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Pre-season Vitamin D and Iron Levels as a Predictor of Musculoskeletal Injury in Division I Athletes

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Pre-Season Vitamin D and Iron Levels as a Predictor of
Musculoskeletal Injury in Division I Athletes

A thesis submitted for the partial fulfillment
of the requirements for the degree of
Master of Science in Athletic Training

by

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Abstract

Context: Pre-season testing that includes the analysis of micronutrient serum levels in the blood has become a routine part of pre-participation examinations in collegiate football. **Objective:**

Assess the predictability of vitamin D and iron serum levels as indicators of future injury during a subsequent competitive season for lower extremity sprains, strains, and fractures. Additionally, determine the effectiveness of vitamin D supplementation on increasing serum 25(OH)D levels and preventing injury. **Design:** Cohort Study. **Setting:** National Collegiate Athletic Association Division I football program. **Participants:** Football athletes (n=349) who underwent pre-participation examinations and participated in at least one of three competitive football seasons.

Main Outcome Measure(s): Vitamin D and Iron serum levels collected during pre-season testing and injury occurrence of the lower extremity or core was analyzed to determine if a predictive association existed. A vitamin D serum level cut-point for high-risk or low-risk athletes was established using a receiver operator characteristic analysis (ROC) for various injury associations including all injuries, fractures, and muscle strains. Cross-tabulation analysis produced sensitivity, specificity, positive and negative likelihood ratios, and odds ratios for each cut-point. Backwards stepwise logistic regression was used to determine the best combination of injury risk factors. Individuals supplemented with vitamin D were analyzed using a paired-samples T-Test to determine if supplementation procedures were effective. **Results:** Muscular injuries were significantly predicted by low vitamin D levels (<23.15 ng/mL; OR=3.042; 90% CI [1.426, 6.486], p=0.019) high game exposure (OR=2.636; 90% CI [1.208, 5.753], p=0.036), previous injury (OR=3.390; 90% CI [1.546,7.431], p=0.010). Based on a three-factor prediction model, athletes with two or more risk factors were at an increased risk of injury with an odds ratio of 6.000 (90% CI [2.639, 13.642], p=0.001). Vitamin D supplementation increased mean pre-season levels by 32.206 ± 15.52 (p<.001). **Conclusion:** Use of a model with multiple risk

factors can help identify athletes who are at risk for injury based on both intrinsic and extrinsic factors. The modifiable factor of vitamin D status may help athletic trainers to decrease injury risk in those with low serum 25(OH)D levels through a short-term supplementation program.

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Thank you to Tim Ridner for providing the inspiration for this project. You motivated me to take a deeper look into the clinical practice around me to determine the effectiveness of what is currently being done to provide the best care for the athletes we treat. A special thank you to Matt Summers for allowing me to access the athlete information necessary to complete this project. Your desire to care for athletes and better understand the clinician's role in injury prevention made this project possible.

Dedication

I would like to dedicate this project to my classmates in the University of Arkansas Athletic Training Education Program. Each of you have encouraged me to analyze every aspect of what I learn as my clinical sites to determine if we are providing the most evidence-based care for our athletes. You inspire me to continually learn every day and be innovative in my clinical practice. The knowledge and experience that you provide to the field of athletic training will only continue to grow as you begin your career and make an impact on athletes and patients all over the country. I dedicate this project to you in the hopes that your thirst for knowledge will never be quenched.

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I. Introduction

Athletic trainers often hear the phrase “keep them healthy” from fans and coaches in regard to the athletes they care for. When injuries start to plague a roster, the athletic trainer is quickly associated with the decrease in team performance that may be due to an increasing number of talented athletes sidelined by injury. As the medical professional who most closely works with a team on a daily basis, the pressure to keep athletes healthy and performing at their best falls on the athletic trainer. For this reason, the area of injury prevention and prediction has become one of particular interest in athletic training.

Injury prediction models have been developed for a variety of factors that could predispose someone to injury risk. These prediction models can be used by athletic trainers to implement interventions in at-risk athletes to decrease their likelihood of sustaining an injury. Injury is a multifaceted issue that can be caused by intrinsic or extrinsic factors. It is often difficult for athletic trainers to address extrinsic factors, such as the weather or duration of competition. Therefore, efforts to prevent injury through intrinsic modifications may be effective in reducing risk.

One intrinsic factor that can be analyzed and modified is vitamin and mineral deficiencies. Normal blood values for many vitamins and minerals have been identified to determine adequate amounts necessary for normal functioning of organ systems. These values are measured through blood draws. Of particular interest in the athletic population are blood markers of vitamins and minerals due to their influence on many organ systems. The side effects of deficiency in vitamin D and iron (ferritin) may predispose an athlete to injury. These micronutrients play a vital role in bone, muscle, blood, immune, and neurological health. Once a deficiency in one of these micronutrients has been identified, supplementation can be used to

reverse the effects. This type of intervention is a noninvasive and efficient way to modify a potential intrinsic factor in athletes that could help to prevent future injury.

Statement of the Problem

Current research has analyzed the effects of vitamin D in a wide variety of athletic and non-athletic populations. The majority of current research focuses on identifying the prevalence of vitamin D deficiency and insufficiency and fracture risk, primarily stress fractures. Minimal research has been done on the effects of vitamin D levels in athletes participating in contact sports. More research is needed to analyze the effects of vitamin D on musculoskeletal injuries as well as the influence of supplementation in decreasing risk. If found to be a predictor of injury, vitamin D supplementation is a time efficient and noninvasive technique for lowering injury risk in athletes.

Additionally, minimal research has been done about the association between other micronutrients and injury risk. Iron plays a role in the maintenance of healthy bone and blood in the body. In the athletic population, this is especially important as these systems are routinely stressed during exercise. Therefore, it is reasonable to suggest that this micronutrient should be analyzed as a possible predictor for athletic injury. Many athletes undergo routine screenings of these blood markers as part of their preseason physical examination. However, these values have not been regarded as musculoskeletal injury predictors as of yet.

The purpose of this study is to identify the predictive value of pre-season serum 25(OH)D and ferritin levels in collegiate football players on musculoskeletal injury risk during the season. Additionally, this study will analyze the effects of vitamin D₃ supplementation in increasing serum 25(OH)D levels and decreasing injury risk. Data will include preseason serum levels of vitamin D, iron, vitamin D supplementation use, subsequent serum 25(OH)D retest levels

following supplementation and occurrence of injury during the corresponding competitive season (August-December). Three competitive seasons will be included in this analysis. The results of this study will seek to identify a serum 25(OH)D level that can serve as a predictor of increased risk for musculoskeletal injury that can be used by healthcare professionals working with athletic populations.

Hypotheses

The hypotheses for the research study above are as follows:

1. More than 50% of athletes will have deficient or insufficient vitamin D levels during preseason testing.
2. Athletes with deficient or insufficient levels of vitamin D levels will be more likely to sustain a musculoskeletal injury during their competitive season.
3. Athletes with deficient or insufficient vitamin D will be more likely to sustain a fracture during the competitive season.
4. Athletes with insufficiencies of one or more micronutrients analyzed will be more likely to sustain a musculoskeletal injury during their competitive season.
5. Vitamin D supplementation will increase vitamin D levels in those with preseason values categorized as insufficient or deficient.

Assumptions

1. All athletes were free of injury and fully cleared for participation during the time of preseason testing.
2. Serum levels were obtained, analyzed, and documented by a licensed medical professional.

3. Athletes sought medical attention for evaluation and care after an athletic injury was sustained.
4. All injuries were sustained during football practices or games while performing football activities.
5. All injuries were accurately documented by medical staff.
6. All supplementation was accurately documented by medical staff.

Limitations

1. All data collected was retrospective in regard to injury and supplementation. Therefore, statistical analysis is limited to the data available and no specific interventions could be enacted.
2. Micronutrient serum levels fluctuate based on nutrition, supplementation, and exposure to sunlight throughout the course of the season. Injuries that occurred late in the season will still be analyzed with regard to data collected from preseason testing.
3. Athletes who sustain an injury may not seek medical attention due to fear of being held from competition or a variety of other factors. Therefore, some injuries may not be accounted for in the data analysis.
4. Supplementation varied based on individual athlete needs and was not strictly enforced or documented in those with adequate levels.

Delimitations

The study was limited to a cohort of NCAA Division I football athletes during three separate competitive seasons. The results of this study should be relevant for athletes that participate in collegiate football, professional football, or other contact sports.

Significance of the Study

One of the five domains of athletic training is injury prevention and there is a wide variety of research seeking to identify the best ways to prevent athletic injury. Insufficient vitamin D levels have shown a strong association with fracture risk in small populations, but more information is needed on its effect on other musculoskeletal injuries and in contact sports. Additionally, minimal research has been done on the effects of other micronutrients on predicting injury risk. The information learned from this study will help to add to the growing body of research surrounding prediction of injury in athletic populations. If found to be significant, blood work results may be used by athletic trainers to identify athletes at increased risk for musculoskeletal injury. Based on the results of this study, athletic trainers can better understand if supplementing athletes is evidence-based in reducing their risk of injury.

II. Literature Review

Vitamin D

Vitamin D is well known for its importance in the regulation of calcium and phosphate absorption for overall bone health. Recently, it has been shown to impact musculoskeletal and immune system functions as well.^{1,2} Vitamin D is synthesized in the skin through ultraviolet B (UVB) radiation exposure from the sun. Radiation with a wavelength between 290-315 nm is absorbed by 7-dehydrocholesterol in the epidermis and dermis and converted to previtamin D₃.³ Previtamin D₃ quickly transforms to vitamin D₃.⁴ Vitamin D obtained from the skin or from dietary intake is transported to the liver to be metabolized to form 25-hydroxyvitamin D (25[OH]D). This is then metabolized in the kidneys to create the active form 1,25-dihydroxyvitamin D (1,25[OH]₂D) that acts through specific vitamin D receptors throughout the body.³⁻⁵ Vitamin D is also obtained through dietary intake of some natural foods such as cod liver oil and meats and others that are fortified to include vitamin D such as some cereals and dairy products.^{2-4,6} Vitamin D₃ (cholecalciferol) and D₂ (ergocalciferol) supplements can also be used; however, ergocalciferol is irradiated from plants and may not have the same biological effects as cholecalciferol.⁵ Although it is classified as a vitamin, research findings have shown that Vitamin D is actually a fat-soluble secosteroid that functions as a prohormone after being synthesized to 25(OH)D.^{5,7}

Vitamin D deficiency is a wide spread issue that has been shown in the general population. Severe deficiency is shown through rickets and osteomalacia.⁴ Signs and symptoms of rickets include bowlegs, bone pain, tetany, swollen joints and convulsions.^{3,7} Vitamin D deficiency in adults may present with back and bone pain, fatigue, recurrent infections, muscle pain, and depression.²²⁻²⁷ Due to its importance in bone and musculoskeletal health, Vitamin D

has become an area of interest in athletic populations. Deficiency may put athletes at an increased risk of injury or illness. Studies have shown that many athletes are at risk for vitamin D deficiency, especially athletes who participate in winter or spring sports, play an indoor sport, or have a dark skin tone.^{6,8,9} Serum 25(OH)D levels have been analyzed in many athletic populations such as gymnasts, professional football and basketball players, ballet dancers, and division I collegiate athletes. These studies report a high prevalence of inadequate vitamin D levels (either deficient or insufficient) as high as 83% and 91%.^{6,10-15} The purpose of this literature review is to discuss the current literature regarding vitamin D deficiency and supplementation, vitamin B12, folate, and iron levels as they relate to injury and illness risk in athletes.

Risk Factors for Vitamin D Deficiency

The majority of risk factors for vitamin D deficiency and insufficiency revolve around receiving adequate UVB radiation because it is the main source by which the body synthesizes vitamin D.³⁷ Dark skin tone is a risk factor for vitamin D inadequacy because those with dark skin tones have more melanin than light skinned individuals.³⁸ Melanin is an ultraviolet filter that absorbs UVB photons.³⁹ In individuals with dark skin, 7-dihydrocholesterol is competing with melanin to absorb UVB photons.³ Even though individuals with dark skin tones have an adequate amount of 7-dehydrocholesterol in their skin to absorb UVB and synthesize previtamin D₃, the high concentration of melanin prevents them from absorbing adequate radiation to achieve vitamin D sufficiency.

This risk factor has been demonstrated in the general public and in athletic populations. One of the first studies to measure the difference in vitamin D absorption in different ethnic populations found that one minimal erythemal dose (MED) of ultraviolet radiation significantly

increased circulating vitamin D levels in Caucasian participants, but had a minimal effect on the African American participants.³ It was found through another treatment of increased dosage that the African American participants required a dose that was six times larger than one MED to achieve significant changes in circulating vitamin D levels.³ Similarly, a study that analyzed the vitamin D levels in a group of Division 1 NCAA athletes found that those with dark skin tones had lower average serum 25(OH)D levels than athletes with light skin tones.⁸

Other factors that can prevent adequate vitamin D absorption from UVB photons include excessive use of sunscreen and wearing clothing that covers most of the skin.³⁸ Additionally, vitamin D absorption is low for individuals who spend the majority of time indoors or do not spend time outdoors between the hours of 10 a.m. and 3 p.m. when UVB radiation is at its peak.⁴⁰⁻⁴¹ Therefore, athletes who play indoor sports or who practice in the mornings or evenings may be at an increased risk for low serum 25(OH)D levels. Living in locations with reduced sun exposure is another risk factor for vitamin D deficiency. Latitudes above 37° north (north of San Francisco, CA) have a decrease in sunlight between November and February.^{38,42} This has been shown to cause seasonal variations in vitamin D from the fall to the winter and spring.^{6,43}

Individuals with high percentages of body fat are also at an increased risk for having low levels of circulating vitamin D. Vitamin D is a fat-soluble vitamin and many researchers believe that it can become sequestered inside of fat tissue instead of circulating in the form of serum 25(OH)D.¹⁵ This is still up for debate among researchers as others believe that obese individuals simply have more volume that dilutes vitamin D and causes levels to be lower in this population.⁴⁴ In a study of 42 collegiate athletes, serum 25(OH)D levels were compared to anthropometric and body composition measures. Vitamin D levels were negatively associated with height, total body mass, BMI, body fat percentage, and fat-free mass. When using a linear

regression mixed model and controlling for sex, they found that fat mass was the greatest predictor of low vitamin D levels and explained approximately 36% of the variation in serum 25(OH)D in individuals with more fat mass.¹⁵ Athletes with higher body fat percentage may be at an increased risk for vitamin D deficiency.

Assessment of Vitamin D Status

Vitamin D levels are measured clinically using serum 25(OH)D levels. This is believed to be a more accurate descriptor of vitamin D levels than the biologically active form, 1,25(OH)₂D, because it is not influenced by homeostatic regulation.⁴⁵ When vitamin D levels are inadequate, parathyroid hormone (PTH) concentration increases to cause the kidneys to release more 1,25(OH)₂D into the bloodstream to maintain homeostasis.⁴⁶ Therefore, 1,25(OH)₂D will still display normal levels when vitamin D is not adequate. Serum 25(OH)D levels are not influenced by PTH and can provide a more accurate vitamin D status.

Vitamin D deficiency and insufficiency are most commonly categorized as less than 20 ng/mL (50 nmol/L) and between 21 and 29 ng/mL (52-72 nmol/L) respectively.⁴ Sufficient levels are defined as beginning at around 30 ng/mL (75 nmol/L).¹⁶ These cut points were determined by the activity of calcium absorption and PTH activity at these levels. Serum 25(OH)D levels between 30-40 ng/mL (75-100 nmol/L) show a leveling of PTH activity. When 25(OH)D levels increased from 20 ng/mL to 32 ng/mL intestinal calcium absorption increased by 20%.¹⁷ While these levels are widely accepted in research, there is not currently agreement on what should be classified as the optimal concentration of serum 25(OH)D.

Vitamin D Status of Athletes

Vitamin D levels have been measured in many athletic populations. Findings vary between populations, but many researchers identify widespread vitamin D deficiency and

insufficiency in athletes. A meta-analysis of 2,313 athletes found that 56% did not have adequate vitamin D levels.⁹ Some athletic populations have greater disparities in vitamin D than others. For example, vitamin D levels analyzed in a group of elite female gymnasts ages 10-17y showed that 83% of athletes were classified as insufficient with levels below 30 ng/mL.¹⁰ Research analyzing other indoor sports have reported similar findings. A group of 279 National Basketball Association athletes undergoing combine training were analyzed and showed 32.3% deficiency (<20 ng/mL) and 47% insufficiency (20-32 ng/mL). Only 20.8% of the athletes had sufficient levels above 32 ng/mL.¹⁴ Similarly, a group of Spanish professional basketball players reported 57% deficiency and insufficiency.¹⁸ Low vitamin D levels in these athletes may be attributed to long hours spent training primarily indoors leading to a lack of sun exposure.

Athletes who participate in outdoor sports or live in areas with abundant sunlight are not immune to vitamin D deficiency either. Serum 25(OH)D levels were obtained from a sample of 449 athletes living in middle eastern countries. Deficiency of < 20 ng/mL was found in 91% of this population while 58% had severely deficient levels < 10 ng/mL. Neither skin coverage, sunlight exposure, nor skin color were shown to be associated with low levels in this population.¹⁹

Seasonal changes in vitamin D levels have also been analyzed in athletes. Sunlight is not abundant between the months of November through February.^{38,42} This is especially true in areas that are located at a latitude > 37° North.³⁸ The decrease in UVB radiation during these months may lead to decreases in serum 25(OH)D levels when compared to measures in May through August. This difference was shown in an analysis of vitamin D levels in a group of elite ballet dancers (n=19). Serum 25(OH)D levels were obtained in February and then again in August. As hypothesized, the average winter serum 25(OH)D levels were significantly lower than summer

levels (14.9 ng/mL vs. 23.9 ng/mL; $p < 0.001$).¹² Similar results were seen in a study of collegiate wrestlers ($n=19$) when vitamin D levels were measured in the fall, winter, and spring. Serum 25(OH)D levels were below sufficient (<32 ng/mL) in 74% of the population. This percentage increased to 94% of athletes with either deficient or insufficient vitamin D levels in the winter and spring.⁴⁷ It is important to recognize that both ballet dancers and wrestlers compete and train indoors. This may have been the reason for the high level of insufficiency in both of these populations. Even with this limitation, both populations experienced seasonal differences in vitamin D levels.

Serum 25(OH)D levels were also analyzed at three different time points throughout the year in a population of collegiate athletes ($n=41$). This sample analyzed both indoor and outdoor athletes and compared their levels. Average serum 25(OH)D levels were 49.0 ± 16.6 in the fall, 30.5 ± 9.4 in the winter, and 41.9 ± 14.6 in the spring.⁶ When comparing indoor and outdoor athletes, serum 25(OH)D levels were significantly greater in outdoor athletes than in indoor athletes (53.1 ± 17.4 vs 39.3 ± 8.9 ng/mL; $P = 0.013$) in the fall, but not in the winter (31.9 ± 10.2 vs 26.3 ± 5.0 ng/mL; $P=0.15$) or spring (44.6 ± 15.6 vs 33.1 ± 4.8 ng/mL; $P=0.09$). This indicates the importance of sun exposure in the summer in outdoor athletes to achieve optimal vitamin D status. Athletes may be at a risk for decreased serum 25(OH) levels during the winter depending on sport and location.

Fracture Risk

Vitamin D has been associated with markers of bone health such as bone mineral density and sufficient rates of bone turnover through osteoblast and osteoclast activity.^{11,48} Therefore, adequate vitamin D levels help to maintain the necessary bone structure needed to function with normal stress. Due to the calcium regulating function of vitamin D and its overall influence on

bone health, vitamin D levels have been measured in association with fracture risk. Serum 25(OH)D levels were measured in 80 professional football players during the 2011 off-season and compared to reported injuries from the 2011-2012 and 2012-2013 seasons. Serum 25(OH)D levels revealed that 68.8% (n=55) of athletes had either insufficient or deficient vitamin D levels. Twenty-one of these athletes sustained a fracture with nine of them sustaining more than one fracture. Analysis showed that players who sustained at least one fracture had significantly lower vitamin D levels compared to those with no fractures after controlling for number of years played.¹¹ However, the mechanism of injury of these fractures was not disclosed and it is unclear whether they resulted from non-contact or contact injuries. Therefore, it is unclear whether adequate vitamin D can protect against macrotraumatic fractures that occur in athletes.

Stress fractures have also been a large focus in research because they are often caused by the inability of the bone to remodel accurately after large, repeated stresses. They can also be caused when abnormal bone is exposed to normal forces.⁴⁹ This is of particular interest in athletic populations due to the high intensity and frequency of training that predisposes them to increased risk for stress fractures and stress reactions. In a group of elite female gymnasts, low serum 25(OH)D levels were significantly associated with stress reactions.¹⁰ A study of collegiate athletes indicated no significant relation to stress reactions and low serum 25(OH)D levels. However, they did find a negative correlation between stress reactions and bone mineral density.¹¹

A prospective study by Ruohola et al. followed Finnish military recruits through the first 90 days of basic training. This period was chosen due to the rapid introduction of high amounts of physical activity that may predispose these individuals to injury. They measured serum 25(OH)D levels at the beginning of basic training. Multivariate analysis revealed that serum

25(OH)D levels below the median for the cohort was significantly associated with an increased risk for stress fractures.⁵⁰ This study design allowed for a homogenous sample that underwent the same training regimen. This was appropriate to determine increased risk for stress fractures between individuals based on serum 25(OH)D levels.

Vitamin D and Musculoskeletal Health

While the main function of vitamin D is often believed to be calcium regulation and bone morphology, recent research suggests that it also plays a role in skeletal muscle function. Receptors for vitamin D have been found in muscle and have been suggested to help increase muscle strength and prevent injury.¹ This finding broadens the influence of vitamin D in the overall musculoskeletal system. Research has been done to measure increases in muscular strength and performance in relation to vitamin D status. Ward et al. performed a cross-sectional study of post-menarchal adolescent girls to determine a relationship between serum 25(OH)D levels and muscle power, velocity, and jump height. They measured baseline serum 25(OH)D concentrations, PTH, and calcium. After controlling for height and weight, they determined a positive relationship between 25(OH)D levels and jump velocity, height, power, and force.⁵¹ Although these findings suggest performance advancements in those with higher serum 25(OH)D levels, increased muscular strength and power may also serve in injury prevention. Additionally, muscles and bones work together as muscles exert forces on bones and bones adapt to these forces.⁵² It is believed that vitamin D may help to maintain this relationship and prevent injury by allowing for adequate remodeling of bone and sustaining muscle strength.⁵²

Although there is believed to be an association, incidence of general musculoskeletal injury has yet to be consistently related with vitamin D levels.⁷ Wolman et al. measured baseline serum 25(OH)D levels in February and August and tracked injuries throughout the six-month

period in a group of elite classical ballet dancers. They observed lower serum 25(OH)D levels in February than in August (14.9 ng/mL vs. 23.9 ng/mL). Additionally, they observed twice as many injuries in the winter than in the summer, 24 and 13 respectively. Despite clinical relevance, statistical analysis failed to show a significant association between serum 25(OH)D levels and injury rates in this population.¹² Similarly, musculoskeletal health was analyzed in a group of adolescent male ballet dancers in relation to vitamin D levels. Serum 25(OH)D levels were measured and injury history was taken for all subjects. There was no significant difference in injury reporting rate between those with sufficient vitamin D levels and those classified as insufficient or deficient. Additionally, no significant association was found between injury, primarily back pain and muscle tears, and vitamin D status.⁵³ Another study of collegiate wrestlers confirms the inconsistency in the research surrounding vitamin D and musculoskeletal injury. Barcal et al. followed a group of collegiate wrestlers throughout their competitive season and found that more injuries were reported in those with a higher vitamin D status.¹⁵ More research is needed to analyze the relationship between serum 25(OH)D levels and musculoskeletal injuries.

Vitamin D and Illness

Vitamin D has also been suggested to play a role in immune function and illness prevention. An association was identified between vitamin D and the up-regulation of antimicrobial proteins and peptides (AMPs). This association was discovered by Liu et al. when they observed that vitamin D receptors are present in human monocytes. These monocytes are phagocytic white blood cells that work as part of the immune system. When treated with vitamin D, these monocytes trigger one AMP known as cathelicidin.⁵⁴ This AMP can play a critical role

in preventing the development of infections.⁵⁵ However, the practical implication of this finding deserves to be studied further.

One main research interest surrounding this finding has been the association between vitamin D and upper respiratory tract infections (URTIs). This association has been studied both at the cohort and nationwide level. Finnish military men were tested for serum 25(OH)D levels at baseline and then followed for 6 months. Researchers tracked the number of days they were absent due to respiratory infection during that time. They found that those with levels below 40 nmol/L (\approx 16 ng/mL) missed significantly more days due to respiratory infection compared to those with sufficient vitamin D levels.²¹ These findings were also confirmed in a diverse population through the secondary analysis of the Third National Health and Nutrition Examination Survey that was originally conducted between 1988 and 1994. Researchers analyzed the association between serum 25(OH)D levels and recent URTIs while adjusting for demographic and clinical factors. They found an inverse relationship between serum 25(OH)D levels and URTIs. Their results showed those URTIs were reported by 24% of individuals with levels below 10 ng/mL, 20% of individuals with levels between 10-30 ng/mL, and 17% of individuals with levels greater than 30 ng/mL.⁵⁶

The association between vitamin D levels and illness, specifically URTIs has also been studied in athletic populations. A group of endurance runners (n=225) were followed for a 4 month period during the winter. Their serum 25(OH)D levels were collected at the beginning and end of the 16-week training period. During this time, researchers kept daily illness logs for each of the participants that included symptoms and severity. They found that those classified as vitamin D deficient ($<$ 30 nmol/L) experienced a greater proportion of URTI symptoms and a greater severity of these symptoms than those in the optimal group ($>$ 120 nmol/L) during the 4

month period.⁵⁷ In contrast to this finding, no association was identified between serum 25(OH)D levels and illness or infection in a cohort of collegiate wrestlers during their competitive season.⁴⁷ Similarly, in a group of collegiate athletes, there was an association in Spring between vitamin D levels and illness, but not in Fall or Winter.⁶ While vitamin D may be shown to play a role in the up-regulation of AMPs, the practical implication for illness prevention in athletes remains inconclusive.

Vitamin D Supplementation

Vitamin D supplementation can be used to achieve adequate vitamin D levels when sun exposure and dietary intake are not sufficient. There are two types of vitamin D supplements: vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Vitamin D₃ is suggested to be more effective in optimizing bone health and more closely aligns with the effects of vitamin D that is obtained through UVB radiation.⁵⁸ In one randomized-controlled trial, Vitamin D₂ was given to a group of adolescent girls with low vitamin D levels to determine if there was a correlation between supplementation and bone mineral density, bone mineral content, muscle strength, and muscle force compared to a placebo group. Although serum 25(OH)D levels improved in the supplementation group, there were no significant correlations with bone health. There was a correlation between increased serum 25(OH)D levels and muscle jump velocity that may show a correlation between vitamin D₂ supplementation and muscular strength and force.⁵¹ Vitamin D₂ may be more useful for vegetarians or vegans because it is not synthesized from animal fat like vitamin D₃.⁴

Injury status has also been correlated with vitamin D supplementation. In a supplementation trial of collegiate swimmers with adequate vitamin D levels, the group that received vitamin D₃ supplementation of 4,000 IU/day over a six-month period saw a mean

increase of +1 ng/mL while the placebo group experienced a mean decrease of 20 ng/mL.⁵⁹ This suggests that vitamin D supplements may help to maintain adequate levels throughout the winter and during periods of low vitamin D exposure. Injury status documented in this study showed that 77% of injuries occurred during periods of decreased serum 25(OH)D.⁵⁹ Elite ballet dancers who were given vitamin D₃ supplements (2000 IU/day) for four months experienced less injuries compared to the control group. All injuries experienced by the control group were muscular strains or tears.¹³ This suggests that vitamin D supplementation may help to improve muscle function and protect against injury.

Vitamin D supplementation has also shown to be effective when adequate calcium levels are present. This occurs because vitamin D regulates calcium for bone and muscle function.⁵² These can also come from dietary intake or through the use of supplements. A randomized-controlled trial including United States Female Navy recruits used vitamin D and calcium supplementation to determine if supplementation would deduce the high rate of stress fractures seen in this population. The intervention group had a 20% lower incidence rate of stress fractures compared to the placebo group.⁴⁹ This finding suggests that vitamin D and calcium supplementation may be useful in conjunction to improve bone health and prevent stress fractures.

Suggestions for supplementation in athletic populations are not consistent because of the previously discussed disagreement on the optimal levels of vitamin D. The Food and Nutrition board currently suggests 600 IU/day and is the official recommendation by the United States government. However, other organizations have suggested much higher supplement intake based on research that current suggestions are not enough. The Endocrine Society suggests 1,500-2,000 IU/day, while the Vitamin D council says to take 5,000 IU/day. Vitamin D toxicity is not

common in most people who take supplements and occurs if 40,000 IU/day or more are taken for several months or from a significantly larger one-time dose. The current recommendations are significantly lower than this level in order to prevent vitamin D that leads to hypercalcemia.

Iron

Iron is an essential mineral that is obtained from food sources such as meat, poultry, fish, cereals, legumes, fruits, and vegetables. Iron plays a major role in red blood cell oxygen carrying capacity through its binding to hemoglobin and myoglobin. About 60% of iron is bound to hemoglobin and 15% is bound to myoglobin.³⁴ The remaining 25% of iron is stored in a readily mobilized form. Iron levels are assessed through the measurement of ferritin, the primary form of iron in the body, in the plasma. Unlike hemoglobin, ferritin is not impacted by living at elevations above sea level or smoking, thus making it a better assessment of iron levels. However, ferritin can increase during inflammation, infection, or liver disease. As defined by the World Health Organization, ferritin levels are categorized as either iron deficient (<12 µg/L) or iron overload (>200 µg/L). Individuals who are iron deficient are also described as being anemic. Other than the two extremes, no optimal level of iron was defined.³⁵

Iron levels analyzed in a group of professional runners revealed that 22% had ferritin levels between 50 and 30 µg/L (first stage iron depletion), 13% had ferritin levels between 30 and 12 µg/L (second stage iron depletion). Iron levels were not analyzed for an association with injury risk in this study.³¹ There is a lack of research regarding the association between ferritin levels and injury risk in athletic populations. A paper about nutritional requirements for swimmers indicated that a diet that contains adequate levels of micronutrients, including iron, was important for overall health and performance. However, they did not discuss any association between high ferritin levels and injury prevention.³⁶

III. Methods

Participants

This study included 349 male football player-years from a NCAA Division I institution. Data includes participants from three separate competitive seasons 2015 (n=120), 2016 (n=115), and 2017 (n=114). All athletes were cleared for participation for the following competitive season during pre-participation examination. Preseason blood work was obtained from all participants as per the standard pre-participation examination protocol at the university. All athletes were cleared by a sports medicine physician as not having any current injuries. Each athlete was considered to be a new participant each year if they participated in more than one season. This approach has been used in previous predictive studies and is regarded as acceptable for use.⁶² Participants demographics for each year are as follows: in 2015, age was 20 ± 1 y., in 2016 age was 20 ± 1 y., and in 2017 age was 20 ± 1 y.

Variables

Vitamin D serum levels were collected through blood samples collected during the pre-participation examination by a licensed phlebotomist. Athletes were instructed to abstain from eating for at least 8 hours and to not participate in any type of physical activity before their blood was sampled. It is standard to collect about 10 ml of venous blood for the purpose of analysis. Based on the results of the serum 25(OH)D blood analysis, athletes were classified as either deficient (≤ 20 ng/mL), insufficient (between 21ng/mL and 29 ng/mL) or sufficient (≥ 30 ng/mL).

Iron level was assessed through serum ferritin concentrations. This has been shown to be a reliable indicator for measuring iron levels and correlates to the results found from taking bone marrow samples.⁶⁰ During the three years of data collected for this study, two separate methods

were used to collect ferritin levels. This resulted in different units that were reported and separate cutoffs for sufficiency and insufficient. For individuals where iron was reported in $\mu\text{g/dL}$, sufficiency was classified as between 50-160 $\mu\text{g/dL}$. Conversely, those with iron reported in units of ng/mL , sufficiency was classified as between 20-345 ng/mL . Individuals with levels below 50 $\mu\text{g/dL}$ or 20 ng/mL were classified as deficient, respectively.

Musculoskeletal injury was defined as any sprain, strain, or fracture to either the lower extremity or core that resulted in the athlete seeking medical attention and missing at least one subsequent practice or competition due to injury. This definition of injury was based on classification from the National Collegiate Athletic Association.⁶¹ After an injured athlete sought medical attention, the certified athletic trainer thoroughly documented evaluation and subsequent treatment. Therefore, all documented injuries were included in this analysis and considered to be a musculoskeletal injury.

Due to the importance of vitamin D in bone health, fractures were considered a separate category in our analysis. The mechanism of the fractures, either acute or stress fracture, was not specified in the study. Additionally, non-fracture musculoskeletal injuries were categorized based on the location of the injury in the lower extremity. These injuries were documented as either foot, ankle, lower leg, knee, quadriceps, hamstring, hip, or core. Those categorized as calf, quad, hamstring, and hip were considered separately in analysis and classified as muscular injuries.

Game exposure was retrospectively recorded in this study due to its previous association as a predictor for lower extremity sprains and strains in a cohort of collegiate football players.⁶² This variable was included in the current study due to its previously investigated value for injury

prediction. Its inclusion in a model with multiple predictive risk factors, including vitamin D, helps to determine if vitamin D is of value in injury prediction beyond game exposure. Game exposure was measured through the variable “starter status” that included individuals who were listed as starters on either offense, defense, or special teams for at least four games during the respective season. These data were collected through coaching and team statisticians’ records. Individuals were dichotomously classified as either a starter or a non-starter for analyses. Game exposure data was available for two out of the three seasons included in this study, the 2016 and 2017 seasons.

Previous injury has been shown to be a predictor of future injury. For the purpose of this study, this criterion was included to better determine the predictive value of vitamin D and iron in the presence of or in spite of previous injury. Previous injury was documented as an injury that occurred the year prior to the current competitive season. Previous injury was also defined by the National Collegiate Athletic Association definition that requires that an athlete miss at least one practice or competition to be considered an injury.⁶¹ Previous injury was collected from the athletic training facility medical database records for the year prior to the current season. Data for this variable was included for upperclassmen only and was limited to the 2016 and 2017 competitive seasons.

Supplementation regimens were prescribed for each individual after pre-season testing. Those who were classified as adequate were provided a maintenance dose of 2,000 IUs (include company brand, location, etc.) that were to be taken 4 times per week. Those who were deficient or insufficient were given 50,000 IUs of vitamin D₃ to take once a week by a member of the sports medicine staff. Each dose was documented after it was given to the athlete if they were prescribed the 50,000 IU vitamin D₃ regimen. For the purpose of the study, those in the 50,000

IU group were categorized as “supplemented”, while those on the 2,000 IUs were categorized as “maintenance.” Athletes in the supplemented group had their blood redrawn after eight weeks of supplementation. They were then reclassified based on serum 25(OH)D levels as either sufficient, insufficient, or deficient. A new supplementation regimen was prescribed to them if they were classified into a new group after testing. The preseason vitamin D serum level was used for analysis for injury risk. However, the eight-week follow up data on those in the supplemented group was used for a separate analysis to determine if the supplementation regimen was effective in increasing vitamin D serum levels to a sufficient value during the eight-week period.

Race demographic data was collected for each athlete based on their pre-participation examination information. Due to the prevalence of low vitamin D in individuals with high melanin, it is reasonable to include this variable in analysis to determine if the association holds true for this cohort. Individuals were dichotomously labeled as either Caucasian or non-Caucasian.

Data Collecting Location, Ethics, and Consent

Blood collection was conducted in the athletic training facility and analyzed at a local laboratory by a licensed professional. Each athlete completed a blood draw as a part of their pre-participation examination. Data was collected from the athletic training facility medical records database. Researchers were allowed to access de-identified medical data for further analysis by the University of Arkansas Institutional Review Board (IRB #17-04-632). All athletic data was de-identified to ensure patient confidentiality. Due to the retrospective nature of this study, participant consent was not necessary.

Data Analysis

Following data entry, athletes were dichotomously classified as injured or uninjured during the season following bloodwork. Receiver operating characteristics (ROC) analyses was used to establish cut points for the creation of dichotomized risk factors (High-risk vs. Low-risk). Subsequently, cross tabulation analysis was used to calculate sensitivity, specificity, and odds ratios with 90% confidence intervals for each potential risk factor. Risk factors analyzed included vitamin D level, iron level, race, game exposure, and previous injury. If multiple significant risk factors were identified, backward stepwise logistic regression analysis was used to determine the best combination of injury risk factors. Additional ROC analysis was done to determine the number of factors that classified individuals at an increased risk for injury. Following this, another cross-tabulation analysis revealed the model's specificity, sensitivity and odds ratio with 90% confidence interval. Data for the 2015 season does not include game exposure or previous injury. For this reason, the 2015 season is not included in analyses related to game exposure, previous injury, or a combination of one or more of these factors with other risk factors (vitamin D level, iron, or race). Finally, individuals supplemented with vitamin D were analyzed using a paired-samples T-Test to determine if supplementation procedures were effective.

IV. Manuscript

Abstract

Context: Pre-season testing that includes the analysis of micronutrient serum levels in the blood has become a routine part of pre-participation examinations in collegiate football. **Objective:** Assess the predictability of vitamin D and iron serum levels as indicators of future injury during a subsequent competitive season for lower extremity sprains, strains, and fractures. Additionally, determine the effectiveness of vitamin D supplementation on increasing serum 25(OH)D levels and preventing injury. **Design:** Cohort Study. **Setting:** National Collegiate Athletic Association Division I football program. **Participants:** Football athletes (n=349) who underwent pre-participation examinations and participated in at least one of three competitive football seasons. **Main Outcome Measure(s):** Vitamin D and Iron serum levels collected during pre-season testing and injury occurrence of the lower extremity or core was analyzed to determine if a predictive association existed. A vitamin D serum level cut-point for high-risk or low-risk athletes was established using a receiver operator characteristic analysis (ROC) for various injury associations including all injuries, fractures, and muscle strains. Cross-tabulation analysis produced sensitivity, specificity, positive and negative likelihood ratios, and odds ratios for each cut-point. Backwards stepwise logistic regression was used to determine the best combination of injury risk factors. Individuals supplemented with vitamin D were analyzed using a paired-samples T-Test to determine if supplementation procedures were effective. **Results:** Muscular injuries were significantly predicted by low vitamin D levels (<23.15 ng/mL; OR=3.042; 90% CI [1.426, 6.486], p=0.019) high game exposure (OR=2.636; 90% CI [1.208, 5.753], p=0.036), previous injury (OR=3.390; 90% CI [1.546,7.431], p=0.010). Based on a three-factor prediction model, athletes with two or more risk factors were at an increased risk of injury with an odds ratio of 6.000 (90% CI [2.639, 13.642], p=0.001). Vitamin D supplementation increased mean

pre-season levels by 32.206 ± 15.52 ($p < .001$). **Conclusion:** Use of a model with multiple risk factors can help identify athletes who are at risk for injury based on both intrinsic and extrinsic factors. The modifiable factor of vitamin D status may help athletic trainers to decrease injury risk in those with low serum 25(OH)D levels through a short-term supplementation program.

Introduction

Injury prediction models have been developed for a variety of factors that could predispose someone to injury risk. These prediction models can be used by athletic trainers to implement interventions in at-risk athletes to decrease their likelihood of sustaining an injury. Injury is a multifaceted issue that can be caused by intrinsic or extrinsic factors. It is often difficult for athletic trainers to address extrinsic factors, such as the weather or duration of competition. Therefore, efforts to prevent injury through intrinsic modifications may be more effective in reducing risk.

One intrinsic factor often analyzed and modified is vitamin and mineral deficiencies. Normal blood values for many vitamins and minerals have been identified to determine adequate amounts necessary for normal functioning of organ systems. These values are measured through blood sample analyses. Of particular interest in the athletic population are blood markers of vitamins and minerals due to their influence on normal function, and perhaps exercise performance. The side effects of deficiency in vitamin D and iron (ferritin) may predispose an athlete to injury. These micronutrients play a vital role in bone, muscle, blood, immune, and neurological health.

Current research has analyzed the effects of vitamin D in a wide variety of athletic and non-athletic populations. Vitamin D deficiency in adults may present with back and bone pain, fatigue, recurrent infections, muscle pain, and depression.²²⁻²⁷ Due to its importance in bone and musculoskeletal health, Vitamin D has become an area of interest in athletic populations. Deficiency may put athletes at increased risk of injury or illness. Studies have shown that many athletes are at risk for vitamin D deficiency, especially athletes who participate in winter or spring sports, play an indoor sport, or have a dark skin tone.^{6,8,9} Serum 25(OH)D levels have

been analyzed in many athletic populations such as gymnasts, professional football and basketball players, ballet dancers, and division I collegiate athletes. These studies report a high prevalence of inadequate vitamin D levels (either deficient or insufficient) as high as 83% and 91%.^{6,10-15} The majority of current research focuses on identifying the prevalence of vitamin D deficiency and insufficiency and associated fracture risk, primarily stress fractures. Minimal research has been done on the effects of vitamin D levels in athletes participating in contact sports.

Additionally, minimal research has been published regarding the association between micronutrients and injury risk. Iron plays a major role in red blood cell oxygen carrying capacity through its binding to hemoglobin and myoglobin. In the athletic population, this is especially important as the cardiovascular system is routinely stressed during exercise. Therefore, it is reasonable to suggest that this micronutrient should be analyzed as a possible predictor for athletic injury due to its possible influence on performance and fatigue. Iron levels have been assessed in athletic populations to determine the prevalence of low iron. Iron levels analyzed in a group of professional runners revealed that 22% had ferritin levels between 50 and 30 $\mu\text{g/L}$ (first stage iron depletion), 13% had ferritin levels between 30 and 12 $\mu\text{g/L}$ (second stage iron depletion). Iron levels were not analyzed for an association with injury risk in this study.³¹ Although many athletes undergo routine screenings of these blood markers as part of their preseason physical examination, there is a lack of research regarding the association between ferritin levels and injury risk in athletic populations. Therefore, this value has not been regarded as a potential intrinsic musculoskeletal injury predictor.

The purpose of this study is to identify the predictive value of pre-season serum 25(OH)D and ferritin levels in collegiate football players on musculoskeletal injury risk during the season.

Additionally, this study analyzed the effectiveness of vitamin D₃ supplementation in increasing serum 25(OH)D levels and decreasing injury risk. Data included preseason serum levels of vitamin D and iron, vitamin D₃ supplementation, subsequent serum 25(OH)D retest values following supplementation and occurrence of injury during the subsequent competitive season (August-December). Three competitive seasons were included in this analysis. The results of this study sought to identify a serum 25(OH)D level that can serve as a predictor of increased risk for musculoskeletal injury to be used by healthcare professionals working with athletic populations.

Methods

Study Design and Participants

The current study retrospectively analyzed a cohort of Division I football players (n=349) from three consecutive competitive seasons in 2015 (n=120), 2016 (n=115), and 2017 (n=114). Demographic information for age was collected from pre-participation exam forms. In 2015, age was 20 ± 1 y., in 2016 age was 20 ± 1 y., and in 2017 age was 20 ± 1 y. Participants were included if they completed pre-season testing that included vitamin D and iron serum levels and participated in the subsequent competitive season. Individuals were considered to be a new athlete for each competitive season if they competed in more than one of the included seasons, which produces 348 player-seasons for analysis due to the classification based on values that can change over time. This approach has been used in previous predictive studies and is regarded as acceptable for use.⁶² Specialists, including kickers, punters, and snappers, were excluded from analysis.

All participants were assumed to be free of injury during pre-season testing. It was also assumed that all injuries that occurred during the competitive season were reported to certified medical staff and documented accordingly. An injury was defined in accordance with the

National Collegiate Athletic Association as a sprain, strain, or fracture to the lower extremity or core that caused the participant to be restricted from activity for at least one practice or competition.⁶¹ Data was collected from the athletic training facility medical records database. The study received approval from the university's Institutional Review Board. All athletic data was de-identified to ensure patient confidentiality. Due to the retrospective nature of the study, participant consent forms were not needed for data collection.

Testing Protocol

Participant demographic information and blood work results were collected as part of the university's pre-participation examination. *Vitamin D levels* were measured using serum 25-hydroxy vitamin D (25(OH)D) levels obtained through a 10 mL blood sample. Serum 25(OH)D are considered the most accurate assessment of vitamin D levels in the body.⁴⁵ Individuals were categorized as either deficient (≤ 20 ng/mL), insufficient (between 21-29 ng/mL), or sufficient (≥ 30 ng/mL).^{4,16} Incoming freshman and new athletes were analyzed at the end of May. Returning athletes were analyzed in the middle of July before the start of pre-season camp. *Iron levels* were assessed through serum ferritin concentrations. This is a reliable indicator for measuring iron levels and correlates to results from bone marrow samples to assess iron.⁶⁰ During the three years of data collected for this study, two separate outputs were recorded to collect ferritin levels. This resulted in different units reported and separate cutoffs for sufficiency classification. For individuals where iron was reported in $\mu\text{g/dL}$, sufficiency was classified between 50-160 $\mu\text{g/dL}$. Conversely, those with iron reported in units of ng/mL, sufficiency was classified as between 20-345 ng/mL. Individuals with levels below 50 $\mu\text{g/dL}$ or 20 ng/mL were classified as deficient, respectively. *Musculoskeletal injury* was defined as any sprain, strain, or fracture to either the lower extremity or core that resulted in the athlete seeking medical attention and missing at least

one subsequent practice or competition due to injury. This definition of injury was used because it is the same definition used by the National Collegiate Athletic Association.⁶¹ After an injured athlete sought medical attention, the certified athletic trainer thoroughly documented the evaluation to include the body part, type of injury, amount of days (practice or games) missed due to injury. During data analysis, a variable of overall injury (OI) was used and included all strains, sprains, and fractures that caused the athlete to miss at least one practice or competition. Due to the importance of vitamin D in bone health, a fracture injury (FI) category was considered separately in analysis. The mechanism of fractures, acute or stress fracture, was not specified in this study. Additionally, those categorized as lower leg, quadriceps, hamstring, and hip were considered separately in analysis and classified as muscular injuries (MI). For the purpose of this study, lacerations, contusions, concussions, dislocations, and subluxations were not included due to their typical mechanism of injury. *Game exposure* was retrospectively recorded through the variable “starter status” that included individuals who were listed as starters on either offense, defense, or special teams for at least four games during the respective season. These data were collected through coaching and team statisticians’ records. Individuals were dichotomously classified as either a starter or a non-starter for the purpose of analysis. *Previous injury* was documented as an injury that occurred the year prior to the current competitive season. Previous injury was also defined by the National Collegiate Athletic Association definition that requires that an athlete miss at least one practice or competition for an incident to be considered an injury.⁶¹ Previous injury was collected from the athletic training facility medical database records for the year prior to the current season. Data for this variable was included for the 2016 and 2017 seasons. *Supplementation* was prescribed to each individual based on their pre-season vitamin D level. Athletes with serum 25(OH)D levels ≥ 40 ng/mL were

recommended a maintenance dose of 2,000 IUs to be taken 4 times per week. Those with vitamin D levels < 40 ng/mL were given 50,000 IUs to take once a week by a member of the sports medicine staff. Each dose was documented after it was given to the athlete if they were prescribed the 50,000 IU regimen. For the purpose of the study, those in the 50,000 IU group were categorized as “supplemented,” while those on the 2,000 IUs were categorized as “maintenance.” Athletes in the supplemented group had their blood redrawn after eight weeks of supplementation. They were then reclassified based on serum 25(OH)D levels as either sufficient, insufficient, or deficient. A new supplementation regimen was prescribed to them if they were classified into a new group after testing. The preseason vitamin D serum level was used for our analysis of injury risk. However, the eight-week follow up data on those in the supplemented group was used for a separate analysis to determine if the supplementation regimen was effective in increasing vitamin D serum levels to a sufficient value during the eight-week period.

Data analysis

Following data entry, athletes were dichotomously labeled as injured or uninjured during the season following bloodwork. Receiver operating characteristics (ROC) analyses were used to establish cut points for the creation of dichotomized risk factors (High-risk vs. Low-risk). Subsequently, cross-tabulation analysis was used to calculate sensitivity, specificity, and odds ratios with 90% confidence intervals for each potential risk factor. The Fisher’s exact (1-sided) value was used to determine significance ($p < 0.025$). Additional cross tabulation analysis was used in place of ROC analysis for iron levels as a risk factor because of two different units of ferritin that were reported in the data. For this reason, individuals were dichotomously classified as either insufficient or deficient for the purpose of analysis. Other potential risk factors, such as

game exposure, previous injury, and race were also dichotomized and analyzed in a cross-tabulation model in relation to injury. If multiple significant risk factors were identified, backward stepwise logistic regression analysis was used to determine the best combination of injury risk factors. Additional ROC analysis was done to determine the number of factors that classified individuals at an increased risk for injury. Subsequent cross-tabulation analysis was performed to determine sensitivity, specificity, odds ratio and confidence interval of the proposed multiple factor model. Data for the 2015 season did not include game exposure or previous injury data. For this reason, the 2015 season is not included in analysis for game exposure, previous injury, or our combination model including risk factors beyond vitamin D level, iron, or race. Finally, individuals supplemented with vitamin D were analyzed using a paired-samples T-Test to determine if supplementation procedures were effective.

Results

In the three seasons of data included in this study, there were a total of 90 (24.8%) injuries that required an athlete to be restricted from activity. These injuries were further classified into categories based on the joint or body segment that was injured. This categorized revealed that there were 14 (3.9%) foot injuries, 28 (7.7%) ankle injuries, 4 (1.1%) gastrocnemius-soleus complex injuries, 25 (6.9%) knee injuries, 4 (1.1%) quadriceps injuries, 18 (5.0%) hamstring injuries, 3 (0.8%) hip injuries (including adductor, hip flexor, and extensor muscle strains), and 9 (2.5%) core injuries (including back musculature and abdominal musculature strains). Further, MI and FI specific data revealed that 19 (5.2%) fractures occurred throughout the three seasons. Of these 19 fractures, 12 (3.3% of all injuries) occurred in the lower extremity. In regard to muscle injury, there were 27 (7.4%) muscle injuries involving either the lower leg, thigh, hip, or core musculature.

Vitamin D and Iron Pre-Season Data

Pre-season vitamin D results indicated that the average vitamin D level for all three years amongst participants was 37.18 ng/mL \pm 14.63. These values were further categorized into groups based on standard norms to reveal that 218 (60.1%) were sufficient, 99 (27.3%) were insufficient, and 22 (6.1%) were deficient. Individuals with serum 25(OH)D levels below 40 ng/mL were re-tested after 8 weeks of 50,000 IU vitamin D supplementation. Before supplementation, the mean vitamin D level amongst this group was 30.01 ng/mL \pm 8.98. After supplementation for eight weeks, the mean vitamin D level significantly increased to 62.31 \pm 15.60 ng/mL (95% CI [30.00,34.61]), $p < .001$). In regard to pre-season iron measurement, participant ferritin levels revealed that 20 (5.5%) of individuals had iron levels considered to be deficient.

Vitamin D and Injury Risk Analysis

The results of ROC and cross-tabulations analyses for vitamin D values are shown in Table 1. The results of the ROC analysis revealed an area under the curve of 0.514 for OI, 0.425 for FI, and 0.610 for MI. Further cross-tabulation analysis was completed only for OI and MI variables and is shown in Table 2. The cut point that was established for OI was 30.05 ng/mL ($p = 0.187$). The cut point for muscular injury was 23.15 ng/mL ($p = 0.019$). Through this analysis, vitamin D was shown to be a significant predictor of muscle injury when including all three years of data.

Vitamin D and Race

A cross-tabulation analysis was done to analyze pre-season vitamin D levels in association to race. Athletes were dichotomized as either sufficient (vitamin D levels \geq 30

ng/mL) or insufficient/deficient (vitamin D levels < 30 ng/mL). A second cross-tabulation analysis was done to using the clinical cut point for vitamin D deficiency (≤ 20 ng/mL). The results of this analyses are shown in Table 3. Both classifications of vitamin D status were clinically significant in relation to race ($p = 0.001$). A greater proportion of non-white athletes were at a greater risk of having both deficient and insufficient vitamin D levels compared to white athletes.

Iron and Injury Risk

Cross-tabulation analysis was performed to analyze iron as a predictive factor for OI, FI, and MI. The various categories of injury were each analyzed to determine a predictive effect. The results of these analyses are shown in Table 4.

Game exposure and Previous Injury

Game exposure data as indicated by “starter status” was included for the 2016 and 2017 seasons. Due to the above finding that there was a significant predictive association between vitamin D and MI, game exposure and previous injury variables were analyzed in association with MI to determine their predictive value for inclusion in a predictive model. The results of cross-tabulation analyses for these variables is included in Table 5. Both game exposure and previous injury were statistically significant in predicting MI ($p < 0.05$).

Vitamin D Prediction Models of Muscle Injury Risk

Due to the exclusion of 2015 data in this analysis, a second cross-tabulation was run for vitamin D and race in relation to injury risk using only the 2016 and 2017 participants. Additionally, a cross-tabulation analysis that analyzed the potential interaction between vitamin D and race in relation to muscle injury was completed. The results of these analyses are shown in

Table 6. The odds ratios of both the vitamin D and interaction between vitamin D and race were the same. Therefore, it can be concluded that vitamin D is of greater predictive value when analyzing muscle injury.

In order to develop a predictive model including starter status, previous injury, vitamin D and race, each were dichotomized as “high risk” or “low risk.” Backward stepwise regression was used to determine the best combination of risk factors that predicted muscle injury. Results of this analysis are shown in Table 7. Race was not shown to be significant based on this analysis and was not include in the prediction model. ROC analysis for the three identified risk factors (low vitamin D, previous injury, and starter status) was completed to establish a risk associated cut-point for factors. A three-factor model was created using all of these variables. Individuals were classified as having 1, 2, or 3 of these potential risk factors. A ROC analysis of these factors in relation to muscle injury revealed an area under the curve of 0.743 with 2 factors as the ideal cut-point. The results of a cross-tabulation using the cut point of ≥ 2 factors are included in Table 8. Footballers who had 2 or more risk factors had 6.000 (90% CI [2.639, 13.642], $p = 0.001$) greater odds of sustaining a MI during the subsequent season than those who had < 2 risk factors.

Discussion

The measurement of vitamin D levels in athletes is a well-studied area of research due to its known importance in maintaining the health of many of the organ systems utilized during exercise. Widespread vitamin D deficiency and insufficiency have been observed in multiple studies. A meta-analysis of 2,313 athletes found that 56% did not have adequate vitamin D levels.⁹ In a group of participants with similar age to the current cohort, an analysis of a group of collegiate athletes reported 74% below sufficient (<32 ng/mL).⁴⁷ The findings of the current

study are not in agreement with previous studies in that 60.1% were sufficient and only 39.1% were either insufficient or deficient. The higher prevalence of sufficient individuals may be due to the fact that vitamin D levels were taken in the month of July when UVB radiation is high.^{38,42} Additionally, the outdoor nature of the sport in which these individuals participate increases their exposure to UVB radiation. In previous studies, athletes who participate in indoor sports are more likely to have lower vitamin D levels.^{10,14,18,47} In a study of collegiate athletes that compared indoor and outdoor athletes, serum 25(OH)D levels were significantly greater in outdoor athletes than in indoor athletes in the fall.⁶ However, a study that analyzed a similar cohort as the current study found a lower percentage of sufficient individuals comparatively. A study analyzing professional football players found that 68.8% were either insufficient or deficient.¹¹ The greater percentage of individuals with sufficient vitamin D levels in the current study may be attributed to year-round supplementation.

Iron levels have not been studied as extensively as vitamin D levels in athletes. Additionally, most research is conducted using runners as the population of interest due to the importance of iron for endurance.⁶³ A study done in a group of professional runners, both male and female, revealed that 35% had ferritin levels categorized as either first or second stage iron depletion ($< 50 \mu\text{g/L}$).³¹ The current study revealed that only 5.5% of individuals were categorized as deficient. The lower prevalence of iron deficiency in this population may be due to the sex of the cohort. It is estimated that females are more likely to be iron deficient than males.⁶³ However, this is not in agreement with a study of triathletes that revealed that male triathletes had a higher incidence of iron deficient anemia than female triathletes.⁶⁴ Due to the disagreement among these studies and a lack of studies done in a wide variety of athletes, more research is needed to determine if there is a gender difference between males and females in

regard to iron levels. Additionally, iron was not shown to be a significant predictor of injury risk. This finding is similar to that of a study done in elite runners that did not find low iron levels to be predictive of injury risk.³¹

Vitamin D supplementation has been observed in a variety of athletic populations in regard to both increasing levels and decreasing injury risk. In the current study, vitamin D supplementation was given in two separate regimens. For those with levels above sufficient (≥ 30 ng/mL), a maintenance dose of 2,000 IU four times per week was recommended. Individuals with levels below 40 ng/mL were recommended to take 50,000 IU once per week. The individuals in this category of supplementation were tested after 8-weeks of supplementation. This supplementation regimen was significantly effective in increasing vitamin D levels compared to a study in a cohort of collegiate swimmers that used 4,000 IU/day of vitamin D₃ supplementation for a 6-month period. Lewis et al⁵⁹ only identified a mean increase of + 1 ng/mL in the supplement group. However, their supplementation was considered effective because the placebo group experienced a decrease of 20 ng/mL during the 6-month period. Current clinical recommendations for vitamin D supplementation is not consistent. The Food and Nutrition board currently suggests 600 IU/day and is the official recommendation by the United States Department of Health and Human Services.⁶⁹ The Endocrine Society suggests between 600-1,000 IU/day.⁶⁸ However, the Vitamin D council suggests a much higher supplementation of to take 5,000 IU/day based on research that current suggestions from previous agencies are inadequate.⁷⁰ All of these values are based on recommendations for an average adult. No specific recommendations were made for an athletic population. The results of this study suggest that the recommendation to increase vitamin D level most closely aligns with the recommendations by the Vitamin D council. In the current study, a high dose of vitamin D given during a shorter

amount of time was more effective in increasing serum 25(OH)D levels. Additionally, the toxicity level of vitamin D is extremely high (40,000 IU/day for several months).⁷¹ The dosage recommended by this study is significantly lower than the toxic level and is considered safe for individuals to use to increase levels efficiently.

Dark skin tone is a widely recognized risk factor for low vitamin D levels due to an increased amount of melanin in the skin compared to those with light skin.^{3,38} In individuals with dark skin, 7-dihydrocholesterol is competing with melanin to absorb UVB photons.³ Even though individuals with dark skin tones have an adequate amount of 7-dehydrocholesterol in their skin to absorb UVB and synthesize previtamin D₃, the high concentration of melanin prevents them from absorbing adequate radiation to achieve vitamin D sufficiency.³⁸ The results of the current study are in agreement with previous research that has observed low vitamin D levels in individuals with dark skin.

The value of vitamin D levels as a predictor of athletic injury has not been firmly established. Previous research identified an association between low vitamin D levels and incidence of stress fractures and stress reactions in gymnasts and military recruits.^{10,50} Additionally, in a cohort of professional football players, individuals with lower vitamin D levels were more likely to sustain at least one fracture during two seasons of data collection.¹¹ In contrast to the previous findings, we identified no significant predictive effect of low vitamin D levels on fracture risk (ROC area under the curve = 0.425). Similarly, when analyzing pre-season vitamin D levels in association with all injuries, there was no significant predictive value. The absence of predictive value may have been due to the inability to collect vitamin D data immediately before injury. The data used in this study was collected at the beginning of the competitive season and may have fluctuated between the time of the blood draw and the injury.

However, it should be noted that the results of this study are similar to those seen in a different cohort of collegiate athletes who also had no significant relation between low vitamin D levels and stress reactions.¹³ Similarly, a study of adolescent male ballet dancers did not show a significantly predictive injury reporting rate in relation vitamin D status.⁵³

While the main function of vitamin D is often believed to be calcium regulation and bone morphology, recent research suggests that it also plays a role in skeletal muscle function. Receptors for vitamin D have been identified in muscle and have been suggested to help increase muscle strength and prevent injury.¹ This finding broadens the influence of vitamin D in the overall musculoskeletal system. In the current study, low vitamin D levels were shown to be a significant predictor of muscle injury in those with levels ≤ 23.15 ng/mL. This was the only type of injury where vitamin D was observed as a statistically significant predictor. This finding may be explained by the results of a study done by Ward et al. in a cross-sectional analysis of post-menarchal adolescent girls. They measured baseline serum 25(OH)D concentrations, parathyroid hormone (PTH), and calcium. After controlling for height and weight, they determined a positive relationship between 25(OH)D levels and jump velocity, height, power, and force.⁵¹ Although these findings suggest performance advancements in those with higher serum 25(OH)D levels, increased muscular strength and power may also serve in injury prevention. Additionally, muscles and bones work together as muscles exert forces on bones and bones adapt to these forces.⁵² It is believed that vitamin D may help to maintain this relationship and prevent injury by allowing for adequate remodeling of bone and sustaining muscle strength.⁵² These findings of the current study correspond to a study done by Wolman et al.¹² in a group of elite classical ballet dancers. They observed lower serum 25(OH)D levels in February than in August (14.9 ng/mL vs. 23.9 ng/mL). Additionally, they observed twice as many muscle injuries in the winter

than in the summer, 24 and 13 respectively. Despite clinical relevance, statistical analysis failed to show a significant association between serum 25(OH)D levels and injury rates in this population.

Inclusion of game exposure and previous injury in this study was due to the known predictive value of these variables in predicting injury risk in similar cohorts.^{62,65,66} In the current study, previous injury was associated in an odds ratio of 3.39 indicating that an individual with an injury occurring in the previous year has 3 times the odds of sustaining a subsequent muscle injury compared to those who were not injured during the previous year. This is an important practical finding for clinicians. The occurrence of subsequent injury may be due to a lack of full rehabilitation of a previous injury, biomechanical compensation, playing in a fearful manner, or a number of other factors that are associated with an athlete returning to competition after an injury. Based on these findings, clinicians should take measures based on individual athlete needs in order to prevent additional injury. Similarly, analysis of game exposure revealed an odds ratio of 2.636. Those who participate more in competition are at an increased risk of sustaining an injury. This finding has also been confirmed in another study of collegiate football players.⁶² It is reasonable to suggest that the more time that an athlete spends playing the sport, the more likely they are to sustain an injury based on an increased exposure, fatigue and amount of physical contact in the sport of football.

All three predictive factors of muscle injury that were observed in this data set were combined to determine if an increased risk was present when multiple risk factors were true of an individual. The results of this analysis showed that individuals with ≥ 2 of the risk factors (low vitamin D, previous injury, and starter status) were at an increased risk for injury. Of these factors, the most easily modifiable by clinicians is vitamin D level. Supplementation results of

the current study suggest that the regimen used for individuals with low vitamin D was efficient in raising levels to ≥ 40 ng/mL. Based on this model, clinicians can seek to identify these high-risk individuals in similar cohorts and implement a non-invasive supplementation intervention to increase vitamin D levels. The increase in vitamin D levels may correlate to a decrease in muscular injuries based on the results of this study.

While vitamin D was shown to be a significant predictor in the current study in both separate ROC and cross-tabulation analysis and with the inclusion of previous injury and game exposure in the prediction model, it is important to note that vitamin D alone may not be the sole reason for injury occurrence. It could simply be a marker for those who are experiencing a deeper issue that places them at an increased risk for injury. Dietary, lifestyle, or metabolic issues can all manifest themselves as low vitamin D in many individuals. Therefore, when identifying those with low vitamin D, clinicians should be vigilant in determining if an underlying condition exists in these individuals that may predispose them to future injury.

Limitations

The biggest limitation of the current study was the retrospective design. This research design did not allow researchers to gather all desired data, such as 8-week vitamin D level follow-up on all individuals, that would have strengthened the associations seen in this study. Some documentation was incomplete in athlete files that excluded participant data from analysis. Similarly, the data that was available did not have vitamin D levels throughout the season. Therefore, it is likely that the pre-season vitamin D level taken in July that was analyzed in relation to injury may not have been truly predictive of an injury that occurred in November. Future research should analyze vitamin D levels at shorter time intervals to strengthen the association of vitamin D levels as a predictor of muscular injury. Additionally, game exposure

and previous injury data were not included for the 2015 year. Analysis of the four-factor model could not include data from this year and led to a decrease in subject number and a decrease in injury that may have strengthened the model. Finally, although the model does include known risk factors to injury, it is not an all-encompassing list of potential injury factors. For example, psychosocial stress has been associated with an increased risk in injury but was not included or analyzed in this model.⁶⁷ Future studies should incorporate vitamin D values to additional known risk factors within models in order to better assess risk.

Conclusion

Injury prevention is a multi-faceted issue that healthcare professionals face on a daily basis. Many of the factors that lead to injury risk may not be modifiable by an athletic trainer or other healthcare professional, such as previous injury and game exposure. However, micronutrient levels that play a role in organ system regulation are modifiable. Therefore, the recognition of low vitamin D level as a predictive factor for injury risk is important because it can be modified through a noninvasive and safe supplement regimen. The regimen proposed in this study was effective at increasing serum 25(OH)D levels within eight weeks. Additionally, due to the high specificity of vitamin D level in relation to injury risk, healthcare professionals do not have to focus a lot of attention on those who do not meet the threshold for low vitamin D. This is also similar in the three-factor model that included low vitamin D level, high game exposure, and previous injury. Those with less than 2 factors have a high likelihood of not sustaining an injury. Therefore, strategies for injury prevention can be focused towards those meeting two or more factors.

V. Discussion and Conclusion

The purpose of this study was to assess the pre-season vitamin D and iron levels of a cohort of Division I NCAA football players to determine if they compare to previous research values in athletic populations and in those with similar risk factors. These values were to be analyzed to determine their predictive relationship to various types of injuries. Secondly, any significant findings were to be included in a model that included the known predictors of game exposure and previous injury to determine a combination of risk factors that increase risk of injury in this population.

In this study, predictive factors of injury were low vitamin D level, game exposure, and previous injury. These three factors were combined in a model to determine how many factors from this combination increase the risk of injury. It was found that having three or more of the four factors increased the risk of injury in this population.

Injury prevention is a multi-faceted issue that healthcare professionals face on a daily basis. Many of the factors that lead to injury risk may not be ones that are modifiable by an athletic trainer or other healthcare professional, such as previous injury and game exposure. Among these modifiable factors are the micronutrient levels that play a role in the regulation of many organ systems. Therefore, the recognition of low vitamin D level as a predictive factor for injury risk is important because it can be modified through a noninvasive and safe supplement regimen. The regimen proposed in this study was effective at increasing serum 25(OH)D levels in a time efficient manner. Additionally, due to the high specificity of vitamin D level in relation to injury risk, healthcare professionals do not have to focus a lot of attention on those who do not meet the threshold for low vitamin D. This is also similar in the four-factor model that included low vitamin D level, non-white race, high game exposure, and previous injury. Those with zero

or one of these factors have a high likelihood of not sustaining an injury. Therefore, strategies for injury prevention can be focused towards those meeting three or more factors.

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Appendix



UNIVERSITY OF
ARKANSAS

Office of Research
Compliance

April 20, 2017

MEMORANDUM

TO: Brendon McDermott Mariellen Veach
 Casey Wagner Derek Worley
 Kristen Peterson Taylor Overton
 Gary Wilkerson

FROM: Ro Windwalker
 IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 17-04-632

Protocol Title: *Observation of Pre-Season Screening and Injury and Illness Incidence in Division I Collegiate Athletes*

Review Type: EXEMPT EXPEDITED FULL IRB

Approved Project Period: Start Date: 04/19/2017 Expiration Date: 04/18/2018

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (<https://vpred.uark.edu/units/rscp/index.php>). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 1,500 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

Tables and Figures

Table 1. ROC Results for Pre-season Vitamin D levels as a predictor of injury (includes 2015 data)

Injury Category	ROC (area under the curve)
Overall Injury	.514
Fracture	.425
Muscular Injury	.610

Table 2. Cross-tabulation Results for Pre-season Vitamin D levels as a predictor of injury (includes 2015 data)

Injury Category	Cutpoint	Fishers Exact (1-sided)	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
Overall Injury	<30.05 ng/mL	0.187	0.4138	0.6475	1.297	0.852 to 1.975
Muscular Injury	<23.15 ng/mL	0.019	0.3200	0.8660	3.042	1.426 to 6.486

Table 3. Cross-tabulation of Race as a predictor of Vitamin D levels (includes 2015 data)

Vitamin D Classification	Cut point	Pearson Chi-Square	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
Cut Point for Deficient	≤ 20	14.487	0.1010	0.9923	14.487	2.662 to 78.829
Cut Point for Insufficient/Deficient	<30	54.184	0.5000	0.8923	8.286	4.935 to 13.913

Table 4. Cross-tabulation of Iron as a predictor of injury (includes 2015 data)

Injury Category	Fisher's Exact (1-sided)	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
Overall Injury	0.556	0.0562	0.9393	0.921	0.384 to 2.208
Fracture	0.717	0.0556	0.9415	0.947	0.167 to 5.379
Muscular Injury	0.530	0.0385	0.9387	0.613	0.109 to 3.428

Table 5. Cross-tabulation of Muscle Injury Risk Factors (excludes 2015 data)

Injury Category	Fisher's Exact (1-sided)	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
Game exposure	0.036	0.500	0.7250	2.636	1.208 to 5.753
Previous Injury	0.010	0.55	0.7350	3.390	1.546 to 7.431

Table 6. Cross-tabulation results for Vitamin D and Race as predictors for muscle injury (excludes 2015 data)

Risk Factor	Fisher's Exact (1-sided)	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
Low vitamin D (<23.15)	.002	0.35	0.92	6.192	2.563 to 14.963
Non-white race	.248	0.75	0.355	1.651	0.365 to 1.357
Vitamin D and Race	.002	0.35	0.92	6.192	2.563 to 14.963

Table 7. Logistic Regression for determination of prediction model factors (excludes 2015 data)

Risk Factor	Significance	Adjusted Odds Ratio	Confidence Interval
Previous Injury	.022	3.148	1.378 to 7.191
Game exposure	.120	2.186	0.956 to 4.998
Low vitamin D (<23.15)	.002	5.923	2.346 to 14.952

Table 8. Cross-tabulation of Three-Factor Model (excludes 2015 data)

Risk Factor	Fisher's Exact (1-sided)	Sensitivity	Specificity	Odds Ratio	Confidence Interval (90%)
2 or more factors	.001	0.45	0.88	6.000	2.639 to 13.642