Association between vitamin D status and testosterone and cortisol in ice hockey players

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ABSTRACT: The identification of the vitamin D receptor in tissues related to testosterone and cortisol production, in conjunction with the observed correlations between vitamin D levels and these hormones in the general population, suggest vitamin D may influence testosterone and cortisol concentrations in athletes. A cross-sectional study design was used to evaluate the association between 25(OH)D and testosterone and cortisol concentrations in young male ice hockey players ($n = 50$). All athletes were recruited during October from the Sosnowiec area, Poland (50° N). Commercially available ELISA kits were used to determine total serum 25(OH)D, testosterone and cortisol concentrations. Serum 25(OH)D concentration was analyzed as both a continuous and dichotomous variable, binned at the criteria for deficiency (< 20 ng·ml^{-1}), to investigate a threshold effect. Neither continuous ($r = 0.18$, $p = 0.20$) nor dichotomous ($r = 0.16$, $p = 0.27$) 25(OH)D concentration was significantly correlated with testosterone concentration. A small, inverse correlation ($r = -0.30$, $p = 0.04$) was detected between 25(OH)D and cortisol concentrations when analyzed as a dichotomous variable only. Serum 25(OH)D concentration was neither associated with testosterone ($p = 0.09$) nor cortisol concentrations ($p = 0.11$) after adjusting for age, fat free mass and fat mass in sequential linear regression. The inability of vitamin D status to independently predict testosterone and cortisol concentrations suggests that any performance-enhancing effects of vitamin D in athletes are unlikely to be mediated primarily through these hormones, at least amongst young male ice-hockey players.

INTRODUCTION

Serum 25-hydroxyvitamin D (25[OH]D) concentration has been associated with superior aerobic [1] and anaerobic [1, 2] exercise performance in athletes. However, evidence to support a causal relationship between 25(OH)D concentration and exercise performance in athletes is lacking, as the majority of controlled trials report no effect of vitamin D supplementation [3-6]. To date, most interventions in athletes lack statistical power, have few severely vitamin D deficient athletes and/or do not standardize training exposure before and during the intervention. These design issues make it difficult to detect small effects of vitamin D supplementation on exercise performance. Small enhancements in exercise performance (e.g., 1-2% improvement) may be practically meaningful for high-level athletes [7]; however, conducting interventions to detect these small effects in athletes

may not be practical for several reasons, including that large sample sizes would be required to detect differences between treatment groups. Given the methodological issues with previous trials and the practical challenges of conducting interventions with elite athletes, it remains unclear whether vitamin D supplementation is a worthwhile strategy to positively influence athletic performance.

Despite the equivocal results from randomized trials in athletes, several plausible biological mechanisms exist to support a mechanistic link between vitamin D status and physical performance in athletes. Traditionally, the identification of the vitamin D receptor (VDR) in cardiac and skeletal muscle has primarily been cited to support the plausibility of the vitamin D system influencing exercise performance [8]. It appears poor vitamin D status may reduce

muscle function due to altered kinetics of the active hormone, 25-hydroxyvitamin D, leading to disruptions in calcium and phosphate control, protein synthesis and phospholipid metabolism [9]. However, the exact mechanism of action remains unclear. The expression of the VDR in other tissues has led to the investigation of alternative mechanisms [10]. The VDR is located in the Leydig cells of the testes [11], the major site of testosterone production in men, and in the paraventricular nuclei within the hypothalamus [12], which are involved in regulatory control of cortisol synthesis in the adrenal gland [13]. In addition, the VDR and glucocorticoid receptor are located adjacent to each other in cells and the results from *in vitro* studies indicate that vitamin D may increase the effectiveness of cortisol signaling [14], which may reduce systemic concentrations needed to achieve function. Thus, the vitamin D system may influence exercise performance over time by modifying testosterone and cortisol levels.

Testosterone and cortisol production are involved with the remodeling of skeletal muscle. Testosterone is an anabolic steroid hormone associated with muscle protein accretion [15] and increased exercise performance [16, 17]. Cortisol is a glucocorticoid known for its catabolic and anti-inflammatory functions, and chronic elevation of this hormone may be detrimental to exercise performance [18]. In the sport science literature, tracking changes in testosterone and cortisol and their ratio (testosterone-to-cortisol) have been used to monitor athletes and evaluate the relative predominance of anabolic or catabolic metabolism during training [19-24]. In theory, demanding exercise training with inadequate recovery may lead to disturbances in hormonal balance, which may negatively impact skeletal muscle regeneration and compromise function [22]. With that said, studies in athletes assessing changes in hormones with respect to exercise training and performance outcomes have yielded conflicting results [19-25]. Never-the-less, disturbance of testosterone levels [10], or hormonal balance, leading to reduced protein synthesis seems to be a plausible mechanism by which poor vitamin D status may negatively influence exercise performance in athletes.

Serum 25(OH)D concentration has been positively associated with resting testosterone concentrations in the general population [26-28], while limited reports exist examining the relation between 25(OH)D and cortisol concentrations [29]. We are aware of only one study in athletes evaluating the association between 25(OH)D, testosterone and cortisol concentrations. In 45 soccer players, 25(OH)D concentration exhibited a positive association with testosterone concentration and an inverse association with cortisol concentration [30]. Replication of these findings in athletes is needed to confirm the relationship, especially in athletes participating in other sports [30]. Examining the association between 25(OH)D concentration and these hormones may provide tentative evidence for an alternative mechanism by which vitamin D may enhance exercise performance in athletes. This information would aid in determining the plausibility of vitamin D influencing exercise performance through the actions of cortisol and testosterone and may inform future inter-

ventions supplementing vitamin D in athletes as a means to manipulate hormonal balance. Establishing mechanisms of action in athletes are especially important given the aforementioned constraints of conducting vitamin D and exercise performance interventions in this population. Therefore, the purpose of this study was to assess the cross-sectional association between 25(OH)D and testosterone and cortisol concentrations in young male athletes of the same sport. We hypothesized there would be a positive association between 25(OH)D and testosterone concentrations and a negative association between 25(OH)D and cortisol concentrations.

MATERIALS AND METHODS

Participants

Fifty male ice hockey players from the Private Athletic High School of the Polish Ice Hockey Federation participated in our study. The anthropometric and demographic data of the participants are presented in Table 1. All athletes were Caucasian and of Polish nationality. The type, intensity and volume of exercise training was similar for all athletes. During the competitive season, athletes participated in five training sessions (120-180 min each) and 1-2 matches per week. Athletes ate at the same school canteen. Ethical approval for this study was provided by the local Ethical Committee. Prior to the study written informed consent was obtained from participants or their parents for cases where the participant was under 18 years of age. The study was conducted according to the Declaration of Helsinki.

Study Design

A cross-sectional study design was used to evaluate the strength of association between 25(OH)D and testosterone and cortisol concentrations in young male ice hockey players. All athletes were recruited during October from the Sosnowiec area, Poland (50°N). The blood collection and anthropometric measurements took place in the morning (between 8 and 9 a.m.), after overnight fasting and a minimum of 12 hours after the last training session.

Blood and Anthropometric Measurements

After blood collection, all blood samples were centrifuged for 10 minutes at 3500 rpm. Serum samples were stored frozen at -20ºC until the analysis.

Total serum 25(OH)D concentration was measured using commercially available ELISA kits (DiA Source, Belgium) according to the manufacturer's protocol, with the average intra-assay coefficient of variation below 4.0%. The 25OH Vitamin D Total ELISA (DiA Source, Belgium) has the Certificate of Proficiency by the Vitamin D External Quality Assessment Scheme (DEQAS) Advisory Panel.

Commercially available ELISA kits (DRG, Germany) were used to determine total cortisol and testosterone concentrations in serum. All samples were assayed in duplicates and the coefficients of variation of the intra-assays were less than 6% for cortisol and testosterone; moreover, the reference material (BIO-RAD, USA) was attached

to each analytical run. The hormonal analyses were carried out in the laboratory of the Department of Biochemistry with an implemented quality system (with accreditation of the Polish Centre for Accreditation no. AB946).

Body mass (BM) and body composition were measured using a Tanita Body Composition Analyser MC-420 (Japan) based on the method of bioelectrical impedance (BIA). BIA has been demonstrated to be a valid predictor of body composition in athletes, with prediction error similar to anthropometric techniques and good reliability [31].

Statistical Analysis
Data were evaluated for normal distributions using the Kolmogorov-Smirnov test and evaluation of the histogram for 25(OH)D concentration, each of which indicated a positive skew. The distribution of the 25(OH)D concentration data was improved with a logarithmic transformation. Serum 25(OH)D concentration was also binned at the criteria for deficiency (< 20 ng·ml^{-1}) to investigate a threshold effect. Means ± standard deviations were used to present descriptive characteristics. Associations between continuous and dichotomous variables were evaluated using Pearson correlational coefficients. The associations between 25(OH)D concentration and testosterone and cortisol were evaluated using sequential linear regression (SLR) to control for potential covariates. Correlations between variables and theory were used to identify potential covariates for use in the control step in SLR. Previous literature has reported associations between maturation [32], body composition [33] and hormone status. Age, fat-free mass (FFM) and fat mass (FM) were used in step one for each SLR model, with 25(OH)D entered in step two as either a continuous or dichotomous variable. Multivariate normality was inspected using residual plots from the SLR analysis. Two-sided p-values and an alpha of 0.05 were used to determine statistical sig-

nificance. All analyses were performed using SPSS version 20.0 (IBM, Armonk, NY). The design of this study allowed for the detection of moderate correlations (0.38) with 80% power.

RESULTS
Descriptive information for the 50 ice hockey players is presented in Table 1. Only 38% ($n = 19$) of athletes had sufficient 25(OH)D concentration (≥ 30 ng·mL^{-1}) [34]. Forty percent ($n = 20$) of athletes had insufficient 25(OH)D concentration (< 30 ng·mL^{-1}), while 22% ($n = 11$) were deficient (< 20 ng·mL^{-1}) [34]. Testosterone and cortisol concentrations for the athletes are shown within Table 1.

Correlations between testosterone and 25(OH)D concentrations and potential covariates are presented in Table 2. Figure 1 depicts the relationship between 25(OH)D and testosterone concentrations. Neither continuous ($r = 0.18, p = 0.20$) nor dichotomous ($r = 0.16$, $p = 0.27$) 25(OH)D concentration was correlated with testosterone concentration. There was a statistically significant bivariate correlation ($r = 0.33, p = 0.019$) between age and testosterone concentration. SLR was used to investigate potential suppression of the bivariate relationship between testosterone and 25(OH)D concentrations due to age and to control for body composition (Table 3). Serum 25(OH)D concentration was not a significant predictor of testosterone concentration after controlling for age, FFM and FM ($R^2 = 0.17$, Finc$(1,45) = 2.99, p = 0.09$). Analyzing 25(OH)D concentration as a dichotomous variable did not substantially change its relationship to testosterone.

Correlations between cortisol and 25(OH)D concentrations and potential covariates are also within Table 2. Figure 2 depicts the relationship between 25(OH)D and cortisol concentrations. A small, inverse correlation ($r = -0.30, p = 0.04$) was detected between 25(OH)D and cortisol concentrations when analyzed as a dichotomous variable only. FM was trending toward a statically significant asso-

TABLE 1. Descriptive information for the athletes ($n = 50$).

	Mean (SD)	Min-Max
Age (years)	17.2 (0.9)	15.6-18.7
Height (cm)	180.6 (6.6)	165.5-194.0
Body mass (kg)	75.6 (10.8)	52.4-100.8
BMI (kg·m^{-2})	23.1 (2.7)	17.2-28.4
Percent body fat (%)	12.9 (3.8)	3.5-20.8
Fat free mass (kg)	65.6 (7.5)	47.1-82.9
Fat Mass (kg)	10.0 (4.1)	2.0-19.4
Vitamin D (ng·ml^{-1})	30.3 (14.9)	12.5-91.4
Testosterone (nmol·L^{-1})	19.2 (4.4)	9.5-28.8
Cortisol (nmol·L^{-1})	493 (62)	366-630

TABLE 2. Correlations between potential predictor variables and testosterone and cortisol concentrations.

	Testosterone *r*	Cortisol *r*
Age (years)	0.332[a]	0.082
Body mass (kg)	0.052	0.224
BMI (kg·m^{-2})	0.010	0.240[b]
Percent body fat (%)	0.018	0.222
Fat free mass (kg)	0.068	0.186
Fat Mass (kg)	0.012	0.253[b]
25(OH)D concentration (log)	0.184	-0.210
25(OH)D concentration (binned)	0.159	-0.296[a]

Note. n= 50. [a] indicates statistically significant at $p < 0.05$. [b] indicates trending at $p < 0.10$.
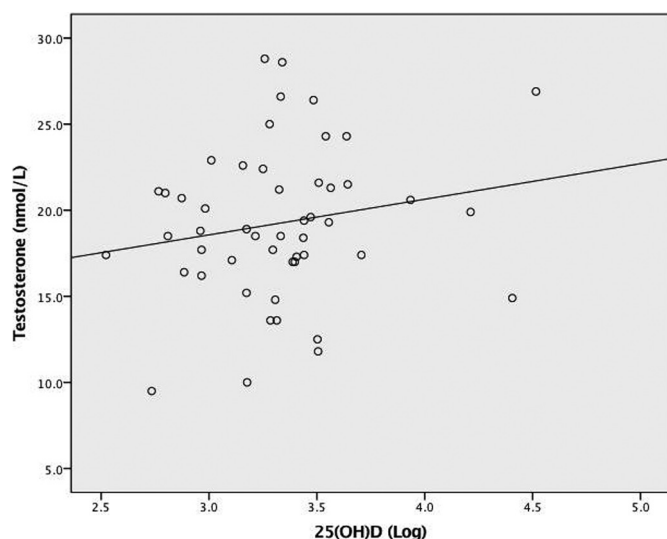
FIG. 1. The relationship between 25(OH)D and testosterone concentrations ($n = 50$). R^2 linear = 0.03, $p = 0.20$.
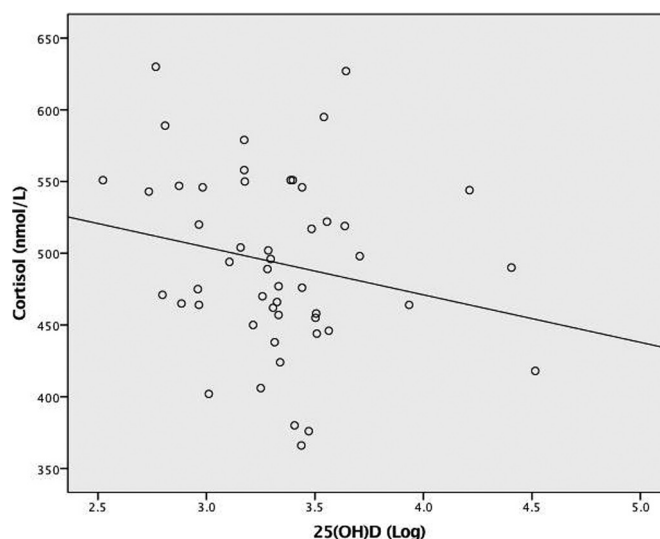

FIG. 2. The relationship between 25(OH)D and cortisol concentrations ($n = 50$). R^2 linear = 0.04, $p = 0.14$.

ciation with 25(OH)D (r = -0.24, p = 0.09; data not shown) and cortisol concentrations (r = 0.25, p = 0.08). In SLR (Table 4), 25(OH)D concentration (dichotomous) was not a significant predictor of cortisol concentration after adjusting for age, FFM and FM

($R^2 = 0.13$, Finc(1,45) =2.75, $p = 0.11$). Analyzing 25(OH)D concentration as a continuous variable did not significantly impact the results.

TABLE 3. Sequential linear regression with (log of) 25(OH)D concentration predicting testosterone concentration

Predictors	Regression Coefficient	Standard Error	Standardized Regression Coefficient	p-value	ΔR2 (adjusted)
Step 1[a]					0.057
Age	2.026	0.775	0.401	0.012	
FFM	-0.085	0.132	-0.145	0.521	
FM	0.189	0.236	0.174	0.429	
Step 2[b]					0.039
25(OH)D	2.747	1.588	0.244	0.090	

Note. Vitamin D status for the athletes was log-transformed. n = 50.
[a] Predictors: Age, FFM, and FM. [b] Predictors: Age, FFM, FM and 25(OH)D.

TABLE 4. Sequential linear regression with (binned) 25(OH)D concentration predicting cortisol concentration.

Predictors	Regression Coefficient	Standard Error	Standardized Regression Coefficient	p-value	ΔR2 (adjusted)
Step 1[a]					0.012
Age	8.745	11.115	0.123	0.436	
FFM	-1.085	1.926	-0.131	0.576	
FM	3.938	3.349	0.259	0.246	
Step 2[b]					0.036
25(OH)D	-37.008	22.337	0.251	0.105	

Note. Vitamin D status for the athletes was binned at deficiency (<20.0 ng·mL^{-1}). $n = 50$.
[a] Predictors: Age, FFM, and FM. [b] Predictors: Age, FFM, FM and 25(OH)D.

DISCUSSION

The primary aim of this study was to evaluate the cross-sectional associations between 25(OH)D and testosterone and cortisol concentrations in young male ice hockey players. Vitamin D status was analyzed as both continuous and dichotomous variables to investigate a threshold effect at the criteria for deficiency (< 20.0 ng·ml^{-1}). Bivariate correlations between variables indicated that the relationship between 25(OH)D and cortisol concentrations was improved when analyzed as a dichotomous variable ($r = -0.30$, $p = 0.04$ vs. $r = -0.22$, $p = 0.12$), whereas the correlation for testosterone concentration was not enhanced ($r = 0.16$, $p = 0.27$ vs. $r = 0.18$, $p = 0.20$). The main finding of this study was that 25(OH)D concentration was neither associated with testosterone ($p = 0.09$) nor cortisol concentrations ($p = 0.11$) after adjusting for age and body composition.

Our failure to detect a statistically significant association between 25(OH)D and testosterone concentrations is not in agreement with the majority of previous literature. Cross-sectional studies, primarily conducted in aging populations, have consistently reported a positive association between 25(OH)D and testosterone concentrations in men [26-28]. One intervention found a small increase in testosterone concentration with vitamin D supplementation [35], though this finding has not been confirmed in other intervention trials [36, 37]. In terms of studies with athletes, a recent observational investigation in soccer players detected a weak, positive association between 25(OH)D and testosterone concentrations along with similar seasonal variation [30]. In contrast to many of these prior observational investigations, we did not detect an association between 25(OH)D and testosterone concentrations in young ice hockey players. It is likely that the association between 25(OH)D and testosterone concentrations is weak and larger cohorts are needed to detect such small correlations. Furthermore, it is also possible that severe vitamin D deficiency (< 10 ng·mL^{-1}) is required to substantially reduce testosterone concentrations in men. Observational evidence indicates that the relationship between 25(OH)D and testosterone concentrations is not linear, but rather the association is strongest in individuals with deficient 25(OH)D concentrations [28]. However, neither sample size nor the prevalence of vitamin D deficiency explain the differing findings in our study and that in Lombardi et al. [30]. While neither study reported athletes with severe deficiency, the prevalence of deficiency (< 20 ng·mL^{-1}) and insufficiency (< 30 ng·mL^{-1}) was greater in our slightly larger cohort of athletes. It should be noted that Lombardi et al. [30] reported a bivariate correlation in athletes competing on different teams and did not control for confounding variables, which tend to augment bivariate relationships.

Similar to the investigation in soccer players [30], we detected a bivariate association between 25(OH)D and cortisol concentrations. However, this association was no longer significant after adjusting for body composition and age. It is not known if the association between 25(OH)D and cortisol concentrations reported in Lombardi et al. [30] would persist after statistical adjustment for confounding, specifically adjustment for body composition and training volume. Evidence suggests adipose tissue and exercise training influence cortisol, testosterone and 25(OH)D concentrations [33, 38-40].

Serum 25(OH)D concentration has been associated with numerous aspects of physical performance in observational studies of athletes [1, 2]. In non-athletes and athletes, 25(OH)D concentration has demonstrated positive associations with resting testosterone levels [26-28, 30]. An inverse association between 25(OH)D and cortisol concentrations has also been reported in athletes [30]. Based on these links, it is plausible that some of the purported benefit of vitamin D on physical performance is mediated through favorable changes in anabolic and catabolic hormones. The inability of 25(OH)D concentration to independently predict testosterone and cortisol concentrations in our athletes suggests that any performance-enhancing effects of vitamin D are unlikely to be mediated, at least primarily, through these hormones amongst young male ice-hockey players. Instead, actions through the VDR in cardiac and skeletal muscle, or other tissues, may represent more plausible mechanisms. Future studies incorporating larger cohorts and measurement of relevant confounding variables are needed to elucidate the relationship between 25(OH)D and testosterone and cortisol concentrations in athletes.

Strengths of this study are that the athletes had similar training regimens and dietary options, statistical adjustment for body composition, and simultaneous assessment of both testosterone and cortisol. Training volume is an important modifier of cortisol and testosterone concentrations [39] and physical activity levels are associated with 25(OH)D concentration [38]. Thus, the relative homogeneity of training within this sample likely reduces any potential impact exercise volume may have on the associations between 25(OH)D and cortisol and testosterone concentrations. With regards to diet, effects on testosterone are most likely mediated through long-term changes in body composition [33]. Given the similar nutritional options available to the athletes, and the statistical adjustment for body composition, it is unlikely that unmeasured dietary variables significantly influenced the association between 25(OH)D and testosterone concentrations. Lastly, the concurrent measurement of cortisol and testosterone may provide a better overall picture of the anabolic/catabolic hormonal environment than testosterone alone [41]. Importantly, the majority of previous studies examining the association between 25(OH)D and testosterone concentrations have failed to incorporate cortisol measurements [26-28].

Although this investigation had strengths, several important limitations need to be acknowledged. While this population of young male ice-hockey players fell within a relatively narrow age range (15.6-18.7 years), maturation differences could confound the associations between 25(OH)D and hormone concentrations, particularly for testosterone, as it is known to increase substantially throughout adolescence [32]. Age was used as a proxy measure for maturation and entered in SLR as a control variable to help minimize this problem, but residual confounding remains a possibility given

the limited accuracy of age for determining physical maturation. The use of BIA to assess body composition is another limitation. The prediction error associated with this technique is higher than that of DEXA and hydrostatic weighing. The reduced accuracy of BIA may have diminished our ability to detect statistically significant associations between body composition and other variables. It may also have impacted our ability to adjust for body composition in SLR. Future investigations should use DEXA or hydrostatic weighing for the assessment of body composition. Furthermore, we only assessed the association between 25(OH)D and hormone concentrations during October and it is possible that the strength of association is modified by season due to changing 25(OH)D concentrations. This study's modest sample size of 50 is a limitation, only allowing for the detection of moderate correlations (0.38) with 80% statistical power. A sample size of greater than 120 would be required to detect correlations less than 0.25. With that said, it seems unlikely that such small associations in a cross-sectional, observational study would translate to meaningful causal effects in randomized trials.

CONCLUSIONS

In the present study, no statistically significant associations were found between 25(OH)D and testosterone concentrations. While there was a small, statistically significant inverse correlation between dichotomous 25(OH)D status and cortisol concentration, this association was not significant after adjusting for body composition and age. Our data suggest that any performance-enhancing effects of vitamin D are unlikely to be primarily mediated through these hormones in young hockey athletes. Genomic and nongenomic signaling through the VDR at cardiac and skeletal muscle seem to be more plausible mechanisms. With that said, monitoring vitamin D status remains a critical strategy to identify athletes that are deficient and that would benefit from supplemental vitamin D intake for other reasons.

Acknowledgements

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