

Article

Associations Between Serum 25-Hydroxyvitamin D Concentration, Sun Exposure Index, Body Composition, and Repeated Sprint Ability in Male Soccer Referees

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ABSTRACT: Despite the high prevalence of hypovitaminosis D among athletes and the proposed role of vitamin D in musculoskeletal health and performance, data on vitamin D status in soccer referees and its relationship with body composition and performance remain scarce. This cross-sectional study evaluated 25(OH)D concentrations and their associations with the sun exposure index (SEI), body composition, and performance in a sample of 21 male referees (30.6 ± 3.4 years) from the Brazilian Football Confederation. Serum 25(OH)D concentration was assessed from blood samples, SEI by questionnaire, body fat percentage (BF%), fat free mass, and bone mineral density (BMD) by dual-energy X-ray absorptiometry, calcium and carbohydrate intake by 24-h recall, and performance by the repeated sprint ability (RSA) test. Associations were examined using linear regression analysis adjusted for (1) age, (2) fat free mass, and (3) carbohydrate or calcium intake. 76% presented serum 25(OH)D concentrations < 30 ng/mL. 25(OH)D was positively associated with SEI ($\beta = 1.17$; $p = 0.04$, Cohen's $f^2 = 0.19$), whereas no significant associations were observed with BMD, BF% or RSA, even after adjustments. There is a high prevalence of hypovitaminosis D among the soccer referees evaluated. Although 25(OH)D was not significantly associated with body composition or performance, its positive association with SEI reinforces the importance of sun exposure.

Keywords: Body composition; Performance; Sports nutrition; Vitamin D; Bone density

1. Introduction

Among the pleiotropic benefits of vitamin D are its key roles in maintaining and promoting musculoskeletal health [1]. The metabolic actions of vitamin D occur through the interaction of its active form (1,25-dihydroxyvitamin D) with the vitamin D receptor (VDR), which is expressed in various body tissues, including bone, skeletal muscle, and adipose tissue. It is well known that vitamin D regulates intestinal absorption and renal reabsorption of calcium and phosphorus and induces bone remodeling. Also, vitamin D appears to be involved in cell proliferation and differentiation, regulation of calcium homeostasis, muscle contractile function, growth, and modulation of damage-induced muscle inflammation [2,3].

However, despite the potential importance of vitamin D for athletes, hypovitaminosis D remains common in sporting populations. A high prevalence of vitamin D insufficiency (30%; $n = 3725$; <20 ng/mL) [4] and inadequacy (56%; $n = 2313$; <32 ng/mL) was found in athletic populations [1]. Factors such as sun exposure, seasonal variation, geographic location [1], age [4], gender [5], and body composition [6–8] have been identified as important determinants of vitamin D status.

Evidence suggests that hypovitaminosis D can impair bone health and muscle function, increase the risk of injury, delay muscle recovery, and compromise exercise performance [9]. In elite sports, particularly professional soccer, players and referees generally differ in body composition, training load, exercise capacity, and nutritional requirements [10]. However, during the game, the physical/physiological demands of the referees are similar to those of the players [11,12]. Thus, soccer referees require adequate physical fitness and appropriate nutrition to cope with the duration and intensity demands of the match and support accurate decision-making [13].

Although a high prevalence of hypovitaminosis D has been observed among athletes [1,4], data on vitamin D status in soccer referees remain scarce. Furthermore, understanding the association of vitamin D with body composition and physical performance may provide relevant information for nutritional monitoring. Therefore, this study aimed to: (i) assess vitamin D levels and (ii) examine the associations between serum 25(OH)D concentration and sun exposure habits, body composition, and sprint capacity in elite national level soccer referees.

2. Materials and Methods

2.1. Sample

The participant selection was conducted through non-probabilistic sampling with members of the Brazilian Football Confederation (CBF) of Santa Catarina–SC. There were 29 male soccer referees eligible to participate in the study. Eight refused or could not participate due to travel or refereeing commitments. The final sample consisted of 21 (72%) elite national level soccer referees.

The study was approved by the Federal University of Santa Catarina (UFSC) Human Research Ethics Committee (protocol number: 2.572.301) and followed the guidelines established by the Declaration of Helsinki. All participants provided informed written consent to participate. The eligibility criteria consisted of not having a metal prosthesis in the body and not using vitamin D supplementation.

2.2. Data Collection

This observational cross-sectional study collected data in July 2018 (winter). Participants attended the UFSC Department of Nutrition Body Composition Laboratory in the morning, following instructions to fast overnight, wear appropriate clothing, and refrain from using any stimulants and from performing moderate or intense physical activity the day before. During a laboratory visit, blood samples were collected for analysis of serum 25(OH)D levels, body composition was assessed, and a sun exposure questionnaire was administered. Food intake was assessed using three 24-h recalls collected on non-consecutive days before the physical test; two were retrieved by phone, and one in person on the day of the laboratory visit.

Repeated sprint ability (RSA) was assessed at the Athletics Center outdoors sports track at UFSC, from 5 pm in the afternoon. All data collection was performed by trained professionals.

2.3. Solar Exposure Questionnaire

Information on participants' SEI was assessed using a simplified, adapted questionnaire [14] consisting of five multiple-choice items related to habitual sun exposure, sunscreen use, and skin response to sun exposure. Each response option was assigned a predefined score, and the final SEI corresponded to the sum of the scores obtained in all items.

The questionnaire included the following items: (1) Frequency of being outdoors for more than 15 min with arms and/or legs exposed and without sunscreen: daily = 8; 4 to 6 times a week = 6; 2 to 3 times a week = 3.5; Once a week = 2; less than once a week or never = 1; (2) Time you normally do outdoor sports without sunscreen: between 7 and 11 a.m. = 1; between 11 a.m. and 3 p.m. = 2; between 3 and 5 p.m. = 1; after 5 p.m. = 0; I do not or rarely do outdoor sports = 0; (3) Frequency of using sunscreen: never = 3; 1 to 2 times a week = 2; 3 to 6 times a week = 1; daily = 0; (4) Sun Protection Factor (SPF) you normally use: never = 3; SPF 15 = 2; SPF 30 = 1; SPF greater than 30 = 0; (5) What is your tendency to tan or burn?: never burns or tans = 0; never burns but tans = 1; rarely burns or tans = 2; burns minimally and easily browns = 3; moderately burning and evenly browning = 4; burns easily and tans minimally = 5; burns easily and never tans = 6.

The final SEI ranged from 22 (high sun exposure, rarely uses sunscreen, burns easily, and does not tan easily) to 1 (has no sun exposure habit, frequent use of sunscreen, never burns or tans).

2.4. Anthropometry and Body Composition

Body mass was recorded on an electronic scale (model RIW200 Welmy[®], São Paulo, SP, Brazil) with a precision of 100 g. Height was measured on a stadiometer (Alturaexata[®], Belo Horizonte, BH, Brazil) with a precision of 1 mm. The collected body mass and height data were used to determine the body mass index (BMI). Whole body bone mineral density (BMD), body fat percentage (BF%), and fat free mass were evaluated by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, General Electric-GE[®], Madison, WI, USA). The device was calibrated before analysis, and the protocol used followed the manufacturer's instructions.

2.5. Food Intake

To assess food intake, three 24-h recalls for non-consecutive days were used. The first two recalls were collected by telephone, and the last was collected in person on the day of the physical test. To reduce potential biases, the Multiple Pass Method [15] and a photo album of home measurements were used as supporting material. The same interviewer collected all recalls of all participants. The data obtained were converted to grams or milliliters using the table of homemade measurements or weighed on an analytical scale (model YP-B20002, Bioscale[®], Paraná, PR, Brazil). Energy, carbohydrate, protein, vitamin D, and calcium were assessed (Nutrition Data System for Research[®] software, Graduate Pack 2017 version; NCC Food and Nutrient Database, University of Minnesota, Minneapolis, MN, USA). Inter- and intra-individual variability in macro and micronutrient intakes was adjusted. In addition, micronutrient intake was adjusted for the total energy value according to the residual method [15,16].

2.6. Vitamin D Serum Level Analysis

Blood was collected from the antecubital vein. Serum 25(OH)D was determined by Chemiluminescent Microparticle Immunoassay (ADVIA-CENTAURXP[®], Siemens, Munich, Germany). 25(OH)D concentration was classified according to the Endocrine Society Clinical Practice Guideline parameters:

adequate (25(OH)D > 30 ng/mL), insufficient (25(OH)D > 21 and <29 ng/mL), and inadequate (25(OH)D < 20 ng/mL) [17].

2.7. Repeated Sprint Ability

The RSA is used by the CBF according to International Federation of Association Football (FIFA) protocols to evaluate referees' physical agility and speed performance in fast runs during the season. To pass the test, the field referees must complete six 40-m sprints within 6.1 s of each sprint, and the assistant referees must perform five 30-m sprints within 4.8 s each. The recovery intervals between sprints were a maximum of 60 and 30 s for the field and assistant referees, respectively. To characterize sprint capacity, were considered the best sprint time (BT) and average sprint time (AT, mean of all sprints). The percentage of time decrease across sprints (RSA) was evaluated according to the formula: $RSA = [(AT/BT) * 100] - 100$, which estimates the ability to resist fatigue during repeated sprint exercise [18].

2.8. Data Treatment and Analysis

Statistical analyses were performed using STATA for Windows, Version 13.1 (StataCorp LP, College Station, TX, USA). Data normality was assessed using the Shapiro-Wilk test and visual inspection of q-q plots and histograms. The referees were categorized according to their position (field or assistant), and Student *t*-test or Mann-Whitney were used to compare the two groups. Linear regression, both crude and adjusted, was used to examine the associations between the dependent variables (25(OH)D, BMD, and RSA) and the independent variables (SEI, BF%, and 25(OH)D). Three adjustment models were tested: (1) age, (2) fat free mass, and (3) food intake (carbohydrate intake for BF% and RSA regressions or calcium intake for SEI and BMD regression). For RSA, all analysis were adjusted for referee position, as the RSA protocols used differed between the two referee categories. The significance level $p < 0.05$ was adopted. In addition, the quality of the models was assessed using the multicollinearity diagnostic techniques (VIF) and the residual method. We verified the regression model adjustment assumptions regarding the residual's normality by Shapiro-Wilk test and visual assessment of graphs of the residuals. Cohen's f^2 was used to estimate the effect size (EF) of the results, and values were ranked as small (between 0.02 and 0.14), moderate (between 0.15 and 0.34), and large (≥ 0.35) [19].

3. Results

The characteristics of the sample (8 field referees and 13 assistant referees) and the results of the physical test and food intake are presented in Table 1. There was no significant difference between field and assistant referees for age, anthropometric and body composition variables, SEI, 25(OH)D levels, food intake, and RSA performance.

Table 1. Sample characterization and description (mean $\pm$ SD) of sun exposure index and vitamin D, food intake, and sprint capacity in elite national level soccer referees ($n = 21$).

	Total N = 21	Field Referee N = 8	Assistant Referee N = 13	<i>p</i> -Value
Sample characterization				
Age (years)	31 $\pm$ 3	31 $\pm$ 4	30 $\pm$ 3	0.41
Body mass (kg)	82 $\pm$ 8	85 $\pm$ 7	81 $\pm$ 8	0.29
Height (m)	180.1 $\pm$ 5.4	181.1 $\pm$ 3.7	179.5 $\pm$ 6.3	0.52
BMI (kg/m ²)	25.3 $\pm$ 1.6	25.8 $\pm$ 1.8	24.9 $\pm$ 1.4	0.26
BF% (%)	20 $\pm$ 7	20 $\pm$ 7	21 $\pm$ 7	0.87
Fat free mass (kg)	66 $\pm$ 8	69 $\pm$ 8	65 $\pm$ 8	0.33
BMD (g/cm ²)	1.30 $\pm$ 0.10	1.30 $\pm$ 0.01	1.30 $\pm$ 0.02	0.55

Sun exposure and 25(OH)D				
Frequency of exposure	4.8 ± 2.3	5.3 ± 2.5	4.5 ± 2.2	0.42
Time of exposure	0.6 ± 0.7	0.6 ± 0.7	0.6 ± 0.8	0.98
Use of sunscreen	2.1 ± 0.6	1.7 ± 0.5	2.4 ± 0.6	0.03
Sun protection factor	1.2 ± 0.9	1.0 ± 0.5	1.4 ± 1.1	0.38
Tendency to tan or burn	3.9 ± 1.1	4.4 ± 1.1	3.7 ± 1.1	0.18
SEI	13 ± 3	13 ± 2	13 ± 3	0.70
25(OH)D (ng/mL)	27 ± 8	29 ± 8	26 ± 8	0.39
Food intake				
Energy (kcal)	2510 ± 230	2580 ± 250	2472 ± 225	0.32
Energy (kcal/kg)	31 ± 4	31 ± 4	31 ± 4	0.86
Carbohydrates (g)	240 ± 70	240 ± 70	230 ± 70	0.73
Carbohydrates (g/kg)	2.9 ± 0.9	2.9 ± 1.0	2.9 ± 0.8	0.8
Protein (g)	150 ± 30	160 ± 40	150 ± 30	0.35
Vitamin D (mcg)	5.4 ± 1.7	5.9 ± 2.4	5.0 ± 1.2	0.26
Calcium (mg)	830 ± 230	820 ± 290	840 ± 210	0.92
Performance				
BT (s)	-	5.6 ± 0.2	4.4 ± 0.2	-
AT (s)	-	5.5 ± 0.2	4.3 ± 0.1	-
RSA (%)	-	1.7 ± 0.8	2.3 ± 1.5	0.36

Legend: 25(OH)D, 25-Hydroxyvitamin D. AT, Average Sprint Time. BF%, Body Fat Percentage. BMD, Bone Mineral Density. BMI, Body Mass Index. BT, Best Sprint Time. RSA, Percentage of Time Decreased in Sprints. SEI, Sun Exposure Index. Differences between groups were tested by the student *t* test.

Five (24%) referees had adequate vitamin D status, while thirteen (62%) and three (14%) had insufficient and inadequate vitamin D status, respectively. Fifteen (72%) self-reported white ethnicity, three (14%) brown ethnicity, and three (14%) chose not to answer.

In regression analysis (Table 2), SEI was positively associated with 25(OH)D concentrations ($\beta = 1.17$; $p = 0.043$), with a moderate ES (Cohen's $f^2 = 0.19$). This association remained statistically significant after adjustment for fat free mass ($\beta = 1.23$; $p = 0.045$), although the EF was small (Cohen's $f^2 = 0.13$). In contrast, 25(OH)D concentrations were not significantly associated with BMD or BF% neither with RSA in all analyses performed (Table 2).

Table 2. Relationships between 25(OH)D and sun exposure variable, body fat percentage and sprint capacity in elite soccer referees ($n = 21$).

Dependent Variable	Independent Variable		β	IC95%	<i>p</i> -Value	R^2	VIF	Cohen's f^2
25(OH)D	SEI	Crude	1.17	0.004–2.308	0.043	0.157	-	0.19
		Model 1	0.93	−0.110–1.976	0.08	0.320	1.04	0.47
		Model 2	1.23	0.028–2.439	0.045	0.117	1.07	0.13
BMD	25(OH)D	Crude	0.001	−0.002–0.005	0.43	0.032	-	0.03
		Model 1	0.001	−0.002–0.006	0.41	0.068	1.37	0.07
		Model 2	0.001	−0.001–0.004	0.38	0.414	1.00	0.71
		Model 3	0.001	−0.002–0.05	0.41	0.032	1.00	0.03
25(OH)D	BF%	Crude	−0.12	−0.677–0.437	0.66	0.045	-	0.05
		Model 1	−0.18	−0.672–0.303	0.44	0.214	1.01	0.27
		Model 2	−0.15	−0.872–0.579	0.68	0.098	1.06	0.11
		Model 3	−0.21	−0.759–0.340	0.43	0.038	1.05	0.04
RSA	25(OH)D	Adjusted ^a	0.02	−0.013–0.054	0.23	0.107	1.04	0.12
		Model 1 ^a	0.02	−0.018–0.063	0.26	0.047	1.27	0.05
		Model 2 ^a	0.02	−0.009–0.049	0.17	0.272	1.06	0.37

Model 3 ^a	0.007	−0.025–0.040	0.63	0.209	1.13	0.26
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Legend: β , regression coefficient. IC95%, 95% confidence interval. 25(OH)D, 25-hydroxyvitamin D. BF%, body fat percentage. BMD, bone mineral density. RSA, the percentage of time decreased in sprints. SEI, Sun Exposure Index. Model 1 adjusted for age. Model 2 adjusted for fat free mass. Model 3 adjusted for food intake (carbohydrate intake for %BF and RSA regressions or calcium intake for SEI and BMD regression). ^a For RSA, all analysis were adjusted according to the referee's position, since the RSA protocols differed between the two referee categories.

4. Discussion

To our knowledge, this was the first study that evaluated the prevalence of hypovitaminosis D and the associations between serum 25(OH)D, SEI, body composition, and sprint capacity in a FIFA-certified physical test in soccer referees.

The majority of the referees evaluated (>70%) had serum 25(OH)D levels < 30 ng/mL, in agreement with a previous systematic review that reported a high prevalence of vitamin D insufficiency (30%; $n = 3725$; <20 ng/mL) [4] and inadequacy (56%; $n = 2313$; <32 ng/mL) [1] in athletes. Similarly, a high prevalence of hypovitaminosis D (<30 ng/mL) has also been reported in soccer players, ranging from 64% ($n = 28$) [20] to 84% ($n = 342$) [21]. The high prevalence of hypovitaminosis D was noteworthy, considering that the study was conducted in a subtropical region and that soccer refereeing is predominantly performed outdoors.

Seasonal variations in serum 25(OH)D concentrations have been reported in soccer players, with vitamin D insufficiency and deficiency occurring more frequently during the autumn and winter months [22], whereas higher concentrations of vitamin D metabolites are typically observed during summer [23]. This seasonal factor may partially explain the high prevalence of hypovitaminosis D observed in our sample. The present study was conducted in the middle of winter (*i.e.*, July), when UVB radiation levels are reduced, and there is an increase in clothing use, and consequently, reduced endogenous synthesis of vitamin D [17]. Notably, although evidence regarding seasonal 25(OH)D variations in Brazilian athletes remains scarce, the present study provides evidence that sun exposure may be an important determinant of serum 25(OH)D in Brazilian soccer referees evaluated during the winter season. In our study, SEI was the only variable that predicted serum 25(OH)D in crude ($\beta = 1.17$; $p < 0.05$; Cohen's $f^2 = 0.19$) and when adjusted for fat free mass ($\beta = 1.23$; $p < 0.05$; Cohen's $f^2 = 0.13$). This finding is consistent with a previous study demonstrating a positive association between sun exposure and serum 25(OH)D concentrations [24] and reinforces the well-established role of sun exposure in endogenous vitamin D synthesis [17]. Additionally, although seasonal variations in vitamin D have been reported in a population-based study of Brazilian adults, which showed lower serum 25(OH)D in winter than in fall, spring, and summer [25], evidence among Brazilian athletes remains scarce. Therefore, our findings contribute to the limited evidence on vitamin D status in Brazilian soccer referees during the winter, highlighting the importance of monitoring its status and sun exposure even in athletes of a predominantly performed outdoor sport in a subtropical region.

The relationship between adiposity and vitamin D has been extensively investigated. One proposed mechanism is that vitamin D may be sequestered within the vast body fat pool, thereby reducing its bioavailability and resulting in obesity-linked hypovitaminosis D [26]. The volumetric dilution has also been proposed as an explanation, suggesting that lower serum 25(OH)D concentrations may reflect distribution across body volume in individuals with greater body size, rather than reduced vitamin D stores [27,28]. Previous studies have identified body fat as an important predictor of 25(OH)D concentrations [29–31], also evidence in collegiate athletes, evaluated by DXA, has also identified body fat as an important predictor of 25(OH)D concentrations, but not other body composition variables [30]. However, in the present study, no statistically significant association was observed between BF% and 25(OH)D concentrations in either crude or adjusted analysis. Although it is still not yet clear whether a high level of body fat is an indicator of risk of hypovitaminosis D in athletes, our findings suggest that BF% is not a major determinant of vitamin D status in this sample of soccer referees.

Previous evidence has demonstrated a positive association between 25(OH)D and bone health in athletes [32], however, other studies have not observed statistically significant associations [24,29,33]. Consistent with these findings, no significant association between 25(OH)D concentration and BMD was observed in the present study. Although adjustment for fat free mass increased the EF (Cohen's $f^2 = 0.71$), the association remained statistically nonsignificant. Nevertheless, the model explained approximately 41% of the variance in BMD ($R^2 = 0.41$), suggesting that factors related to body composition may contribute to bone health outcomes. This finding is plausible considering that (1) bone health is strongly influenced by mechanical loading and exercise-related adaptations [34], and fat free mass is one of the determinants of skeletal loading; and (2) during winter months, the period when data collection took place, muscle mass is suggested to play an important role in vitamin D homeostasis acting also as a reservoir [35,36].

However, the relationship between vitamin D and BMD is likely influenced by many factors, such as age, sex, sports modality, genetics, BMD measurement sites [37], calcium intake, and energy and carbohydrate availability [38]. In the present study, eleven (52%) referees had calcium intakes lower than the recommended by the Dietary Reference Intakes (DRI) (<800 mg/day) [39], while mean energy and carbohydrate intakes were approximately 30 kcal/kg/day and 3 g/kg/day, respectively. Although dietary adequacy was not specifically evaluated, these findings highlight the multifactorial nature of bone health and suggest that the relationship between serum 25(OH)D and BMD in athletes warrants further investigation, particularly considering body composition and dietary factors.

As is well established, during the game, the physical/physiological demands of the referees are similar to those of the players [11,12]. Thus, soccer referees' performance is crucial for sustaining the match's duration and intensity and supporting accurate decision-making [13]. Through a match, referees are submitted to repeated periods of high-intensity running, acceleration, deceleration, and sprinting [11,12]. In this context, in our study, the performance was evaluated by RSA, considered a relevant indicator of match-related performance in athletes of team sports [40,41] and soccer [40]. The RSA consists in the evaluation of physical agility and speed performance in fast runs suggested to provide a relevant measure of explosive power, maximal oxygen consumption and muscle buffer capacity [40,41], also this test is included in the CBF protocol for evaluating referees according to FIFA [42]. However, the mechanisms underlying the influence of vitamin D and muscle function are not fully understood [3]. Other authors have investigated associations between serum 25(OH)D and performance in intense, short-term efforts, whether performed once or repeatedly, but findings have been consistent [9,43,44]. Partially consistent with our result, ref. [44] evaluated 50 ice hockey players (62% with hypovitaminosis D, 25(OH)D < 30 ng/mL) and found no associations between 25(OH)D and performance in a 5×6 repeated sprint cycle ergometry test and the vertical jump test. In contrast, in 67 male soccer players (55% with hypovitaminosis D, 25(OH)D < 30 ng/mL), 25(OH)D was inversely associated with times in single sprints of 10 m and 30 m and directly associated with performance in vertical jumps [43]. Interestingly, another study observed that the association between 25(OH)D and performance is influenced by indoor or outdoor training and by season. The authors evaluated the association between physical test and serum 25(OH)D in spring and autumn in a sample of indoor ($n = 22$) and outdoor ($n = 25$) athletes and did not find an association between performance parameters and 25(OH)D in the indoor group, although they observed a positive association between 25(OH)D and countermovement jumps in only outdoor group and during autumn [45]. In the present study, serum 25(OH)D was not a predictor of RSA, even after adjusting for the referee position and models 1, 2, and 3. Nevertheless, the model adjusted for fat free mass showed a large EF (Cohen's $f^2 = 0.37$), and besides not being statistically significant ($p = 0.16$), the model explained 27% of the variability in RSA performance ($R^2 = 0.27$). Although the association remained nonsignificant, this finding suggests that body composition should be considered when investigating the association between 25(OH)D and sprint performance.

The present study has limitations. First, its cross-sectional design precludes causal inferences and does not allow the evaluation of seasonal variations in vitamin D status. The data collection was in winter, which

limits the generalization of these findings to other seasons. The relatively small sample size ($N = 21$) may have reduced the statistical power to detect small to modest associations, increasing the risk of type II error. Therefore, the interpretation of associations should be approached with caution, particularly regarding the generalizability of the findings. In addition, sun exposure habits were assessed through self-reported measures, which may be subject to recall bias. Despite these limitations, the study has important strengths: (i) assessment of body composition using the DXA reference method; (ii) assessment of food intake using the Multiple Pass Method and NDSR software (Nutrition Data System for Research[®]); (iii) the use of the residual method [16] to adjust inter- and intra-personal variability of nutrients and to adjust micronutrients by the total energy value; and (iv) the application of official RSA protocols referenced by FIFA and CBF in the assessment of the sprint capacity of soccer referees.

5. Conclusions

The study found a high prevalence of hypovitaminosis among Brazilian soccer referees. Despite 25(OH)D was not significantly associated with body composition or performance, its positive association with sun exposure reinforces the importance of sun exposure, mainly during the winter season. We recommend routine screening of 25(OH)D in this population, and we suggest that referees would benefit from dietary advice in order to improve health.

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Author Contributions

Conceptualization, Methodology and Investigation: M.P.G., C.R., J.d.C. and F.H. Data Curation, Formal Analysis: M.P.G., C.R., V.S.P.P., G.Z.L. Validation: J.d.C., D.A.S.S. and F.H. Writing—Original Draft Preparation: M.P.G. and C.R. Writing—Review & Editing: M.P.G., V.S.P.P., G.Z.L., J.d.C., D.A.S.S. and F.H. All authors approved the final version of the paper and agree with the authorship order.

Ethics Statement

The study was approved by the Federal University of Santa Catarina (UFSC) Human Research Ethics Committee (protocol number: 2.572.301, approval date 30 March 2018) and followed the guidelines established by the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Farrokhyar F, Tabasinejad R, Dao D, Peterson D, Ayeni OR, Hadioonazadeh R, et al. Prevalence of Vitamin D Inadequacy in Athletes: A Systematic-Review and Meta-Analysis. *Sports Med.* **2015**, *45*, 365–378. DOI:10.1007/s40279-014-0267-6
- Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The Roles of Vitamin D in Skeletal Muscle: Form, Function, and Metabolism. *Endocr. Rev.* **2013**, *34*, 33–83. DOI:10.1210/er.2012-1012
- Pojednic RM, Ceglia L. The Emerging Biomolecular Role of Vitamin D in Skeletal Muscle. *Exerc. Sport Sci. Rev.* **2014**, *42*, 76–81. DOI:10.1249/JES.0000000000000013
- Harju T, Gray B, Mavroedi A, Farooq A, Reilly JJ. Prevalence and Novel Risk Factors for Vitamin D Insufficiency in Elite Athletes: Systematic Review and Meta-Analysis. *Eur. J. Nutr.* **2022**, *61*, 3857–3871. DOI:10.1007/S00394-022-02967-Z
- Segheto KJ, Juvanol LL, da Silva DCG, de Carvalho CJ, Hansen F, Gabiatti MP, et al. Does the Relationship between 25-Hydroxyvitamin D Status and Bone Mass Vary According to Skin Color in Adults? Results of a Brazilian Population-Based Study. *Arch. Osteoporos.* **2021**, *16*, 31. DOI:10.1007/s11657-021-00876-y
- Zürcher SJ, Quadri A, Huber A, Thomas L, Close GL, Brunner S, et al. Predictive Factors for Vitamin D Concentrations in Swiss Athletes: A Cross-Sectional Study. *Sports Med. Int. Open* **2018**, *2*, E148. DOI:10.1055/A-0669-0791
- Ip TST, Fu SC, Ong MTY, Yung PSH. Vitamin D Deficiency in Athletes: Laboratory, Clinical and Field Integration. *Asia. Pac. J. Sports Med. Arthrosc. Rehabil. Technol.* **2022**, *29*, 22–29. DOI:10.1016/j.asmart.2022.06.001
- Dominoni LAdC, Gabiatti MP, Piazza FRG, Streb AR, Del Duca GF, Hansen F. Vitamin D Is Associated with Body Composition and Fat Intake, but Not with Cardiometabolic Parameters in Adults with Obesity. *Nutr. Res.* **2022**, *105*, 97–104. DOI:10.1016/j.nutres.2022.06.006
- Close GL, Russell J, Cobley JN, Owens DJ, Wilson G, Gregson W, et al. Assessment of Vitamin D Concentration in Non-Supplemented Professional Athletes and Healthy Adults during the Winter Months in the UK: Implications for Skeletal Muscle Function. *J. Sports Sci.* **2013**, *31*, 344–353. DOI:10.1080/02640414.2012.733822
- Schenk K, Bizzini M, Gatterer H. Exercise Physiology and Nutritional Perspectives of Elite Soccer Refereeing. *Scand. J. Med. Sci. Sports* **2018**, *28*, 782–793. DOI:10.1111/SMS.12989
- Vieira CMA, Costa EC, Aoki MS. O Nível de Aptidão Física Afeta o Desempenho do Árbitro de Futebol. *Revista Brasileira de Educação Física* **2010**, *24*, 445–452. DOI:10.1590/S1807-55092010000400002
- Weston M, Drust B, Gregson W. Intensities of Exercise during Match-Play in FA Premier League Referees and Players. *J. Sports Sci.* **2011**, *29*, 527–532. DOI:10.1080/02640414.2010.543914
- Reilly T, Gregson W. Special Populations: The Referee and Assistant Referee. *J. Sports Sci.* **2006**, *24*, 795–801. DOI:10.1080/02640410500483089
- Palacios C, Gil K, Pérez CM, Joshupura K. Determinants of Vitamin D Status among Overweight and Obese Puerto Rican Adults. *Ann. Nutr. Metab.* **2012**, *60*, 35–43. DOI:10.1159/000335282
- Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of Dietary Recall Using the USDA Five-Step Multiple-Pass Method in Men: An Observational Validation Study. *J. Am. Diet. Assoc.* **2004**, *104*, 595–603. DOI:10.1016/j.jada.2004.01.007
- Willett W. *Nutritional Epidemiology*; Oxford University Press: Oxford, UK, 2013; ISBN 9780199979448.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: An Endocrine Society Clinical Practice Guideline. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1911–1930. DOI:10.1210/jc.2011-0385
- Rampinini E, Bishop D, Marcora SM, Ferrari Bravo D, Sassi R, Impellizzeri FM. Validity of Simple Field Tests as Indicators of Match-Related Physical Performance in Top-Level Professional Soccer Players. *Int. J. Sports Med.* **2007**, *28*, 228–235. DOI:10.1055/s-2006-924340.
- Cohen J. *Statistical Power Analysis for the Behavioral Sciences Second Edition*, 2nd ed.; Lawrence Erlbaum Associates: New York, NY, USA, 1988.
- Galan F, Ribas J, Sánchez-Martínez PM, Calero T, Sánchez AB, Muñoz A. Serum 25-Hydroxyvitamin D in Early Autumn to Ensure Vitamin D Sufficiency in Mid-Winter in Professional Football Players. *Clin. Nutr.* **2012**, *31*, 132–136. DOI:10.1016/j.clnu.2011.07.008
- Hamilton B, Whiteley R, Farooq A, Chalabi H. Vitamin D Concentration in 342 Professional Football Players and Association with Lower Limb Isokinetic Function. *J. Sci. Med. Sport* **2014**, *17*, 139–143. DOI:10.1016/j.jsams.2013.03.006
- Perrone MA, Pieri M, Caminiti G, Ali W, Bernardini S, Parisi A, et al. Vitamin D Deficiency in Professional Football

- Players during Competitive Season of Italian First Division (Serie A). *Sports* **2024**, *12*, 153. DOI:10.3390/SPORTS12060153
23. Książek A, Zagrodna A, Lombardi G, Słowińska-Lisowska M. Seasonal Changes in Free 25-(OH)D and Vitamin D Metabolite Ratios and Their Relationship with Psychophysical Stress Markers in Male Professional Football Players. *Front. Physiol.* **2023**, *14*, 1258678. DOI:10.3389/FPHYS.2023.1258678
 24. Allison RJ, Farooq A, Hamilton B, Close GL, Wilson MG. No Association between Vitamin D Deficiency and Markers of Bone Health in Athletes. *Med. Sci. Sports Exerc.* **2015**, *47*, 782–788. DOI:10.1249/MSS.0000000000000457
 25. Longo GZ, da Silva DCG, Gabiatti MP, Hansen F, Ferreira FG, Silva DAS, et al. The Interaction between Higher 25(OH)D Concentrations and Physical Activity on Inflammatory Biomarkers: Evidence from a Cross-Sectional Brazilian Population. *Nutr. Res.* **2026**, *153*, 32–40. DOI:10.1016/j.nutres.2026.06.008
 26. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased Bioavailability of Vitamin D in Obesity. *Am. J. Clin. Nutr.* **2000**, *72*, 690–693. DOI:10.1093/ajcn/72.3.690
 27. Savastano S, Barrea L, Savanelli MC, Nappi F, Di Somma C, Orio F, et al. Low Vitamin D Status and Obesity: Role of Nutritionist. *Rev. Endocr. Metab. Disord.* **2017**, *18*, 215–225. DOI:10.1007/s11154-017-9410-7
 28. Drincic AT, Armas LAG, Van Diest EE, Heaney RP. Volumetric Dilution, Rather than Sequestration Best Explains the Low Vitamin D Status of Obesity. *Obesity* **2012**, *20*, 1444–1448. DOI:10.1038/OBY.2011.404
 29. Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW, Larson-Meyer DE. Vitamin D Status Relative to Diet, Lifestyle, Injury, and Illness in College Athletes. *Med. Sci. Sports Exerc.* **2011**, *43*, 335–343. DOI:10.1249/MSS.0b013e3181eb9d4d
 30. Heller JE, Thomas JJ, Hollis BW, Larson-Meyer DE. Relation between Vitamin D Status and Body Composition in Collegiate Athletes. *Int. J. Sport Nutr. Exerc. Metab.* **2015**, *25*, 128–135. DOI:10.1123/ijsnem.2013-0250
 31. Peeling P, Fulton SK, Binnie M, Goodman C. Training Environment and Vitamin D Status in Athletes. *Int. J. Sports Med.* **2013**, *34*, 248–252. DOI:10.1055/s-0032-1321894
 32. Vlachopoulos D, Ubago-Guisado E, Barker AR, Metcalf BS, Fatouros IG, Avloniti A, et al. Determinants of Bone Outcomes in Adolescent Athletes at Baseline: The PRO-BONE Study. *Med. Sci. Sports Exerc.* **2017**, *49*, 1389–1396. DOI:10.1249/MSS.0000000000001233
 33. Ducher G, Kukuljan S, Hill B, Garnham A, Nowson S, Kimlin M, et al. Vitamin D Status and Musculoskeletal Health in Adolescent Male Ballet Dancers: A Pilot Study. *J. Danc. Med. Sci.* **2011**, *15*, 99–107. DOI:10.1177/1089313X1101500301
 34. de la Puente Yagüe M, Yurrita LC, Cabañas MJC, Cenzual MAC. Role of Vitamin D in Athletes and Their Performance: Current Concepts and New Trends. *Nutrients* **2020**, *12*, 579. DOI:10.3390/NU12020579
 35. Mason RS, Rybchyn MS, Abboud M, Brennan-Speranza TC, Fraser DR. The Role of Skeletal Muscle in Maintaining Vitamin D Status in Winter. *Curr. Dev. Nutr.* **2019**, *3*, nzz087. DOI:10.1093/cdn/nzz087
 36. Rybchyn MS, Abboud M, Puglisi DA, Gordon-Thomson C, Brennan-Speranza TC, Mason RS, et al. Skeletal Muscle and the Maintenance of Vitamin D Status. *Nutrients* **2020**, *12*, 3270. DOI:10.3390/nu12113270
 37. Allison RJ, Farooq A, Cherif A, Hamilton B, Close GL, Wilson MG. Why Don't Serum Vitamin D Concentrations Associate with BMD by DXA? A Case of Being “bound” to the Wrong Assay? Implications for Vitamin D Screening. *Br. J. Sports Med.* **2018**, *52*, 522–526. DOI:10.1136/bjsports-2016-097130
 38. Dolan E, Dumas A, Esteves GP, Takarabe LL, Perfeito LAM, Keane KM, et al. The Influence of Nutrition Intervention on the P1NP and CTX-I Response to an Acute Exercise Bout: A Systematic Review with Meta-Analysis. *Sports Med.* **2024**, *54*, 2889–2906. DOI:10.1007/s40279-024-02087-0
 39. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*; National Academies Press: Washington, DC, USA, 2005; ISBN 030908525X.
 40. Rampinini E, Sassi A, Morelli A, Mazzoni S, Fanchini M, Coutts AJ. Repeated-Sprint Ability in Professional and Amateur Soccer Players. *Appl. Physiol. Nutr. Metab.* **2009**, *34*, 1048–1054. DOI:10.1139/H09-111
 41. Buchheit M, Bishop D, Haydar B, Nakamura FY, Ahmaidi S. Physiological Responses to Shuttle Repeated-Sprint Running. *Int. J. Sports Med.* **2010**, *31*, 402–409. DOI:10.1055/S-0030-1249620
 42. FIFA FITNESS TESTS FOR MATCH OFFICIALS. 2024. Available online: https://digitalhub.fifa.com/m/4b958c43c4b415a1/original/Fitness-tests-for-Match-Officials_EN.pdf (accessed on 25 August 2026)
 43. Koundourakis NE, Androulakis NE, Malliaraki N, Margioris AN. Vitamin D and Exercise Performance in Professional Soccer Players. *PLoS ONE* **2014**, *9*, e101659. DOI:10.1371/journal.pone.0101659
 44. Orysiak J, Mazur-Rozycka J, Fitzgerald J, Starczewski M, Malczewska-Lenczowska J, Busko K. Vitamin D Status and Its Relation to Exercise Performance and Iron Status in Young Ice Hockey Players. *PLoS ONE* **2018**, *13*, e0195284. DOI:10.1371/journal.pone.0195284

45. Wilson-Barnes SL, Hunt JEA, Williams EL, Allison SJ, Wild JJ, Wainwright J, et al. Seasonal Variation in Vitamin D Status, Bone Health and Athletic Performance in Competitive University Student Athletes: A Longitudinal Study. *J. Nutr. Sci.* **2020**, 9, e8. DOI:[10.1017/JNS.2020.1](https://doi.org/10.1017/JNS.2020.1)