State of The Art on The Main Randomized Clinical Studies, Meta-Analysis, and International Consensus on The Influence of Serum Levels and Supplementation of Vitamin D on Athletes Performance

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Abstract: Introduction: Vitamin D (VD) deficiency (serum 25-hydroxyvitamin D (25 (OH) D) <50 nmol/L or 20 ng/mL) is more common than is thought in the majority of the world population. In this context, athletes have the same predisposition to low levels of vitamin D, with the majority of their concentrations below 20 ng/mL in a wide variety of sports, especially in the winter months. RV is also essential in extra-skeletal functions, including skeletal muscle growth, immune and cardiopulmonary functions, and inflammatory modulation, which influence athletic performance. Vitamin D can also interact with extra-skeletal tissues to modulate injury recovery and also influence the risk of infection. Objective: Performed a wide analysis of the world literature to compose the State of the Art on the main effects of vitamin D supplementation on the performance of athletes through randomized clinical studies, meta-analysis, and the latest international conferences and consensus. Methods: The present study followed a broad literature review of randomized clinical studies, meta-analysis, and the latest international consensus. The Cochrane instrument was adopted to assess the quality of the studies. Main findings and conclusion: One of the main aspects that must be taken into account is important evidence that suggests that free (bioavailable) 25(OH)D may be a better marker of vitamin D status. Many researchers do not take into account that athletes may need a greater supply of vitamin D to meet the requirements of muscle metabolism due to the potential routes of vitamin D use. A significant debate seems to be needed to determine and standardize the classification of vitamin D deficiency. There are still information gaps on the correlation between vitamin D supplementation and athletes' exercise performance. However, because vitamin D induces myogenesis and muscle protein synthesis, causing an increase in the percentage of rapidly contracting muscle cells, and because vitamin D receptors (VDR) play a significant role in muscle regeneration after injury, supplementation in athletes is recommended.

Keywords: Athletes, Vitamin D, Performance, Injuries

1. Introduction

Deficiency of vitamin D (serum 25-hydroxyvitamin D (25(OH)D) <50 nmol/L or 20 ng/mL) is more common than is thought in the majority of the world population. It occurs in <20% of the population in northern Europe, in 30-60% in western, southern, and eastern Europe, and up to 80% in the countries of the Middle East [1, 2]. Severe deficiency (serum 25 (OH) D <30 nmol/L or 12 ng/mL) is found in > 10% of Europeans. The European Society of Calcified Tissues (ESCT) recommends that the measurement of serum 25 (OH) D be standardized by a Vitamin D Standardization Program [3].

In this regard, the consequences of vitamin D deficiency include mineralization defects and lower bone mineral density, causing fractures [4, 5]. The extra-skeletal consequences can be muscle weakness, falls, and acute respiratory infection [6, 7]. Fortification of foods by adding vitamin D to dairy products, bread, and cereals can improve the vitamin D status of the entire population, but it is necessary to monitor quality assurance to prevent poisoning [8]. Future research should include genetic studies to better define...
individual vulnerability for vitamin D deficiency and Mendelian randomization studies for the effect of vitamin D deficiency on long-term non-skeletal outcomes, such as cancer [9, 10].

In this context, parathyroid cells express the enzyme 1-alpha-hydroxylase and can synthesize the active form, 1.25 (OH) 2D intracellularly, from the serum pool of 25 (OH) D [11]. In situations of hypovitaminosis D, the lower intracellular synthesis leads to secondary hyperparathyroidism that is associated with increased bone resorption. There is an inverse correlation between PTH and 25 (OH) D, described in children and the elderly. Several cutoff values of 25 (OH) D for PTH normalization have been published, and the majority are concentrated between 28 and 40 ng/mL (70 to 100 nmol/L) [11].

The absorption of calcium by the intestine is dependent on the action of active vitamin D in the duodenum, through a saturable transcellular process, whose stimulus leads to the synthesis of proteins such as calbindin-D9k (CaBP-9k) and the epithelial apical channel TRPV6. However, there is evidence that the non-saturable transport that occurs with part of the absorption of calcium in the human ileum also influences vitamin D [7].

In this context, athletes have the same predisposition to low levels of vitamin D, with the majority of their concentrations below 20 ng/mL in a wide variety of sports, especially in the winter months. Vitamin D is important in bone health, but recent research also points to its essential role in extraskeletal functions, including skeletal muscle growth, immune and cardiopulmonary functions, and inflammatory modulation, which influence athletic performance. Vitamin D can also interact with extraskeletal tissues to modulate the recovery from injuries and also influence the risk of infection [12].

The active form of vitamin D (calcitriol) exerts its biological effects by binding to the nuclear vitamin D receptors (VDR), which are found in most human extraskeletal cells, including skeletal muscles. Thus, vitamin D deficiency can cause strength deficits and lead to fatty degeneration of type II muscle fibers, which is negatively correlated with physical performance. Vitamin D supplementation has been shown to improve vitamin D levels and can positively affect skeletal muscles in athletes [13].

Therefore, the present study aimed to make a comprehensive analysis of the world literature to compose the State of the Art on the main effects of vitamin D supplementation on the performance of athletes through randomized clinical studies, meta-analysis, and the latest international conferences and consensus.

2. Methods
2.1. Study Design

The present study followed a broad literature review of randomized clinical studies, meta-analysis, and the latest international consensus, following the rules of PRISMA [14]. Available at: http://www.prisma-statement.org/.

Table 1 shows the main variables of the present study that will be addressed according to the classification of the acronym PICOS (P = Patients; I = Intervention; C = Control (O); O = Outcomes; S = Study Design (Type of studies).

<table>
<thead>
<tr>
<th>TABLE 1 Table of PICOS (Patients; Intervention; Control; Outcomes; Study Design).</th>
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2.2. Study eligibility criteria

Only randomized clinical studies, systematic reviews, meta-analysis and latest international conferences and consensus were included.

Selection of studies and risk of bias in each study

The Cochrane instrument was adopted to assess the quality of the included studies [15].

Data sources and research strategy

The search strategies for this systematic review were based on the keywords (MeSH Terms) "Vitamin D; (25(OH)D); Athlete; Performance;
Hypovitaminosis; Immunity; Inflammatory Process”, with publications from the year 2010 to 2020, in order to analyze the most recent scientific publications. The SCOPUS (Elsevier and non-Elsevier database), COCHRANE Library, PUBLISH (MEDLINE biomedical literature, life science journals and online books) and SCIENCE DIRECT (Elsevier database) databases were used, including the National Institutes of Health RePORTER Grant database and clinical trial records. In addition, a combination of keywords with the Booleans “OR”, AND and the operator "NOT" were used to target scientific articles of interest. The title and abstracts were examined under all conditions. The research structure used in the databases is shown in Table 2.

Table 2. Example of the search structure in PubMed, the same search strategy was used in the other databases.

<table>
<thead>
<tr>
<th>PubMed</th>
<th>Athletes OR Vitamin D OR (25 (OH) D) OR Hypovitaminosis</th>
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<td>Immunity OR Inflammatory Process OR Performance</td>
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<td>Case reports OR Editorials OR Letters to the editor</td>
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3. Development and Discussion

3.1 Major current outcomes

In recent years, interest in research that investigates the status of vitamin D in athletes [10] has increased. Scientific reports suggest a pleiotropic nature of vitamin D, suggested by the demonstration of vitamin D receptors (VDR) in almost every nucleated cell in our body. After binding to the nuclear membrane and VDR, calcitriol can perform several significant functions in the body, including effects on bone mineralization, normal function of the nervous, immune, endocrine, and cardiovascular systems, as well as hormone production, regulation of the expression of more than 900 gene variants and the normal function of the muscular system [10].

In this sense, calcitriol affects the function of osteoblasts through several mechanisms, for example, the regulation of phosphate homeostasis, increasing the synthesis of fibroblast growth factor 23 (FGF23), and the stimulation of active mitogenic protein kinase signaling, which can improve the response to mechanical load [10].

As a corollary, current evidence documents that bone cells can produce 1,25-dihydroxyvitamin D from the precursor 25 (OH) D and that this activity may be responsible for the skeletal effects of circulating 25(OH)D. Athletes are known to have a higher bone mineral density (BMD) compared to people who lead a sedentary lifestyle. Thus, any increase in body weight caused by training contributes to the bone remodeling process and mechanically creates an adequate bone structure [16].

Thus, it is proposed to stimulate the musculoskeletal load through dynamic and high-intensity physical activity to compensate for the low levels of 25 (OH) D, with bone health problems in athletes. However, athletes are more susceptible to the effects of low BMD when vitamin D deficiency is encountered. Also, vitamin D affects innate and adaptive immunity. VDRs are found in most immune cells, including Treg cells, neutrophils, dendritic cells, B cells, and macrophages. The activation of the immune system can be regulated by the circulation of 25 (OH) D and induced by activation [17].

In this scenario, according to the 2nd International Conference on Vitamin D Controversies, it was held in Monteriggioni (Siena), Italy, in 2018 [2]. The purpose of this meeting was to address controversies and timely topics in vitamin D research, to review the available data related to these topics and controversies, to promote discussions to help resolve remaining problems, and ultimately to suggest a research agenda to clarify areas of uncertainty. Several questions from the first conference (2017) were revised, such as assays used to determine the serum concentration of 25-hydroxyvitamin D (25 (OH) D), which remains a critical and controversial issue for defining vitamin D status.

Besides, definitions of the nutritional status of vitamin D (ie, sufficiency, insufficiency, and deficiency) were also revised. New areas have been revised, including the vitamin D threshold values and how they should be defined in the context of specific diseases, sources of vitamin D, and risk factors associated with vitamin D deficiency. Also discussed were non-skeletal aspects related to vitamin D, including to reproductive system, neurology, chronic kidney disease, and falls. The therapeutic role of vitamin D and the findings of recent clinical trials have also been addressed [2].

As a supporting example of the 2nd International Conference, a systematic review study was carried out on the effects of vitamin D supplementation on muscle strength in athletes. A
conducted a computerized bibliographic search of 3 databases (PubMed, MEDLINE, and Scopus) was carried out. Randomized controlled trials (RCT) that measured serum vitamin D concentrations and muscle strength in healthy athletic participants aged 18-45 years were included in the review. Five randomized controlled trials and one controlled trial were identified, and the quality assessment showed that 5 trials were of "excellent quality" and 1 was of "good quality". The trials lasted from 4 weeks to 6 months and dosages ranged from 600 to 5,000 international units (IU) per day. Vitamin D2 was found to be ineffective in the impact of muscle strength in both studies in which it was administered. In contrast, vitamin D3 has been shown to have a positive impact on muscle strength. In 2 studies, the strength outcome measures improved significantly after supplementation (p<0.05). In the other 2 studies that administered vitamin D3, there were tendencies to improve muscle strength. Specifically, improvements in strength ranged from 1.37 to 18.75% [16].

### 3.2 Major Studies - State of the Art

A cross-sectional study examined the association of vitamin D status with athletic performance and blood markers in adolescent athletes. 47 Taekwondo athletes, aged between 15 and 18 years, were included. Athletic performance was assessed using maximum oxygen consumption (VOmax), Wingate anaerobic power test, vertical jump, agility T-test, lower limb muscle strength, and fatigue resistance. Blood samples were collected to assess the dosage of 25-hydroxyvitamin D [25(OH)D], free testosterone, cortisol, creatine kinase, and urea. The 25 (OH) D concentration of the participants ranged from 16 to 73.25 nmol/L, indicating that 74.5% of adolescent athletes have vitamin D insufficiency or deficiency. Vitamin D status did not show any significant effects on the factors performance indicators or blood markers. The concentration of 25 (OH) D in the serum was positively correlated with the mean potency (r = 0.359, p <0.05) and the relative mean potency (r = 0.325, p <0.05) after adjustment for bone age, height, weight, training experience, lean body mass, and fat mass. However, the concentration of 25 (OH) D was not associated with other factors related to performance and blood markers. Also, multiple analyses of linear regressions revealed that the serum concentration of 25 (OH) D was not a significant predictor of athletic performance in adolescent athletes. Therefore, this study showed that the level of vitamin D is weakly correlated with an anaerobic capacity [17].

Subsequently, a randomized, placebo-controlled study looked at the effects of vitamin D3 supplementation on physical performance during winter training in vitamin D-deficient taekwondo athletes. Thirty-five male and female college-age taekwondo athletes between 19-22 years old with a low serum concentration of 25 (OH) D (28.8 ± 1.10 nmol/L), were randomly assigned to a vitamin D group (n = 20) or a placebo group (n = 15). Participants received a vitamin D3 capsule (5,000 IU/day) or a placebo during 4 weeks of winter training. Blood samples were collected for analysis of 25 (OH) D serum concentrations. Physical performance tests included Wingate's anaerobic test, isokinetic muscle strength and endurance, countermovement jump test, sit-ups, agility test, and 20 m pacemaker. Serum concentrations of 25 (OH) D increased significantly in the vitamin D group (96.0 ± 3.77 nmol/L) after 4 weeks of supplementation, but no changes were found in the placebo group. There were significant interaction effects for peak anaerobic power and isokinetic knee extension at 180 degrees/s. Changes in the serum concentration of 25 (OH) D were positively associated with changes in peak power and isokinetic extension of the knee at 180 degrees/s. Therefore, it is suggested that 4 weeks of vitamin D supplementation raises the serum concentration of 25 (OH) D to sufficient levels. Correcting vitamin D insufficiency improves some aspects of performance, but not all. Thus, the effectiveness of vitamin D supplementation for improving performance remains unclear [18].

Also, a study examined the vitamin D status of professional volleyball players and determined its correlation with shoulder muscle strength. 52 healthy male professional volleyball players (23.2 ± 4.5 years) were included, who were categorized by vitamin D status (<20 ng / mL: deficiency, 20-30 ng / mL: insufficiency, and> 30 ng / mL: sufficiency). The strength of the internal (IR) and external (ER) shoulder muscles was examined using an isokinetic dynamometer. Fourteen players (26.9%) had vitamin D deficiency, 24 players (46.2%) were deficient in vitamin D and 14 players (26.9%) had enough vitamin D. There was no significant correlation between vitamin D level and shoulder muscle strength at 60 ° / s and 180 ° / s. Also, the isokinetic forces of the shoulder were not significantly different between the three groups in all configurations. In conclusion, vitamin D insufficiency was common in elite volleyball players. Although not associated with isokinetic muscle
weakness, vitamin D levels should be monitored regularly, and vitamin D should be provided to young elite athletes, considering its importance for musculoskeletal health [19].

Besides, a systematic review and meta-analysis study investigated the effects of vitamin D3 supplementation on skeletal muscle strength in athletes. Vitamin D3 supplements or vitamin D3 fortified foods always have claims to bring benefits to people's health, including bone and muscle health. Randomized Clinical Trials (RCTs) on the effects of vitamin D3 supplementation on muscle strength in healthy athletes were analyzed using three databases (PubMed, Embase, and Cochrane Library). A serum 25(OH) D above 30 ng/mL was considered sufficient in this study. As a result, five RCTs with 163 athletes (vitamin D3 n = 86, placebo n = 77) met the inclusion criteria. Fourteen athletes were lost to follow-up and 149 athletes (vitamin D3 n = 80, placebo n = 69) were documented with complete results. Among athletes with baseline serum 25 (OH) D suggesting insufficiency, a daily dosage of vitamin D3 in 5000 IU for more than 4 weeks led to a serum 25 (OH) D concentration of 31.7 ng/mL. Athletes with a sufficient serum level of 25 (OH) D at the start of the study were recruited in only one study, and participants were assigned to receive vitamin D3 at a daily dosage of 3570 IU or placebo for 12 weeks, their 25 (OH) D serum levels, sufficiency was well maintained above the cut-off limit. A maximum bench press repetition (1-RM BP) did not improve significantly and there was no significant increase in maximum quadriceps contraction. Also, there was no significant overall effect of vitamin D3 intervention on muscle strength in this meta-analysis. Therefore, although serum 25(OH)D concentrations after supplementation has reached sufficiency, muscle strength has not significantly improved at this point in the current meta-analysis. Further well-designed randomized controlled trials are needed with a large number of participants examined for the effect of vitamin D3 supplementation on serum concentrations of 25(OH)D, muscle strength in a variety of sports, latitudes, and diverse multicultural populations [20].

Also, a retrospective review study of the records was conducted to determine whether vitamin D status was associated with diagnoses of depression in military athletes and whether the diagnoses differed by geographic location. Results: Depression (defined through diagnostic codes) was more prevalent in individuals diagnosed with vitamin D deficiency (20.4%) than in individuals without deficiency (4.2%). After adjustment, diagnoses of vitamin D deficiency remained significantly associated with diagnoses of depression. In addition, diagnoses of vitamin D deficiency were strongly associated with geographic latitude ($r^2 = 0.92$, $p = 0.002$). Therefore, athletes in northern latitudes may be at increased risk for vitamin D deficiency, as well as being at increased risk of developing depression [21].

Another study examined the prevalence of vitamin D deficiency and deficiency in young Russian football players and the effectiveness of their treatment. The participants were 131 young male soccer players (age 15.6 ± 2.4 years). Low levels of vitamin D (below 30 ng/mL) were observed in 42.8% of the participants analyzed. These athletes were divided into two groups composed of people with vitamin D deficiency (serum vitamin D below 21 ng/mL) and insufficiency (serum vitamin D in the range 21-29 ng/mL). A dietary supplement of 5,000 IU cholecalciferol per day was administered for two months. After treatment, an average increase of 92% in the concentration of vitamin D was observed (before treatment - 19.7 ± 5.4 ng/mL, after treatment - 34.7 ± 8.6 ng/mL, $p < 0.001$) and 74% of the post-treatment values were within the reference range (30-60 ng/mL). Serum vitamin D concentration increased by 200% ± 98% ($p <0.001$) during the first month of treatment with vitamin D deficiency and failure treated successfully in 83% of soccer players [22].

Furthermore, the role of vitamin D in the regulation of immune responses may increase during periods of high psychological and physiological stress. Due to the high demands placed on US Marine Corps recruits undergoing 12 weeks of basic military training, it is hypothesized that vitamin D status would be related to mucosal innate immunity markers, and daily vitamin D supplementation would increase immune responses during training. Thus, a randomized, double-blind, placebo-controlled study was formed by men (n=75) and women (n=74) who started basic recruitment training during summer and winter. The subjects received 1,000 IU of vitamin D3 + 2,000 mg of calcium/d (n=73) or placebo (n=76) for 12 weeks. Saliva samples were collected pre-training, during (weeks 4 and 8) and post-training (weeks 12) to determine salivary SIgA and cathelicidin (mucosal immunity indexes) and α-amylase (stress indicator). The initial (baseline) and post-training serum levels of 25(OH)D were measured. As a result, serum 25(OH)D levels were 37% higher in recruits who entered training in summer compared to winter. A positive relationship was observed between the baseline levels...
of 25(OH)D and the rates of SIgA secretion. When stress levels were high during summer training, baseline levels of 25(OH)D contributed to an increase in secretory rates of salivary secretory immunoglobulin (SIgA) and cathelicidin, the latter only in men. Vitamin D supplementation contributed to changes in SIgA and cathelicidin, specifically SIgA was greater in the treatment group. These data highlight the importance of vitamin D and mucosal immune responses during strenuous basic military training when stress levels are increased [23].

A prospective, non-interventionist and observational study determined the seasonal changes in the total serum concentration of 25(OH) vitamin D (RV) and its influence on upper respiratory tract infection (RTI) morbidity among elite water sports athletes. Study participants included 40 elite athletes and 30 control subjects. The serum levels of 25 (OH) VD and TNF-α, IFN-γ, IL-4, and IL-6 were detected by the ELISA technique. RV deficiency/insufficiency dominated in both groups of elite athletes, mainly in synchronized swimmers (100%) compared to controls (63.3%) (p≤0.05). The prevalence of RV deficiency/insufficiency depends on the season, but regardless of the season, the highest values were observed among athletes. RV sufficiency was detected in 30% and 13.3% of control subjects in August and February and only in 10% of swimmers in August. More than 3 episodes of RTI were detected only in elite athletes in the winter-spring. Increased levels of TNF-α, IL-4, IL-6 and decreased levels of IFN-γ were detected in all athletes but were more expressed in swimmers. Therefore, RV insufficiency is prevalent among elite athletes involved in synchronized swimming and swimmers. It is accompanied by a decrease in IFN-γ, an increase in TNF-α, IL-4, and IL-6 levels, and an increase in RTI morbidity [24].

Also, studies have suggested that vitamin D supplementation may increase serum fibroblast growth factor 23 (FGF23) among patients with vitamin D deficiency. Thus, a systematic review and meta-analysis were performed to summarize all available data. Nine studies were eligible for meta-analysis. Seven studies measured serum intact FGF23 and two studies measured serum C-terminal FGF23. Meta-analyses found that intact serum FGF23 increased significantly after oral vitamin D3 supplementation in participants with vitamin D deficiency. Serum C-terminal FGF23 also increased after vitamin D3 supplementation in participants with vitamin D deficiency [25].

Recent studies have shown that vitamin D supplementation decreases the parameters of oxidative stress (OE). This systematic review and meta-analysis aimed to investigate the effect of vitamin D supplementation on the parameters of OE. In thirteen clinical trials, vitamin D supplementation increased serum levels of total antioxidant capacity (TAC) and glutathione. Also, the concentration of malondialdehyde (MDA) decreased significantly after vitamin D supplementation compared to placebo. There was no difference between the experimental and placebo groups in the subset of studies that administered vitamin D at less than 100,000 IU per month. However, a significant increase in TAC levels was found in the subset of studies that administered vitamin D between 100,000 to 200,000 IU and those that administered vitamin D between more than 200,000 IU monthly [26].

Since the 1970s, several reports have described reversible morphological changes in the skeletal muscles of patients with severe vitamin D deficiency that express atrophy of type II muscle fibers. The changes resulted in vitamin D supplementation. Vitamin D induces myogenesis and muscle protein synthesis causing an increase in the percentage of fast-twitch muscle cells (type II fibers). This type of fiber is responsible for high power, rapid muscle contraction, and muscle development [27].

Vitamin D receptors (VDR) appear to play a significant role in muscle regeneration after injury when VDR expression increases. The local presence and activation of 1α-hydroxylase in the injured muscle fibers allow the muscle to synthesize an active form - 1,25 (OH) 2D. The signaling axes of VDR mediation are not well defined. However, smad3 (decapentaplegic homolog 3) which implies bone morphogenetic protein (BMP), transforming growth factor (TGF-β1), Src (proto-oncogene tyrosine-protein kinase Src), phosphoinositide 3 kinase (PI3K), and cAMP cascades of responsible element-binding proteins (CREB) appear to have significant predictive significance in differentiating the muscle parent and regenerating the muscle after damage. The role of vitamin D in muscle intracellular pathways in, for example, inducing mitochondrial function, has been documented. Mitochondrial oxidative phosphorylation is the main ATP resource for the contraction of skeletal muscle in individuals with severe vitamin D deficiency. Sinha et al. proved that cholecalciferol therapy increased the mean serum status of 25 (OH) D and simultaneously caused an improvement in maximum
oxidative phosphorylation and a reduction in phosphocreatine recovery half-life [28].

3.3. Limitations and Biases

The accuracy of vitamin D dosages varies widely between laboratories and between different assays and can reach up to 17 ng/mL. There are still differences in the extraction of vitamin D from its binding protein, a cross measurement of 25 (OH) D2, 25 (OH) D3, and other metabolites in addition to the lack of standardization and, for this reason, quality control tools such as DEQAS (International Vitamin D External Quality Assessment Scheme) in an attempt to reduce these variations in data analysis.

The most used methods today are competitive assays based on specific antibodies and non-radioactive markers. The aim is to improve the comparability between the results obtained with different methodologies. Whichever method is employed, a precise definition of the normal range is essential. It is also noteworthy that the intra-individual variability can be from 12.1 to 40.3%. Clinical conditions that interfere with serum 25 (OH) D levels are highly dependent on environmental and lifestyle factors, particularly exposure to UVB rays.

Also, randomized controlled and multicenter clinical studies are still lacking to better understand the relationship of vitamin D supplementation in the performance of athletes.

4. Conclusion

One of the main aspects that must be taken into account is important evidence that suggests that free (bioavailable) 25(OH)D may be a better marker of vitamin D status. Many researchers do not take into account that athletes may need an increased supply of vitamin D to meet muscle metabolism requirements due to the potential routes of vitamin D use. A significant debate seems to be needed to determine and standardize the classification of vitamin D deficiency. There are still information gaps about the correlation between vitamin D deficiency, vitamin D supplementation and athletes’ exercise performance. However, because vitamin D induces myogenesis and muscle protein synthesis, causing an increase in the percentage of rapidly contracting muscle cells, and because vitamin D receptors play a significant role in muscle regeneration after injury, supplementation in athletes is recommended.

References


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Informed written consent obtained from the participant

Conflict of interest
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