COMPARING BLOOD SERUM VALUES OF VITAMIN D AND CALCIUM IN DIVISION I FEMALE ATHLETES

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The purpose was to compare blood serum levels of vitamin D and calcium to bone mineral density and previous fracture in Division I women's basketball and soccer athletes. We recruited thirty athletes (19.53 ± 1.105 years); NCAA Division I soccer (n=21) and basketball (n=9). The calcium average was 9.61 ± .39 mg/dL for soccer and basketball was 10.01 ± 0.33 mg/dL. Vitamin D average for soccer was 44.48 ± 12.09 ng/DL and basketball 52.00 ± 17.44 ng/dL. Total bone mineral density measurements for the two groups did not differ at a statistically significant level ($t[11.9] = 0.74$, $p = .472$, $g = 0.36$). The results indicated bone mineral density and previous stress fractures were not statistically significant ($p = .663$, $g = 0.65$).

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CHAPTER 1. INTRODUCTION

Overview of the Problem

Deficient intake of vitamins and minerals are a common problem in athletes that can lead to musculoskeletal injuries, such as stress fractures. Vitamin D and calcium are critically important to the development and function of the skeletal system as well as other processes throughout the body (Wacker, 2013). Blood serum levels of vitamin D and calcium are a major concern for athletes who participate in indoor sports as well as outdoor athletes who participate in the Midwest, as the amount of sun exposure is limited (Ross, 2011). Due to the lack of sun, the vitamin D and calcium levels are potentially lower for these athletes. This can lead to increased risk of injury and could negatively affect physical performance.

To analyze vitamin D and calcium serum levels, a single blood draw is the standard. To measure bone mineral density, GE Lunar full body Dual-energy X-ray absorptiometry (DEXA) has been proven to be reliable and valid. DEXA is also considered the gold standard in measuring bone mineral density (Branski, 20019). Vitamins and minerals and their relationship to bone mineral density has been analyzed mainly in male athletes. However, female athletes, specifically Division I soccer and basketball players, has not been extensively investigated. Examining the vitamin D and calcium levels in female athletes can help with injury prevention and increase physical performance.

Statement of Purpose

The objective of this study was to compare blood serum levels of vitamin D and calcium to total body bone mineral density of Division I female athletes who participate in basketball and soccer.

Research Questions

1) What are the differences in blood serum values for vitamin D and calcium when compared between-groups (i.e., Women's Basketball and Women's Soccer)?
2) What is the relationship between blood serum values and total body bone mineral density?

Dependent and Independent Variables

The dependent variables of this study are the blood serum values of vitamin D and calcium and total body bone mineral density results. The independent variable of this study was the group assignment.

Limitations

This research study had its limitations. First, the study was conducted only women's soccer and basketball players. There are other sports teams at the university that could have been recruited to get a better perspective on vitamin and mineral inadequacies. A convenience sample size of only 30 Division I athletes was used, which is a small sample size. There were 21 female soccer players and nine female basketball players. Due to the uneven numbers, comparisons between the sports were difficult. The study was conducted in August; thus, there is more sun available to be absorbed than in the winter months. Therefore, researchers cannot generalize the results for winter months. Data were collected once; therefore, there was no comparison to post-season levels of vitamin D, calcium, or total body bone mineral density.

Delimitations

After a thorough analysis of previous research, as well as a consideration of participant time, participants only had the DEXA and blood draw done once. It is recognized there may be a change in blood serum levels post season, however, it is beyond the scope of this project to do the DEXA scan and blood draw twice. It is not commonly used in the clinical setting; however, it is the gold standard and gives immediate results. Thus, the DEXA was conducted on all participants to measure total body bone mineral density.

Assumptions

It was assumed that participants reported their nutrition and supplement use honestly on the demographics form. It was also assumed that the participants recorded their tanning bed usage as well as their medications. Finally, it was expected that each participant did not take calcium supplements 24 hours prior to the scan.

Significance of Study

Musculoskeletal injuries are common in Division I athletes due to the amount of stress placed on their bodies (McCabe, 2012). Limited vitamin D and calcium can cause a greater risk of a decreased bone mineral density level. Analyzing vitamin D, calcium, and bone mineral density allows athletes to be aware of their levels and potentially reduce the risk of injuries. The results of this study will be used to determine if there is a correlation between vitamin D and calcium levels with bone mineral density levels. A further result of this study will be used to clarify if there is a correlation between vitamin D levels and indoor versus outdoor sports. Lastly, the results will be used to reveal if there is a significance between blood serum values of calcium and vitamin D when compared between Women's Basketball and Women's Soccer.

CHAPTER 2. LITERATURE REVIEW

Current researchers seek to find, treat and prevent disease and injuries in athletes. Elite athletes undergo rigorous training schedules to obtain their highest level of physical fitness and compete to their fullest. Unfortunately, athletes are affected by injuries that impede their ability to perform (Heaney, 2014). Vitamin D and calcium are two components of nutrition needed to maintain bone health. By understanding the biochemistry of vitamin D and calcium, researchers and physicians can provide better care to their athletes assist the prevention of sport-related injuries.

Both vitamin D and calcium are known to be critically important to the development and function of the skeletal system, in addition to other processes throughout the body. Considering the relationship between vitamin D, calcium, and musculoskeletal injuries, researchers are interested in exploring a possible correlation between blood serum levels of vitamin D and calcium and the rate of stress fractures (McCabe, 2012). The purpose of this literature review is to examine previous research on how vitamin D and calcium can affect bone health in athletes.

Vitamin D

Introduction to Vitamin D

An understanding of biochemistry and dosage of vitamin D is essential to recognize the skeletal and extra-skeletal health benefits. Knowledge of vitamin D levels is important for all individuals, especially female athletes in the northern United States due to increased loading patterns and limited sun exposure. Because vitamin D plays a critical role in numerous processes throughout the body, the current research in this field is expansive and covers many pathways and disorders relating to vitamin D.

Biochemistry

The biochemistry of vitamin D plays an important role in understanding the human physiology and how it connects to optimal dosage. Unlike other vitamins, which must be ingested, vitamin D can be creased through ultraviolet radiation being absorbed through human skin (Aksnes, 1982). During sun exposure, 7-dehydrocholesterol in the skin absorbs ultraviolet B, then 7-dehydrocholesterol is converted to pre-vitamin D3 in the epidermis (Wacker, 2013). Whether the Vitamin D3 is originally absorbed through sunlight or through ingestion, it is metabolized sequentially in the liver and kidneys. Next, the vitamin D3 attaches to binding proteins that move vitamin D3 to the liver to undergo hydroxylation to arrange into the active vitamin D form (Ross, 2011). The active form of vitamin D is 1,25(OH)2D, and researchers use the levels of the active form for research purposes. Scientists who examined gene array in cells and tissues have found 1,25(OH)2D regulates hundreds of genes throughout the body (Wacker, 2013). The activate form of vitamin D, 1,25(OH)2D interacts selectively with an intestinal binding protein. When the body has the correct levels of vitamin D, the cells around the body can communicate properly. Conversely, vitamin D deficiencies can result in inadequate skeletal mineralization (Wacker, 2013). Along with increased autoimmunity and increased susceptibility to infection, the biochemistry of vitamin D and its effect on human physiology is of fundamental importance to optimizing dosage (Aranow, 2011).

Vitamin D Dosage

To measure how much vitamin D an individual has absorbed, researchers and doctors evaluate vitamin D serum concentrations in the blood. There are several choices of vitamin D serums available; the two most utilized are 25(OH)D and 1,25-dihydroxyvitmain D (1,25(OH)2D), and the main difference between these two are their half-lives. The most frequently used serum, 25(OH)D, has a half-life of 15 days, and the second most frequently used serum, 1,25-dihydroxyvitmain D (1,25(OH)2D), has a half-life of about 15 hours (Cranney, 2007). Half-lives are important for researchers and doctors because it is the amount of time until half of the quality of that vitamin still exists in the human body. In turn, these levels can indicate vitamin D deficiency or intoxication (Jones, 2008). Thus, the most frequently used serum, 25(OH)D, has a half-life of 15 days, which means that after 15 days, half of the vitamin D from an oral supplement has left the body. The scientific community agrees vitamin D is essential for normal physiological functions of the body. The most plentiful source of vitamin D is through the sun. However, a combined intake of vitamin D through diet, ultraviolet exposure, and supplementation is necessary to obtain the desirable serum vitamin D levels (Ogan, 2013). However, there is controversy regarding the proper amount of vitamin D individuals should receive each day.

Dosage Controversies

The levels of measurable vitamin D are divided into three categories: deficient, insufficient, and sufficient. However, there is still some debate regarding optimal vitamin D levels. Deficient vitamin D leads to symptoms of bone pain, muscle weakness, and frequent infections ("Vitamin D Council," 2017). Other symptoms include: increased risk of cardiovascular disease, cognitive impairment in older adults, severe asthma in children, cancer, and depression ("Vitamin D Council," 2017). These symptoms of deficient vitamin D levels can alert doctors and patients to the serious nature of the vitamin deficiency. What may be more concerning is that some people do not experience any symptoms. Furthermore, those with insufficient levels can have similar symptoms as those with deficient levels (Gallagher, 2010). Indeed, even individuals with insufficient vitamin D levels may be producing extra parathyroid hormone and not absorbing calcium, which can affect their bones. Again, like those with truly deficient levels of vitamin D, those with insufficient levels may also experience infections, have depressive symptoms, and anxiety ("Vitamin D Council," 2017). In contrast, sufficient levels of vitamin D allow the body to maximize the amount of calcium circulating in the body, strengthen the bones and increase muscle metabolism.

Vitamin D dosage for an individual is not standardized by all organizations and can lead to confusion for the lay population. In fact, even many recognized, legitimate organizations recommend various levels of vitamin D. Table 2.1 displays the differences in the recommended vitamin D levels from five separate organizations. The table below shows the discrepancies among each organization in deficient, insufficient, sufficient, and toxic vitamin D levels. The only similarity is between insufficient and sufficient levels for Food and Nutrition Board and the Institute of Medicine, Food and Nutrition. These differences can lead to confusion among the lay population as to which recommendations to follow. It is equally frustrating for researchers who attempt to draw correlations between vitamin D and injuries because each researcher must establish the parameters of sufficiency and insufficiency.

Table 2.1

Recommended Vitamin D Levels

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Academy of Nutrition and Dietetics	Institute of Medicine, Food and Nutrition
Deficient	0-30 ng/mL	0-20 ng/mL	0-11 ng/mL	<20 ng/mL	<12 ng/mL
Insufficient	31-39 ng/mL	21-29 ng/mL	12-20 ng/mL	20-30 ng/mL	12-20 ng/mL
Sufficient	40-80 ng/ml	30-100 ng/mL	>20 ng/mL		≥20 ng/mL
Toxic	>150 ng/mL				>50 ng/mL

Furthermore, in addition to confusion about an appropriate vitamin D dosage for the general population, appropriate vitamin D dosage for specific populations is also inconclusive.

For example, the Endocrine Society recommends a daily value of vitamin D ranging from 400-800 IU a day for adult women (Harris, 2017). In contrast, the National Institute of Health recommends 600 IU a day (New Recommended Daily Amounts of Calcium and Vitamin D, 2011). The Vitamin D Council recommends 900-5,700 IU/day, which is an unusually large range ("Vitamin D Council," 2017). The question of dosage becomes even more concerning for female athletes because they have higher demands on their bodies than the general population of women. In fact, in a meta-analysis of several randomized clinical trials, researchers concluded 700-800 IU of vitamin D per day was required to achieve adequate levels in the blood to prevent a decline in bone health (Bischoff-Ferrari, 2005). Indeed, these vitamin D dosage discrepancies can lead to confusion as to the correct amount of vitamin D for both athletic and non-athletic populations alike.

Vitamin D and Bone

Bone is an active structure that continuously undergoes remodeling throughout life. Biochemical markers that are used for assessment of bone turnover include enzymes and nonenzymatic peptides located in the cellular and noncellular compartments of the bone (Shetty, 2016). There are two kinds of bone turnover markers and they are grouped based on the metabolic phase in which they are produced: bone formation markers or bone resorption markers. Bone turnover markers are important to measure collagen breakdown products along with other molecules released from osteoclasts and osteoblasts during bone resorption and formation. Bone is constantly being remodeled. Both resorption and reformation are important for repair of micro-fractures and allows for modifications of structure in response to stress and biomechanical forces. Formation and resorption work closely so that bone mass does not change

(Ross, 2011). Having sufficient vitamin D levels to sustain high levels of bone mineral density is important for all individuals.

Vitamin D is necessary for healthy bones; without vitamin D the body cannot effectively absorb calcium, which is essential for proper bone health. A combined analysis of 12 fracture-prevention studies found evidence that supplementation of about 800 International Units a day reduced hip and non-spinal fractures by approximately 20% (Nair, 2012). More specifically at the cellular level, Kuchuk et al. (2009) examined the bone turn over markers relating to 25(OH)D. The study consisted of 2,798 participants of two German cohorts in which participants began the study at birth. Each participant had blood samples drawn between November 2006 and May 2009 during all seasons and months. The results indicated there was a clear seasonal dependency with the measurement of 25(OH)D. The range of 25(OH)D was 21.8 nmol/L to 142.4 nmol/L with a mean of 63.1 nmol/L in the winter months, which included November through April. The summer months had a range from 23.2 nmol/L to 224.6 nmol/L with the mean at 83.9 nmol/L. For all three parameters, there was a significant ($p < 0.001$), non-linear effect of months in a year. Males had higher physical activity levels ($p < 0.001$), and higher 25(OH)D concentrations ($p = 0.009$). One result from the study was a clear seasonal variation of bone turnover markers. Another result was an association between bone turnover markers and 25(OH)D, which are dependent on serum calcium levels. Thus, athletes who participate in a winter or spring sport may have limited exposure to the sun which can negatively impact vitamin D levels.

Vitamin D Deficiency in Athletes

Approximately 1 billion people are vitamin D deficient or insufficient, including elderly, children and young adults (Holick, 2007). This equates to 13.5% of the world has inadequate levels of vitamin D. Vitamin D is a crucial component of muscle function, muscle strength, muscle mass, as well as bone mineral density. Muscle and bone health are important for the athletic population to perform at their peak level. More specifically, athletes depend on a healthy and complete diet to have adequate nutrients required to promote and uphold physical performance of their sport and protect against injuries. The Academy of Nutrition and Dietetics (2019) states vitamin D is capable of improving athletic performance related to injury prevention, rehabilitations, increasing muscle fiber size, reducing inflammation, improving neuromuscular function and decreasing the risk of stress fractures. Athletes across the world intake an average of 100 IU to 250 IU/day of vitamin D through food. In a systematic review and meta-analysis of 23 studies that included 2,313 athletes, proportions of vitamin D inadequacy were compared using chi-squared analysis. Of the 2,313 athletes, an average of 56% had vitamin D inadequacy that significantly varied by geographical location ($p < 0.001$). The risk increased significantly for winter and spring sports (RR 1.85; 95% CI [1.03-6.26]), and indoor sports (RR 1.19; 95% CI [1.09-1.30]). Inadequacy leads to musculoskeletal injuries, which may impede athletic performance. The authors of the meta-analysis reported 19% of athletes had bone-related injuries 95% CI [7-36], and muscle and soft-tissue injuries occurred in 37.5% of those athletes 95% CI [11.5-68.5] (Farrokhyar, 2015). Based on the results of this systematic review, vitamin D levels in the majority of athletes were lower than the adequate amount they need to compete. The athletes that compete in the winter and spring need to be especially cautious of vitamin D levels due to decreased sun exposure.

In a study that included athletes from a variety of sports, Halliday et al. (2011) tracked the vitamin D status of 41 athletes from the University of Wyoming (18 men, 23 women); 12 of these athletes were indoor athletes and 29 outdoor athletes. Blood samples were collected in the fall (September/October), winter (February/March), and spring (April/May). Participants also answered a vitamin D-specific questionnaire at the same time as the blood draws. During the spring blood draw, body composition and bone density were evaluated using DEXA. Illness and injury that occurred throughout the year were documented by athletic training staff and team physician. Vitamin D status changed significantly across time ($p = .001$) and averaged 49.0 ± 16.6 ng/mL in the fall, 30.5 ± 9.4 ng/mL in the winter, and 41.9 ± 14.6 ng/mL in the spring. The number of athletes who were vitamin D insufficient increased greatly during the winter months. However, athletes who were vitamin D deficient was highest during the spring. The percentage of athletes at insufficient and deficient levels by season can be found in Table 2.2.

Table 2.2

Vitamin D Level by Season

Vitamin D level	Fall	Winter	Spring
Insufficient	9.8%	60.6%	16.0%
Deficient	2.4%	3.0%	4.0%

Vitamin D status was not correlated with total bone density or bone density in the lumbar spine. Seven athletes developed injuries due to overuse, including five stress reactions (two in the shin, two in the foot, and one in the femur). Frequency of injury was not related to vitamin D level; however, injury was significantly negatively correlated with total bone mineral density (BMD) (r= -0.50, $p = 0.02$). Overall, vitamin D levels varied across the year with higher percentage of athletes with insufficient or deficient levels in winter compared to fall or spring. Athletes who participated in indoor sports had lower circulating vitamin D in the fall compared to outdoor sports. Overuse injuries are potentially higher for athletes who practice and compete during winter months were vitamin D levels proved to be the lowest based on this study.

One athletic population at risk for vitamin D deficiency would be hockey players because their sport takes place during the winter. Mehran et al. (2016) conducted a study consisting of 105 athletes from three professional hockey teams in the National Hockey League. All players underwent a preseason physical examination and laboratory work in September. The physical exam included height, weight, body mass index, and race/ethnicity. The laboratory value included serum 25-hydroxy (OH)vitamin D. The blood serum 25(OH)D levels were defined as deficient (<20 ng/mL), insufficient (20-31.9 ng/mL), sufficient (≥ 32 ng/mL), and ideal (≥ 40 ng/mL). During pre-season, no players were considered deficient. The average vitamin D level was 45.8 ± 13.7 ng/mL. However, fourteen of the players (13.3%) were considered insufficient during the pre-season scan. A total of 91 players were sufficient and 68 of the players had ideal serum vitamin D levels. When examining vitamin D compared to age, those players who were insufficient were younger (23.1 years) than those who were sufficient (25.9 years) ($p = 0.018$). Age was the only parameter that was statistically significant different between groups (Mehran, 2016). Although these athletes participate in an indoor sport, the majority of vitamin D levels were sufficient. A potential explanation could be that the blood draws done in September allowed the athlete to obtain vitamin D throughout summer thereby influencing the vitamin D levels. Conducting blood draws in January could yield different results.

In another study focused on vitamin D levels in hockey players, Orysiak et al. (2018) examined the relationship between vitamin D concentrations, isometric strength, agility tests, and an endurance test. Fifty male hockey players (17.2 ± 0.9 years) participated in the study. Each participant was tested on maximal joint torque of the elbow, shoulder, hip, knee and trunk. Vertical jump was conducted on the Kistler force platform to measure power output. The last performance test was repeated sprint ability. Each participant underwent the 5x6 sprint cycle test, which comprised of five, six-second maximal sprints every 30 seconds. Blood samples were collected after an overnight fast. The mean vitamin D concentration was 30.4 ng/mL. A total of eleven participants had vitamin D deficiency and twenty participants had vitamin D insufficiency. There was significantly correlation between vitamin D levels and joint torque of the right shoulder (r= -.029, $p = 0.04$). This was the only isometric strength test that was correlated with vitamin D. Counter movement jumps and vitamin D were correlated after adjusting for age. There was not a significant correlation between the sprint test and vitamin D. Vitamin D and physical performance was not significantly correlated, except for the strength test, in this study even though 62% of the athletes were either deficient or insufficient (Orysiak, 2018).

Jung et al. (2018) also investigated the effects of vitamin D supplementation on physical performance, but instead of using hockey players as the participant population, Jung et al. (2018) investigated taekwondo athletes. Thirty-five collegiate male and female TKD athletes participated in the study that took place from January to February. Participants were between the ages of 19-22 years old. Only participants that were vitamin D insufficient (<50 nmol/L) were included in the study. Participants received either a 5,000 IU vitamin D supplement or a visually identical placebo. Athletes in this study trained five times a week for at least four hour/day. For the duration of the study, participants lived in a dormitory with regulated living conditions and nutrient supply. Blood samples were taken in the morning after an overnight fast. Physical performance was tested using the Wingate anaerobic test, isokinetic muscle strength and endurance, and field-based fitness battery tests. There was significant interaction effect for group by time for serum 25(OH)D concentrations ($p < .001$). The group receiving the Vitamin D supplement increased serum 25(OH)D concentrations (96.0 ± 3.77 nmol/L) after four weeks of supplementation compared to placebo group (32.7 ± 2.40 nmol/L). There were also significant correlations between serum 25(OH)D concentrations and anaerobic peak, isokinetic knee extension at 180 degrees ($p = 0.019$). The researchers also highlighted injury rate of the placebo group was four times higher than the group receiving supplementation during the winter training (Jung, 2018). These athletes train indoors, therefore, adding vitamin D supplementation into the routine of athletes who train during the winter months or indoor could be useful for preventing injury.

Another population that is at risk for vitamin D deficiency are professional basketball players due to the sport taking place inside and during the winter months. Vitamin D levels were obtained for 279 athletes who participated in the 2009-2013 National Basketball Associated (NBA) Combine. The study used the Endocrine Society's guidelines to categorize vitamin D levels. These included deficient levels of vitamin D as <20 ng/mL and sufficient levels as >30 ng/mL. A limitation of the study included no explanation for the range of 21-29 ng/mL that was not mentioned, however, it can be concluded the range of 21-29 ng/mL was considered insufficient. Vitamin D deficiency was found in 32.3% of players and insufficient vitamin D levels were found 41.2% of the players. A total of 118 players had a history of fracture. History of fracture was present in 40% of the athletes with deficient levels of vitamin D. A total of 42.6% of athletes with insufficient vitamin D levels had a history of fractures, and 45.6% of players with sufficient levels of vitamin D also had a risk of fractures. Therefore, vitamin D level and fracture history was similar across all groups. Stress fractures were present in 23 of the 118 total fractures; the mean level of vitamin D was 30.7 ng/mL. This value was higher than the level for athletes with no history of fractures (25.3 ng/mL) (Grieshober, 2018). Overall, there was no significant correlation between vitamin D level and stress fracture risk for NBA players.

However, the methods of this study limited the researchers' ability to control for potential influencing variables. Another limitation was that the researchers did not collect data on information regarding vitamin supplementation prior to the study. Thus, because the study was conducted in May, the seasonal variation of vitamin D could be a factor in its results.

Lewis et al. (2013) conducted a six-month randomized, placebo-control trial to examine the effects of vitamin D supplement on the changes of vitamin D status in Division I swimmers and divers. The study consisted of both male (n=19) and female (n=13) athletes. A supplement of 4,000 IU of vitamin D was administered to 19 participants and 13 participants received placebo. At the start of the study, all athletes had vitamin D levels ≥32 ng/mL. The initial mean vitamin D level for those assigned to receive the 4,000 IU vitamin D supplements (52 ± 13.7 ng/mL: p = 0.03) was lower than those receiving placebo (64 ± 16.7 ng/mL). At the midway point of the study, the group of athletes receiving supplementation had an increase of 8 ng/mL from their original value (60 ± 19.9 ng/mL). The placebo group of athletes had a decrease in vitamin D levels of 12 ng/mL (52± 18.4 ng/mL) (Lewis, 2013). In addition to measuring the level of vitamin D, the researchers noted a correlation of higher levels of vitamin D to greater bone mineral density (BMD) and bone mineral content (BMC) in female participants. In the study, 92% of women reported right leg dominance, which corresponded with a positive change in bone density in the right femur neck (0.003 ± 0.04 g/cm$_2$). The increase in vitamin D levels for the swimming and diving athletes who received supplementation and the decrease in those who received the placebo is important because they participate in an indoor sport and are unable to receive vitamin D from the sun during their sports participation.

The research conducted by Lewis at el. (2013) compliments a similar study conducted with female athletes that examined vitamin D and bone mineral density. Participants included cheerleaders (*n*=9), dancers (*n*=10), and a non-athlete control group (*n*=10) between the ages of 19 and 22 to determine if bone mineral density and nutrition factors were affected by different training regimens. Bone mineral density was examined using a Dual Energy X-ray Absorptiometry (DEXA) for full body, spine and dual femur. Each skeletal site of measurement was compared to a reference database of the same age, ethnicity, and sex. Vitamin D was assessed using a finger prick blood sample for each participant (<1 mL). The participants also completed a calcium and vitamin D questionnaire. There were no significant differences between the three groups in total body bone mineral density ($p = 0.134$). Nevertheless, there was a significant difference between the non-athlete control group and the dance team for total body BMD z-scores (dance 1.46 ± 1.23 vs. control 0.19 ± 1.22, $p = 0.033$). The z-score for bone mineral density was not significantly different for the dance and cheer team ($p = 0.441$). There was also no statistical significance for the z-score between the cheer team and non-female control group ($p = 0.441$). However, there was a statistically significance difference for insufficient vitamin D levels (10-29 ng/mL) in 74% of the athletes (dance 27.45 ± 4.26 ng/mL, cheer 24.59 ± 7.61 ng/mL). Nonetheless, there was no significant difference for either dance or cheer for daily calcium intake ($p = 0.91$) or the estimated daily vitamin D intake ($p = 0.82$). The daily vitamin D intake for the dance team was 256 ± 335 IU and the cheer team 228 ± 145 IU (Kenny, 2017). Table 2.3 displays the spine and dual femur BMD results and Table 2.4 designates vitamin D status and questionnaire results. The results of this particular study suggest the majority of female athletes have insufficient or deficient levels of vitamin D.

Table 2.3

Spine and Dual Femur BMD Results

	Dance (*n*=10)	Cheer (*n*=9)	Control (*n*=10)
Spine BMD (g/cm^2)	1.39 ± 0.16	1.36 ±0.16	1.15± 0.11
Spine Z-score	1.48 ± 1.07	0.56 ± 0.62	0.19± 1.22
Dual Femur BMD (g/cm^2)	1.2 ± 0.14	1.11 ± 0.16	
Dual Femur Z-score	0.48 ± 0.41	-0.2 ± 1.44	

Table 2.4

Vitamin D Status and Questionnaire Results

	Dance (*n*=10)	Cheer (*n*=9)
Serum Vitamin D (ng/mL)	27.45 ± 4.26	24.59 ± 7.61
Daily Calcium Intake (mg)	503.6 ± 723.55	531.33 ± 236.22
Daily vitamin D Intake (IU)	255.9 ± 335.07	228.22 ± 144.86

Gymnasts are another group of female athletes who compete almost entirely in an indoor arena and thus are predisposed to low levels of Vitamin D. This sport places emphasis on lower body weight, which can lead to limited calories. When an individual has limited calories, this can subsequently lead to inadequate intake of vitamins, such as vitamin D. Lovell et al. (2008) studied the results of 18 female gymnasts (10-17 years old) who competed as part of the Australian Institute of Sport (AIS). A dietitian reported the levels of calcium each gymnast consumed through the use of a food frequency questionnaire. The vitamin D mean level was 56 nmol/L with levels ranging from 29-84 nmol/L. There was only one athlete with a level less than 30 nmol/L. Five athletes had vitamin D levels between 30-50 nmol/L and nine athletes had vitamin D levels 50-75 nmol/L. Only three gymnasts had vitamin D levels above 75 nmol/L.

Case notes over the twelve months before the study began were reviewed by the researchers. During a clinical examination using either an x-ray, bone scan, or magnetic resonance imaging, 13 of the gymnasts suffered from a bone stress injury. The tests also revealed stress reactions in 12 of the gymnasts and a stress fracture in one gymnast (Lovell, 2008). A stress reaction is the precursor to a fracture. During the reaction stage, the bone structure is breaking down and ultimately becoming weaker. According to the Vitamin D Council, a total of 15 of the participants in this study were considered to have vitamin D deficiency. Although the researchers did not provide results of inferential statistical analyses, they concluded that they found a relationship between low levels of vitamin D and bone related injuries in elite gymnasts. Future research should include more gymnasts from other countries to explore the consideration of geographical locations.

The Lovell et al. (2008) study is similar to a study conducted by Bloom et al. (2010) that assessed the vitamin D levels in females who participated in the NCAA Division II volleyball, softball, or gymnastics. A total of 36 athletes completed the study including outdoor athletes (n=19) and indoor athletes (n=17). All athletes completed demographics and health history questionnaires along with a sunlight exposure questionnaire and a blood spot test. DEXA was used to measure bone mineral density of the total body, lumbar spine and femoral neck. The athletes completed a three-day food recall in both the fall and spring. Person product-moment correlation was used to analyze relationships between serum vitamin D levels, body fat percentage, vitamin D intake and BMD. The reports of the three-day food recall indicated there was no significant difference in vitamin D intake for indoor and outdoor athletes (indoor: 1.8 ± 1.2, outdoor: 1.4 ± 1.1 μg/d) (Bloom, 2010). Also, there was no significant difference for serum calcium concentrations between indoor and outdoor athletes (indoor: 50 ± 20, outdoor 48 ± 15

ng/mL; $p = .52$). However, 36% had vitamin D serum concentrations equal to or less than 40 ng/mL, which was considered insufficient for this study. Considering 36% of participants in this study had insufficient vitamin D levels, all athletes need to monitor their intake of vitamin D to ensure peak performance is maintained.

The biochemistry and physiology of vitamin D are important concepts in understanding how vitamin D works with other vitamins, minerals and hormones. Vitamin D is an essential part for ensuring proper bone health, immune function, muscle function, cardiovascular health, maintenance of the respiratory system, brain development, and preventing against cancer (Ross, 2011). Female athletes are one group who are at risk for vitamin D insufficiency or deficiency, which can impact athletic performance. The research indicates athletes are one population who have inadequate levels of vitamin D. Athletes are required to perform at optimal levels to be competitive in their sport; having inadequate levels of vitamin D can inhibit their performance due to muscle and skeletal injuries, as well as increases susceptibility to illness. However, existing research is inconclusive, and each study has limitations. Thus, future research is necessary to have a better understanding of how vitamin D related to both injury and performance.

Vitamin D and Geographic Location

Geographic location effects the amount of vitamin D the skin can produce. The amount of UV exposure necessary to synthesize an adequate amount of vitamin D depends on UV index, season, and latitude (Freedman, 2013). The UV index determines the risk of overexposure of UV radiation and compares it with ozone concentrations, altitude, cloud coverage, time of day, and wavelength (D'Orazio, 2013). The further from the equator, the greater the angle the sun will hit the atmosphere, which leads to less UV available (Wacker, 2013). Farrokhyar et al. (2015)

explored how latitude effects vitamin D status in athletes. Studies from Australia, Middle East, Spain and United States were conducted on 2,313 athletes. Of those athletes, 56% had vitamin D inadequacy that varied by geographical location ($p < 0.001$). The United Kingdom and the Middle East had significantly higher levels of inadequacy. The researchers explained the risk for inadequacy of vitamin D was slightly higher for latitudes of > 40° N (RR 1.14 95% CI [0.91-1.44)]. However, the risk increased considerably after removing the Middle East, which was an outlier [RR 1.85 (1.35-2.53)]. There was a significant risk of inadequacy of vitamin D for indoor sports' participants living in latitudes of ≥40° (RR 1.19; 95% CI [1.09-1.30]). The risk of vitamin D inadequacy increased for athletes who participate in winter and spring sports (RR 1.85; 95% CI [1.27-2.70]). The risk of inadequate vitamin D levels increases with higher latitudes in winter and early spring months.

In contrast to those winter and spring sports that restrict the opportunities for vitamin D metabolism because they must be held inside, in some geographical locations there is enough sun light for the skin to produce vitamin D year-round. A study conducted in Baton Rouge, LA, examined vitamin D in 19 distance runners ages 19-45 (Willis, 2012). Participants gave a blood sample after an overnight fast three days following a prescribed diet (15% protein, 25% fat, and 60% carbohydrate) and an exercise regimen. Baton Rouge is one geographical location where vitamin D synthesis is possible year-round, however 42% (five males and three females) of the runners were vitamin D insufficient (Willis, 2012). Two participants (both males) had concentrations below 20 ng/mL. Athletes regardless of sport and geographical location are susceptible to having inadequate levels of vitamin D. Geographical location is a key component to allow the skin to produce adequate vitamin D. However, athletes who live in locations close to the equator that synthesize vitamin D most likely train in the early morning or evening when vitamin D is not likely to be synthesized (Willis, 2012).

Conclusion

In summary, vitamin D deficiencies are present in a majority of athletes despite the knowledge of how crucial vitamin D is to musculoskeletal injuries. However, since dosage requirements vary by established organizations, it is difficult for athletes and the lay population to know the correct amount of vitamin D they need. Furthermore, athletes place high loading patterns on their body and train during periods of the day when vitamin D cannot be synthesized. In addition, athletes who participate in sports that occur during the winter or take place indoors may have limited exposure to vitamin D levels, which can impair their physical performance. Staying healthy for peak performance is an athlete's main objective; vitamin D is one of the key components to help that athlete avoid injuries.

Calcium

Calcium, the most abundant mineral in the body, is essential for everyday functions and long-term bone formation and remodeling (Florencio-Silva, 2015)). In addition to being vital for the building and maintenance of bones, calcium also supports both blood clotting and nerve transmission, thus enabling muscles to contract. Although the body stores the majority of its calcium in the teeth and bones, calcium is lost through sweat, hair, nails, skin, urine and feces (Ross, 2011). Because the body is not capable of producing new calcium, it must be incorporated through the daily diet.

Sources of Calcium

There are two main categories of calcium sources. First, calcium is available through food sources such as milk, yogurt, cheese, kale, and broccoli. Some grains are fortified and therefore, their calcium content can be significant. The second category of calcium is supplementation. The two main forms of calcium supplements are carbonate and citrate. Calcium carbonate is more commonly available due to convenience and cost. Calcium carbonate is most efficient when taken with food. However, calcium citrate can be absorbed equally well when taken with or without food (Straub, 2007). Calcium citrate is useful for people with inflammatory bowel disease and absorption disorders. The percentage of calcium absorbed depends on the amount of calcium consumed at one time.

Absorption

Factors negatively affecting calcium absorption include high levels of sodium, insufficient vitamin D, and a diet high in phytic acid (Committee of Review of Dietary Reference Intakes, 2010). Phytic acid is a natural substance found in plant seeds and impairs mineral absorption. When there is an increase in serum calcium levels, the parafollicular cells of the thyroid gland secrete calcitonin. The calcitonin blocks bone calcium resorption helping keep serum calcium levels in the normal range of 8.5 and 105 mg/dL (Ross, 2011). Calcium absorption decreases 15%-20% in adulthood (Committee of Review of Dietary Reference Intakes, 2010). The body's demand for calcium relative to skeletal growth fluctuates by life stage. The major physiological activities include bone accretion, which starts as an infant during skeletal growth and maintenance of bone mass after growth has stopped. As adults, net calcium is lost from the body when bone formation does not maintain with the rate of bone resorption. Another factor that affects calcium absorption is vitamin D intake. Higher levels of vitamin D in the body improves calcium absorption (Ross, 2011).

Calcium is absorbed through two different pathways: a transcellular mechanism (active transport) and paracellular mechanism (passive diffusion). The transcellular mechanism is located in the duodenum, and the paracellular mechanism is the diffusion process that occurs throughout the intestine (Bronner, 2003). The active transport of calcium is dependent on calcitriol and vitamin D receptors. The activation of the transcellular mechanism is initiated by calcitriol and is the primary form of absorption of calcium at moderate or low intake levels. The duodenum is the site of the majority of absorption due to high concentrations of vitamin D receptors. Transcellular transport is dependent on the up-regulation of the responsive genes, which includes the transient receptor potential cation channel (Xue & Fleet, 2009). The absorption of calcium impacts bone health, muscle contraction, transmitting nerve messages and hormone release.

Recommended Dietary Allowance

The National Institutes of Health (NIH) established recommended dietary allowances (RDA) for vitamins and minerals to meet the nutrient requirements of nearly all healthy people. Calcium is one of the minerals the NIH has set recommended dietary allowances to ensure the proper amount of calcium required for bone health and maintain proper metabolic functions. Males and females ages 14-18 should consume 1,300 mg a day. Because bone growth slows once individuals reach adulthood, the RDA for calcium drops to 1,000 mg per day. These recommendations were established to maintain adequate rates of calcium retention and bone health. However, calcium deficiency has no acute symptoms because circulating blood levels of calcium are regulated. The body deposits calcium into the bones when calcium levels are too high. The bones then release calcium when blood levels drop too low. This process is regulated by vitamin D, calcitonin, and parathyroid hormone (PTH). On the other hand, hypocalcemia occurs when calcium levels in the blood are less than 2.12 mmol/L. Common symptoms include numbness and tingling in hands and feet, muscle cramps, fatigue, irritability, dysphagia, and depression (Weaver, 2006). In contrast, hypercalcemia occurs when serum calcium levels are 10.5 mg/dL or greater (Moe, 2008). Clinical signs and symptoms include anorexia, weight loss, polyuria, heart arrhythmias, fatigue, soft tissue calcifications, renal insufficiency, and nephrolithiasis (Jones, 2008). Both hypocalcemia and hypercalcemia can cause physiological issues throughout the body.

To determine if bone mass continued to increase in female college aged students Recker et al. (1992) examined 156 healthy college-aged women. Participants completed nine visits every six months. At each visit they underwent a DEXA scan, turned in their physical activity monitor, and completed a seven-day food diary. The set of bone mineral values for each subject was regressed on corresponding time to obtain a rate of change in bone mineral density. There was significant increase in bone mass over the period of study including 4.8% for the forearm, 5.9% for lumbar BMC, 6.8% for lumbar BMD, and 12.5% for total bone mass. The forearm and spine sites and total body bone mineral (TBBM) measurements all indicated significant increase in bone mass during the study. Bone density gain was positively correlated with calcium intake and physical activity ($r=.31$; $p = .004$). Physical activity and dietary calcium intake both have a positive effect on bone gain (Recker, 1992). The results of this study indicate that bone gain did occur in young women in the spine, total body, and forearm. Calcium/protein intake ratio had the strongest influence on rate of gain in spinal BMD. This study reveals the importance of continuing proper nutrition throughout early adulthood due to the fact that increasing bone mass is still occurring.

The Recker et al. (1992) study looked at bone gain in young adults, whereas, Li et al. (2015) conducted a study to investigate the potential correlation between types of hip fractures and serum calcium level in the elderly. Each patient admitted into the Daping Hospital for hip fracture between January 2011 and December 2013 was included into the study. The study included 101 cases of femoral neck fractures (80.20% female, age 73.85 ± 11.88) and 95 cases of femoral intertrochanteric fractures (57.90% female, age 76.27 ± 11.93). Patients had fasting blood glucose taken at the time of admission, post operation, and at discharge. Serum calcium levels are presented in Table 2.5. Overall, patients with femoral neck fractures were generally younger and involved more women than those with intertrochanteric fractures. Calcium levels were lowest at admission and discharge for patients with intertrochanteric fractures. Decrease in bone mineral density and a decrease in the sensitivity of calcium-sensing receptors in the lower limb muscles can lead to more falls, which can result in fractures. Ensuring our elderly have adequate calcium intake is important to help maintain the bone and lower limb muscle strength to reduce balance issues resulting in falls.

Table 2.5

Serum Calcium Levels

	Admission	Post Operation	Discharge
Femoral Neck Fracture group	2.19 ± 0.12	2.01 ± 0.12	2.15 ± 0.13
Femoral Intertrochanteric Fracture group	2.13 ± 0.15	2.01 ± 0.14	2.10 ± 0.16
P value	0.002	1.000	0.019

Military Recruits

A population at risk for calcium deficiencies related to bone health is female members of the military due to increased loading patterns during recruitment. Researchers examined 3,700 female Navy recruit’s prevalence of stress fractures, vitamin D and calcium supplementation (Lappe, 2008). The recruits were divided equally into a treatment group (200 mg calcium 800IU vitamin/day) and control group (placebo). During the eight-week study, subjects took four pills per day, which were given during breakfast and dinner. Participants completed a risk factor questionnaire, which was used to determine fracture risk in bones. This risk of fracture in subjects who had amenorrhea, absence of periods, was 91% higher than those with one or more menstrual cycles during training (RR, 1.91, 95% CI [1.47-2.47]; $p = <0.0001$). In the treatment group, the risk of stress fracture in individuals with amenorrhea decreased 83% ($p = 0.0035$). The recruits in the treatment group had 20% lower incidence of stress fracture than the control group (5.3% verse 6.6%, respectively; $p = 0.026$). Under mechanical loading as intense as basic military training, micro-fractures can develop and can lead to remodeling to repair the micro-fractures. Optimal levels of circulating calcium are needed to repair the micro-damage and inhibit an increase of non-targeted remodeling to maintain serum calcium concentrations. Physical activity in the presence of low calcium intake can cause additional stress on bones because of the need to offset the significant calcium loss in sweat. During heavy sweating and low calcium intake, calcium is taken from the bone reservoir. This loss of calcium can weaken the skeleton over short periods (Parfitt, 2002). Overall, supplementation of calcium for female military recruits should be considered for reducing the risk of stress fractures.

In comparison to the Lappe et al. (2008) where researchers looked at female Navy recruits, while Garakani et al. (2016) conducted a cross-sectional study on male military personnel from January 2012 to May 2013. A total of 335 healthy males without bone or joint disease ages 35-55 were selected and grouped into operative military personnel (168 active field soldiers) and non-operative personnel (167 military official staff). The operative group was involved in regular exercise and training programs, walking at least 30 minutes a day and field operations at least four times a year. The non-operative group included those without any regular training program. Each participant filled out a questionnaire with topics involving smoking, calcium intake and physical activity. BMD was measured using a dual x-ray and laser (DXL)

Calscan. A total of 219 participants (65.4%) had normal BMD, 114 (34%) had osteopenia, and two (0.6%) had osteoporosis. There was a significant association between osteopenia and age ($p < 0.05$). The linear regression reveled that BMD is significantly associated with calcium intake ($p < 0.05$). The operative military personnel had lower prevalence of osteopenia and osteoporosis ($p < 0.001$). The operative military personnel also had higher dietary intake and physical activity and lower BMI compared to the non-operative group. Overall, active military personnel with higher dietary intake have decreased risk of osteopenia and osteoporosis.

Based on the results of the previous study, implementing a supplementation of calcium and vitamin D could potentially be beneficial to reducing the risk of stress fractures. However, more research should be completed to ensure supplementation is the proper choice for Navy recruits. The physical demand of military training and low serum calcium levels proliferates the risk of musculoskeletal injuries. However, physical activity and proper nutrition is vital for reducing the risk of osteopenia and osteoporosis. Monitoring dietary intake to ensure proper nutrients such as calcium, which is essential for bone formation will reduce fracture risk.

Athletes

Optimal intake of nutrients is essential for energy and maintenance, and adequate micronutrients enhance recovery and improves performance. Conversely, long-term inadequate calcium intake can increase the risk of bone fracture and can cause osteopenia, which can lead to osteoporosis (Committee of Review of Dietary Reference Intakes, 2010). Osteoporosis, characterized by porous and fragile bones, effects 10 million U.S. adults, 80% of whom are female.

Researchers examined athletes in a single sport, Leinus et al. (1998) assessed the energy expenditure and nutrient intake of male and female basketball players compared to students who did not participate in sports. The study included seven male and seven female basketball players and ten female students who did not participate in sports. The researchers collected data for two training days and two resting days. Both male and female basketball players had higher energy expenditure and lower energy intake on training days compared to rest days. The researchers reported the recommended calcium level for these athletes was 1,200 mg/d. Interestingly, this recommendation is 100 mg/d lower than NIH recommendation of 1,300 mg/d. The mean calcium intake was low, between 597 and 857 mg. Calcium levels for male athletes were 857 ± 251 mg, and female levels 597 ± 280 mg. Both male and female calcium levels were considered low compared to the NIH recommendation. In contrast, the control group who did not participate in sports had calcium levels of 806 ± 421 mg. Dietary calcium intake was moderately related to energy intake (r=0.57). On training days, the female basketball players only consumed 46.8% of the recommended intake of calcium. This study demonstrated that energy intake and nutrients are low overall for athletes, and on training days the energy intake and nutrient deficit is even greater (Leinus, 1998).

In contrast to the Lienus et al. (1998) who only looked at a single sport, Nunes et al. (2018) conducted a study evaluating 242 elite athletes (63% males) at the beginning of their preparatory phase of competition. The sports involved in the study included basketball (*n*=29), judo (*n*=24), rowing (*n*=3), handball (*n*=10), volleyball (*n*=26), swimming (*n*=92), karate (*n*=1), triathlon (*n*=23), tennis (*n*=18), athletics (*n*=4), sailing (*n*=6), shooting (*n*=2), fencing (*n*=2), and horseball (*n*=2). Of those athletes, 77 (*n*=47 for males and *n*=30 for females) participated in the evaluation during the competition phase of their respective season. The sports involved in the evaluation in the competitive phase induced basketball (*n*=13), handball (*n*=10), volleyball (*n*=15), swimming (*n*=35), and triathlon (*n*=4). The athletes completed seven-day food records, underwent a DEXA scan, and body composition was measured after a twelve-hour fast. Bone mineral content for females was 1.61-3.29 kg and 1.77-4.75 kg for males during preparatory phase. For the competitive phase, females' BMC was 1.82-3.79 kg and males was 1.93- 4.60 kg. Between the preparatory phase and competition, females had a decrease in calcium -132 (-284 to 59) mg/d ($p = 0.021$). However, males had an increase in calcium 175 (-112 to 322) mg/d ($p = 0.017$). In the preparatory phase, females had a higher percentage of mismatch for vitamin B12, calcium, iron, selenium, and zinc, compared to males ($p = 0.05$). In the competitive phase, there was a higher percentage of mismatch for thiamin, vitamin B12, calcium and iron for females ($p < 0.05$). Most of the vitamins and minerals examined during this study are related to muscle contraction and energy expenditure. Calcium levels decreased between preparatory stage and competition. Between preparatory stage and competition is the time when training loads are the heaviest for athletes. Therefore, nutrient levels should be monitored to ensure athletes maintain adequate levels for optimal performance. Future research should compare nutritional intake and performance by conducting performance variables.

In comparison to Nunes et al. (2018) who examined 14 different sports, Madden et al. (2017) examined the micronutrient intake of 18 male and 22 female Paralympic athletes. Each participant recorded dietary intakes using a Three-Day Food Record and dietary supplement questionnaire. All micronutrient intakes were presented as the percent of the RDA or adequate intake (AI). Not surprisingly, males had a significantly higher total energy intake than females (2,092 kcal/day, 1,602 kcal/day respectively) ($p = 0.013$). However, male athletes did not meet the RDA for folate and vitamin A. Furthermore, females did not meet the RDA for iron or calcium. Only 27.2% of the RDA's recommendation for calcium was consumed by female athletes. Females also had a lower percent of the recommendation for calcium ($p = 0.031$)

compared to males. A total of 15% of participants recorded using a multivitamin/multimineral formulation. The top reasons females used supplements were "stay healthy" (59.1%) and "medical" (50.0%). On average, females did not meet the calcium recommendations. Calcium plays a major function in bone health and muscle strength and function. Although no standard is available, it has been suggested athletes may require higher levels of micronutrients. With females lacking calcium, it could lead to decreases in performance or increase in injury.

Another population that undergoes an intensive training schedule is cross country runners. Barrack at el. (2010) designed a study to evaluate diet, menstrual history, serum hormone concentrations and bone mass in female runners with normal and abnormal bone turnover. A total of 39 cross country runners between ages of 14 and 17 participated. The study included a seven-day dietary assessment, twenty-four-hour dietary recall, serum measurements, DEXA, and questionnaire to assess their participation in other sports. Serum concentrations of bone-specific alkaline phosphatase (BAP) and Cross-linked C-telopeptides of type 1 collage (CTX) were used to classify runners with normal or abnormal bone turnover. Runners with elevated bone turnover (EBT) had lower mean intake of each micronutrient determined by the seven-day dietary assessment compared to those with normal bone turnover. Runners with EBT consumed a lower amount of calcium daily ($p = 0.09$). A total of 13 runners met the criteria for abnormal bone turnover. All of the 13 runners had BAP and CTX values that fell within the elevated range, which indicates EBT. Compared to normal turnover, those runners with EBT were younger, had significantly lower mean body weight, fat mass, lean tissue mass, and body mass index. Those runners with EBT had lower mean daily intakes of energy ($p = 0.07$), carbohydrates ($p = 0.09$), and dietary fat ($p = 0.09$) than those runners with normal turnover. BMC at total body, femoral neck, total hip, and lumbar spine was lower in runners with EBT.

The findings of this study suggest that runners with EBT are consuming inadequate calories, which can lead to energy deficiency due to high levels of physical activity. Energy deficiency can lead to complications with BMC and micronutrient levels in the athlete.

Endurance athletes have high aerobic capacity to endure long-lasting physical loads. Baranauskas et al. (2015) conducted a study on 146 endurance athletes; men (n=108) and women (n=38) in Lithuania during general training from 2009-2012. The sports included in the study were: rowing (n=24), cycling (n=40), swimming (n=43), skiing (n=14), biathlon (n=20), and long-distance running (n=43). Each participant underwent a 24-hour dietary survey and anthropometric measures. It was concluded that endurance athletes consumed 33.6% less calcium than the RDI. Moreover, female athletes compared to males consumed less calcium than the RDI (55.3% vs. 25.9%, $p = 0.013$). Athletes in general need proper calcium levels due to the fact that athletes are more likely to lose calcium through perspiration. Without proper amounts of micronutrients, athletes will not be at peak physical shape to compete at the highest level. Specifically, calcium is important for strong bones, as well as muscle contraction. Without proper calcium athletes are more likely to experience muscle cramping.

An additional population at risk for deficiencies are vegetarians due to their diets containing lower amounts of calcium (Tucker, 2014). Kaur et al. (2017) conducted a study to investigate the nutritional status between vegetarian and nonvegetarian sportspersons. The study included 120 participants, 60 vegetarians and 60 non-vegetarians between the ages of 16 and 25 years old. Each participant completed a 24- hour recall for three days, anthropometric measurements, and blood samples. Based on the 24-hour food recall, consumption of milk and milk products was significantly higher among vegetarian males compared to non-vegetarian males ($p \leq 0.05$). Calcium was also higher among vegetarian females compared to nonvegetarian females, but the difference was not statistically significant. Average daily consumption of calcium was higher in vegetarian males and females compared to non-vegetarians. However, the results were not statistically significant. Compared to the RDA, both vegetarians and non-vegetarians who participated in this study were found to consume low amounts calcium. Through a well-balanced diet, vegetarians can consume all required nutrients in sufficient amounts, which is vital for optimal athletic performance.

Female Athletes

Female athletes at a high risk of calcium deficiencies are those who have a low energy intake or those who do not meet the calcium recommendations (Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine, 2000). Low energy intake has been defined as ≤30 kcal/kg/day (Ross, 2011). The causes for low energy intake could include eating disorders, rational intentions to decrease body weight for a sport, and unintentional failure to meet energy requirements (Ross, 2011). Female athletes who do not consume the dietary recommendations of three to four servings of dairy foods per day or have low energy intake are at high risk for calcium deficiencies. Clark et al. (2003) assessed the pre-and post-season dietary intake of NCAA Division I female soccer players. A total of 13 athletes completed a three-day food recall and anthropometric measurements including height, weight and percent body fat. The results of the study indicated energy intake during pre-season was significantly higher than the post-season average (2,290 ± 310 g/kg and 1,865 ± 530 g/kg, respectively). The calcium level for pre-season was 931 ± 223 g/kg and post season was 695 ± 289 g/kg (p = .005) (Clark, 2003). This study indicates female athletes were not consuming the RDA for calcium during pre-season with a significant reduction in the post-season. Female athletes report unintentional failure of meeting energy requirements include time management, lack of knowledge and food availability (Clark, 2003). Thus, female athletes who do not meet energy requirements are at risk for mineral inadequacy. Lack of calcium can lead to deficiencies in vascular contraction and vasodilation as well as muscle function (Beto, 2015).

Unlike Clark et al. (2017) who conducted a study using only 13 female soccer players, Nieves et al. (2010) conducted a study using only cross-country runners. The two-year study included 125 female athletes ages 18 to 26 years old and measured longitudinal changes in bone density and incident stress fractures. Dietary calcium was linked to significant reductions in fracture incidence. Women who consumed less than 800 mg of calcium a day had approximately six times the stress fracture rate of women who consumed more than 1,500 mg. Calcium intake was positively related to BMD of the hip ($p < .05$) and total body ($p < .005$) as well as total body BMC ($p < .005$). For every standard deviation increase in calcium intake (~600 mg), women gained .0016 g/cm2 in spine BMD ($p = .07$). Therefore, even with the increase in calcium and a small bone gain, this was not statistically significant. Women also gained .0022 g/cm2 in hip BMD, along with .0025 g/cm2 in total body BMD. Calcium intake is related to greater bone mineral density thereby potentially reducing the risk of stress fracture.

In contrast to the study conducted by Nieves et al. (2010) who examined multiple sports, Nabatov et al. (2016) assessed the relationship between sports-related factors on tissue mineral status on field hockey and fencing. A total of 48 females ages 12-17 participated; there were split three groups: field hockey, fencing, and non-athlete group. Each athlete had to give hair and saliva samples. Calcium level in the hair for the control group was 846.1 ± 109.7, field hockey was 1,039.4 ± 61.1, and fencing was 698.0 ± 93.8. However, these numbers were extremely high compared to the saliva levels of calcium. The control was 45.6 ± 2.3, field hockey 50.2 ± 2.4, and fencing 32.2 ± 3.4. The difference in calcium levels in saliva compared to hair deems further research on the reliability and validity of both measurements. Athletes who participated in fencing had significantly decreased calcium levels in saliva compared to the control. The high physical activity in stressful conditions of competition increase metabolism and turnover of nutrients (Castronovo, 2013). Ensuring proper levels of calcium will be imperative to remaining healthy for female athletes. The results of calcium levels from hair verse saliva samples were very different. Research should be conducted to determine which method is most accurate for further research.

In summary, inadequate calcium levels are a concern for the female population. Calcium is an important mineral for building and maintenance of bones. Adequate calcium is essential for reducing stress fractures in athletes, particularly female athletes with restricted calories.

Female Athletes: Combination of Calcium & Vitamin D

Figure skaters are a group of athletes who compete primarily indoor. In 2002 during the US National Synchronized Skating Championships, 132 skaters (mean age 15.7 ± 2.4 years) participated in a study that assessed the energy, macronutrient and micronutrient intakes of competitive figure skaters. Each participant self-reported their height and weight, collected a three-day dietary food recall, which consisted of two weekdays and one weekend day. Macronutrient and micronutrient intakes were analyzed using the Food Processor Nutrition Analysis software. Consumption of less than two thirds of the estimated average requirements was considered inadequate intake. Each participant also completed a supplement survey questionnaire to determine prevalence of dietary supplement use, frequency of use of dietary supplements, type of supplement use, and the reason for use of dietary supplements. Total energy intakes of participants were 1,656 ± 497 kcal/d, which is lower than the recommended 2,200 to 2,500 kcal/d intake for female athletes who exercise more than 10 to 20 hours weekly.

The results for vitamin D were 3.6 ± 3.0 ug, which is 72% of the recommended dietary allowance (RDA)/adequate intake (AI). Calcium intake was 849 ± 378 mg, which is 65% of the RDA/AI. For dietary supplements, 40% of the participants reported using supplements, with 50% using these supplements daily. The researchers concluded it is vital to educate skaters about maintaining adequate dietary intake to ensure the athlete understands the importance of these key vitamins. Considering that vitamin D acts to increase the amount of calcium that can be absorbed through the intestine, the calcium intakes of the athletes presented in this study indicate the skaters are not getting adequate vitamin D are also not absorbing enough calcium to meet their body's needs. Without proper calcium and vitamin D, bone health and muscle function may be compromised (Ziegler, 2006).

Similar to the research conducted by Ziegler (2006), 11 soccer athletes completed a three-day food record during two weekdays and one weekend day (Mullinix, 2003). The records were also analyzed using the Food Processor Nutrition software. However, the study conducted on soccer athletes included a menstrual history, body image perception, supplement usage, and attitude toward sports nutrition practices to understand the mentality of athletes toward nutrition. A total of 59% of the players found that it was "somewhat easy" to maintain their in-season body weight. Meanwhile, 41% found it "very easy" to maintain their in-season weight. The attitudes to sports nutrition practice questions indicated the athletes had a good understanding of proper hydration and adequate understanding of macronutrients. Lastly, they had a poor understanding of the role of micronutrients in performance. Total energy results for the study was 2,015 ± 19 kcal/d. The recommended energy intake using the Harris-Benedict equation plus activity factor was 2,716 kcal/d. The results for vitamin D were 2.6 g ± 2.4 and calcium 887 mg ± 510. The results indicated for vitamin D the athletes consumed 52% of the recommendation and calcium

89%. Calcium and vitamin D were two of the seven that were less than the 100% of the recommendations. Considering supplementation, 55% of the athletes consumed a supplement occasionally while 33% consumed a supplement daily. The study indicates elite soccer players have inadequate energy intake, which subsequently leads to inadequate vitamins and minerals. Having inadequate vitamin and minerals consumption can inhibit performance and lead to injury. In addition, the Dietary Reference Intake's (DRI) were not designed for athletes or individuals who exercise regularly. Based off the results that athletes had a poor understanding of the role of micronutrients in performance and calcium and vitamin D levels were not met by majority of the athletes, it may be beneficial to first teach athletes the importance of micronutrients (Mullinix, 2003).

Birth Control and Bone Mineral Density

Peak bone mass in early adulthood is a major determinant of osteoporosis. Young women with hypothalamic dysfunction manifest as amenorrhea and oligomenorrhea are likely to have reduced bone mineral density (Braam, 2003). This leads to a greater risk of osteoporosis later in life (Drinkwater, 1984). Physicians typically treat amenorrhoeic athletes with hormone therapy or an oral contraceptive (OC) (Haberland, 1995). Because oral contraceptives play a critical role in numerous processes throughout the body, the current research in this field is expansive, however, research regarding oral contraceptives in female soccer and basketball players is limited.

In a two-year randomized study, researchers examined 127 female runners between the ages of 18 and 26 years old. The participants had to run at least 40 miles a week and compete in competitive races. Throughout the study women who stopped taking OC had significantly lower percent body fat (adherent to treatment:23.7 $\pm$ 4.8, switched from treatment to control 19.5 $\pm$

6.1), fewer menstrual periods per year (adherent to treatment: 10.8 ± 2.3, switched from treatment to control 6.3 ± 5,1), and more disordered eating (adherent to treatment 10.9 ± 11.2, switched from treatment to control 17.4 ± 16.2) than women who adhered to the OC (Cobb, 2007). Randomized OC had no effect on changes in BMC or BMD. The exception is in the oligomenorrheic group; total hip BMD was significantly reduced in the OC group compared to the control group (*p* -0.04). Regardless of the treatment group, bone changes were correlated to initial menstrual status. Overall, the amenorrhoeic and oligomenorrheic groups had increased in spine BMD and whole-body BMC.

In a similar study conducted by Hergenroeder et al. (1997), evaluated 15 amenorrhoeic subjects and 9 oligomenorrheic subjects. Participants who had amenorrhea were randomly assigned to receive one of three medications: (1) oral contraceptive, 0.035 mg of ethinyl estradiol and 0.5 to 1.0 mg of norethindrone per day on 21 days of each 28-day cycle; (2) medroxyprogesterone group, medroxyprogesterone, 10 mg/day on the last 12 days of the calendar month; or (3) placebo group, placebo identical in appearance to the medroxyprogesterone tablet, one tablet per day for the last 12 days of the calendar month. Oligomenorrheic subjects were randomized to receive medication regimen 2 or 3 as described for the amenorrhoeic subjects. The results indicated that in amenorrhoeic subjects, lumbar spine bone mineral density content and bone mineral density at 12 months were greater in the oral contraceptive group than in the medroxyprogesterone group (p = 0.002 & p = 0.003 respectively) and the placebo group (p = 0.05 & p = 0.009 respectively). Total body bone mineral content and bone mineral density at 12 months were greater in the oral contraceptive group than in the medroxyprogesterone group (p=0.05) and the placebo group (p=0.05) when controlled for baseline total body bone mineral content or bone mineral density and body weight.

Overall, treatment with oral contraceptives was associated with improved lumbar spine and total bone mineral density after 12 months of treatment in young women.

Overall, reducing the risk of osteoporosis begins by taking measure when females are in their early teens to ensure proper nutrients to build total bone mineral density. Some athletes may benefit from trying oral contraceptives to improve their bone mineral density especially in sports that involve endurance, weight classes, and restricting calories.

Conclusion

In summary, future research is warranted to determine whether, and how, vitamin D and calcium effect physical performance and musculoskeletal injuries. While we do know that deficiencies can cause major health issues like cardiovascular disease, depression, osteopenia, and osteoporosis, we do not know the extent of musculoskeletal injuries in athletes. Furthermore, these consequences are exacerbated for athletes because of increased loading patterns and indoor athletes who receive limited sun exposure. In turn can result in impaired physical performance and injury. Indeed, because these adverse health consequences of vitamin D and calcium deficiencies across the spectrum of competitive sports, it is vital that stakeholders have reliable information to ensure the health of their athletes. More specifically, to date little research has been conducted focusing on Division I women’s soccer or basketball players.

CHAPTER 3. METHODOLOGY

Purpose

The primary purpose of this project was to compare current blood serum values of vitamin D and calcium in Division I females who participate in basketball and soccer at a midsized, Midwestern institution. A secondary purpose was to compare the results of the blood serum values to total body bone density by way of a dual-energy x-ray absorptiometry (DEXA) scanning. This chapter describes the population of the study, setting of the study, data collection instrumentation, procedures, and the data analysis. The research questions are as follows:

1) What are the differences in blood serum values for vitamin D and calcium when compared between-groups (i.e., Women's Basketball and Women's Soccer)?
2) What is the relationship between blood serum values and total body bone mineral density?

Participants

A sample of 30 Division I Women's Soccer (n=21) and Women's Basketball (n=9) between the ages of 18 and 24 were recruited by presenting information to potential participants at team meetings. Inclusion criteria consisted of being a student athlete at a pre-identified mid-sized NCAA Division I institution who were listed on the official roster for Women's Basketball and Women's Soccer. Informed written and verbal consent were obtained from each participant before enrollment. Because of the potential issues with the DEXA, all participants took a urinary pregnancy test on the day of the scan. Exclusion criteria for DEXA scan included participant was greater than 350 lbs in body weight (weight tolerance of the machine), height greater than 78 inches (due to the height limitation of the machine), shoulder width of greater than 23.5 inches Participants could not have taken calcium supplementations <24 hours of the DEXA scan due to the fact it may interfere with bone mineral density values. Previous contrast agents such as ones used for a MRI or CT scan >7 days is not permitted due to interference with bone mineral density value. Participants were not allowed to complete the scan if they had an x-ray or computed tomography procedure in the last two weeks due to limited radiation exposure. Lastly, if the participant had previous treatment of cancer and/or pregnant, they were unable to complete the scan.

Setting

The blood draws were conducted at the Bentson Bunker Fieldhouse Human Performance Laboratory by a trained phlebotomist from a local hospital. DEXA scans were conducted in the Biomechanics Laboratory also located in Bentson Bunker Fieldhouse. This laboratory was used because the DEXA (GE Healthcare Lunar Prodigy) scanner is located at this site and blood draws could be safely obtained from the phlebotomist.

Equipment and Instruments

To measure vitamin D and calcium levels, a single venipuncture was conducted by a trained phlebotomist employed by a local hospital. All blood serum was analyzed at a local hospital for vitamin D and calcium values. Following the blood draw, athletes took a pregnancy test to determine exclusion criteria. Lastly, participants completed their DEXA scan to measure bone mineral density.

Procedures

Once participants arrived to the laboratory, they completed the IRB-approved informed consent and demographics questionnaire. The demographics questionnaire gave additional information about age, gender, race, position, years of playing experience, years of collegiate experience, supplement use, tanning bed use, sunscreen use, and eating habits.

Next, the phlebotomist employed by the local hospital, collected blood samples. A single venipuncture allowed the phlebotomist to obtain separate vials of blood to be analyzed for vitamin D and calcium content. All blood serum was analyzed by a local hospital for vitamin D and calcium values in accordance with the Clinical Laboratory Improvement Amendment (CLIA) accreditation. The alias for the vitamin D used by the hospital was 25-OH Vitamin D. The hospital used Chemiluminescent Microparticle Immunoassay (CMIA) which is the modified and advanced form of enzyme linked immune Sorrbant Assay (Ilyas, 2014). The hospital used tube that contained serum gel and collected a minimum of 0.50 mL of blood. Calcium methodology through the hospital included using Arsenazo III Calcium Dye Complex. The test tubes included Lithium heparin gel and 0.5 mL of blood was collected. The hospital separated plasma from cells within two hours of the draw. Following the blood draws, participants provided a urine sample for the Clinical Guard pregnancy test. If the pregnancy test was determined to be negative, participants were able to complete the DEXA scan. For the scan, participants removed all jewelry, heavy clothing, shoes, and socks. A research team member who was trained to use the DEXA helped position the participant on the scanner. Two Velcro straps were placed on the lower limbs to help keep the lower body in correct position during the scan. Participants were asked to remain still as the scanning arm moved from the top of the head to the feet and back to the head. This took approximately 5-12 minutes depending on height and weight of each participant. When the scan was complete, the Velcro straps were removed, and the participant was assisted off the DEXA table.

Data Analysis

Statistical analysis for research question one was conducted two ways. The first method includes using an independent samples t-test, using continuous variables. The second method for research question one is a chi-squares test of homogeneity, based on discrete categorization of sufficiency. The second research question will be analyzed using various correlation coefficients to test for relationships. Finally, the relationships to injuries will be explored with logistic and Poisson regression analysis.

Conclusion

The primary purpose of this project was to compare current blood serum values of vitamin D and calcium in Division I females who participate in Basketball and Soccer at a midsized, NCAA Division I institution. A secondary purpose was to compare the results of the blood serum values to total body bone density by way of a DEXA scanning. With bone injuries common in female athletes, the results of this study may allow clinicians to incorporate supplements into their practice to help avoid musculoskeletal injuries. Overall, this study was used to determine if Division I athletes have low vitamin D and calcium levels, as well as if those correlated to bone mineral density.

CHAPTER 4. MANUSCRIPT

Deficient intake of vitamins and minerals is a common problem in athletes and has the potential to increase the risk of sustaining musculoskeletal injuries (Holick, 2008; Jung, 2018; McCabe, 2012). Vitamin D and calcium are vitally important to the development and function of the skeletal system as well as other processes throughout the body (Wacker, 2013). In addition, limited intake of vitamin D and calcium have been documented as major contributors to decreased bone mineral density levels (Laird, 2010; Reid, 2013). The nutritional status of an athlete plays a critical role in his or her ability to excel in sports (Burke, 1998; Economos, 1993; Leinus, 1998). Thus, ensuring optimal vitamin D and calcium intake is of great importance to ensure peak performance for the athlete, as well as potentially reduce the risk of musculoskeletal injuries.

Calcium, the most abundant mineral in the body, is essential for everyday functions and long-term bone formation and remodeling (Florencio-Silva, 2015). Although the body stores the majority of its calcium in the teeth and bones, calcium is lost through sweat, hair, nails, skin, urine, and feces (Ross, 2011). Because the body is not capable of producing new calcium, it must be incorporated through the daily diet. Optimal levels of circulating calcium are needed to repair micro-damage and inhibit an increase of non-targeted remodeling to maintain serum calcium concentrations (Feng, 2011). Physical activity in the presence of low calcium intake can cause additional stress on bones because of the need to offset the significant calcium loss in sweat. During heavy sweating and low calcium intake, calcium is taken from the bone reservoir. This loss of calcium can weaken the skeleton over short periods (Lappe, 2008; Mahajan, 2017; National Institutes of Health Office of Dietary Supplements, 2016; Parfitt, 2002). It has been suggested that calcium, which is needed for bone mineralization, can be protective against stress fractures (Pepper, 2006). In some studies utilizing participants with limited calcium intake, the researchers reported decreased body weight as well as reduced bone strength, length, weight, and calcium content compared to individuals with adequate calcium intake (Iwamoto, 2003; Kunkel, 1990; Persson, 1993; Peterson, 1995; Peterson, 1992; Thomas, 1991;Thomas, 1988; Welch, 2005). Since physical activity places additional stress on bones and calcium is lost through sweating, it is important for calcium levels to be monitored in athletes to ensure they are healthy and able to compete at optimal levels.

Similar to calcium, vitamin D is critical for injury prevention and enhancing athletic performance by increasing muscle fiber size, reducing inflammation, improving neuromuscular function, and decreasing the risk of stress fractures (The Academy of Nutrition and Dietetics, 2019). Athletes across the world intake an average of 100 IU to 250 IU/day of vitamin D through food; however, the recommended intake of vitamin D for females 14-50 years old is 600 IU/day (National Institutes of Health Office of Dietary Supplements, 2016). Sufficient vitamin D intake is necessary for healthy bones; without vitamin D the body cannot effectively absorb calcium, which is essential for proper bone health. Researchers indicate there is a positive correlation between vitamin D levels and bone mineral density (BMD) and bone mineral content (BMC) in females (Lappe, 2008; Lewis, 2013; Lovell, 2008). In a study of athletes from a variety of sports, Halliday et al. (2011) tracked the vitamin D status of 41 athletes from the University of Wyoming (18 men, 23 women); 12 of these athletes were indoor athletes and 29 were outdoor athletes. Blood samples were collected in the fall (September/October), winter (February/March), and spring (April/May). Additionally, participants answered a vitamin D-specific questionnaire at the same time as the blood draws. During the spring blood draw, body composition and bone density were evaluated using DEXA. Illnesses and injuries that occurred throughout the year were documented by the athletic training staff and team physician. Vitamin D status changed significantly across time (p = .001) and averaged 49.0 ± 16.6 ng/mL in the fall, 30.5 ± 9.4 ng/mL in the winter, and 41.9 ± 14.6 ng/mL in the spring. The number of athletes who were vitamin D insufficient increased greatly during the winter months. However, the number of athletes who were vitamin D deficient was highest during the spring. Similar to athletes, members of the military perform physically demanding tasks that place additional stress on the bones. One study by Lappe et al. (2008) examined the prevalence of stress fractures in 3,700 female Navy recruits' when given vitamin D and calcium supplementation. The recruits were divided equally into a treatment group (200 mg calcium 800IU vitamin D/day) and control group (placebo). At the end of the eight-week study the recruits in the treatment group had 20% lower incidence of stress fracture than the control group (5.3% verse 6.6%, respectively; p = 0.026). Conclusively, vitamin D levels are of great importance to athletes due to the fact that vitamin D increases muscle fiber size, reduces inflammation, and aids in improving bone mineral density.

Geographic location is one factor that affects the amount of vitamin D the skin can produce. The amount of UV exposure necessary to synthesize an adequate amount of vitamin D depends on UV index, season, and latitude (Freedman, 2013). The UV index determines the risk of overexposure to UV radiation and compares it with ozone concentrations, altitude, cloud coverage, time of day, and wavelength (D'Orazio, 2013). The further from the equator, the greater the angle the sun will hit the atmosphere, which leads to less UV availability (Wacker, 2013). In a systematic review and meta-analysis of 23 studies, researchers examined differences between vitamin D content in the blood in different geographical locations. In this study, the researchers reported that 54% of the 2,313 athletes had insufficient vitamin D levels, which varied by geographical location (Farrokhyar, 2015). The researchers explored how latitude effects vitamin D status in athletes across the world and explained the risk for inadequacy of vitamin D was slightly higher for latitudes of > 40° N (RR 1.14 95% CI [0.91-1.44)]. However, the risk increased considerably after removing the Middle East, which was an outlier [RR 1.85 (1.35-2.53)]. Furthermore, there was a significant risk of inadequacy of vitamin D for indoor sports' participants living in latitudes of ≥40° (RR 1.19; 95% CI [1.09-1.30]). The risk of vitamin D inadequacy increased for athletes who participated in winter and spring sports (RR 1.85; 95% CI [1.27-2.70]). Inadequacy leads to musculoskeletal injuries, which may impede athletic performance (Hamilton, 2011). However, because Division I women's soccer in the United States is a fall sport, which is active from August through November, the geographical location of the Midwest restricts the amount of vitamin D that athletes can produce despite participating in an outdoor sport.

In contrast to outdoor sports, indoor sports restrict the opportunities for vitamin D metabolism due to being held indoors. Researchers in previous studies have examined vitamin D insufficiency and deficiency in indoor sports; the results indicated that in four studies with a total of 366 indoor athletes, 101 (27.5%) were vitamin D deficient and 153 (41.8%) were insufficient (Griescholber, 2018; Kenny, 2017; Lovell, 2008; Orysiak, 2018). The season for Division I women's basketball is October through April (Levinson, 2018). Therefore, the majority of their season takes place when the UV exposure is limited, thus limiting their vitamin D. Because women's basketball takes place indoors and during a time of limited UV exposure, monitoring nutrient levels in these athletes could help decrease injury risk by ensuring nutrient levels are adequate for the workload being performed.

However, even with the knowledge on the importance of vitamin D and calcium, the research lacks on its relationship to current levels and injury predisposition in Division I female athletes, especially soccer and basketball. The literature on female basketball players is nonexistent in terms of vitamin D, calcium, and total BMD. There are few researchers that have studied female soccer players, yet, there is still a gap in the literature using Division I soccer players in the Midwest, examining vitamin D, calcium, and total BMD. Furthermore, the comparison of the relationship of blood serum values between indoor and outdoor athletes is not well recognized.

Vitamin D and calcium are vital for the development and function of the musculoskeletal system. Because Midwest and indoor athletes have inadequate vitamin D levels, more research needs to be conducted specific to female athletes to ensure optimal performance in their respective sports.

Methodology

A total of thirty collegiate female athletes (basketball (*n*=9) and soccer (*n*=21) aged 18-24 years (19.53 ± 1.105 years), who were on the roster for a midsized, Division I NCAA institution, were recruited for this study. During recruitment at respective team meetings, the aim of the study, procedures, benefits, and possible risk factors were discussed. It was explained in the initial recruitment meetings that a pregnancy test would be administered because it was a contraindication to be pregnant and be exposed to a DEXA scan. In addition, all data were deidentified to all coaches and athletic trainers. Athletes were told they could share their individual results, but the research team blinded the data to reduce coercion on the part of any coaches.

A written consent form approved by the institutional review board of North Dakota State University was obtained from each participant when they attended their assigned data collection time. Participants also completed a self-reported demographics questionnaire that examined supplement use, fracture history, oral contraception, tanning bed exposure, and the use of sunscreen. Next, a phlebotomist employed by a local hospital collected blood samples using a single venipuncture, which allowed the phlebotomist to obtain separate vials of blood to be analyzed for vitamin D and calcium content.

All blood serum was analyzed by a local hospital for vitamin D and calcium values in accordance with the Clinical Laboratory Improvement Amendment (CLIA) accreditation. The hospital's alias for the vitamin D was 25-OH Vitamin D. The hospital used a Chemiluminescent Microparticle Immunoassay (CMIA), which is the modified and advanced form of enzyme linked immune Sorrbant Assay (Ilyas, 2014). The tubes contained serum gel and collected a minimum of 0.50 mL of blood. Arsenazo III Calcium Dye Complex was used for calcium analysis. Another test tube collected 0.5 mL of blood and had Lithium Heparin gel which acts as an anticoagulant. The hospital separated plasma from cells within two hours of the draw. Analysis of the blood serum was conducted by the CLIA-approved hospital and all results were reported to the research team with the deidentified patient information.

Following the blood draws, participants provided a urine sample for the pregnancy test (ClinicalGuard® HCG Strips). If the pregnancy test was negative, participants were able to complete the DEXA scan. No participants were excluded from the analysis based on the results of the pregnancy test. For the scan, participants removed all jewelry, heavy clothing, shoes, and socks. A research team member who was trained to use the DEXA helped position the participant on the scanner. Two Velcro straps were placed on the lower limbs to help keep the lower body in correct position during the scan. Participants were asked to remain still as the scanning arm moved from the top of the head to the feet and back to the head. This took approximately 5-12 minutes depending on height and weight of each participant. When the scan was complete, the Velcro straps were removed, and the participant was assisted off the DEXA table. The DEXA provided immediate results for bone mineral density and bone mineral content of the head, arms, legs, trunk, ribs, spine, pelvis, and total body.

Statistical Analysis

Correlation analysis was used to describe several within subject relationships among measured variables. Pearson correlation coefficients were calculated between BMD and calcium, as well as BMD and Vitamin D. Similarly, analysis explored the relationship between self-reported fractures and birth control with the same two factors of calcium and Vitamin D. All of these statistics were calculated for the overall sample and also for the two sub-samples of soccer and basketball players. Additionally, a regression model was estimated with total BMD as the dependent variable and calcium, Vitamin D, birth control, and sport as independent predictor variables. Finally, a regression model was estimated with total BMD as the dependent variable and calcium, Vitamin D, birth control, and sport as independent predictor variables.

Results

Demographic information for all participants is depicted in Table 4.1 below. Table 4.1 represents both averages and frequencies.

Table 4.1

Demographics of Study Population

	Soccer (*n*=21)	Basketball (*n*=9)
Age (years)	M=19.52 ± 1.21	M=19.55 ± 0.88
Height (cm)	M=66.67 ± 2.71	M=70.13 ± 3.44
Weight (kg)	M=64.04 ± 13.01	M=70.31 ± 22.63
Total experience in the sport (years)	M=14.57 ± 2.93	M=11.49 ± 2.93
Number of Caucasian	19 (90%)	8 (89%)
Number of African American	2 (10%)	1 (11%)
Number of Freshman	4 (19%)	2 (22%)
Number of Sophomores	7 (33%)	3 (33%)
Number of Juniors	4 (19%)	3 (33%)
Number of Seniors	6 (29%)	1 (11%)
Tanning bed use	0 (0%)	0 (0%)
Sunscreen use	11 (52%)	4 (44%)
Calcium supplementation	1 (5%)	3 (33%)
Vitamin D supplementation	2 (10%)	2 (22%)
Fish oil supplementation	1 (5%)	0 (0%)
Birth control	10 (48%)	0 (0%)

The relationship between total BMD and both calcium and vitamin D blood serum levels was assessed with Pearson correlation coefficients, and associated *p*-values, which appear in Table 4.2. For the overall sample, the relationship is minimal. For basketball players, the correlation between BMD and measured calcium is negative, indicating that as calcium levels were higher total BMD was lower. Although, not statistically significant given the small sample size (p = .091). For vitamin D, the reported correlation is positive for soccer players but negative for basketball players. Signifying for basketball as vitamin D levels was high, total BMD was lower.

Table 4.2

Correlations with BMD

	Calcium	Vitamin D
Overall	-0.079	0.007
	(.689)	(.972)
Soccer	-0.001	0.171
	(.997)	(.484)
Basketball	-0.635	-0.377
	(.066)	(.317)

Correlations were also calculated between number of self-reported fractures and levels of calcium and vitamin D. Results appear in Table 4.3 with p-values appearing in parentheses. None of the correlations were statistically significant, but the largest coefficients were negative.

Table 4.3

Correlations with Reported Fractures

	Calcium	Vitamin D
Overall	-0.121	-0.243
	(.540)	(.213)
Soccer	-0.094	-0.135
	(.702)	(.582)
Basketball	0.068	-0.654
	(.862)	(.056)

Correlations were also estimated between total BMD and birth control. For the overall sample, the correlation was -.128 (p=.516), and for soccer players the correlation was -.084 (p=.732). Therefore, as use of birth control increased, total BMD increased. However, we did not specifically ask the type of birth control the participant was taking. Therefore, we cannot conclude if it had hormones or not. None of the basketball players reported taking birth control.

Finally, a regression model was estimated with total BMD as the dependent variable and calcium, Vitamin D, birth control, and sport as independent predictor variables. The model was not statistically significant ($F[4, 24]=.316$, $p=.864$). BMD does not appear to be altered substantially by any of the independent measures. In particular, the difference between an indoor and outdoor sport does not affect BMD ($t[26]=.71$, $p=.484$).

Discussion

Given the established role of vitamin D and calcium in bone health, immunity, and inflammation, it is imperative that coaches, athletic trainers, and athletes routinely asses the levels of vitamin D and calcium to make appropriate dietary recommendations and changes. Vitamin D deficiency is recognized as a worldwide epidemic, with athletes not being spared (Hamilton, 2011). Recent research has provided evidence suggesting that maintaining adequate vitamin D and calcium may reduce injuries (Owens, 2018). Furthermore, research is needed specifically regarding female athletes to determine the extent in which vitamin D and calcium can improve athletic performance.

Interestingly, the vitamin D recommendation for an individual is not standardized across all organizations, which can lead to confusion for the lay population. In fact, even many recognized, legitimate organizations recommend various levels of vitamin D. Table 4.4 displays the differences in the recommended vitamin D levels from five separate organizations, indicating the discrepancies among the organizations for deficient, insufficient, sufficient, and toxic vitamin D levels.

Table 4.4

Recommended Vitamin D Levels

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Academy of Nutrition and Dietetics	Institute of Medicine, Food and Nutrition
Deficient	0-30 ng/mL	0-20 ng/mL	0-11 ng/mL	<20 ng/mL	<12 ng/mL
Insufficient	31-39 ng/mL	21-29 ng/mL	12-20 ng/mL	20-30 ng/mL	12-20 ng/mL
Sufficient	40-80 ng/ml	30-100 ng/mL	>20 ng/mL		≥20 ng/mL
Toxic	>150 ng/mL				>50 ng/mL

The local hospital that completed the vitamin D analyses used the reference range value for sufficient vitamin D of 27-80 ng/mL. The local hospital categorized the athletes and indicated that all but three had sufficient vitamin D levels. The vitamin D level ranged from 23-95 ng/mL with an average of 46.73 ng/mL (*SD* = 14.03). If we used the recommendations from Table 4.4 for vitamin D, the participants included in this study could be in a different category of sufficiency based on which organization's recommendations. Table 4.5 organizes the results of our participants' vitamin D levels according to the organization's parameters. The results indicate that if we used the Vitamin D Council, two athletes would be considered in the "Deficient" category and 18 in the "Insufficient" category. Another key difference is that our results only showed one athlete in the toxic level. Tables 4.6 and 4.7 organize the results of our participants' vitamin D levels according to the organization's parameters by sport.

Table 4.5

Athletes Vitamin D Levels based on Different Organizations

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Academy of Nutrition and Dietetics	Institute of Medicine, Food and Nutrition	Local Hospital
Deficient	2	0	0	0	0	0
Insufficient	8	2	0	2	0	2
Sufficient	19	29	31		21	27
Toxic	1	0			10	1

Table 4.6

Soccer Athletes Vitamin D Levels based on Different Organizations

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Academy of Nutrition and Dietetics	Institute of Medicine, Food and Nutrition	Local Hospital
Deficient	2	0	0	0	0	0
Insufficient	6	2	0	2	0	2
Sufficient	13	19	21		15	19
Toxic		0			6	

Table 4.7

Basketball Athletes Vitamin D Levels based on Different Organizations

	Vitamin D Council	Endocrine Society	Food and Nutrition Board	Academy of Nutrition and Dietetics	Institute of Medicine, Food and Nutrition	Local Hospital
Deficient	0	0	0	0	0	0
Insufficient	2	0	0	0	0	0
Sufficient	6	9	9		6	8
Toxic	1	0			3	1

Having an understanding of the recommendations of all of the organization is vital. If allied health care professionals or coaches are concerned about the vitamin D of an athlete, they should not solely rely on the data from the hospital to make a determination of their athlete being healthy. Based on the results reported from the local hospital, the majority of our athletes are considered to be in a healthy range, however, based on the recommendations from the Vitamin D Council, a large proportion of our athletes do not meet recommended levels, which could impede their ability to perform at the highest level.

Our methodology is similar to a study conducted by Bloom et al. (2010) that assessed the differences between indoor and outdoor athlete's vitamin D levels. Bloom et al. (2010) conducted the study using participants who were female NCAA Division II volleyball, softball, or gymnastics athletes. A total of 36 athletes completed the study including outdoor athletes (n=19) and indoor athletes (n=17). The reports of the three-day food recall indicated there was no significant difference in vitamin D intake for indoor and outdoor athletes (indoor: 1.8 ± 1.2, outdoor: 1.4 ± 1.1 μg/d) (Bloom, 2010). However, 36% of the athletes who participated in this study had vitamin D serum concentrations equal to or less than 40 ng/mL, which was considered insufficient for this study. In comparison to our results, 6% of our athletes were considered insufficient. It is important to note that the recommended amount considered sufficient in this study was ≥ 40 ng/mL, which is high compared to the various organizations listed above.

Researchers (Grieshober, 2018) who had similar vitamin D findings as Bloom et al. (2010), albeit in a different population, used the Endocrine's Society Guidelines to categorize 279 National Basketball Association (NBA) athletes who participated in the 2009-2013 Combine. The Endocrine Society provides the following range for sufficient and deficient blood serum values: <20 ng/mL and sufficient levels as >30 ng/mL. Grieshober et al. (2018) reported 32.3% of participants were deficient and 41.2% as insufficient. Although similar findings as Bloom et al. (2010), it is again worth noting that the guidelines for categorizing athletes differed. The results of our study differed significantly from either Grieshober et al. (2018) and Bloom et al. (2010); however, our range for sufficiency was much larger.

Similar to vitamin D, calcium recommendations for an individual are not standardized by all organizations. However, calcium recommendations are separated by ages rather than deficient, insufficient, sufficient, and toxic. The RDA and Dietary Reference Intakes (DRI) both have the same recommendations in comparison to the National Osteoporosis Foundation whose recommendations are lower for ages 9-18 and higher than RDA and DRI for ages 19-50. The Dietary Reference Intake (DRI) is the general term for a set of reference values used to plan and assess nutrient intakes of healthy people (National Institutes of Health Office of Dietary Supplements, 2016). The RDA is the average daily level of intake sufficient to meet the nutrient requirements of nearly all healthy people (National Institutes of Health Office of Dietary Supplements, 2016). Table 4.8 displays the differences in the recommended calcium levels from three separate organizations. The table also illustrates the discrepancies among each organization by age. The local hospital that did the calcium analyses used the reference range values of 0-1 years: 8.4-11.1 mg/dl, 1-18 years: 9.1-10.6 mg/dl, and adult: 8.4-10.5 mg/dl.

Table 4.8

Recommended Calcium Levels

	RDA (mg)	DRI (mg)	National Osteoporosis Foundation (mg)
9-13 years old	1,300	1,300	1,000
14-18 years old	1,300	1,300	1,000
19-50 years old	1,000	1,000	1,200

When the results were analyzed by age based on the local hospital parameters, we found that for soccer athletes ages 1-18 years old, four were in the recommended reference range values for calcium and two were below the recommended reference range values. For soccer athletes 19 and older, there were 15 out of the 30 athletes in the recommended reference range values for calcium. For basketball athletes ages 1-18, only one participant was in the recommended reference range; the remaining basketball players were 19 and older and all eight were in the recommended range for calcium.

The results of the study we conducted found that there was no correlation between vitamin D, calcium, and total BMD. Our results contrast those of a study by Recker et al. (1992), which indicated bone density was positively correlated with calcium intake and physical activity ($r=.31$; $p = .004$). This discrepancy may be in part because the Recker et al. methodology included participants completing nine visits every six months. This is in contrast to our study, which only had a single set of measurements for vitamin D, calcium, and total bone mineral density. Overall, there were differences in the methodology used in our study compared to other studies, which could lead to the discrepancies in results. However, more research should be conducted on female athletes, vitamin D, calcium, and total BMD.

When analyzing the relationship between fracture history and vitamin D and calcium levels, our results did not show a statistically significant correlation. However, Lappe et al. (2008) examined vitamin D, calcium, and stress fractures on 3,700 female Naval basic trainees during a 24-month recruitment period. Participants completed a risk factor questionnaire as well as maintained a record of menstrual periods to ascertain amenorrhea and use of contraception throughout the study. Participants were separated into two groups the control (placebo) and the treatment group (2000 mg calcium and 800 IU vitamin D/day). The results of the study indicated that participants who used depomedroxyprogesterone oral contraception had a 48% greater risk of fracture than non-users. However, there was no significant difference in fracture incidence between those who used contraceptive types and nonusers. The results also revealed the treatment group had a 20% lower incident of stress fractures than the control group. This is similar to Nieves et al. (2010) who reported that women who consumed less than 800 mg of calcium a day had approximately six times the stress fracture rate of women who consumed more than 1,500 mg. Therefore, there are studies that show the relationship between increasing vitamin D and calcium and reducing the fracture rate in females. More research should be conducted to examine if vitamins and minerals of female athletes are correlated to stress fractures.

Our study indicated that outdoor sports compared to indoor sports had no effect on vitamin D, calcium, or total BMD. In contrast to our study where we found no correlation, the Maruyama-Nagao et al. (2016) study found that vitamin D concentrations were lowest in March and highest in September for both indoor and outdoor athletes. Yet, the concentrations levels of vitamin D were lower in the Maruyama-Nagao et al. (2016) study for indoor athletes compared to outdoor athletes. One of the potential reasons we did not have the same results could be due to the fact that we only did measurements in August. Our results differed from theirs in terms of being low in March and high in September because we only measured vitamin D in August. The results may have been different in late fall and spring.

Our results indicated oral contraception had no effect on BMD. Nevertheless, previous studies have shown that oral contraception reduces the rate of stress fractures. Amenorrhoeic runners who took oral contraception for at least six months gained more spine BMD and whole-body BMC than amenorrhoeic runners who did not take oral contraception (Cobb, 2007; Hergenroeder, 1997). Conversely, researchers have concluded that combined hormonal contraceptives (CHC) had significantly less positive adjustment BMD change at the total hip after two years (Brajic, 2018). By contrast, adjusted femoral neck 2-year BMD changes were significantly more positive in e-CHC users compared to n-CHC users (Brajic, 2018). Because oral contraceptives play a critical role in numerous processes throughout the body, the current research in this field is expansive, however, research regarding oral contraceptives in female soccer and basketball players is limited. Interestingly, no basketball players in our study reported taking oral contraception.

Athletes depend on a healthy and complete diet to have adequate nutrients required to promote and uphold physical performance in their sport and protect against injuries. Approximately 13.5% of the world has inadequate levels of vitamin D (Holick, 2007). Vitamin D is a crucial component of muscle function, muscle strength, muscle mass, as well as BMD (Ceglia, 2009). Muscle and bone health are important for the athletic population to perform at their peak level. Calcium is another nutrient that is essential to help bones and for nervous system function, muscle contraction, and secretion of hormones. Yet, despite all the information supporting the importance of vitamin D and calcium for optimal performance, we have yet to establish a common set of guidelines by which allied healthcare professionals can base decisions and nutritional guidance.

Evidence supports the role for vitamin D and calcium in skeletal muscle with potentially significant impacts on the performance and injury risk of athletes (Hamilton, 2011). Further research is indicated to evaluate the required levels of vitamin D and calcium for optimal musculoskeletal function due to the discrepancies among organizations. Meanwhile, clinicians and coaches working with athletes should be aware of the impact vitamin D and calcium deficiency have on the athlete. The results of our research do not show a correlation with vitamin D, calcium, and BMD. However, the results of our study should serve as a pilot to future researchers interested in comparing indoor and outdoor athletes as well as a focus on female, Division I athletes. Therefore, future research on vitamin D, calcium, and BMD should focus on soccer and basketball athletes and do a follow-up analyses mid- and post-season to consider the differences over an entire calendar year. Furthermore, given the different recommendations for vitamin D, future researchers should consider the recommendation levels they use for their particular study.

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