a different level of risk. Research has determined that joint loading does play a significant part in developing osteoarthritis. While this may not be the only cause, it will provide a similar factor to mimic what is seen in NFL athletes. In order to induce OA in the mouse model that mimics joint loading, an Intron Electro Pulse E1000 will be used. This is an instrument designed to test dynamic and static force for a variety of materials. Wu et al. conducted a study on a mouse model in 2015 using this machine.[102] The study aimed to determine what force load and cycle length most closely mimicked

the joint loading effects known to induce OA. The research conducted by Wu et al. determined that 6 Newtons of force for 60 cycles induced OA as evidenced by chondrocyte apoptosis, cartilage matrix degradation, disruption of cartilage collagen fibril arrangement, and increased levels of serum cartilage oligomeric matrix protein.[102] Based on those results, the mice in this study will also be subjected to 60 cycles of 6 Newton joint force to the knee. The knees will be placed hyper flexed in the Intron Electro Pulse E1000, as shown in Figure 6 below. The mice will also be sedated under anesthesia to ensure proper treatment.

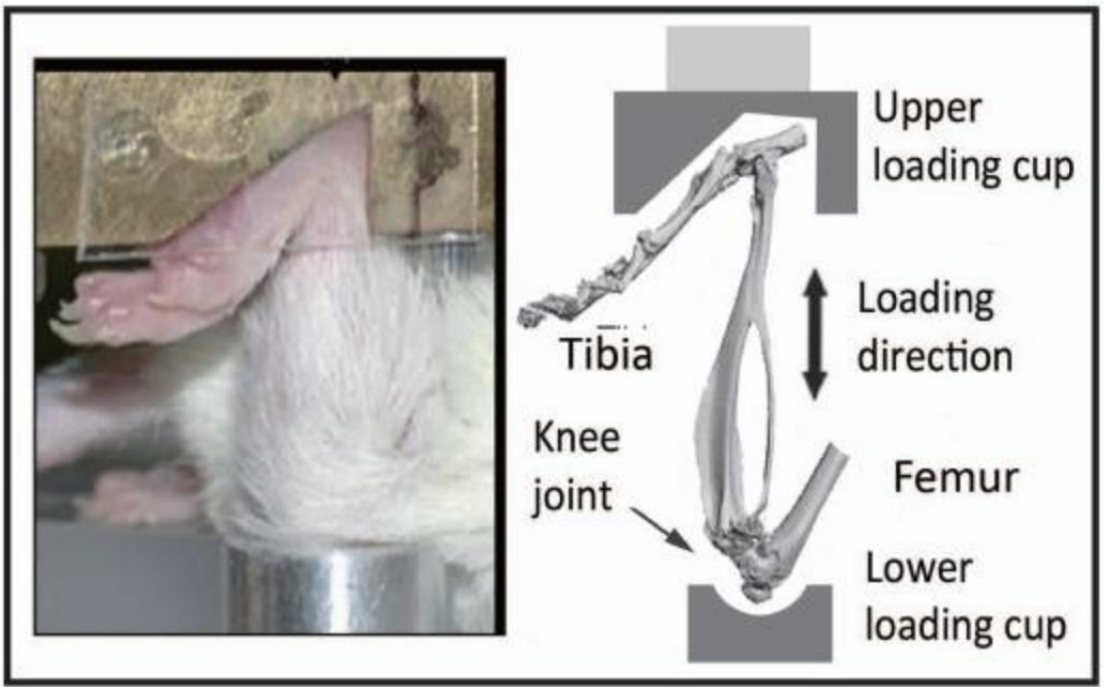

Figure 6. The Intron Electro Pulse E1000 that will be used to induce OA in the mouse model.[102]

5.1.4 Osteoarthritis Testing

During the study, the progress, or lack thereof, of OA will be consistently monitored. This will occur during the duration of the study by noninvasive means. At the conclusion of the study, the animals will be sacrificed so that histological studies can be conducted on the joint to more adequately determine the efficacy of the KD. While the study is ongoing, inflammation and

oxidative stress will be monitored to observe the potential increases and/or decreases of these levels throughout the study. Inflammation will be monitored using enzyme-linked immunosorbent assay. In this assay, levels of the NLRP3 inflammasome will be monitored, as well as cytokines IL-1β and IL-18. For oxidative stress, a urinary assay will be used to monitor the levels of nitric oxide as well as the antioxidant system in the body that works to deplete them.

5.2 Specific Aim One

The first aim of the study is to determine if the ketogenic diet can prevent the onset of OA in NFL athletes. For the first aim, 24 mice will be used. 12 of the mice will be used in the test group while the other 12 will be used in the control group. The test group will be fed the KD that is described above for 3 weeks. During this time, the blood levels will be determined after each meal to ensure the optimal level is achieved. The blood ketone levels should reach their optimal levels within the first 3 days, but the full 3 weeks will allow the mice to adapt adequately to the metabolic changes that occur during ketogenesis. The control group will be fed a regular chow diet but every other variable will remain the same for the control.

Following the 3 weeks, both the test and control group will undergo OA induction via the Intron Electro Pulse E1000. In the following weeks, both the inflammation and ROS will be tested using the analysis outlined above. These results will be monitored and recorded for 5 months following the induction. Once completed, the animals will be sacrificed in order to compare histological findings in the knees of both the control and test group. Analysis of the knee joint will include observation of any potential chondrocyte apoptosis, osteophyte formation, joint space width, and

cartilage degradation. With these parameters, the study will be able to determine if the ketogenic diet prevented the induction of OA as compared to the control group.

5.3 Specific Aim Two

The second aim of the study is to determine if the ketogenic diet could be used to treat OA once degradation has begun. This will require two groups as well, one test and one control. 24 mice will be used with 12 in each group. For this aim, the mice in both groups will undergo OA induction via the Intron Electro Pulse E1000 first. Following induction, both the inflammation and ROS will be tested daily using the analysis outlined above. This will provide a baseline level for both components in order to compare future data. OA progression will be monitored and once the disease has shown degradation signs, the test group will be fed a KD. The control group will be fed a regular chow diet. Again, the blood ketone levels will be monitored for the test group to ensure the mice stay within the ketosis range. During this time, inflammation and ROS species will continue to be monitored in order to see any increases or decreases in the levels. The mice will be continuously monitored for 5 months following the introduction of the KD. At the completion of the study, the animals will be sacrificed in order to compare histological findings in the knees of both the control and test group. The knee joint will be analyzed and the parameters such as chondrocyte apoptosis, osteophyte formation, joint space width, and cartilage degradation will be observed. With these parameters, the study will be able to determine if the KD treated the developed OA as compared to the control group.

5.4 Experimental Pitfalls and Future Aims

These experimental designs are intended to determine if there is a potential for using the ketogenic diet in NFL athletics. This is the first step to an alternative option for either preventing or treating a disease that is pervasive amongst these professional athletes. Although this research may shed some light on unanswered questions, there are some pitfalls that this research cannot overcome. First, the mice model used in this research is one that does not directly correlate to NFL athletes. The risk factors that are prevalent amongst NFL athletes are multidimensional with no perfectly clear path. The research attributes OA development to many factors in NFL athletes such as weight, joint loading, injuries, knee positioning, biomechanics, and much more. With that being said, a universal model for OA development in NFL athletes is next to impossible to create. That is why this research serves to be a starting point for further research. Additionally, research is inconclusive on how exactly ROS and inflammation are associated with OA. There is some research to suggest that inflammation and ROS produce the initial insult while other research suggests that they only potentiate it once it has begun. It may also be a combination of both or varying in different cases. That being said, this model cannot answer that question specifically but can only provide experimental outcomes for both cases. Lastly, the KD itself still has much research that needs to be completed. Currently, research studies have been unable to determine how the KD, and ketone bodies specifically, work to produce the results that are so often seen with individuals on the diet. It is not clear whether they work to competitively bind, increase transcription, or something completely different. This research design does not seek to answer the question of how it works but only if it may work in this specific population.

There is still so much research to be had on this topic. OA is a very prevalent disease in the NFL population but with many outstanding questions to be answered. In the future, this research study should be use as a launching point for more studies targeting high-risk populations. As stated above, the mice model does not adequately represent the multidimensional factors of OA progression in NFL athletes. With that being said, this study should be completed in NFL athletes themselves to determine if the KD can actually provide a benefit to these professional athletes. Likewise, a similar study should be carried out to various mice models to determine if there is a potential for using the diet in other high-risk populations.

6. Conclusion

Osteoarthritis is a debilitating joint disease that greatly affects so many. It is currently the most prevalent type of joint disease and the third most rapidly increasing disease associated with disability.[4] Individuals diagnosed with OA are greatly affected in every part of their lives as activity levels likely decrease because of the pain and inflammation associated with the disease. Although the disease is a localized to the joint, the diagnosed individual is likely to be affected holistically as research suggests that 1 in 3 patients over the age of 45 will suffer from depression or anxiety.[6] Other research has even suggested that there is a 55% increase in mortality associated with the disease.[5] These outcomes are quite alarming with no clear end in sight. Treatment currently consists of NSAIDs or eventual joint replacement. NSAIDs only provide pain relief and joint replacements are extremely costly and provide a burden on the individual. There needs to be a better way for those so greatly affected by the disease.

Treatment needs to improved universally but especially for those that are known to be at a high risk for developing the disease. High-risk groups may include the elderly population or individuals with occupations that produce strain on the joint. Athletes are one population that are greatly affected, specifically those that participate in athletics that involve running, jumping, weight-lifting, or wrestling. Football involves many of these activities and has continually shown to put an athlete at high-risk for developing OA. The risk is even higher for football athletes that participate for long periods of time and extend their participation through professional football in the NFL. Research showed that 40.6% of 2,538 retired NFL athletes under the age of 60 had been diagnosed with some form of arthritis.[57] This high risk for OA in NFL athletes cannot be pinpointed to one direct cause but is believed to be a variety of factors that may include joint

loading, weight, and injury prevalence. Due to the lack of treatment or preventative measures, OA continues to be a result of pursuing football at the highest level. NFL football continues to be a beloved sport in the United States, so there needs to be a way to counteract this prevalent OA development.

After researching the ketogenic diet, it was determined that it could be an option for treating or preventing OA in NFL athletes. Research shows that oxidative stress and inflammation is greatly associated with OA. It is currently not known whether these two factors start the production of OA or if another insult, such as a knee ligament injury, starts the development of OA and ROS and inflammation are a way that the body responds and subsequently potentiates OA. In any case, the KD is also associated with a decrease in ROS and inflammation. The high fat, low carb diet produces ketone bodies that are later used for energy in the body. It is the production of those ketone bodies and their circulation through the blood that positive effects ensue. It is currently not known how these ketone bodies produce their results but research has discussed some theories for their mechanism. Some research suggests that the ketone bodies competitively bind and inhibit the NLRP3 inflammasome which prevents rapid, uncontrollably inflammation production. Other research suggests that ketone bodies bind to GPR109A which later inhibits proinflammatory NF-κB. Ketone bodies also work to decrease ROS by activating Nrf2 for antioxidants or by inhibiting HDAC so that transcription of antioxidant molecules may be increased.

The research that is currently available on topics of OA, NFL athletes, and the KD is inconclusive in many ways. That is the downfall of this research. There are so many questions left unanswered. This research was only a literature review, so no new answers could be provided, but only a

summation of what is currently being discussed in research. With this in mind, research must progress. OA is a costly and burdensome disease that affects so many. Research needs to progress in order to provide the answers necessary to find more and better treatment and preventative measure options. Additionally, the KD has the potential for treating so many diseases, and maybe that is the downfall for research on this diet. There is so much research being conducted on how the KD can be used for treatment in Parkinson's, Alzheimer's, concussions, cancer, etc. that OA may be overlooked as a disease it can benefit. In any case, research needs to progress in determining how the KD works to create these positive outcomes. This will help progress the use of the diet in multiple diseases. Lastly, there needs to be more focused research on the prevalence of OA in NFL athletes. OA is rampant amongst retirees of the sport. This is a sad reality for so many that are pursuing a dream that they have worked so hard to achieve. OA should not be the common outcome for them. This literature review and research proposal serves as a starting point for more focused research. If the proposal were to be conducted, the results would provide interesting information for how research on OA in NFL athletes may proceed.

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