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The Effects of Season-Long Vitamin D Supplementation on Collegiate Swimmers and Divers

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Abstract

The purpose of this 6-month randomized, placebo-controlled trial was to determine the effect of season-long (September–March) vitamin D supplementation on changes in vitamin D status, which is measured as 25(OH) D, body composition, inflammation, and frequency of illness and injury. Forty-five male and female athletes were randomized to 4,000 IU vitamin D ($n = 23$) or placebo ($n = 22$). Bone turnover markers (NTx and BS AP), 25(OH)D, and inflammatory cytokines (TNF- α , IL-6, and IL-1 β) were measured at baseline, midpoint, and endpoint. Body composition was assessed by DXA and injury and illness data were collected. All athletes had sufficient 25(OH)D (> 32 ng/ml) at baseline (mean: 57 ng/ml). At midpoint and endpoint, 13% and 16% of the total sample had 25(OH)D < 32 ng/ml, respectively. 25(OH)D was not positively correlated with bone mineral density (BMD) in the total body, proximal dual femur, or lumbar spine. In men, total body ($p = .04$) and trunk ($p = .04$) mineral-free lean mass (MFL) were positively correlated with 25(OH)D. In women, right femoral neck BMD ($p = .02$) was positively correlated with 25(OH)D. 25(OH)D did not correlate with changes in bone turnover markers or inflammatory cytokines. Illness ($n = 1$) and injury ($n = 13$) were not related to 25(OH)D; however, 77% of injuries coincided with decreases in 25(OH)D. Our data suggests that 4,000 IU vitamin D supplementation is an inexpensive intervention that effectively increased 25(OH)D, which was positively correlated to bone measures in the proximal dual femur and MFL. Future studies with larger sample sizes and improved supplement compliance are needed to expand our understanding of the effects of vitamin D supplementation in athletes.

Keywords

25(OH)D; athletes; body composition; inflammation; injury

Vitamin D is a secosteroid hormone that is primarily obtained via cutaneous synthesis following exposure to ultraviolet B (UVB). Vitamin D can also be acquired in smaller quantities from fatty fish, eggs, fortified foods, and dietary supplements (Halliday et al., 2011; Holick, 2007; Larson-Meyer & Willis, 2010). However, despite natural food sources and fortification efforts, the Institute of Medicine's adult intake recommendation of 600 International Units (IU) per day (IOM, 2011) is rarely met through diet alone (Holick, 2007). Due to these findings, coupled with limited UVB exposure and accumulating reports

of suboptimal vitamin D status, vitamin D supplements are often warranted to maintain healthy concentrations throughout the year (Holick, 2007; Willis et al., 2008).

Although vitamin D supplementation is becoming more common, vitamin D deficiency (generally defined as 25-hydroxy vitamin D [25(OH)D] concentrations below 20 ng/ml) remains a worldwide epidemic affecting every demographic, including athletes (Cannell et al., 2009; Halliday et al., 2011; Holick, 2007; Larson-Meyer & Willis, 2010; Willis et al., 2008). Vitamin D deficiency is known to contribute to decreased bone mineral density and increased fracture prevalence (Holick, 2007; Larson-Meyer & Willis, 2010; Zittermann et al., 2002). In addition, accumulating evidence suggests that vitamin D insufficiency (<32 ng/ml) is associated with adverse nonskeletal outcomes that include but are not limited to muscle weakness, decreased physical performance, inflammation, increased incidence of injury, and impaired immune function (Berry et al., 2011; Cannell et al., 2009; Holick, 2007, 2012; Weisenberger, 2011; Wicherts et al., 2011).

While adverse health outcomes related to vitamin D status have been well documented in the elderly, (Muir & Montero-Odasso, 2011) they have only recently been identified in athletes (Willis et al., 2008). A high prevalence of vitamin D insufficiency has been documented in athletes, and seasonal fluctuations leading to decreases in 25(OH)D are more significant in athletes who primarily train indoors (Constantini et al., 2010; Halliday et al., 2011; Hamilton, Grantham et al., 2010; Lovell, 2008; Willis et al., 2008). Recent studies in athletes have suggested that higher 25(OH)D concentrations are associated with improved health, body composition, and performance (Cannell et al., 2009; Larson-Meyer & Willis, 2010). However, few prospective studies have examined the health implications of seasonal decreases in vitamin D status (Halliday et al., 2011; Larson-Meyer & Willis, 2010) and randomized controlled trials designed to increase vitamin D status over the course of a competitive season are lacking. While many athletes may have 25(OH)D concentrations that support bone health immediately following the summer months, it is not known how supplement-induced increases in 25(OH)D or maintenance of summer concentrations effect health or body composition during 6 months of training and competition.

The primary objective of this study was to determine how 25(OH)D concentrations respond to 6 months of vitamin D supplementation (4,000 IU) in collegiate swimmers and divers. An additional objective was to determine how changes in 25(OH)D were related to changes in body composition, markers of inflammation and frequency of illness and injury. We hypothesized that increasing 25(OH)D concentrations would be positively correlated with improved bone formation and inversely associated with markers of inflammation.

Methods

Subjects

Men and women NCAA Division I collegiate swimmers and divers from a university in the southeastern United States (latitude 38°N) were recruited to participate. At screening, potential volunteers completed a medical history eligibility form that inquired about preexisting health conditions and the use of supplements and medications. Although athletes were generally healthy, they were excluded if they were under the age of 18 or reported

medical conditions that could compromise safety or confound study results. The university institutional review board approved this study and all eligible athletes gave written informed consent before participating. All institutional and governmental regulations concerning the use of human subjects were followed during this study.

Study Design

This study was designed as a 6-month double-blind, randomized, placebo-controlled vitamin D supplement intervention. Baseline measurements were collected immediately before the start of the swimming/diving athletic season (August) and consisted of a blood draw, body composition assessment, anthropometric measures, and a lifestyle questionnaire. Following baseline measures, athletes were randomly assigned to one of two dietary supplement intervention groups: 4,000 IU vitamin D daily (VIT D) or placebo (PLA). Supplementation for the intervention began in mid-September and continued throughout the study (6 months). Midpoint data were collected during the third month of the intervention (December) and included blood measures and a lifestyle questionnaire. Endpoint study measures were performed 3 months following midpoint data collection (March) and consisted of blood measures, body composition assessment, anthropometric measures, and a lifestyle questionnaire.

Blood Collection and Analysis

For baseline measures, blood samples were obtained between 2:00 p.m. and 5:00 p.m. from participating athletes by trained registered nurses at the university health clinic as part of a routine athletic department preseason physical. Measurements included 25(OH)D, PTH, bone turnover markers, and inflammatory cytokines. Standard guidelines for collecting blood samples were followed and approximately 20ml of whole blood was collected from the antecubital vein to assess N-telopeptide (NTX; Inverness Wampole, Princeton, NJ) and bone specific alkaline phosphatase (BSAP; Quidel, Santa Clara, CA). 25(OH)D and intact parathyroid hormone (iPTH; Siemens, Tarrytown, NY) were also measured along with a Millipore Milliplex luminex panel 100 platform for three inflammatory cytokines: tumor necrosis factor (TNF- α), interleukin-6 (IL-6), and interleukin-1-beta (IL-1 β). Midpoint and endpoint measures were collected at the university's Clinical Research Development and Operations center between 10:00 a.m. and 1:00 p.m. and 8:00 a.m. and 4:00 p.m., respectively. Midpoint blood measures included all baseline blood measures and ionized calcium. Endpoint blood measures included the same blood markers as baseline. The University Biospecimens Core of the Center for Clinical and Translational Science analyzed all blood samples at all three time points.

Anthropometric and Body Composition Measurements

Height and weight were measured at baseline and end-point to calculate body mass index (BMI). Separate dualenergy X-ray absorptiometry (DXA) scans of the total body, AP spine (L1-L4), and proximal dual femur (DXA; GE Lunar-Prodigy; software version 10.0) measured body composition. Before DXA scanning, height and weight (without shoes) were measured with a wall-mount stadiometer (DETECTO 3P, Webb City, MO) and Tanita 800S scale (Tokyo, Japan) at the clinical laboratory by study personnel. One trained laboratory technician completed all DXA scans to assess bone mineral density (BMD), and bone

mineral content (BMC) for the proximal dual femur (left femur (LF) and right femur (RF)), lumbar spine (LS; L2-L4), and total body. Mineral-free lean mass (MFL) and fat mass (FM) were assessed for the arms, legs, trunk, and total body.

Lifestyle Questionnaire

Immediately before each study blood draw, an adapted vitamin D questionnaire (Halliday et al., 2011) was used to assess lifestyle habits that may contribute to vitamin D status. Food sources of vitamin D, dietary supplement use, and UVB exposure were all measured. All indoor and outdoor activities were recorded by the team athletic trainer. Female athletes were asked to report menstrual status and oral contraceptive use. Athletes were also asked to report the date, intensity, and duration of their last training session to assess the impact of these variables on inflammatory cytokines.

Illness and Injury

All documented injuries and illnesses were collected by a certified athletic trainer and reported to study coordinators on a monthly basis. Illnesses of interest included upper respiratory infections (URI), influenza, gastroenteritis, and the common cold. Injuries of interest included bone, connective, and muscle injuries not caused by acute trauma. Baseline, midpoint, and endpoint 25(OH)D was documented to assess how vitamin D status changed in relation to injury onset.

Intervention

Following baseline measures, a clinical research pharmacist randomized all participating athletes into VIT D or PLA supplement groups. Study coordinators distributed supplements to athletes during their morning strength training sessions. Supplement assignments were blinded to both athletes and all study personnel until endpoint measurements were completed. Athletes in each supplement group were instructed to take their assigned capsules daily for the duration of the study. Each month, old bottles were collected and new bottles were distributed. The remaining supplements were counted to calculate compliance. In the month of December (Month 3), athletes were given two supplement bottles to accommodate for the 3-week academic break. Both bottles were returned at the end of January, and compliance for Months 3 and 4 were combined. The vitamin D and placebo gel capsules were provided by Nature Made Pharmavite LLC (Northridge, CA). The placebo contained small quantities of vegetable oils common in the American diet (corn and soybean). Both the vitamin D and placebo gel capsules were certified for content by the manufacturer and placebo capsules were visually indistinguishable from the vitamin D gel capsules.

Statistical Analysis

We estimated that taking 4,000 IU of vitamin D daily would significantly increase mean 25(OH)D by approximately 40 ng/ml (Holick, 2010) while the placebo group would be expected to drop approximately 7 ng/ml from the fall to spring season (Halliday et al., 2011). Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 20.0). Repeated measures analysis of variance (RMANOVA) was proposed to

assess the relationship between changing 25(OH)D concentrations and outcome variables; however, due to research subject “self-treatment” with tanning (both VIT D and PLA groups), it was statistically appropriate to examine Pearson correlations to assess relationships between 25(OH)D and outcome variables of interest instead of RMANOVA. Differences in baseline measurements for height, weight, BMI, and 25(OH)D were compared between supplement groups by independent *t* tests. Factors known to contribute to 25(OH)D were analyzed in a linear regression model. Pearson correlations analyzed relationships between variables of interest, and linear regression was used to assess 25(OH)D as a predictor variable of inflammation. Significance was determined at $p \leq .05$. During the design phase of this study, there were no other trials examining body composition and vitamin D supplementation in athletes to base a formal power calculation. We recruited from a maximum pool of approximately 60 swimmers and divers.

Results

Forty-five athletes met eligibility requirements and completed baseline measures. Following randomization, 13 athletes withdrew from the study before completing the 6-month protocol for personal reasons ($n = 3$) and time conflicts ($n = 10$). A total of 32 athletes completed the 6-month intervention (VIT D $n = 19$; PLA $n = 13$). Baseline characteristics were not different between dropouts and those who completed the study; all data were analyzed to include athletes who completed the protocol ($n = 32$).

Athletes were primarily White (Table 1). There were no significant differences by supplement group in age, weight, height, or BMI at baseline. Swimmers were significantly taller ($p = .03$) and weighed more ($p = .03$) than divers, but both groups were similar in all other demographic characteristics.

Vitamin D Status

Overall Status—At baseline, no athlete was deficient (25(OH)D < 20 ng/ml) and all athletes had 25(OH)D concentrations greater than or equal to 32 ng/ml. Mean 25(OH)D was 57 ± 16 ng/ml at baseline, 57 ± 19 ng/ml at midpoint, and 50 ± 16 ng/ml at endpoint. Eighty-eight percent ($n = 28$) of athletes maintained 25(OH)D concentrations greater than 40 ng/ml in the fall (baseline), 81% ($n = 26$) in the winter (midpoint), and 69% ($n = 22$) in the spring (endpoint). Mean supplement compliance was 70% for VIT D (range: 29–88%) and 76% for PLA (range: 64–87%).

25(OH)D by Group—Mean vitamin D status was significantly lower at baseline in athletes assigned to vitamin D treatment (52 ± 13.7 ng/ml; $p = .03$) compared with PLA athletes (64 ± 16.7 ng/ml; Figure 1). At midpoint, 13% had concentrations less than 32 ng/ml ($n = 3$ PLA; $n = 1$ VIT D). Midpoint 25(OH)D increased 8 ng/ml from baseline in VIT D (60 ± 19.9 ng/ml) and decreased 12 ng/ml in PLA (52 ± 18.4 ng/ml). Midpoint to endpoint mean decreases of 7 ng/ml (VIT D) and 8 ng/ml (PLA) in 25(OH)D were observed in both supplement groups, (VIT D 53 ± 16.9 and PLA 44 ± 14.4 ng/ml) but were not statistically different. Sixteen percent dropped below 32 ng/ml ($n = 1$ VIT D; $n = 4$ PLA) at endpoint.

6-Month Changes in Status by Group—When 6-month changes in 25(OH)D were assessed in quartiles, the top 25% (who had increased 25(OH)D) consisted of athletes in the VIT D group. The bottom 25% (largest loss of 25(OH)D) included 1 VIT D athlete whose compliance dropped to zero in the last month of the trial and 7 PLA athletes. Mean changes in supplement group 25(OH)D (Table 2) over the course of 6 months were +1 ng/ml (VIT D) and -20 ng/ml (PLA). Individual changes in 25(OH) D are documented in Figure 2. VIT D athletes who fell below 50% compliance to the supplement intervention ($n = 2$) and PLA athletes who tanned more than 120 min over the course of the 6-month intervention ($n = 3$) were excluded from Figure 2.

Dietary and Behavioral Factors

Supplement group was a significant predictor of changes in 25(OH)D from baseline to endpoint ($p < .001$). Leisure time spent in the sun ($p = .001$) was the only significant behavioral predictor of 25(OH)D at baseline, while time spent tanning ($p = .007$) was significant at midpoint. At endpoint, there were no significant behavioral predictors of 25(OH)D. With the exception of seven athletes ($n = 4$ VIT D; $n = 3$ PLA), all athletes recorded 120 min (approx. 5 min a week) or less of tanning over the 6-month intervention. There were no significant differences found between supplement groups at any time point for consumption of vitamin D-containing foods ($p = .5$), tanning exposure ($p = .6$), or amount of leisure time spent in the sun ($p = .2$). At endpoint, time spent walking to class was significantly different in VIT D (41 ± 19.3 min) than PLA (27 ± 12.5 min; $p = .02$); however, time spent walking to class was not a significant predictor of 25(OH)D at any time point. There were no differences in intensity or duration of exercise before blood draw between groups. All women reported normal menstrual status, and of the 13 women, 9 reported oral contraceptive use (VIT D $n = 5$; PLA $n = 4$).

Vitamin D Status and Body Composition

Due to self-treatment with tanning (both VIT D and PLA groups), Pearson correlations were used to assess relationships between 25(OH)D and outcome variables of interest instead of RMANOVA. There were no significant differences between supplement groups in baseline MFL ($p = .8$), FM ($p = .6$), BMD ($p = .3$) or BMC ($p = .4$). There were no significant differences between changes in MFL ($p = 1.0$), FM ($p = .9$), BMD ($p = .4$) or BMC ($p = .9$) between swimmers and divers. Six-month changes in 25(OH)D were significantly correlated with total MFL in the trunk region ($p = .05$). FM was not significantly associated with 25(OH)D, but an inverse trend was observed ($p = .06$). Changes in 25(OH)D were not significantly correlated with changes in total body, proximal dual femur, or lumbar spine BMD or BMC (Table 3).

In men, both total MFL ($p = .03$; Table 3) and MFL of the trunk ($p = .04$) were positively correlated with increases in 25(OH)D. Also in men, increases in 25(OH) D were positively correlated with left femoral neck ($p = .03$) and android region ($p = .02$) BMC. In women, increases in 25(OH)D were positively correlated with right femoral neck BMD and BMC ($p < .05$) and BMD of the right femoral Ward's region ($p = .02$). Although, right versus left leg dominance was not a predictor of proximal dual femur findings in the total sample ($p > .05$), 92% of women reported right leg dominance corresponding with a positive change in bone

density in the right femur neck ($0.003 \pm 0.04\text{g/cm}^2$) compared with a loss ($-0.006 \pm 0.03\text{g/cm}^2$) in the bone density of the left femur neck.

Blood Marker Changes

All athletes had normal midpoint ionized calcium concentrations (4.6mg/dL to 5.6mg/dL). Changes in iPTH concentrations were not associated with changes in 25(OH)D, and iPTH did not correlate with 25(OH)D concentration at any time point. Changes in 25(OH)D did not significantly correlate with changes in BSAP or NTx at any time point during the competitive season (Table 4). NTx was significantly higher in PLA ($23 \pm 8.0\text{nMBCE}$) than VIT D ($18 \pm 4.8\text{nMBCE}$) at midpoint ($p = .04$). There were no differences in inflammatory cytokine measures by time or between supplement groups throughout the 6-month intervention. Inflammatory cytokines did not correlate with 25(OH)D at any time point, and changes in 25(OH)D did not correlate with changes in inflammation (Table 4).

Illness and Injury

Only one athlete reported an illness during the course of the intervention, which precluded additional analysis. Thirteen athletes developed at least one injury (VIT D $n = 9$; PLA $n = 4$) over the 6-month intervention (Figure 3). The majority of injuries were to connective tissue ($n = 9$) and muscle ($n = 7$) and occurred in the winter and spring months; no injuries to bone were reported. Three athletes developed multiple injuries over the course of the intervention and maintained average 25(OH)D concentrations between 40 ng/ml and 60 ng/ml. When examining the timeframe of injury occurrence in relation to 25(OH)D concentration 77% ($n = 10$) of reported injuries occurred after an observed decrease in 25(OH)D (-11 ng/ml to -47 ng/ml loss).

Discussion

The primary purpose of this study was to examine how serum 25(OH)D responded to vitamin D supplementation in collegiate swimmers and divers. In addition, we aimed to determine how changes in 25(OH)D were related to changes in body composition and inflammation. We found that increasing 25(OH)D concentrations in the vitamin D treatment group were not correlated with either total body BMD, proximal dual femur or lumbar spine, or with markers of inflammation. A major strength of this study was that it is the first to administer substantial vitamin D supplementation (4000IU, the IOM upper limit) to indoor collegiate athletes as part of a well-designed randomized placebo-controlled intervention to assess the impact of vitamin D on bone, muscle, inflammation, and injury in athletes.

In this 6-month randomized-placebo controlled trial with indoor athletes, we found a low prevalence of vitamin D insufficiency (25(OH)D < 32 ng/ml) and did not observe athletes with deficient status (25(OH)D < 20 ng/ml). Our findings were somewhat unexpected since previous studies have documented a high prevalence (up to 61%) of athletes with 25(OH)D concentrations below 32 ng/ml (Bescós García & Rodríguez Guisado, 2011; Halliday et al., 2011). Several lifestyle factors may have contributed to our observed high baseline 25(OH)D concentrations. These factors include a primarily Caucasian sample, baseline 25(OH)D measures taken immediately following summer UVB exposure, tanning practices,

and preseason outdoor training. At 38° latitude, vitamin D is readily synthesized from March to October following cutaneous solar UVB exposure (Webb et al., 1988); therefore, elevated 25(OH)D concentrations may have been maintained due to 2–3 hr per week of early afternoon outdoor team training during September and October. Despite high baseline concentrations, we observed that 16% of our athletic sample had 25(OH)D concentrations below 32 ng/ml at endpoint, which suggests that vitamin D insufficiency (25(OH)D < 32 ng/ml) remains a concern as the role of vitamin D in promoting nonskeletal health continues to be investigated.

In our study, we found that the top quartile of athletes with the greatest increases in 25(OH)D were all in the vitamin D supplementation group which had estimated compliance generally greater than 50%. However, two VIT D athletes experienced appreciable increases in 25(OH)D despite being less than 50% compliant. The observed increases in 25(OH)D for these athletes may have been explained by underreported intake of a women's multivitamin or due to regular UVB exposure from tanning (725 min over 6-month intervention). In contrast, the bottom quartile of athletes who experienced the greatest drop in 25(OH)D concentration was mostly in the PLA group with the exception of one VIT D athlete who stopped supplementation in the last month of the intervention. All PLA athletes experienced decreases in 25(OH)D and 92% of PLA athletes lost more than 7 ng/ml, which is similar to that observed in a sample of college athletes of mixed sports (Halliday et al., 2011). In our study, the PLA athletes averaged a loss of 20 ng/ml over the 6-month trial, which is approximately a threefold difference from the loss experienced by athletes in the Wyoming cohort (Halliday et al., 2011). These differences may be attributed to the fact that our study included only indoor athletes, whereas the Halliday et al. sample consisted of primarily outdoor athletes (Halliday et al., 2011). Our findings are in accordance with a recent cross-sectional study by Constantini et al. who documented the 25(OH)D concentration of indoor and outdoor athletes and found that almost twice as many indoor athletes (80%) were below 30 ng/ml compared with outdoor athletes (48%; Constantini et al., 2010). Additional studies that examined indoor athletes have found that vitamin D deficiency rates have ranged from 44% to 83%. (Bescós García & Rodríguez Guisado, 2011; Ducher & al., 2011; Lovell, 2008). This trend suggests that indoor athletes are at a greater risk of lower vitamin D status than outdoor athletes, and may explain the unexpected decrease in 25(OH)D that we observed. These findings also suggest that suboptimal supplement compliance (< 80%) despite weekly reminders may present a significant challenge to maintaining or improving athlete vitamin D status.

Similar to previous findings, changes in 25(OH) D were not correlated with changes in total FM, but an inverse trend was observed (Halliday et al., 2011; Larson-Meyer & Willis, 2010). Several studies agree that loss of FM is associated with mobilizing 25(OH)D from fat stores, resulting in an inverse relationship (Holick, 2007; Larson-Meyer & Willis, 2010; Wortsman et al., 2000). However, the variability in FM loss between subjects and the nature of our study design likely prevented the observation of a significant relationship between these variables. For lean tissue, we observed a positive association between higher vitamin D concentrations and increased MFL in men, but not women. Previous studies suggest that vitamin D is important for supporting muscle mass in part due to its association with Type II muscle fibers (Larson-Meyer & Willis, 2010; Sato et al, 2002; Sato et al., 2005). A recent

randomized controlled trial in older women found that vitamin D supplementation increased the number and size of Type II muscle fibers which may have responded to increased protein synthesis mediated by interactions with vitamin D (Sato et al., 2005). In our study, changes in 25(OH)D and training load were not significantly different between men and women, thus sex differences likely contributed to this observed finding. The ability to gain muscle mass is greater in men and higher vitamin D concentrations may maximize secosteroid interaction with muscle tissue that maximizes accretion of muscle (Alway et al., 1989).

In this study, total BMD and BMC were not related to changes in 25(OH)D. This is contrary to several studies that have shown the benefits of vitamin D on bone in various populations (Sonneville et al., 2012; Zittermann et al., 2002). Although we did not observe significant changes in BMD, due to the nonweight bearing nature of the sport, it is not uncommon for swimmers to lose bone mass over the course of the season (Greene et al., 2011; Taaffe et al., 1997). In the total sample, all athletes maintained normal BMD which suggest that vitamin D supplementation may have played a role in preserving bone mass in athletes. Based on our findings, this may be particularly important in female athletes in the proximal dual femur, an area rich in trabecular bone and sensitive to changes in the hormonal environment.

In an effort to use a more sensitive method to assess changes in bone, we examined 3-month changes in bone turnover markers. BSAP and NTx did not significantly change in VIT D supplemented athletes or correlate with changes in 25(OH)D concentration. In previous studies, PTH has been shown to positively correlate with increased bone degradation as expressed by NTx (Rajakumar et al., 2008). However, in accordance with Halliday et al., we did not observe any statistically significant correlations between PTH and 25(OH)D (Halliday et al., 2011), nor was PTH correlated to NTx, or BSAP throughout the study. The lack of correlation between 25(OH) D and bone turnover markers may be due to the lack of vitamin D deficiency in our sample. When deficiency is not present, PTH concentrations will not significantly rise due to sufficient vitamin D status, thereby partially promoting bone preservation. In addition, persistent resistance training may have encumbered the ability to assess the benefits of vitamin D on bone accretion in 6 months (Kelley et al., 2001). We did observe a significant decrease in NTx concentration from baseline to midpoint. Previous literature suggests that diurnal variation influences bone turnover markers which peak in the morning and drop to their lowest point in the afternoon (Clemens et al., 1997; Clowes et al., 2002). The high concentration of bone turnover markers at midpoint could be due to the associated morning peak since most athletes provided samples during the morning hours. We also observed an increased loss of 25(OH)D from baseline to midpoint in PLA athletes, which may have had an additional impact on NTx concentrations. Conflicts with athlete schedules inhibited our ability to obtain blood at similar times as at baseline and midpoint. This may have contributed to the lack of significant associations between 25(OH)D and bone turnover markers at endpoint.

Vitamin D is also known to play a role in the regulation of inflammatory cytokines such as down-regulating the expression of TNF-alpha and IL-6 in the general population. However, few studies have described this relationship in athletes. Although a recent cohort of collegiate athletes and recreational runners observed increases in TNF-alpha after 25(OH)D

dropped below 32 ng/ml (Willis et al., 2012), we did not observe a significant relationship between 25(OH)D and inflammatory cytokines. Our findings may be explained by several factors including a large proportion of our sample (84%) maintaining 25(OH)D concentrations above 32 ng/ml and significant variability in measured inflammatory cytokine concentrations. TNF-alpha and IL-6 concentrations were highest at baseline in both PLA and VIT D athletes and consistently decreased over the 6-month intervention. High baseline concentrations may have been attributed to the physical stress of starting a new training regimen and observed decreases in midpoint and endpoint inflammatory cytokines may be due to training adaptation that followed (Pedersen & Toft, 2000). A major contributor to cytokine concentration is the time of the blood draw in relation to the last bout of exercise (Gleeson, 2007). During midpoint and endpoint measures, the majority of athletes had exercised within 24 hr of the blood draw. In contrast, the majority of athletes had not exercised within a 24 hr time span at baseline.

In this 6-month intervention, we observed that the majority of documented injuries occurred after an observed decrease in 25(OH)D from December to March; this suggests that vitamin D status may be of concern during the winter and early spring months. Although this observation does not imply cause and effect, recent findings to support this observation are equivocal. A recent study found that the incidence of injury was greater in football players with the lowest concentrations of 25(OH) D (mean 19.9 ng/ml; Weisenberger, 2011), which supports the premise that vitamin D may play an important role in injury prevention. However, other studies including athletic samples did not find significant relationships between 25(OH)D and incidence of injury (Ducher & al., 2011; Halliday et al., 2011). Therefore, more research aimed at determining the effects of 25(OH)D on risk of injury in athletes is needed.

Despite the strengths of this well-controlled study in collegiate swimmers and divers, there were limitations. A major limitation was that all athletes had 25(OH)D greater than 20 ng/ml, which likely inhibited our ability to document adverse skeletal and extraskelatal findings associated with vitamin D deficiency. The use of tanning beds was documented by a questionnaire at all study time points, however individual exposure to UVA or UVB bulbs was not collected. In addition, relying on recall memory with the use of our vitamin D lifestyle questionnaire may have contributed to under- or overreporting of actual UVB exposure. Although we have recognized this study limitation, reported tanning bed exposure between supplement groups was not significantly different throughout the intervention. Moreover, poor compliance to the supplement protocol limited our study findings. A final limitation of this study was the small sample size and large variability in inflammatory cytokines and bone turnover markers. During study design, a power analysis indicated that a sample of 60 athletes randomized to PLA ($n = 30$) or VIT D ($n = 30$) would have 80% power to detect an effect size of 0.8 when the 6-month change in 25(OH)D is compared between study arms. However, tanning self-treatment limited our ability to investigate group comparisons, and our final sample size of 32 would limit our ability to detect significant differences between changes in 25(OH)D and outcome variables in the VIT D and PLA groups.

Conclusion

This study indicates that vitamin D supplementation was effective at increasing 25(OH)D in athletes who primarily trained and competed indoors. We found that 25(OH)D was associated with increased MFL in men and increased bone mass in the proximal dual femur in both sexes. Although not significant, we also observed a greater number of injuries as 25(OH)D concentrations decreased. Future randomized controlled trials should recruit larger athletic samples to assess the “cause and effect” of vitamin D supplementation on bone, muscle, inflammation, and incidence of injury. Continuing clinical trials addressing the impact of vitamin D on athletes will help to expand our knowledge of the most advantageous 25(OH)D concentration needed to promote optimal health in athletes. Sufficient vitamin D supplementation could prove to be an easy and affordable method to preserve bone, decrease risk of injury, and maintain 25(OH)D.

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Figure 1.

Changes in serum 25(OH)D concentration with vitamin D or placebo treatment. Note. a=significant difference between supplement groups; $p = .03$ (independent samples t -test)

Figure 2.

A: Each line represents an individual's change in 25(OH)D of VIT D athletes ($n = 17$); PLA athletes ($n=10$). B: Top quartile (gain) 25(OH)D changes ($n = 6$); Top quartile (loss) 25(OH)D changes ($n = 7$).

Figure 3.
Frequency of reported injuries per athlete.

Table 1 Baseline Characteristics, M(*SD*)

	VIT D, <i>n</i> = 19	PLA, <i>n</i> = 13	Total <i>n</i> = 32
Male/female	11/8	8/5	19/13
Swimmer/diver	14/5	10/3	24/8
Age (y)	19(1.6)	19(1.1)	19(1.4)
Height (cm)	179(10.2)	178(8.6)	178(9.5)
Weight (kg)	73(11.0)	72(4.8)	73(9.7)
BMI (kg/m ²)	23(1.7)	23(2.0)	23(1.8)
25(OH)D (ng/ml)	*52(13.7)	*64(16.7)	57(16.0)
Race (n)			
Asian	0	1	1
Latino	2	0	2
White	17	12	29

* Significantly difference between supplement groups; *p* < .05

Table 2

The 6-Month Pattern of Change in 25(OH)D and Compliance to Supplement Protocol

25(OH)D change [VIT D/PLA]		25(OH)D change from Baseline to Midpoint (ng/ml)	25(OH)D change from Midpoint to Endpoint (ng/ml)	25(OH)D change from Baseline to Endpoint (ng/ml)
Gainers (<i>n</i> = 12) [12/0]	mean (<i>SD</i>)	9.42(15.62)	−1.08(14.72)	8.33(5.48)
	range	−6 to 50	−36 to 25	2 to 19
	% compliance	75.40	53.98	66.80
>7 ng/ml loss (<i>n</i> = 16) [4/12]	mean (<i>SD</i>)	−7.63(18.86)	−11.94(13.16)	−19.56(10.42)
	range	−41 to 33	−47 to 7	−42 to −9
	% compliance	88.67	48.41	72.56

Table 3

Pearson Correlations for 6-Month Changes in 25(OH)D and Body Composition

	Total MFL	Total FM	Total BMD	Total BMC
Male (<i>n</i> = 19)	0.48 (<i>p</i> = .03)*	-0.27 (<i>p</i> = .27)	-0.11 (<i>p</i> = .67)	-0.22 (<i>p</i> = .36)
Female (<i>n</i> = 13)	-0.25 (<i>p</i> = .40)	-0.46 (<i>p</i> = .12)	-0.08 (<i>p</i> = .79)	-0.52 (<i>p</i> = .07)
Total (<i>N</i> = 32)	0.26 (<i>p</i> = .15)	-0.03 (<i>p</i> = .06)	-0.15 (<i>p</i> = .41)	-0.31 (<i>p</i> = .08)

Note. MFL=mineral free lean mass, FM=fat mass, BMD=bone mineral density, BMC=bone mineral content.

* Significant correlation $p < .05$.

Table 4
Pearson Correlations for 6-Month Changes in 25(OH)D and Bone Turnover Markers and Inflammatory Cytokines

Bone turnover	Baseline to Midpoint			Midpoint to Endpoint			Baseline to Endpoint		
	VIT D	PLA		VIT D	PLA		VIT D	PLA	
BSAP	0.01 ($p = .98$)	0.31 ($p = .30$)		0.02 ($p = .95$)	0.23 ($p = .44$)		-0.11 ($p = .67$)	0.03 ($p = .94$)	
NTx	0.91 ($p = .71$)	-0.03 ($p = .92$)		0.21 ($p = .39$)	0.04 ($p = .90$)		0.16 ($p = .51$)	-0.27 ($p = .37$)	
Inflammatory cytokines									
TNF- α	0.18 ($p = .47$)	0.16 ($p = .60$)		-0.11 ($p = .64$)	-0.55 ($p = .05$)		-0.38 ($p = .88$)	0.24 ($p = .43$)	
IL-6	0.10 ($p = .68$)	-0.15 ($p = .61$)		-0.02 ($p = .95$)	-0.05 ($p = .87$)		-0.02 ($p = .94$)	-0.16 ($p = .61$)	
IL-1- β	-0.88 ($p = .72$)	-0.42 ($p = .16$)		0.25 ($p = .31$)	-0.32 ($p = .28$)		0.05 ($p = .83$)	-0.07 ($p = .83$)	

Note. BSAP=bone specific alkaline phosphatase; NTx=N-telopeptide; TNF- α =tumor necrosis factor alpha; IL=Interleukin.