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SERUM VITAMIN D STATUS AND THE IMPACT OF HIGH-DOSE SUPPLEMENTATION ON ATHLETES PERFORMANCE

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Abstract

Background: *Vitamin D is a considered significant bone health and play a pivotal role in skeletal muscle growth, and other physiological processes. However, Vitamin D deficiency is t global health issue, and athletes may be susceptible particularly. The current study aimed to evaluate the serum concentration of 25(OH) $_2$ D3 in university athletes and assess the effects of bolus supplementation of vitamin D3 on their physical performance.* ***Materials and methods:*** *A total of 40 athletes (18-23 years old, 70 ±4kg, and 170±12 cm) from Abdul Wali Khan University voluntarily participated in the trial. The participants were randomly divided in two groups .The treatment group received 200,000 IU of vitamin D3 (Vit- D3) in milk, whereas the placebo group received sterile water in milk. The coach, who administered the supplementation was unblinded, while participants were kept blinded. Serum vitamin D concentration were measured before and after supplementation .The physical performance was evaluated via sprint 30m, standing broad jump and vertical jump tests.* ***Results:*** *Following supplementation, treatment group demonstrated a significant increase in total serum 25(OH) D concentration ($p< 0.05$). Notably, prior to supplementation 58% of athletes were identified as deficient. In the 30 meter sprint the average time for the treatment group improved from 4.0 ± 0.09 to 3.9 ± 0.09, while placebo group showed no significant change ($p>0.05$). Similarly, for the standing broad jump the distance achieved by the treatment group increased from (2.6 ± 0.1 to 2.7 ± 0.1), whereas placebo group exhibited no change (2.7 ± 0.09 -2.7 ± 0.09). The vertical jump height test also significantly improved in the treatment group (10.0 ±0.44-10.6 ± 0.47), in contrast no significant change was observed in the placebo group (10.0 ± 0.41-10.0 ±0.42). Conclusion: A bolus intervention of 200000 IU of Vit- D3 significantly increased serum 25(OH)$_2$ D3*

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levels and reversed deficiency (Vit- D3) in the athletes. Therefore, high-dose supplementation contributed in physical performance tests. Further studies with large sample size and longer duration for trials are required to validate these findings.

Introduction

Vitamin D (Vit-D) is a important fat-soluble vitamin conventionally recognized for its fundamental role in calcium homeostasis and bone mineral metabolism [1]. However, recently studies revealed its consequence surpassing skeletal health, forming it as a significant secosteroid hormone that has receptors in almost every tissue, such as skeletal muscle [2]. While its non-classical roles include regulation of inflammatory processes and immune responses [3]. These mechanisms are significant for athletes, because of effective recovery, optimum muscle function, and mitigated inflammation are crucial factors that influence competitive performance and training adaptation. Subsequently, maintaining adequate Vit D status is now regarded as vital constituent of an athlete's physiological preparedness [4].

Despite the well-established significance of Vit D, deficiency and insufficiency continue to be a global public health concern [5]. In lined with certain athletic communities are not exempt to this challenge, with a high incidence of Vit D deficiency documented across variety sports [6]. Certain athlete-specific factors including heavy usage of sunscreen, schedules of indoor training, or training during low sun exposure, can drastically least exposure to sunlight is the prime source of Vit D synthesis [7].

Mechanistically, the physiological association between physical performance and Vit D conceivable. The skeletal muscle contains Vit D receptors (VDRs), and it is argued that vitamin is responsible for the production of proteins that involved in contraction of muscle, especially in Type II (fast-twitch) muscle fibers [8, 9]. Consequently, these fibers are mainly essential for power generation, speed and strength, supplementing Vit D deficiency in individuals and improve power-interdependent tasks, including jumping and sprinting. Indeed, studies reported that rugby players, have improved in power dependent activities such as sprinting and vertical jump height post supplementation [10, 11]. Nevertheless, the overall body of evidence remains unpredictable, this inconsistent variations are ascribed due to dosage regimens, athletic caliber and Vit D status of the participants [12]. A further common limitation in the previous studies are reliance on extended supplementation protocols such as daily or weekly [12, 13]. While certain regimens pose significant challenges for observance in the real-world athletic team settings [14].

Therefore, the current study was aimed to evaluate the serum concentration of 25-hydroxyvitamin D [25(OH) D], and also to assess the effects of a single large dose(200,000 IU) of Vit D3 supplementation (200,000 IU) on their physical performance (30m sprint, broad jump, and vertical jump) in athletes at Abdul Wali Khan University, Mardan (AWKUM).

Materials and Methods

Study Design and Data Collection Criteria

Current study was one week randomized placebo controlled single blind trial conducted in January 2022. A total of 44 male athletes (18-23 years old) from Sports department were initially evaluated for this study. The participants were divided into two groups through draw from an envelope i.e. 1) treatment and 2) placebo group. The exclusion criteria included; (a) usage of Vit D supplements (b) any medical disorder affecting Vit D levels (c) in-communication or lack of contact with healthcare professionals distributing Vit D supplements; and (d) refusal to the study procedure. Out of all (n=44), four participants were excluded from the study based unfulfilled the inclusion criteria (a) 2 and (d) 2). Current study evaluated serum 25(OH) D and physical performance of the athletes using stand broad jump (SBJ), vertical jump (CJ), and 30 m sprint pre and post intervention of Vit- D supplementation.

Sample Collection and Supplementation Procedure

The informed consent was provided to the athletes who met the baseline criteria. The blood samples were collected from cubical vein by trained medical personnel, and physicals tests were performed by

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the participants. Afterward, the treatment group was supplemented with bolus dose of Vit-D3 (200,000 IU, D-tress injections, Sami pharmaceuticals)) in milk, and control group (placebo) received 5 mL of sterile water in milk. At the same time, the study respondents were also educated to avoid extra supplements particularly performance-enhancing drugs for (48h), coffee, and alcoholic beverages before each trial session. Blood samples were collected for serum vitamin D3 concentration on fourth day of supplementation between 10:30am to 11:30am and physical performance tests (sprinting 30m, SBJ, VJ) were measured between12:00 pm and 2:30 pm. Players were asked not to use any vitamin D supplements while participating in the trial.

Anthropometric Measurements

The height of the athletes was measured using Seca stadiometer (Hamburg, Germany) with participants standing barefoot and their heads in the Frankfort horizontal plane. Body mass was measured to the nearest 0.5 kg using a Secabeam balance 710 (Humburg, Germany), with participants wearing minimal clothing.

Vitamin D Measurement

A total of 5 mL of venous blood was collected in gel tube (without anticoagulation) between 10:30 am and 11:30am on each testing day. The blood was centrifuged for 10 minutes at 1500 rpm, while 0.5 mL of serum was aliquoted into 2mL microtube for testing. The measurement was performed using automated immune-assay system (Mini VIDAS) based on enzyme-linked fluorescence assay (ELFA). The assay determined serum 25(OH) D [15].

Physical performance tests

The athletes completed a series of tests to evaluate their physical performance. A 30 meter (m) sprint test was performed through a hand-held stopwatch. The time began when the respondent crossed the starting point followed by crossing the finishing line. Similarly, the athletes performed standing broad jump, and distance was measured from takeoff line to landing mark by the heel. Correspondingly, the jump-and-reach method was used to assess vertical jump height. The athletes were instructed to coat their fingertips with chalk, and stand flat-footed to develop a baseline. Afterward, executed highest vertical jump, marked the point they touch, and vertical jump was measured based highest jump touch and the standing reach. The study used the average time in triplicates for the analysis.

Statistical Analysis

Data analysis was statistically analyzed using SPSS (version. 20). The continuous data are expressed as mean ± standard deviation while the data normality was assessed using Shapiro-Wilk test Moreover, independent samples t-tests were performed to evaluate baseline characteristics between the experimental and placebo groups. The 2x2 mixed-model ANOVA was used to analyze primary and secondary outcomes between treatment/placebo groups and time (pre/post), with post-hoc analysis performed using paired and independent samples t-tests where appropriate. A p-value of < 0.05 was considered statistically significant for all tests.

Ethical Approval

Current study was ethically approved by AWKUM Ethical Committee, which was conducted in accordance with principles off Helsinki Declaration.

Results

Demographic Characteristics

All 40 participants successfully completed the study protocol. Basic anthropometric characteristics such as age, height, and weight. The aerobic fitness were similar between treatment and control group at baseline with insignificant association (Table 1). Data normality was evaluated using Shapiro Wilks test which revealed normal data distribution in treatment group for parametric analysis ($p < 0.005$) not significantly different and physical activity parameters. Between the groups at baseline basic anthropometric aspects.

Table 1: ***Physical and Demographic characteristics.***

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	Experimental Group (Treatment) n= 20	Placebo Group (Control) n= 20	p-value
Variable(s)			
Age	20.8 ± 1.5	19.8 ± 1.5	>0.05
Height	174.3 ± 4.2	172.8 ± 3.1	>0.05
Weight	71.1 ± 2.9	70.6 ± 2.2	>0.05

Note: The variables Age, Height and Weight are measured in year, cm and kg respectively.

Serum (25(OH) D) Levels

The initial serum 25(OH) D levels treatment group's considerably lower (19.27±5.0) as compared to the placebo group (25.65±6.90). The week after supplementation, treatment group observed 101.7% increase (19.27±5.0-38.88±5.40, p=<0.05) in serum 25(OH) D levels, whereas the placebo group showed negligible change (Table 2 & Fig. 1).

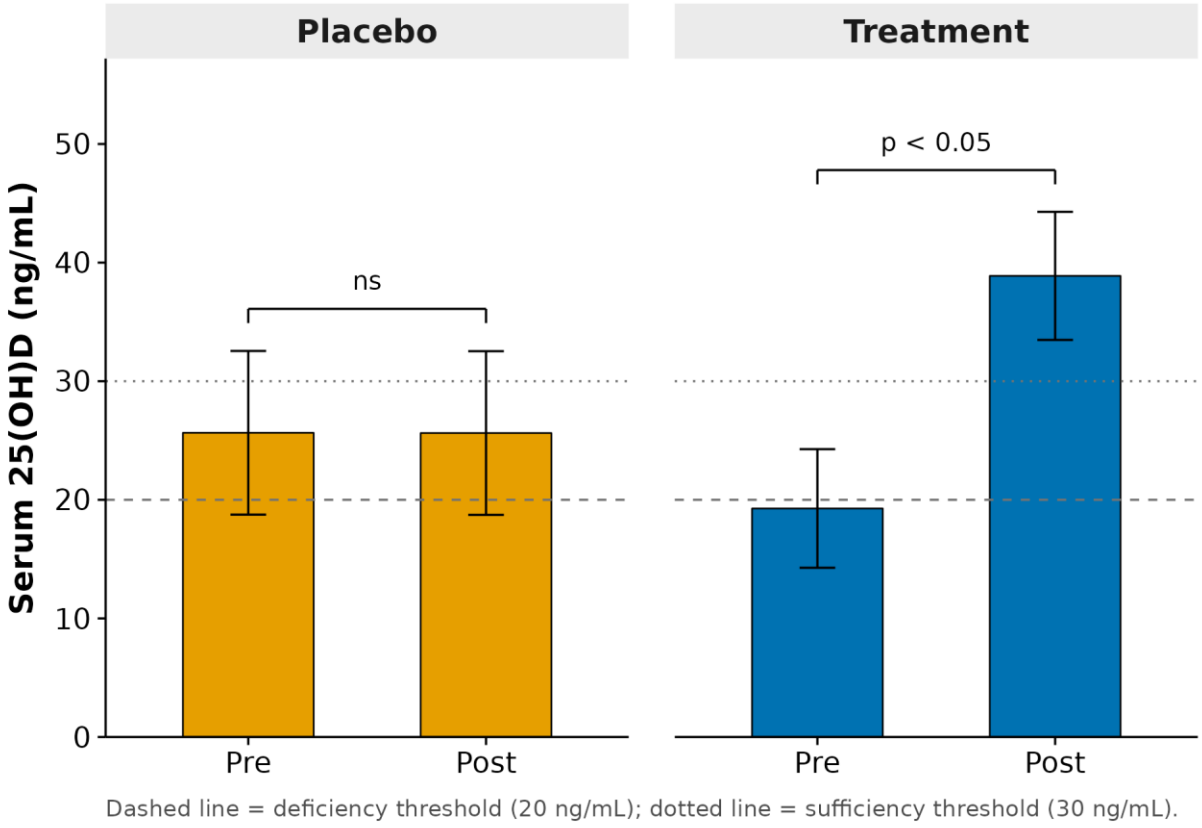


Figure 1: Demonstrates Serum 25(OH) D levels, Pre-supplementation of Vit D and Post-supplementation in placebo and treatment group(s)

Table 2: *Pre and Post supplementation of Serum (25(OH) D)*

Group	Pre (ng/mL)	Post (ng/mL)	Change (%)	p-value
Treatment/ experimental	19.27±5.0	38.88±5.40	101.7	<0.05
Placebo	25.65±6.90	25.63±6.90	0	>0.05

Effects of Vit D on Sprint performance (30m)

The supplementation of Vit D (25(0H) D) resulted in a significant improvement sprinting duration. The treatment group's was noted with significant ($p < 0.05$) decreased (4.0 ± 0.09 to 3.9 ± 0.09 seconds) in the sprinting time. While no significant change was observed in placebo group (Table 3 & Figure 2). According linear regression model,

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serum $25(OH)_2D_3$ levels significantly (F 1, 38) = 25.49, $p < 0.001$), enhanced 30 m sprint performance of athletes after Vit D supplementation. The model analysis revealed that 40.1% ($R^2 = 0.401$) of the variance in sprint (30 m) time was described by Vit D levels in the treatment. The regression coefficient for serum 25(OH)D was negative and statistically significant (B = -0.007, SE = 0.001, β = -0.634, t = -5.05, $p < 0.001$), suggesting that higher vitamin D concentrations are related to more rapid sprint performance (i.e. lower sprint times).

Table 3: *Sprint performance (30 m) pre and post intervention*

Group	Pre (sec)	Post (sec)	Δ Mean	p-value	R (r)	Regression model (R_2)	F(1, 38)	p-value	β (Vitamin D → Sprint Time)	p-value
Treatment	4.00 ± 0.09	3.90 ± 0.09	-0.10	0.032	**0.634**	**0.401**	**25.49,**	0.001	−0.634	0.001
Placebo	4.08 ± 0.10	4.08 ± 0.11	0.00	0.88		-	-	-	-	-

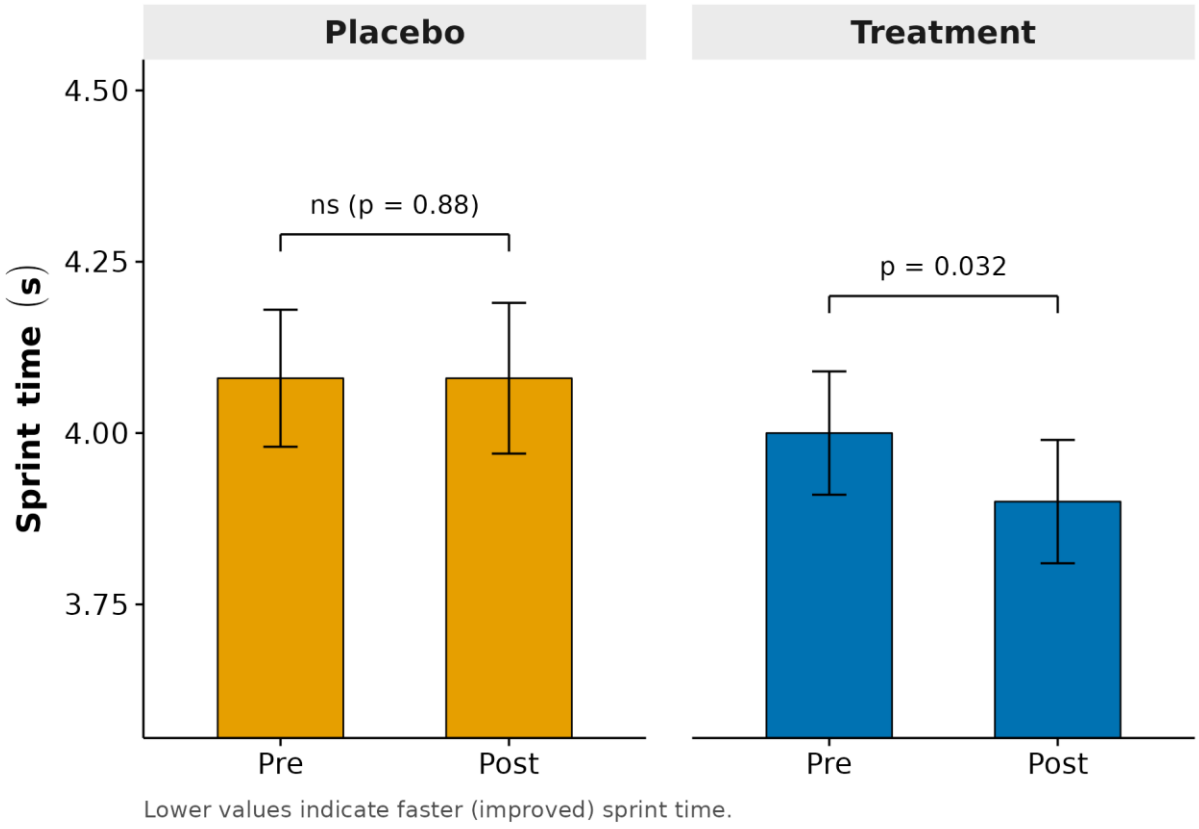


***Figure 2:** Illustrates performance of athletes for 30 m sprinting in treatment and placebo group(s) (n=40, 20, & 20), pre and post supplementation. The supplemented group (treatment group) significantly improved (4.00 ± 0.093.90 ± 0.09, p= 0.032), its performance compared placebo group pre and post supplementation.*

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Effect of Vit D on broad and vertical Jump performance

The participants of the treatment group between day 1 to 8 significantly) observed broad Stand Jump distance improvement (2.6 ± 0.1 to 2.7 ± 0. 1 m, <0.05), while the placebo groups have no insignificant improvement (2.7 ± 0.09-2.7 ± 0.09 m, >0.05). Similarly, the participants were also tested for vertical jump test and observed a significant change in the treatment group (10.0 ± 0.44-10.6 ± 0.47,<0.05) whereas the placebo group have no significant differences (10.0 ± 0.41-10.0 ± 0.42, >0.05) in pre and post treatment of Vit D (Table 4 & Figure 3). While, Receiver Operating Curve (ROC), analysis revealed that levels serum (25(OH)2 D3 and vertical jump after treatment. The concentration of 25(OH) 2D3 showed good discriminative power, with the area under the curve (AUC) of 0.924, which indicates good capacity to discriminate individuals with positive and negative outcome groups. Similarly, the vertical jump height had an AUC of 0.806, which implies a statistically significant predictive value of the specified outcome (Figure 4).

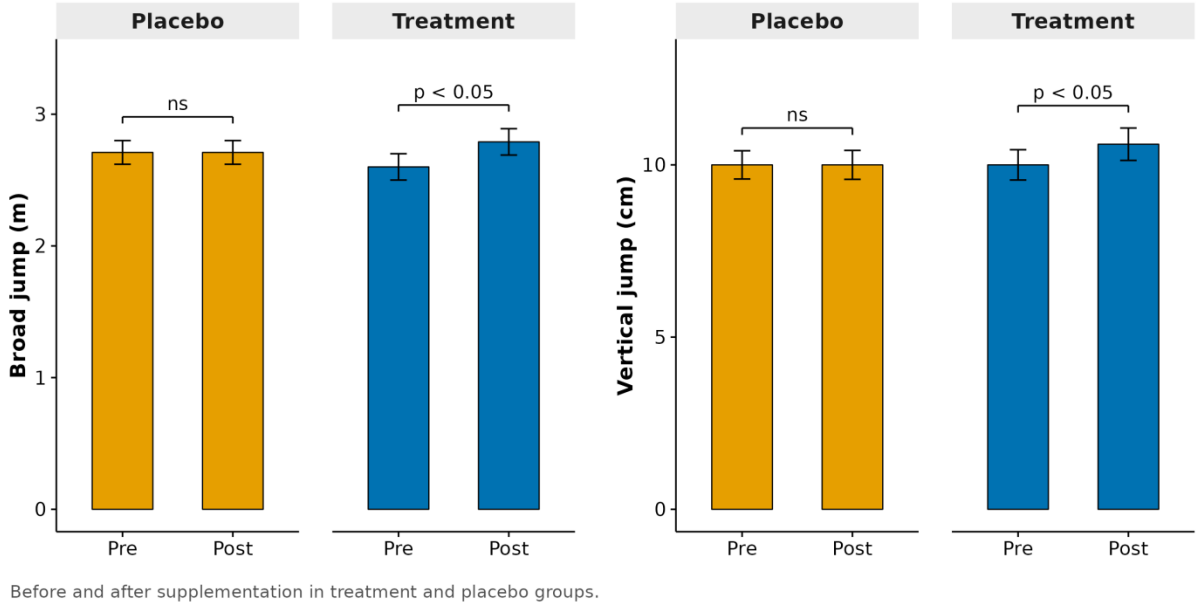


Figure 3: Standing broad jump and vertical jump performance Pre and post supplementation. The treatment group displayed significant (p < 0.05), improvement in both broad jump (2.6 ± 0.1 to 2.79 ± 0.1 m) and vertical jump height (10.0 ± 0.44 to 10.6 ± 0.47 cm), whereas the placebo group showed insignificant (p > 0.05) difference in either measure. Bars represent group means; error bars represent SD.

Table 4: ***Broad and Vertical Jump performance pre and post supplementation***

Broad Jump			**Vertical Jump**			
Groups			Groups			p-value
Treatment	Pre (m)	Post (m)		Pre (m)	Post (m)	<0.05
	2.6 ±0.1	2.79± 0. 1	Treatment	10.0 ± 0.44	10.6 ± 0.47	
Placebo	2.71± 0.09	2.71± 0.09	Placebo	10.0 ± 0.41	10.0 ± 0.42	>0.05

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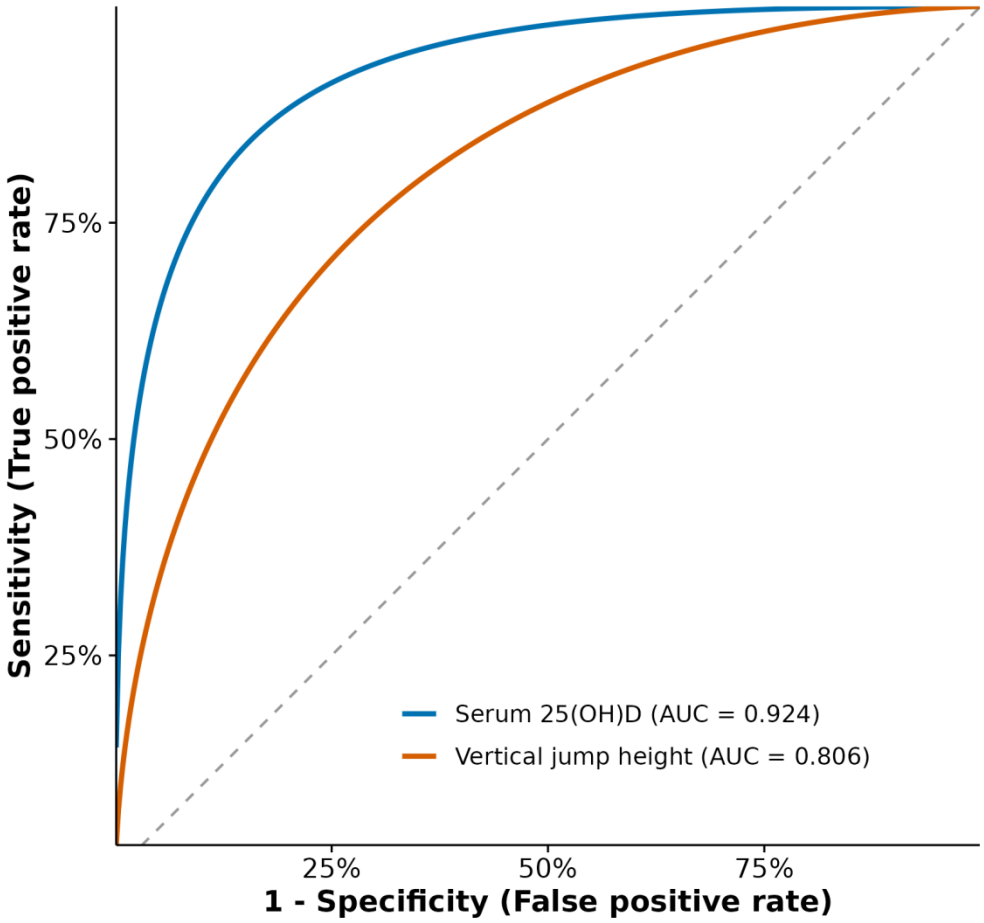


Figure 4: Demonstrates ROC for serum (25(OH)2 D3 and vertical jump after treatment. The concentration of 25(OH) 2D3 showed good discriminative power, with the area under the curve (AUC) of 0.924, which indicates good capacity to discriminate individuals with positive and negative outcome groups. Similarly, the vertical jump height had an AUC of 0.806, which implies a statistically significant predictive value of the specified outcome.

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Discussion: The Vit D deficiency is a common health concern amongst athletes, as it is known to be associated with poor muscle function and performance. While the importance of Vit D and skeletal health is well established, its ergogenic potential and ability to improve skeletal health and address such skeletal deficiency is a topic of ongoing research in the sports medicine field [16]. Current aimed to assess whether a single high-dose Vit D supplementation treatment could rapidly overcome deficiency in athletes and subsequently provoke measurable improvements in power-based and explosive performance metrics.

The main results of this investigation are two revelations: 1) that a high-dose vitamin supplementation regimen (200 IU of Vit D) weekly causes a significant and rapid correction of serum 25(OH)D levels amongst athletes with the deficiency; 2) the resultant biochemical correction yields a noticeable ergogenic effect, which is translated into superiority in tasks with explosive and power-based requirements. The current study observed elevated level of serum 25(OH) D in the treatment group (19.27ng/mL to 38.88 ng/mL), and observed 101.7% increase. Our findings are strongly aligned with previously conducted study which demonstrated that high dose of Vit D supplementation (50,000 IU/week), significantly increased serum 25(OH) D in Vit D deficient athletes in a short period of time [17]. Similarly, Fassio et al. randomized study revealed that optimized high dosage elevated Vit D metabolites in athletes [18]. The results of our study have a good reason to believe that the seemingly ineffective results found in some sports nutrition research might have been a direct result of improper dosing. While our one-week high dose performance provides an effective and effective protocol to team doctors and sports nutritionists to quickly deal with high deficiency levels.

The present study revealed that Vit D supplementation had significantly contributed in all key performance indicators including 30m sprint time broad and vertical jump. Effects of Vit-D on 30m sprint showed that 25 (OH)D levels has increased athletes capability of running and significant changes were observed between pre and post mean values of treatment group. Similarly, Vit- D supplementation has also showed a significant (p-value= <0.05) impact on broad stand jump test (2.6 ±0.1-2.79± 0. 1), and vertical Jump test 10.0 ± 0.4410.6 ± 0.47) of treatment and placebo groups. Our findings in context of athletes performance particularly 30m sprint aligned with previous studies, where Vit D supplemented groups significantly improved its sprint time in young athletes [19, 20]. Similarly, our findings regarding broad and vertical jumps are in agreement with previous authors[16, 19, 20]. Our findings nevertheless, was noticeably deficient, these evidence is consistent with "Sufficiency model" hypothesis, which suggests that athletes performance are mainly observed when fixing deficiency alternatively supplementing sufficient levels that are already adequate [21].

In line with improvements, we observed in athletes performance such as sprinting and jump are constant with Vit D physiological role in skeletal muscles. Vitamin D receptor (VDR) is also expressed in human muscular tissue, the activation of which has been reported to facilitate transcription of genes in protein synthesis and proliferation of type II (fast-twitch) muscle fibers. These fast-twitch fibers are specifically used in the performance tests that we undertake. Therefore, the results of our study contribute practical evidence in support of the hypothesis that vitamin D replenishment is the main factor which maximizes the neuromuscular system adaptive response to the explosive strength [11]. Similarly, compared to study by Michalczyk et al. [22] that found enhanced performance (sprint and jump) in elite soccer players after consuming Vit D, roves the fact that our findings are not only similar to their findings, but also clear results have been documented on the starkness of the difference between a deficient group which performed well enough to rise to the rank and one that did not improve at all. The advocacy of the minimal difference in the placebo group which despite their high baseline did not result in any change, further supports the idea that the change in the vitamin D status is the vital determinant.

Conclusion

In conclusion, this research demonstrates that high-dose Vit-D (200,000 IU/week)

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supplementation is significantly effective intervention for normalizing serum 25(OH)D levels in deficient athletes efficiently. Moreover, the statistical significance of this correction is associated with subsequent improvements in several major areas of athletic performance, including sprint speed, broad-jump distance, and vertical-jump height. Hence, these observations highlight the crucial importance of monitoring and maintaining appropriate Vit D status within sporting communities. Therefore, for coaches and sports-medicine practitioners, confirming the emergence of Vit D adequacy as a direct and successful strategy to supplement explosive power and overall performance in athletes.

Conflict of interest

The authors declared no conflict of interest

Author's Contribution

SA and MA conceptualized, development of methodology, and wrote the manuscript. MS helped in data collection and arrange athletes, and supported in main activities. MTK performed data analysis, and helped in editing and reviewing, and MA provided supervision and primary conceptualization. All the authors reviewed, and agreed for publication of this manuscript.

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