A QUASI-EXPERIMENTAL TRIAL OF THE EFFECTS OF CHOLECALCIFEROL SUPPLEMENTATION ON VITAMIN D STATUS AMONG A DIVERSE POPULATION OF COLLEGIATE BASKETBALL ATHLETES

by

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A Quasi-Experimental Trial of the Effects of Cholecalciferol Supplementation on Vitamin D Status Among a Diverse Population of Collegiate Basketball Athletes

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

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DEDICATION

Affectionately dedicated to Dad, Mom, Kendall, Skittles, and Shade.

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LIST OF ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D, nmol/L
$25(OH)D_2$	25-hydroxyvitamin D2
$25(OH)D_3$	25-hydroxyvitamin D3
aBMD	Areal Bone Mineral Density
ANOVA	One-Way Analysis of Variance
AT	Athletic Trainer
BF%	Body Fat Percentage, %
BM	Body Mass, kg
BMC	Bone Mineral Content
BMI	Body Mass Index
BMD	Bone Mineral Density, g/cm
CI	Confidence Interval
DBP	Vitamin D Binding Protein
DRIs	Dietary Reference Intakes
DSAM	Dietary Supplement Assessment Module
DSQ	Dietary Screener Questionnaires
DXA	Dual X-Ray Absorptiometry
FM	Fat Mass, kg
FFM	Fat Free Mass, %
FFQ	Food Frequency Questionnaire
Hx	History
IT	Illiotibial
IU	International Units
LBM	Lean Body Mass, kg
MBB	Men's Basketball
NCC	Nutrition Coordinating Center
NDSR	Nutrition Data Systems for Research
NFL	National Football League
NHANES	National Health & Nutrition Examination Survey
NIH	National Institutes of Health
RDA	Recommended Daily Allowance
RR	Risk Ratio

SD	Standard Deviation
SEQ	Sun Exposure Questionnaire
SPF	Sun Protection Factor
T1	Time Point 2: Pre-Season; October
T2	Time Point 3: Post-Season; March-April
WBB	Women's Basketball
USDA	United States Department of Agriculture
Yrs	Years of Age

ABSTRACT

A QUASI-EXPERIMENTAL TRIAL OF THE EFFECTS OF CHOLECALCIFEROL SUPPLEMENTATION ON VITAMIN D STATUS AMONG A DIVERSE POPULATION OF COLLEGIATE BASKETBALL ATHLETES

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Background: Vitamin D is a fat-soluble micronutrient commonly found to be clinically deficient in a young, athletic, and otherwise healthy population. A gap remains in scientific literature pertinent to why this discrepancy exists, if particular modalities perpetuate this discrepancy and in what quantity vitamin D_3 supplementation beneficially affects vitamin D status.

Objective: To assess the prevalence of vitamin D insufficiency in a diverse sample of collegiate basketball athletes and to define the required dosage of vitamin D_3 supplementation in order to beneficially affect serum 25-hydroxyvitamin D (25(OH)D), the major circulating metabolite in the human body indicative of vitamin D levels and consequently, one's current status.

Design: This was a quasi-experimental vitamin D intervention trial. Participants were allocated to one of three groups based on their baseline vitamin D status as follows: insufficient (<75 nmol/L) were allocated to 10,000 IU of vitamin D_3 daily, sufficient (75-125 nmol/L) to 5,000 IU of vitamin D_3 daily, and optimal (>125 nmol/L) to no supplementation. Baseline assessments were completed at the beginning of pre-season training and at ~5 months follow-up at post season. Demographics, body composition via dual x-ray absorptiometry, skin pigmentation via spectrophotometer and blood sampling for the assessment of serum 25(OH)D were completed.

Results: The majority of participants (n=13) were allocated to the high dose supplementation group (10,000 IU daily) vs. n=5 allocated to 5,000 IU daily and n=2 to no supplementation. Overall, 77% of participants allocated to the high dose supplementation group (10,000 IU daily) were male (p=0.005), with olive to dark skin tone (p=0.022), and 85% self-reported as African American (p=0.027). Differences among groups were noted for whole body BMD Z-score (p=0.027) and lean body mass (p=0.004). A dose-response emerged in regard to the change of 25-hydroxyvitamin D concentrations from baseline to follow-up, wherein the 10,000 IU daily group exhibited the greatest change in 25(OH)D concentrations (35.01± 26.96 nmol/L) vs. the 5,000 IU daily group (-9.34± 9.62 nmol/L) and the no supplementation group (-41.57± 11.66 nmol/L, p<0.01). Among those allocated to 10,000 IU daily group, 3 (23%) remained insufficient, 9 (69%) climbed to sufficient status and 1 (8%) reached optimal status at follow-up. No participant in the 5,000 IU group reached optimal status and one of the two participants in the no supplementation group remained at optimal status at follow-up. A

significant correlation between the change in 25(OH)D concentrations was observed with baseline 25(OH)D (r_s=-0.78, p=0.01) as well as with lean and fat mass percent r_s=0.83 and r_s=-0.80 respectively, p=0.01).

Conclusion: A dosage of 10,000 IU of vitamin D_3 supplementation taken daily and allotted sufficient time to see marked improvement will help to mitigate the high prevalence of vitamin D deficiency among collegiate basketball players by beneficially impacting serum 25(OH)D levels. However, this dosage was not enough for all participants to reach vitamin D sufficiency. Further research encompassing a larger sample size of indoor, male and female collegiate athletes with varying degrees of adiposity is needed. Improving vitamin D status may have the potential benefits in maximizing sports performance and efficaciously reduce injury risk among collegiate athletes.

CHAPTER 1: LITERATURE REVIEW

Introduction

Micronutrient deficiency in a young, athletic and otherwise healthy population has reached pandemic proportions, specifically in regard to vitamin D status.[1] Contributing to this disparity is the lack of scientific research to support viable treatment and maintenance protocols. Concurrently, the effect of vitamin D on athletic and exercise performance continues to be a matter of debate as emerging literature has produced conflicting findings.[2] Novice literature supports a positive correlation between sufficient circulating 25-hydroxyvitamin D (25(OH)D) and musculoskeletal health in athletes, particularly relevant to an athletic population with heightened physical and physiological demands.[3]

Vitamin D Physiology and Sources

Vitamin D is a fat-soluble micronutrient that occurs in two dietary forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Though similarly metabolized, vitamin D_2 derives from a plant sterol known as ergosterol while vitamin D_3 originates from 7-dehydrocholesterol, a precursor of cholesterol after synthesis in the skin.[4,5] The biologically active hormone form of vitamin D is 1,25-dihydroxyvitamin D, abbreviated

[1,25$(OH)_2$D]. Vitamin D has two potential absorption routes: skin synthesis facilitated by sunlight or dietary ingestion. Vitamin D (calciferol) functions include cellular metabolism and aiding in intestinal absorption of micronutrients like calcium and phosphorus, that subsequently contribute to proper bone health.[4,5] Vitamin D deficiency, also known as hypovitaminosis D, can become detrimental to bone and musculoskeletal health. This concern is of utmost importance to highly trained athletes whose sports necessitate strenuous, physiological demands. Conversely, too much vitamin D, also referred to as hypervitaminosis D, can disturb calcium homeostasis and subsequently lead to increased blood calcium. This altered state can cause the calcification of soft tissues, including certain organs and blood vessels.[4,5]

Endogenous Sources

The major source of vitamin D is provided through interaction of the skin-more specifically, the deep layer below the epidermis called the dermis - with ultraviolet beta (UVB) light. UVB light is a medium-wavelength, biologically active radiation type with the ability to superficially penetrate the skin.[6] In the dermis, 7-dehydrocholesterol is converted into previtamin D_3 by UVB radiation. 7-dehydrocholesterol is provitamin found in the skin and synthesized from dietary cholesterol.[4] The previtamin D_3 is slowly isomerized into vitamin D_3 (called cholecalciferol) and then bound by vitamin D binding protein (DBP) and transferred to the liver with its metabolites. Once in the liver, the cholecalciferol is hydroxylated to 25- hydroxyvitamin D (25(OH)D), referred to as calcidiol, which is the major circulating metabolite in the human body. Calcidiol is then

further metabolized in the kidney to its biologically active form called calcitriol (1,25-dihrdroxyvitamin D). Other important factors that facilitate this process include calcium, phosphate, and parathyroid hormone levels. Both vitamin D_2 and vitamin D_3 represent viable options for oral supplementation, however, research suggests that vitamin D_2 is approximately 30% as effective as vitamin D_3 in achieving desired serum levels. [7,8] According to the National Academy of Medicine, several factors can limit the skins ability to synthesize vitamin D efficiently. Such examples include: the usage of sun screen, high levels of skin melanin (dark pigmentation) in the skin, the distance one is from the Equator, the time of day, and the season of the year.[5]

Exogenous Sources

While skin synthesis disproportionately contributes to vitamin D status to a higher extent than exogenous sources such as dietary intake, for dietary vitamin D, primary sources are a viable option.[9,10] Naturally occurring vitamin D is available through limited exogenous, dietary sources. Such examples include the fatty fish flesh, some fish-liver oils, and eggs from hens fed vitamin D.[5] Conversely, many foods such as milk products, breakfast cereals, and some fruit juices are vitamin D fortified.[5] In addition to naturally occurring sources and fortified sources, vitamin D can also be supplemented. According to the National Academy of Medicine, the median supplement dose is currently set at for vitamin D at 400 IU.[5]

VITAMIN D FUNCTIONS RELEVANT TO THE ATHLETE

Bone Health

Strenuous activity and the physicality of collegiate sports leaves highly trained athletes susceptible to a myriad of injuries including stress fractures. Vitamin D status is directly indicative of bone health, specifically of adequate calcium absorption and bone mineralization.[11] Vitamin D affects bone health physiologically through the bone remodeling process. Vitamin D regulates the osteoclast and osteoblast activity-the functional mediators of proper remodeling. This process is of particular importance to athletes who consistently undertake mechanical loading that is associated with an increase in bone mineral density (BMD) during training.[11] Research suggests that any training-induced increase in body mass aids in the process of bone remodeling and thus, proper bone structure. In athletes with poor bone health as a result of poor vitamin D status, the stimulus of loading the musculoskeletal system through high-intensity dynamic sporting activity is proposed to compensate for 25(OH)D deficiency.[11] However, like the ability of vitamin D to influence muscle function beneficially, optimum levels of serum 25(OH)D for the best possible skeletal and bone health remain a controversial topic of debate.

Muscle Function

Whether or not vitamin D has the capacity to have any measurable effect on skeletal muscle function remains a topic of debate. Particularly in young, trained athletes the available data on this topic remains both limited and highly underpowered.[11] Some research does exist, though, attempting to mitigate this discrepancy. Wyon et al. (2014)

conducted a controlled study intending to determine the influence of vitamin D supplementation during the winter months on both muscle function and injury occurrence within a cohort of elite classical ballet dancers wherein 24 elite classical ballet dancers (intervention n = 17; control n = 7) were supplemented with oral vitamin D3 of 2,000 IU per day for the duration of 4 months.[12] The team measured variables including isometric muscular strength and vertical jump height at two intervention points, pre and post intervention. Injury occurrence during the intervention period was also recorded by the in-house medical team. As a result, they found a significant increase[12] within the intervention group regarding isometric strength (18.7%, $p< 0.01$) and vertical jump (7.1%, $p < 0.01$). The intervention group also sustained significantly less[12] injuries when compared to the control group over the duration of the study period ($p < 0.01$). In conclusion, the authors summarized their results with the assertion that oral supplementation of vitamin D3 during the winter months has beneficial effects on muscular performance and injury occurrence in elite ballet dancers.[12]

Vitamin D status may also correlate with musculoskeletal health in populations other than athletes. Vitamin D supplementation may be a key factor in symptomatic treatment of musculoskeletal pain and weakness.[13,14] Biscoff-Ferrari et al. (2004) investigated the association between 25(OH)D concentrations and lower extremity functionality in an ambulatory elderly population between the ages of 60-90[13] Concurrently, they were interested in deciphering whether or not this perceived association would vary based on activity level. Conclusively, they found a significant positive association[13] between 25(OH)D concentrations and lower-extremity

functionality. This finding remained consistent after adjustments for potentially confounding variables including: age, sex, race or ethnicity, use of a walking aid, BMI, number of comorbid conditions, self-reported arthritis, month of assessment, and activity level.[13] Lower-extremity function improved continuously with higher 25(OH)D concentrations throughout the reference range.

Similarly, Houston et al. (2007) conducted a study with the objective of assessing whether or not an association existed between vitamin D status and physical performance in an elderly population. Within an representative sample of 976 persons aged 65 or older, physical performance was quantitatively assessed using short physical performance battery and handgrip strength as its variables. [14] As a result, approximately 28.8% of women and 13.6% of men had vitamin D levels indicative of deficiency (serum 25(OH)D < 25.0 nmol/L) and 74.9% of women and 51.0% of men had vitamin D levels indicative of vitamin D insufficiency (serum 25(OH)D < 50.0 nmol/L).[14] Furthermore, Vitamin D levels were significantly associated with both performance battery score (β coefficient [standard error (SE)]: 0.38 [0.18], $p = 0.04$) and handgrip strength in men (2.44 [0.84], $p < 0.01$) and women (1.33 [0.53], p =0.01).[14] While these studies pertain to a different population than collegiate athletes, literature continues to supports the protective effect of vitamin D on bone health, specifically fractures, a potentially season-ending injury in collegiate sports. This has been attributed to the established benefit of vitamin D pertinent to calcium homeostasis and bone mineral density. Emerging literature also supports an alternative explanation to these benefits that include that vitamin D affects factors

directly related to muscle strength and function thus reducing fracture risk through fall prevention.[13]

Muscle Strength

Maintenance of muscle strength is an emerging function of vitamin D. Emerging literature supports that musculoskeletal injuries-including tendonitis, a ligament sprain, muscle/tendon strain or muscle tear-sustained may be negatively correlated with lower vitamin D status in athletes.[7] Owens et al. (2018) conducted a randomized controlled trial to distinguish whether or not vitamin D played a beneficial role in skeletal muscle repair and remodeling in an athletic population. As a result, the laboratory found that elevating serum 25(OH)D concentrations to >75 nmol/L with supplemental vitamin D_3 at 4000 IU/day has a positive effect on the recovery of force following a bout of damaging eccentric exercise.[11] Authors observed similar findings in correlative studies between serum 25(OH)D and force recovery following intense exercise. These results suggest that adequate vitamin D exposure can optimize the acute adaptive response to damaging physical work.

Agergaard et al. (2015) conducted a randomized controlled trial in 2015 intending to decipher if vitamin D intake during resistance training improves the skeletal response in two demographics: young and elderly men.[15] The subjects of the study included healthy untrained young ($n = 20$, age 20–30) and elderly ($n = 20$, age 60–75) men. The two demographics were then randomized to a 16-week intervention or placebo group of either 1,920 IU of vitamin-D in addition to 800 mg calcium or 800 mg calcium (placebo

group). Additionally, they performed the study at a period and at a latitude of low sunlight (December-April in Copenhagen, Denmark, 56°N). To test muscular response, during the last 12 weeks of the supplementation the subjects underwent progressive resistance training of the quadriceps muscle. After which, muscle hypertrophy and isometric strength were measured. As a result, while the researchers found no additive effect of vitamin D intake during 12 weeks of resistance training on either whole muscle hypertrophy or muscle strength yet, improved muscle quality in elderly and fiber type morphology in young were observed, indicating a positive effect of vitamin-D on skeletal muscle remodeling.[15]

Hildenbrand et al. (2016) conducted a cross-sectional study of 103 collegiate, male and female athletes residing in the southern United States from three separate NCAA athletic programs.[16] Athletes from three different universities, representing 12 NCAA collegiate sports (8 women's and 4 men's teams) participated in the intervention. 66% percent of the population competed in NCAA Division II, while 34% competed in NCAA Division I athletics.[16] Anthropometric data, dietary vitamin D and calcium intake, sun exposure data, serum 25(OH)D were collected. Researchers also utilized physical performance measures including the following tests: Vertical Jump Test, Shuttle Run Test, Triple Hop for Distance Test and the 1 Repetition Maximum Squat Test[16] in order to determine the influence of vitamin D status on muscular strength and anaerobic power. The findings of this intervention indicate that with decreasing 25(OH)D, there is a concurrent decrease in performance scores ($p < 0.01$) representing measurable indicators of muscle strength and anaerobic power. Specifically, a decrease of 15% for the Vertical

Jump Test, 18% for the Shuttle Run Test, 80% for the Triple Hop for Distance Test, and 77% for the 1 Repetition Maximum[16] Researchers also explain their results in respect to odds ratios for serum 25-OH D in relation to performance measures: 0.85 (95% CI 0.03–0.24) for the Vertical Jump Test, 0.82 (95% CI 0.03–0.25) for the Shuttle Run Test, 0.20 (95% CI 0.03–0.11) for the Triple Hop for Distance Test, and 0.23 (95% CI 0.04–0.42) for the 1 RM Test.[16] Similarly, von Hurst et al. (2013) found a significant correlation ($p < 0.001$) between vitamin D status and hand-grip strength in young adult women (19-29 years) living in New Zealand. Authors concluded that, independent of recreational physical activity, serum 25(OH)D was significantly associated with dominant hand grip strength (R^2=0.13, p=.02) and non-dominant hand grip strength (R^2=0.11, p =0.02).[17]

FACTORS THAT PREDISPOSE TO VITAMIN D DEFICIENCY

Limited Exogenous Intake

Evidence suggests that vitamin D consumption within the general public tends to be low, particularly in groups with specialized eating practices such as vegans, vegetarians or those consuming too few dairy products or fortified foods.[18] Furthermore, college-aged students have been reported to consume under 50% of the current RDA recommendation of vitamin D.[19] In regard to collegiate athletes specifically, research suggests the prevalence of vitamin D insufficiency is no different from the general public-despite athletes greater energy needs.[18] While current literature remains limited, there is thought to be a correlation between insufficient vitamin D levels in athletes and their inadequate dietary intake of both vitamin D and calcium. Bescos Garcia et al. (2011)

investigated serum vitamin D levels after wintertime in 21 male basketball players from a professional Spanish team. Additionally, athletes completed a 4-day dietary recall and food frequency questionnaire in order to assess energy consumption, vitamin D and calcium intake. Their results indicated that the participants vitamin D intake (139 ± 78) IU/day) was below recommended values despite consumption of a high-caloric diet (4,284± 701 kcal/day).[20] As a result, researchers found that 57% of participants were within the deficient window in regards to vitamin D serum concentrations (defined as 25(OH)D < 50 nmol/L).[20] Conclusively, serum 25(OH)D levels correlated with the daily dietary intake of vitamin D ($r=0.65$, $p < 0.01$) and calcium ($r=0.82$, $p <0.01$).[20] The research team concluded that while more research with larger sample sizes is necessary to fully understand this disparity, professional basketball players are at higher risk of low vitamin D status (hypovitaminosis D), particularly after wintertime. Intake of dietary calcium and vitamin D is required if athletes are to avoid low serum 25(OH)D levels, particularly when exposure to sunlight is limited, such as in the winter months.

Limited Endogenous Exposure

Cutaneous previtamin D_3 synthesis and production is highly variable at different latitudes, times of the day, and seasons.[21] Thus, aathletes competing in indoor sports-with subsequent less exposure to sunlight- have been shown to be at risk.[16] Looker et al. (2007) hypothesized that further variation is also modulated by race and level of physical activity.[22] Differences in skin pigmentation and thus dermal production of vitamin D may contribute to this discrepancy as there is substantial evidence to support that synthesis of

vitamin D in darker skin tones is lower when compared to lighter skin tones.[22] This is thought to be due to greater amounts of melanin in darker skin tones that less efficiently absorbs UV wavelengths required to convert 7-dehydrocholesterol to vitamin D.[22,23] Secondly, to this protective nature, African Americans may require increased sun exposure than lighter skin tones in order to produce a similar amount of vitamin D.[22,23] Udowenko et al. (2010) affirms this notion, discussing the reasoning behind the discrepancy is in part due to faster vitamin D skin synthesis in individuals with fair complexions compared to their darker, more melanin-rich counterparts. Athletes with more melanin require increased UVB radiation exposure in order to garner the same 25(OH) D levels as fair-skinned athletes.[8]

Hidlebrand et al. (2016) reported serum 25(OH)D was lower in athletes of African American (n = 12), Hispanic (n = 8), Latin (n = 3) and Asian Pacific (n = 2) descent compared with Caucasians ($p < 0.01$).[16] Peeling et al. (2013) assessed the "associations between gender, anthropometry, predominant training environment and Vitamin D status in 72 elite athletes."[24] While the research generated no significant differences in vitamin D status of injured versus uninjured elite athletes they did in fact find evidence to support the prior notion that indoor sport athletes show the highest rate of musculoskeletal injury when compared to their outdoor counterparts exhibiting significantly lower 25(OH)D concentrations than the outdoor training group (90±28 nmol/L and 131±35 nmol/L, respectively, $p < 0.01$).[24]

Adiposity

Vitamin D status may also be related to an athlete's body mass index (BMI) and more specifically, their adiposity. Vitamin D status as it relates to body composition largely originated in scientific literature with the fat sequestration hypothesis. This hypothesis resulted from a study conducted in 1988 wherein researchers reported a significant correlation between white, obese participants and low circulating serum 25-hydroxyvitamin D.[25] A myriad of subsequent studies have supported this hypothesis including one performed by Wortsman et al. (2000) wherein researchers found that the subsequent increase in vitamin D_3 levels post UV exposure was 57% less in obese subjects when compared to their non-obese counterparts.[26] Heller et al. (2015) supports this notion publishing results that suggest that larger athletes with corresponding excess adiposity may be at higher risk for both vitamin D insufficiency and deficiency, even after controlling for sex in a mixed model.[27] Hidelbrand et al. (2016) also published similar findings suggesting that athletes with body composition in the overweight or obese category had lower serum 25(OH)D ($p < 0.05$) compared with those who were normal or below recommended fat percentages.[16] Although the mean for each group was within the adequate range. Further, the exact mechanism by which the relationship between body fat and serum 25(OH)D remains elusive.

VITAMIN D STATUS AMONG ATHLETIC POPULATIONS

In order to accurately assess vitamin D status accurately, validated methods must exist to reliably quantify such results. Vitamin D status is currently measured byway of 25(OH)D and $1,25(OH)_2D$ metabolites in the circulation. Of the two, serum 25(OH)D represents the more accurate barometer for vitamin D sufficiency and the only vitamin D

metabolite that is used to determine whether a subject is deficient, sufficient or within the hypervitaminosis D category. Unlike serum 25(OH)D, however, serum $1,25(OH)_2D$ provides little to no pertinent information in regard to an individual's vitamin D status. Furthermore, the serum level is often normal or even elevated due a comorbidity of vitamin D deficiency, secondary hyperparathyroidism.[28] The metabolite 25(OH)D represents the major circulating form of vitamin D with an accompanying half-life of approximately 2-3 weeks.[28] This metabolite is most ideal as it represents the summation of both vitamin D intake and vitamin D that is produced from sun exposure.

Farrokhyar et al. (2015) conducted a systematic review and meta-analysis with the objective to study the pervasiveness of vitamin D inadequacy in athletes residing in different countries with varying sun exposure at varying latitudes. Compiling 23 studies published between 2008 and 2014, performed in 9 countries including the US and included 2313 athletes total. Of that total sample, 56% were classified as vitamin D inadequate (< 79.872 nmol/L) with significant risk accompanying athletes competing in either winter or spring sports (Risk Ratio (RR) 1.85; 95% CI 1.27-2.70). Additionally, the risk significantly increased for indoor sport activities (RR 1.19; 95% CI 1.09-1.30). Seven of these accumulated studies included injury data with a prevalence of 43%, 19% of which being bone related and 37.5% being muscle and soft-tissue related (95% CI: 11.5-68.5). Conclusively, authors state that the prevalence of vitamin D inadequacy is significantly higher for indoor sports during both the winter and early spring seasons, and in higher latitudes.[3] Further, Angeline et al. (2013) cite data from the Hospital for Special Surgery in New York that examined 89 players from a single National Football League

(NFL) team. Their findings included that 30% of the sample were deficient (< 50 nmol/L) and 51% insufficient (50-77.5 nmol/L) [7]

RECOMENDED INTAKES OF VITAMIN D FOR ATHLETES

Whiting et al. (2006) summarized the Dietary Reference Intakes (DRIs) for micronutrients including vitamin D but did not consider increased metabolic needs for physically active athletes.[29] With increased physical activity, increased physiological stress and increased metabolic requirements, a highly trained athletic population such as collegiate athletes, micronutrient requirements expectantly increase relative to an inactive state that may apply to the general public. While no consensus currently exist, many researchers have proposed that the recommended daily allowance (RDA) be set upwards of 800-2,200 IU for an athletic population in order to ensure optimal vitamin D status and subsequent improved health outcomes.[28,30–33] While these guidelines are currently under revision by the National Institute of Medicine, **Table 1.1** displays the current, empirical and evidence-based range guidelines of vitamin D status.

EFFECTS OF SUPPLEMENTATION ON VITAMIN D STATUS

More research is necessary to support the effects of supplementation on vitamin D status within an athletic population, however, existing research largely endorses supplementation efficacy. Backx et al. (2016) examined vitamin D deficiency from 128 elite Dutch athletes over the course of one year. Based on their degree of insufficiency at baseline, the athletes falling within the deficient (< 50 nmol/L) or insufficient (50-75

nmol/L) category were then randomly assigned to one of three dosage groups: 400, 1,100 or 2,200 IU of capsulated oral vitamin D_3. Conclusively, serum 25(OH)D concentration increased more in the 2,200 IU/day group (+50 ± 27 nmol/L) than the sufficient group receiving no supplements (+4 ± 17 nmol/L; $p < 0.01$) and the 1,100 IU/day group (+25 ± 23 nmol/L; $p < 0.05$) after 12 months.[34] Cumulatively, the 2,200 IU/d dosage resulted in a sufficient 25(OH)D concentration in 80% of the athletes over the duration of 1 year. This was the result after 70% of those athletes were categorized as insufficient or deficient at baseline based upon the defined intervals above.[34]

Similarly, Close et al. (2013) conducted a study examining the vitamin D concentrations in non-supplemented, UK-based, male professional athletes over an 8-week duration during the winter months. Sixty-one male athletes and thirty male healthy control participants were recruited for the study. The 61 athletes came from four different sports-including rugby, soccer, flat jockeys and jump jockeys- whilst the remaining 30 were otherwise healthy, non-athletic controls.[35] Additionally, the athletes typically train under excessive cloud cover that would contribute to their daily sun exposure. The intervention group received a daily supplement over the duration of 8 weeks of 5,000 IU of vitamin D_3 (called cholecalciferol) whereas the control group received an inert placebo. As a result of the intervention, serum total 25(OH)D concentration significantly increased serum total 25(OH)D from baseline (29 ± 25 to 103 ± 25 nmol, $p < 0.01$), whereas the placebo showed no significant change (53 ± 29 to 74 ± 24 nmol, $p=0.12$).[35] Seven of the ten participants were classified as insufficient (25(OH)D < 50 nmol/L) and two of the ten participants had concentrations low enough to be associated with

deficiency (12.5–30 nmol/L) prior to supplementation. None of the participants in the study exhibited optimal vitamin D concentrations prior to the intervention. Following supplementation however, 60% of the vitamin D supplemented group had vitamin D concentrations greater than 100 nmol/L and could therefore be classified as having reached optimal status.[35]

CONCLUSIONS

Vitamin D has become a micronutrient of interest in sports nutrition. Emerging studies continue to demonstrate the prevalence of hypovitaminosis D among the general population but literature specifically examining this discrepancy within an athletic population remains limited. Of the existing studies, many are pertinent to two ends of a wide spectrum: professional athletes and the elderly. A prior notion exists that sunlight exposure provides adequate plasma levels of vitamin D. While UVB radiation does contribute to vitamin D status, a multitude of factors can hinder this endogenous pathway including the indoor training environment typical of a collegiate basketball player and darker skin pigmentation. Exogenous sources also pose a threat to adequate vitamin D status particularly to highly-trained athletes with increased energy needs.[36] Scientifically proven and empirical research supported supplement protocols and maintenance guidelines need to be both determined and disseminated within the sports nutrition community at large in order to best engineer top athletic performance and health.

Table 1.1: Vitamin D Status Guidelines

	Vitamin D Council[37]	Endocrine Society[37,38]	National Institutes of Medicine[39]	Food and Nutrition Board[37]	Athlete-specific Research[18,21,38]
Deficient	0-75 nmol/L	0-50 nmol/L	0-30 nmol/L	0-27.5 nmol/L	0-50 nmol/L
Insufficient	77.5-97.5 nmol/L	52.5-72.5 nmol/L	30-50 nmol/L	30-50 nmol/L	50-80 nmol/L
Sufficient	100-200 nmol/L	75-250 nmol/L	>50 nmol/L	> 50 nmol/L	> 100 nmol/L
Toxic	> 375 nmol/L				

CHAPTER 2: RATIONALE, OBJECTIVES & HYPOTHESES

Rationale

Accurately measuring and maintaining adequate vitamin D status in highly trained, collegiate athletes is of paramount importance. Low status, whether through insufficient exogenous sources or endogenous pathways has been linked to potentially catastrophic injury including musculoskeletal and bone trauma.[8,12,15,16] Endogenous sources specifically, present a complex disparity in status among darker-skinned athletes referred to as the "Black Athlete Paradox."[11,23] In examination of current literature, there appears to be a paradoxical relationship between ethnicity and vitamin D concentration. When examining 25(OH)D deficiency in ethnically diverse populations, studies demonstrate that Black and Hispanic men are at elevated risk of 25(OH)D deficiency while concurrently at lower risk of osteoporosis, rapid bone loss, and associated fractures compared to Caucasian counterparts.[11] Not only does race seemingly heighten an athletes risk of deficiency, the environment in which basketball players train can also negatively contribute as Kuhn et al. (2014) discusses, 25(OH)D values were characterized by a distinct seasonal variation and season was the most significant predictor of serum 25(OH)D status.[10] Whether or not vitamin D has the capacity to have any measurable effect on skeletal muscle function in young, trained athletes also remains a highly

debatable topic. Contributing to this discrepancy are limited and highly underpowered studies pertinent to young, athletic populations.[11] Prior studies that examine this topic often report data from non-athletic, often elderly populations.[13–15] As natural dietary sources of vitamin D are limited, supplementation remains a safe, fiscal, and efficacious method to combat insufficient status and return athletes to a healthy, sufficient state. The precise dosage amount required for most pronounced efficacy in improvement in serum 25(OH)D status remains elusive and of controversial debate. More data directly observing highly trained, athletic populations is necessary to provide meaningful results that can then be translated to updating current nutrition and dietary standards and guidelines for collegiate athletes.

This study aims to identify the daily dosage of daily cholecalciferol supplementation which improves vitamin D status to sufficient levels (>125 nmol/L) among collegiate basketball players. While optimal levels remain controversial, this parameter of sufficiency is based on current clinical practice as supported by the Vitamin D Council as well as the Endocrine Society.[37,38] However, these parameters are higher than both the National Institute of Medicine as well as the Food and Nutrition Board.[37] Given the heightened needs of a diverse, athletic population participating in an indoor sport, these increased thresholds are necessitated.[8,22,24,40]

Objectives & Hypotheses

Objective 1.1 To assess the prevalence of vitamin D deficiency in a diverse population of male and female collegiate basketball players.

Objective 1.2 To define the appropriate dosage of vitamin D_3 supplementation in order to beneficially affect serum 25(OH)D levels (>125 nmol/L) among collegiate basketball players.

Null (H_O): Athletes with categorized insufficient (< 75 nmol/L) or sufficient (75-125 nmol/L) at baseline and randomized to the intervention group will not see a marked improvement in their serum 25(OH)D levels or gain optimal status (>125 nmol/L) after vitamin D_3 supplementation for the duration of a 5-month intervention period. Given the relatively long circulating half-life of 15 days, this intervention period represents sufficient time to see a marked improvement in vitamin D status.[41]

Alternative (H_a): A dose-response relationship will exist between those athletes receiving higher supplementation (10,000 IU) that will positively affect their serum 25(OH)D levels (> 125 nmol/L) when compared to lower supplemented (no supplement-5,000 IU) group.

CHAPTER 3:
A QUASI-EXPERIMENTAL TRIAL OF THE EFFECTS OF CHOLECALCIFEROL SUPPLEMENTATION ON VITAMIN D STATUS AMONG A DIVERSE POPULATION OF COLLEGIATE BASKETBALL ATHLETES

Introduction

Vitamin D is a fat-soluble micronutrient that occurs in two dietary forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Research supports that vitamin D_3 is more potent and exerts a longer duration of action physiologically than does D_2, rendering it more efficacious in terms of beneficially affecting vitamin D status.[42,43] Examining vitamin D status among collegiate, indoor athletes is of particular relevance as research supports that not only are indoor athletes at greater risk[40,44] of suffering from insufficient vitamin D status due to limited sun exposure but college-aged young people generally under consume a micronutrient-rich diet, proliferating risk factors of vitamin D deficiency.[18,19] Further, demanding and strenuous activity in conjunction with the physicality of collegiate sports leaves highly trained athletes susceptible to a myriad of injuries including sprains, tendinitis, broken bones, knee injuries, and iliotibial band syndrome (IT).[7,18,30] This study aims to address this literature discrepancy byway of the following primary objectives: the first involving defining and examining the prevalence of vitamin D deficiency among a diverse cohort of male and female collegiate basketball

athletes. Second, to decipher the appropriate dosage of vitamin D_3 supplementation requited to impact an athlete's vitamin D status. This study aims to address these objectives while exploring potentially confounding factors including adiposity, endogenous sources including adequacy of sun exposure, exogenous sources including both dietary and supplement intake.

Methods

Participants

The participants in this study were all collegiate male and female basketball athletes from George Mason University Women's Basketball Team (WBB) (n=10) or George Mason University Men's Basketball Team (MBB) (n=10). Participation was voluntary and participants could autonomously withdraw from the study at any time and for any reason at no penalty or loss of benefits. Participants were required to be over the age of 18 years old and a healthy collegiate athlete belonging to one of the two aforementioned teams. The George Mason University Institutional Review Board for Human Subjects approved all procedures and participants provided informed consent prior to participation in the study.

Study design

This was an unblinded quasi-experimental trial with participants allocated to a vitamin D intervention regimen based on their baseline circulating 25-hydroxyvitamin D (25(OH)D) status. Assessments were conducted at baseline during the competition season

(in October 2018), and follow-up, post-season (between March and April 2019) and consisted of a blood draw, body composition assessment, anthropometric measures, and questionnaires.

Vitamin D intervention regimen

The intervention regimen utilized in this trial were derived from the Sports Nutrition Care Manual under the supervision of a Registered Dietitian/Sports Nutritionist.[45] At baseline, 25(OH)D status was determined and participants were allocated to one of three groups in an unblinded fashion based on the supplementation regimen included in **Table 3.1**. Participants were followed for ~ 5 months (length of the competition season), a sufficient time to see changes in vitamin D status.[41] Compliance was assessed by asking participants to return empty supplement bags to their respective Certified Athletic Trainer before picking up the next week's supply.

Measurements

Demographics

Demographic data was collected on age, ethnicity, nationality, basketball position, years of resistance training experience, years of basketball experience, and current pregnancy status. Female participants were required to disclose pregnancy status as those with positive status were not permitted to participate in the study in order to mitigate unintended risk from DXA scan. Race self-reported as African American, Mexican American/Latino, White/Anglo, Asian, Native American and other.

Anthropometrics

Weight was collected using a digital floor scale and measured to 0.1 kg (BOD POD; Cosmed USA, Concord, CA, USA). Standing height was measured to the nearest millimeter via a wall-mounted stadiometer (Detecto, Webb City, MO, USA). These measurements were then used to calculate, body mass index (BMI): weight in kilograms was divided by height in meters squared (kg/m^2). BMI was categorized as "underweight" ($BMI < 18.5\ kg/m^2$), "normal weight" ($BMI > 18.5$ and $< 25.0\ kg/m^2$), "overweight" ($BMI > 25.0$ and $< 30.0\ kg/m^2$) and "obese" ($BMI > 30.0\ kg/m^2$) in accordance with National Institutes of Health guidelines.[46] Dual x-ray absorptiometry (DXA) (Hologic, Horizon A model, Hologic Inc., Waltham, MA) was used to assess body composition including body fat and lean mass. Additionally, bone area, bone mineral content (BMC) and areal bone mineral density (aBMD) of the whole body were measured. Participants were scanned using the whole body scan mode (Hologic APEX software, ver. 5.5.3.1). Calibration and procedures were performed to manufacturer specifications. The percent coefficient of variation (%CV) over the study period for the spine phantom (#26436) was 0.3% for BMD and for the whole body phantom (#1104) was 1.5% for fat mass, 1.5% for lean mass, 0.1% for total mass, and 1.7% for % body fat. All tests were performed under the supervision of a trained technician.

Skin pigmentation

Skin pigmentation was measured at baseline via a portable, computerized spectrophometer (CM-600D, Konica Minolta). This measurement will be performed on

participants' upper underarm. Athletes were classified into 5 skin phototypes: dark (≤10°), olive (10–28°), medium (28–41°), fair (41–55°), and very fair (>55°). However, based on small numbers of participants in each group, these groups were reclassified as: dark- olive (≤10°–28°), medium (28–41°), and fair-very fair (41-55°<) [47,48]

Exogenous Intake of Vitamin D

In order to assess vitamin D intake from dietary sources, between 1 to 3, 24-hour recalls were conducted by a Registered Dietitian via phone call and in-person with each participant. If more than 1 recall was conducted, results pertaining to the same participant were averaged to assess usual intake. The Nutrition Data System for Research (NDSR) was utilized to quantify the total vitamin D (IU/day) intake based on the 24-hour recall results. NDSR's Dietary Supplement Assessment Module (DSAM) captures information pertinent to supplements.[49] NDSR collects information via USDA databases, product labels, scientific literature, foreign food composition tables, and National Health & Nutrition Examination Survey (NHANES) (2013-2014) Dietary Screener Questionnaires (DSQ) Database and supplements added by the Nutrition Coordinating Center (NCC) an establishment of the National Institutes of Health (NIH).[49] Missing foods or supplements typically utilized by our participants were added to the NDSR database (ie. CorePower, Orgain Protein Shake) prior to analysis.

Endogenous Intake of Vitamin D

A sun exposure questionnaire (SEQ) was utilized to asses sun exposure, winter travel and the usage of sunscreen prior to study onset. Sun exposure data were collected

including recent (within the past 3 months), travel to a warmer climate, duration of stay in a warmer climate, hours of direct sunlight, body part most exposed to direct sunlight, sunscreen usage, frequency of application and application site, time spent outdoors, residence over the winter and summer months, and Sun Protection Factor (SPF) brand and frequency of usage.

Outcome Assessment: of Serum 25(OH)D

Upon arrival to the laboratory, participants were seated in an upright position and a blood sample was collected from an antecubital vein using standard sterile phlebotomy procedures. Blood for the analysis of 25(OH)D and albumin was drawn into a 5-ml vacutainer tube that contained no additive (BD Biosciences, San Jose, CA). Samples were allowed to coagulate in cooling beds for ~30 minutes, and subsequently centrifuged at 2,500 rpm for 15 minutes (Eppendorf 5702R, Eppendorf North America, Hauppauge, NY). After centrifugation, the serum was stored at -80 ºC until analysis. A second 5-ml vacutainer tube containing the anti-coagulant ethylenediaminetetraacetic acid (EDTA) was collected, centrifuged as previously described, and the resultant supernatant was stored at -80 ºC. The serum concentration of 25(OH)D was measured in duplicate using a commercially available ELISA kit (Monobind, Lake Forest, CA) and a plate reader (Epoch, BioTek, Winooski, VT). The plasma concentration of VDBP was measured in duplicate using a commercially available bead-based assay kit (EMD Millipore; Billerica, MA) and a CCD-based Luminex Magpix (Austin, TX) multiplex system. The serum concentration of albumin was measured using a colorimetric end point assay (Pointe

Scientific Inc. Canton, MI) and a ChemWell auto-analyzer (Awareness Technology, Palm City, FL). Intra-assay coefficient of variation for 25(OH)D was 4.5%. Bioavailable vitamin D was calculated according to previously published methods.[11,50,51] The following cutoffs were used to determine 25(OH)D (nmol/L) status based on current literature[18,21,38]: ≤75=insufficient;75-125=sufficient; ≥125=optimal.

Statistical Analysis

SPSS Version 24.0 (IBM, Armonk, NY) was used for data analysis. The Shapiro-Wilks test was used to test normality of all variables. Mean ± SD were used to describe continuous and n (%) for categorical variables. A one-way analysis of variance (ANOVA) or chi square (X^2) test were used to assess mean differences in characteristics across intervention groups. ANOVA was used to assess the change in 25(OH)D from baseline to follow-up and X^2 to assess differences in vitamin D status at follow-up across intervention groups. A Bonferroni post-hoc test was performed to assess for differences among groups. A Spearman's correlation was performed to assess for correlations between the change in 25(OH)D and baseline 25(OH)D as well as body composition indices.

Results

Table 3.2 describes participant characteristics overall and by intervention group. The mean age was 20.25±0.85 years old, 12 (60.0%) self-reported as African American and 10 (50.0%) were female. The majority of participants (n=13) were allocated to the high-dose supplementation group (10,000 IU daily) vs. n=5 allocated to 5,000 IU daily

and n=2 to no supplementation. Overall, 10 (76.9%) participants allocated to the high dose supplementation group (10,000 IU daily) were male and 11 (84.6%) African American and similarly 10 (90.91%) were dark or olive skin tone ($p<0.05$). Differences among groups were noted for whole body BMD Z-score ($p=0.027$) and lean body mass ($p=0.004$). No other differences were noted among groups.

Table 3.3 shows no statistically significant differences in vitamin D status at follow-up ($p=0.395$). In the non-supplemented group, one athlete remained at optimal status while the other athlete fell to sufficient status. Among the 5,000 IU daily group, 3 (75%) participants remained at insufficient status while 1 athlete (25%) fell to insufficient status at follow-up. Among the high dose intervention group (10,000 IU daily), 3 (23%) remained insufficient, 9 (69%) achieved sufficient status, 1 (8%) attained optimal status.

Figure 3.1 displays the change of 25-hydroxyvitamin D concentrations from baseline to follow-up by intervention group. A statistically significant increase in 25(OH)D was noted between the 10,000 IU group (+35.0 ± 27.0 nmol/L) and the non-supplemented group (-41.6±11.7 nmol/L) and 5,000 IU group (-9.3±9.6 nmol/L, $p=0.001$). A statistically significant correlation was observed between the change in 25(OH)D (from baseline to follow) and four the body composition indices as follows, fat mass (r_s=-0.65, $p=0.01$), LBM (r_s=0.53, $p=0.05$), LBM percentage (r_s=0.83, $p=0.01$), and body fat percentage (r_s=-0.80, $p=0.01$). Additionally, there was a significant correlation between change in 25(OH)D and baseline 25(OH)D (r_s=-0.78, $p=0.01$).

Figure 3.2 displays the aforementioned difference of 25-hydroxyvitamin D concentrations at baseline and follow-up by individual and group (panels **A-C**). Panels A

and B show the majority of participants allocated to no supplementation or 5,000 IU daily decreased serum 25(OH)D over the course of the trial. Panel **C** shows only 1 of the 13 participants allocated to 10,000 IU daily decreased serum 25(OH)D, the remaining 12 participants (92%) all increased their serum 25(OH)D.

Discussion

Overall, 13 of the 20 (65%) participants were vitamin D insufficient at baseline (based on 25(OH)D of <75 nmol/L). This result is consistent with a recent systematic review and meta-analysis wherein 56% of a total sample of 2,000 athletes residing in 9 different countries including the US were vitamin D inadequacy (based on < 80 nmol/L).[3] The current study provides further evidence of the high prevalence of vitamin D insufficiency among a sample of highly-trained, US collegiate basketball athletes. It is well documented that limited sun exposure, latitude at which you reside, and seasonal variations may inhibit subcutaneous synthesis of vitamin D.[16,21,40] Baseline tests were performed in October, and due to the half-life of vitamin D_3 were indicative of the participants' vitamin D status during the summer months.[52] Hence the decreased 25(OH)D concentrations observed among the no supplementation and 5,000 IU/day groups were likely reflective of a seasonal decline. The 10,000 IU daily was the only dosage which appeared to be protective against this decline in 25(OH)D concentrations among participants **(Figure 3.2)**. Further, basketball is an indoor sport and the majority of this sample were of darker skin pigmentation which further reduces dermal production of

vitamin D_3 and hence, predisposes to vitamin D deficiency.[8,16,22] Darker-skinned athletes, 10 (90.9%) among our sample exhibited heightened risk of vitamin D insufficiency at baseline, and none of the participants with fair or very fair skin fell into the insufficient category at baseline.

Current results indicate a positive association between baseline 25(OH)D concentrations and bone mineral density (p=0.029) and a negative association between 25(OH)D and lean body mass (p=0.004). Prior evidence among male athletes supports a positive relationship between physical activity, lean body mass, and bone mineral density.[53] This inconsistency may be due to the high degree of leanness in the current sample, particularly among male participants. Current average values for body fat percentage for college age men is approximately 15%.[54] In the current sample, the male participants exhibited an average percent fat of just 13.5%. Current results also indicate an inverse association between the change in 25(OH)D observed with baseline 25(OH)D status, fat mass and percentage body fat (p=0.01). Hence, higher body fat and fat mass, were associated with a lower change in 25(OH)D in response to the intervention. Among participants allocated to the 10,000 IU group, 3 remained categorically insufficient at follow-up, but only 1 of these participants decreased in serum 25(OH)D from baseline to follow-up. All three of these participants exhibited higher fat mass compared to the rest of the sample. These results were: 15.44 kg for the male participant (the average for all male participants was 11.7 kg) and between 29.6-42.5kg for the two female participants (the average for all female participants was 21.2 kg). This result suggests that the lack response in 25(OH)D, particularly among the 10,000 IU supplemented group, may have

been inhibited due to increased adiposity. Overweight and obese participants exhibiting low serum 25(OH)D concentrations is well supported in scholarly research.[16,25–27]

According to the National Academy of Medicine, the Recommended Dietary Allowances (RDA) for vitamin D, set for a healthy North American population, is 600 IU.[41] This recommendation does not target athletes, which may have increased needs and further does not address those who may be deemed vitamin D deficient. Previous research suggests a wide range of vitamin D supplementation with as high as a single dose of 300,000 IU.[5,55] Vitamin D is a fat-soluble vitamin hence, there is a potential risk of toxicity and caution must be exerted when establishing supplementation recommendations. In this current study, a treatment dosage of 10,000 IU of vitamin D_3 daily led to increases in 25(OH)D concentrations (+35.1 nmol/L) while a dosage of only 5,000 IU daily led to a mean decrease (-9.34 nmol/L). In addition, only 1 of the 13 (8%) allocated to the 10,000 IU group achieved optimal status, 9 participants of 13 (69%) achieved sufficient status after the duration of ~5 months The most efficacious dosage to impact an individual's status beneficially is difficult to ascertain based on difference in skin pigmentation, level of adiposity, season and baseline vitamin D status. Further optimal status as defined as serum 25(OH)D concentrations >125 nmol/L were difficult to achieve and maintain, only two participants in our sample were able to achieve optimal status at follow-up. Our current results suggest 10,000 IU daily was more efficacious in preventing declines in 25(OH)D as observed among those in the 5,000 IU daily or no supplementation groups however, suggests guidelines pertaining to collegiate basketball athletes are needed.

Strengths and Limitations

This study was a quasi-experimental trail with treatment dosages based on clinical practice guidelines in conjunction with a Registered Dietitian. Further, participants were allocated to one of three intervention groups based on baseline status of 25(OH)D concentration, which has not been the case in the majority of previous studies. This discrepancy in relevant research is exemplified in Heaney (2012) who in conjunction with results from a meta-analysis performed by Bischoff-Ferrari et al. (2012) states that among over 30,000 participants included in randomized controlled trials pertinent to vitamin D status, baseline 25(OH)D concentrations were only available for a total of 14% of them.[56,57] The other trials supplemented participants based on a standard dosage. As baseline status will affect response, failing to assess an individual's baseline status and subsequently issuing a standard dosage may not be efficacious for all.[56,57] Additionally, results from the current study contribute to an emerging pool of literature pertinent to American, indoor, collegiate athletes of diverse skin-tones, sex, and adiposities. Further, this study contributes to scarce literature involving human subjects that serum 25(OH)D concentrations may exert a modulating effect on bone mineral density among collegiate basketball athletes. The primary limitations were the small sample size and recall bias necessitated by dietary, supplement, and sun exposure recall. Compliance represented another limitation, potentially affected by frequent team travel. Prior research indicates a positive association between serum 25(OH)D concentrations and daily dietary vitamin D intake.[20] Yet, high variability as a result of self-disclosure for these measures among our sample may have affected results.

Conclusion

There was a high prevalence of insufficiency among athletes and a dosage of 10,000 IU of vitamin D_3 supplementation, taken daily and allotted sufficient time to see marked improvement, may beneficially impact serum 25(OH)D levels among indoor collegiate basketball athletes. However, supplementation as high as 10,000 IU daily was unable to achieve sufficient status among all participants although appears to be protective against seasonal declines in 25(OH)D concentrations. High adiposity and the lack of ability to achieve a categorically optimal concentration of 25(OH)D above 125 nmol/L may help explain the results. Further research is required to aid in the development of screening protocols which will enable medical and sports nutrition staff to identify key risk factors of hypovitaminosis D including many of the variables explored in this study. These include practice and training location, ethnicity and race, and indices of body composition, particularly body fat %. Ultimately, these findings support that vitamin D should be considered an essential component of an optimal training regimen designed to maximize sports performance and minimize the physiological risks associated with insufficient vitamin D status, specifically among collegiate, indoor athletes.

Table 3.1: Vitamin D Supplementation Regimens

Vitamin D status definition	Baseline 25(OH)D concentration	Supplementation regimen (IU/d)
Insufficient	< 75nmol/L (30 ng/mL)	10,000 IU/cap/day
Sufficient	75-125 nmol/L (30-50 ng/mL)	5,000 IU/cap/day
Optimal	> 125 nmol/L (50 ng/mL)	No Supplementation

[1]Treatment dosages based on clinical practice guidelines in conjunction with a Registered Dietitian

Table 3.2. Characteristics of Participants by Intervention Groups. Presented as Mean ± SD for Continuous Variables and n (%) for Categorical Variables.

	Overall (N=20)	**Intervention Groups**			***p* value**[7-8]
		No Supplement (n=2)[1]	**5,000 IU / day (n=5)**[2]	**10,000 IU / day (n=13)**[3]	
Serum 25(OH)D (nmol/mL)	75.56 ± 31.95	153.38 ± 23.16	89.4 ± 15.89	58.27 ± 8.62	0.000
Sex, male	10 (50)	0 (0)	0 (0)	10 (76.9)	0.005
Age (yrs.)	20.25 ± 0.9	21 ± 0.0	20.6 ± 0.9	20 ± 0.8	0.175
BMI (kg/m^2)	25.1 ± 3.7	22.55 ± 5.3	24.82 ± 2.1	25.6 ± 4.1	0.571
% Normal weight	11 (55)	1 (50)	2 (40)	8 (61.5)	0.794
% Overweight	8 (40)	1 (50)	3 (60)	4 (30.8)	
% Obese	1 (5)	0 (0)	0 (0)	1 (7.7)	
Self-reported race					0.027
White/Anglo	6 (30.0)	2 (100)	2 (40)	2 (15.4)	
African American	12 (60)	0 (0)	1 (20)	11 (84.6)	
Latino	1 (5)	0 (0)	1 (20)	0 (0)	
Mixed	1 (5)	0 (0)	1 (20)	0 (0)	
Skin pigmentation (Inner, upper arm)					0.022
Dark or olive (≤10°–28°)	12 (70.6)	0 (0)	2 (50)	10 (90.9)	
Medium (28–41°)	4 (23.5)	2 (100)	1 (25)	1 (9.1)	
Fair or very fair (41°<)	1 (5.9)	0 (0)	1 (25)	0 (0)	
Body composition[4]					
Whole body BMD (g/cm)	1.28 ± 0.1	1.21 ± 0.0	1.26 ± 0.0	1.3 ± 0.1	0.447
Whole body BMD Z-Score	1.1 ± 0.8	1.6 ± 0.0	1.78 ± 0.7	0.76 ± 0.7	0.029
Fat Mass (kg)	16.45 ± 8.2	16.62 ± 6.5	18.23 ± 4.5	15.73 ± 9.7	0.859

Lean Mass (kg)	63.82 ± 11.3	54.25 ± 4.9	53.1 ± 5.2	69.44 ± 9.6	0.004
Lean mass (kg)/total mass (kg) x 100 (%)	76.8 ± 6.7	74.04 ± 4.9	71.88 ± 3.0	79.12 ± 7.0	0.094
Body Fat (%)	19.45 ± 7.1	22.2 ± 5.3	24.4 ± 3.3	17.13 ± 7.5	0.124
Dietary intake[5]					
Vitamin D, total (IU / day)	350.02 ±333.0	367.07 ±211.4	359.41 ±304.1	343.78 ±375.3	0.994
Sun exposure[6]					
Time spent outdoors (weekday), < 40 min.	17 (85)	2 (100)	4 (80)	11 (84.6)	0.798
Time spent outdoors (weekend), < 40 min.	12 (60)	1 (50)	1 (20)	10 (76.9)	0.083
Average minutes/d of direct sunlight exposure, < 30 min.	15 (75)	1 (50)	4 (80)	10 (76.9)	0.684

[1]Participants allocated to group at baseline if fell within optimal range (>124.8 nmol/L)
[2]Participants allocated to group at baseline if fell within sufficient range (75-124.8 nmol/L)
[3]Participants allocated to group at baseline if fell within insufficient range (< 75 nmol/L)
[4]Based on dual-energy x-ray absorptiometry (DXA)
[5]Based on Nutrition Data Systems for Research (NDSR) data at follow-up
[6]Self-reported at baseline
[7]A one-way analysis of variance (ANOVA) or chi square (X^2) test were used to assess mean differences in characteristics across intervention groups
[8]P Value of ≤ 0.05 determines statistical significance

Table 3.3: 25(OH)D Status at Follow-Up by Intervention Dosage. Presented as n (%).

		Status at Baseline			
		No Supplement (n=2)	5,000 IU (n=5)	10,000 IU (n=13)	*p* value[2-3]
Status at Follow-Up	Insufficient <75 nmol/L	0 (0)	1 (25)	3 (23.1)	0.395
	Sufficient 75-125 nmol/L	1 (50)	3 (75)	9 (69.2)	
	Optimal >125 nmol/L	1 (50)	0 (0)	1 (7.7)	

[1]Total of 5 participants were allocated to 5,000 IU D_3 at baseline but only 4 remained at follow-up due attrition

[2] Chi square (X^2) test were used to assess differences in vitamin D status at follow-up across intervention groups

[3]P Value of ≤ 0.05 determines statistical significance

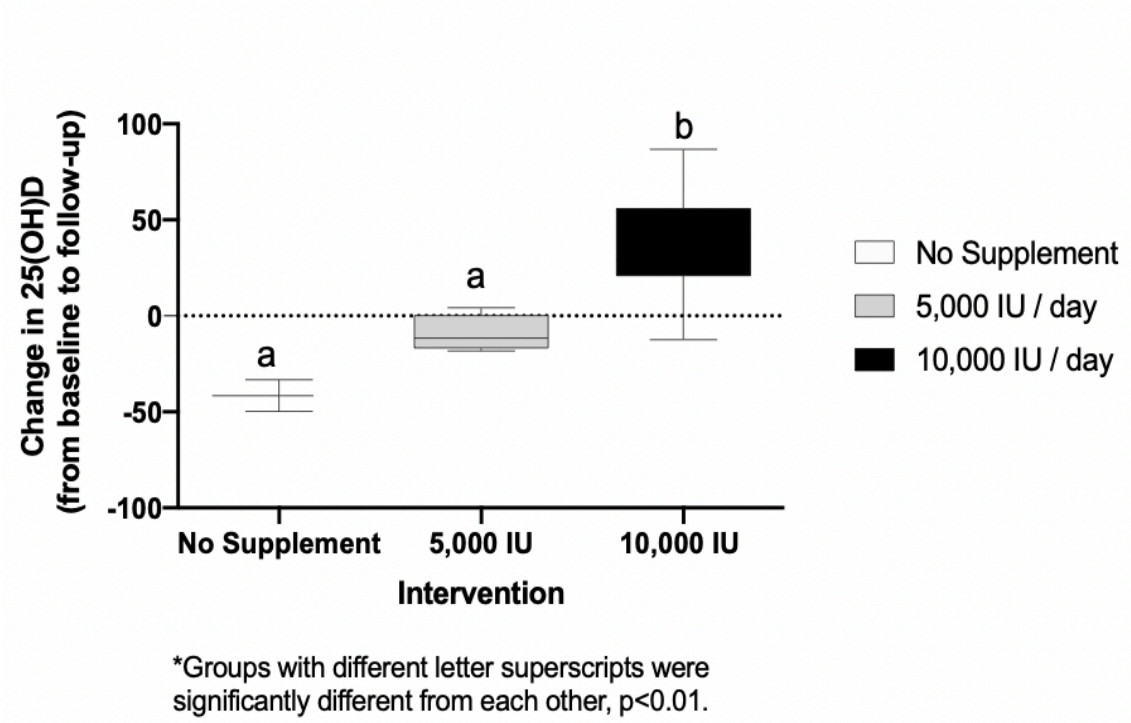


Figure 3.1. Change of 25-hydroxyvitamin D Concentrations by Intervention Group

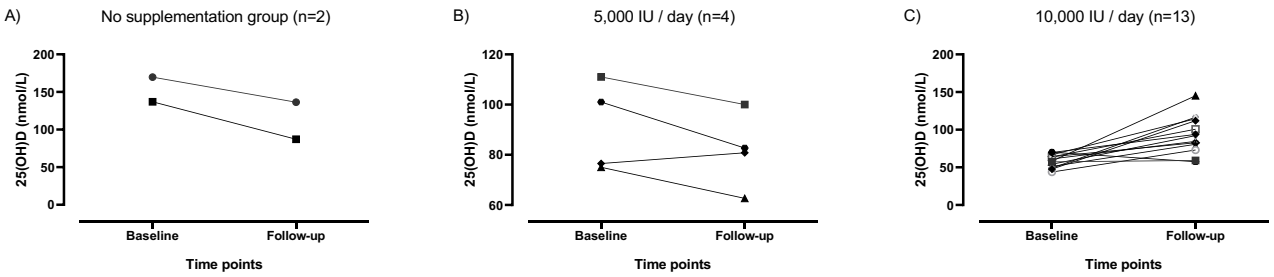
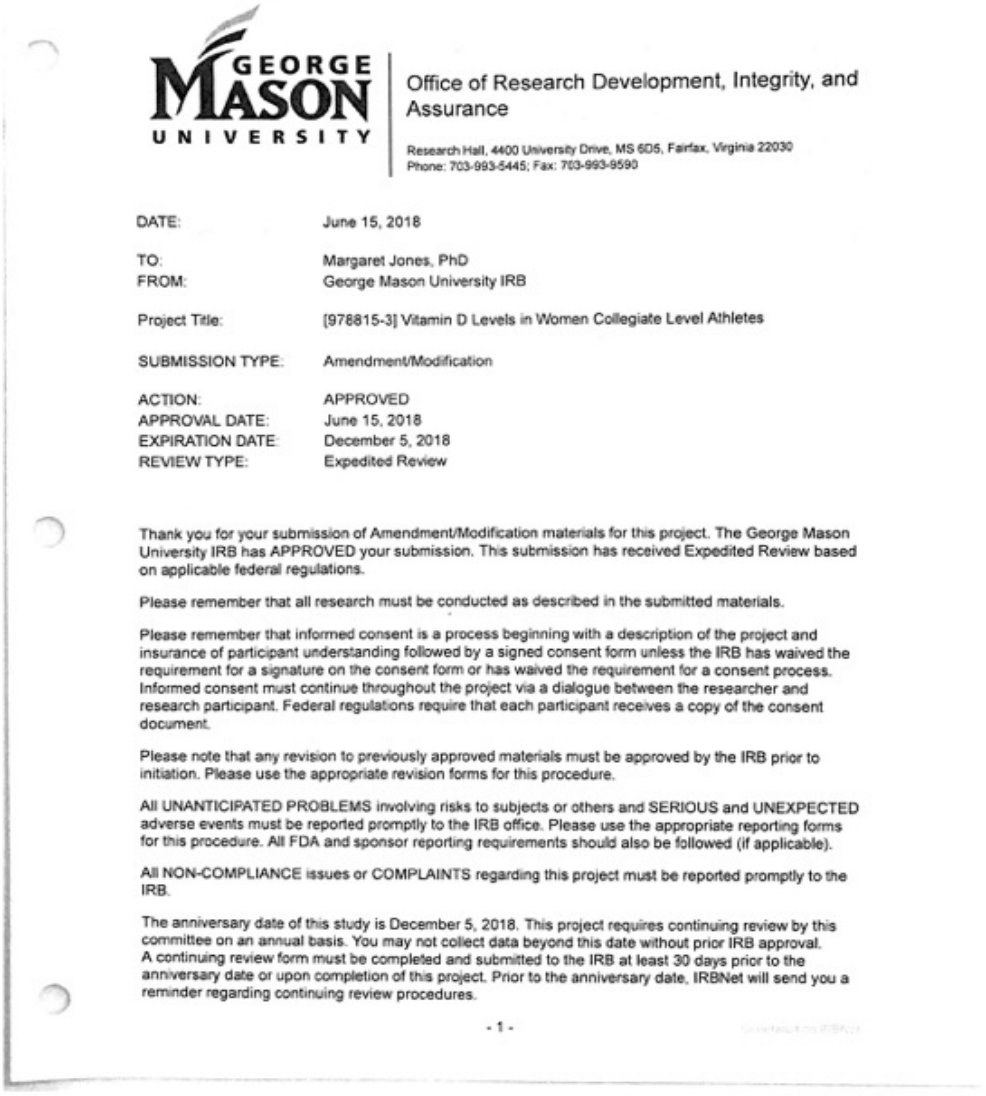


Figure 3.2. 25-hydroxyvitamin D Concentrations at Baseline and Follow-Up by Group and Individual

APPENDIX A. GEORGE MASON UNIVERSITY IRB APPROVAL



GEORGE MASON UNIVERSITY

Office of Research Development, Integrity, and Assurance

Research Hall, 4400 University Drive, MS 6D5, Fairfax, Virginia 22030
Phone: 703-993-5445; Fax: 703-993-9590

DATE:	June 15, 2018
TO:	Margaret Jones, PhD
FROM:	George Mason University IRB
Project Title:	[978815-3] Vitamin D Levels in Women Collegiate Level Athletes
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
APPROVAL DATE:	June 15, 2018
EXPIRATION DATE:	December 5, 2018
REVIEW TYPE:	Expedited Review

Thank you for your submission of Amendment/Modification materials for this project. The George Mason University IRB has APPROVED your submission. This submission has received Expedited Review based on applicable federal regulations.

Please remember that all research must be conducted as described in the submitted materials.

Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding followed by a signed consent form unless the IRB has waived the requirement for a signature on the consent form or has waived the requirement for a consent process. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require that each participant receives a copy of the consent document.

Please note that any revision to previously approved materials must be approved by the IRB prior to initiation. Please use the appropriate revision forms for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to the IRB office. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed (if applicable).

All NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to the IRB.

The anniversary date of this study is December 5, 2018. This project requires continuing review by this committee on an annual basis. You may not collect data beyond this date without prior IRB approval. A continuing review form must be completed and submitted to the IRB at least 30 days prior to the anniversary date or upon completion of this project. Prior to the anniversary date, IRBNet will send you a reminder regarding continuing review procedures.

- 1 -

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