



The Effect of Vitamin D Supplementation After Resistance Training on Physiological Characteristics in Futsal Players with Vitamin D Deficiency

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Received 2022 April 13; Revised 2022 May 01; Accepted 2022 June 16.

Abstract

Background: Recent research indicates the prevalence of vitamin D deficiency worldwide and is conflicting evidence as to whether vitamin D supplementation actually improves physical performance.

Objectives: The purpose of this study was to investigate the effect of vitamin D supplementation on improving muscle strength, muscle volume and cardiorespiratory fitness through resistance training in male athletes with vitamin D deficiency.

Methods: This study was conducted with pre-test and post-test series design and quasi-experimental method. The population included 36 male futsal players with vitamin D deficiency that were randomly divided into four groups of nine: exercise (EX), exercise-supplement (EXS), supplement (SUP) and control (CON). SUP and EXS groups received vitamin D₃ (50,000 intramuscular injections) every two weeks for 8 weeks and performed three resistance training (RT) sessions per week at a rate of 75% 1RM. Before and after intervention, blood sampling were drawn and measurements performed for 1RM, muscle volume (cm²), and VO_{2max} by standard Bruce test. Correlated *t*-test was used to compare pre-test and post-test results and to measure the differences between groups, one-way analysis of variance and LSD post hoc test were used using SPSS statistical software.

Results: Muscle volume increased significantly (*P*-value = 0.001) only in EX and EXS groups. Cardio-respiratory fitness did not change significantly in any of the groups (*P* > 0.05). There were no significant differences between EXS and SUP groups for any of the measured variables (*P* ≥ 0.05).

Conclusions: It seems that simultaneous application of vitamin D supplementation and resistance training for 8 weeks does not have a significant effect on the improvement of the strength and endurance of futsal players.

Keywords: Cardiorespiratory Fitness, Muscle Strength, Resistance Training, Vitamin D

1. Background

Recent research indicates the prevalence of vitamin D deficiency worldwide (1). Vitamin D deficiency can cause adverse effects, including poor muscle strength and consequent poor physical function, joint pain, and osteoporosis. Many studies have also reported a significant correlation between vitamin D deficiency and the prevalence of chronic illnesses such as cardiovascular disease, hypertension, diabetes, stroke, etc. (2). It should be noted that this is also true for athletes. In addition to the role of exercise therapy for health improvement

(3-5), the role of nutrition would not be ignored. In this regard, there is debate in the sports community over whether vitamin D supplementation is beneficial for athletic performance because of the controversy over whether vitamin D supplementation improves physical function. While some cross-sectional or interventional studies provide persuasive arguments in favor of supplementation, there is no strong evidence that vitamin D has a direct effect on body function (1). On the other hand, one report suggests that only 44% of athletes consume an adequate amount of vitamin D (6). Although

vitamin D deficiency has been reported as a major cause of functional weakness, muscle pain, and delayed healing of wounds, few studies have proven that vitamin D deficiency can be a significant problem in sports communities (7). On the other hand, vitamin D may affect the cardiovascular system's ability to carry oxygenated blood and the ability of skeletal muscle to use oxygen (8).

The importance of Vitamin D is supported by the fact that receptors are found in almost every tissue in the body. Discovery of these receptors in muscle cells also indicates its special role as one of the important regulators of muscle tissue function (9, 10). Studies have found that D vitamin metabolites affect muscle by stimulating protein synthesis, increasing the ratio of fast twitch muscle fibers, improving cell function and proliferation (9, 11). Recent studies have also reported a relationship between vitamin D and cardiac respiratory capacity through increasing energy production in cardiac myocytes when sufficient levels are present (12).

Factors such as poor diet habits without vitamin D supplementation, unhealthy lifestyle, and a lack of sunlight have the greatest impact on vitamin D deficiency (13). However, other studies performed on non-athletes or athletes who are active in the athletic fields and exposed to sunlight shown contradictory results. Saremi et al. (14) investigated the effect of an endurance training course with vitamin D supplementation on muscle functional indices in middle-aged postmenopausal women. The results of that study showed that vitamin D supplementation intake and endurance exercises increased muscle strength and volume in these individuals. Markin (15), on the other hand, examined the effect of taking vitamin D supplementation of 50,000 IU for eight weeks on muscle function in active vitamin D deficient individuals. They concluded that although vitamin D supplementation use for 8 weeks significantly increased serum levels of this vitamin, it had no effect on improving physical function. Koundourakis et al. (16) examined vitamin D levels and their effect in male professional soccer players aged 20 - 30 years. They found that vitamin D levels were associated with muscle strength and maximal oxygen consumption (VO_{2max}). In general, their results showed a two-way interaction between the performance indicators of football players and vitamin D levels (16). Considering all of these factors and the role of vitamin D in skeletal muscle function, the question arises whether taking vitamin D supplements can improve the performance of athletes who have previously had vitamin D deficiency. Furthermore, can combining effective exercises to increase skeletal muscle force output and taking vitamin D supplements double the expected muscle-strengthening results? Therefore, the aim of this study was to investigate the effect of vitamin D supplement injection on muscle strength and volume

increases and enhancement of cardiorespiratory fitness through resistance training in male futsal players with vitamin D deficiency.

2. Objectives

The present study was conducted using a pretest-posttest design of time series and quasi-experimental method on male futsal players in Parsabad county (Ardabil province, Iran). Initially, 152 athletes referred to the football board and sports halls association were screened by measuring plasma levels of vitamin D, with more than two-thirds of them showing symptoms of vitamin D deficiency.

3. Methods

Forty athletes were severely deficient in vitamin D, according to the Endocrine Society' Work Index, exhibiting a serum level of vitamin D less than 20 ng/mL, with at least three years of training experience were selected in late summer (17). The registration of athletes for the current study sample was done after they provided informed consent. Participants were assessed by completing a health history questionnaire, a three-day food registration questionnaire, and daily exercise activity summary. At this stage, 4 participants were eliminated based on the required level of activity and physical fitness inclusion criteria and the Global Physical Activity Questionnaire (GPAQ). The GPAQ has questions revolving around three domains: Occupational, transport-related, and leisure-time PA (18). For each domain, there is a pre-set PA list to help participants recall PA, which ensures the reliability and validity of the questionnaire (19). The remaining participants were then divided into four groups of nine athletes each using a random allocation method: exercise (EX), exercise-supplement (EXS), supplement (SUP) and control (CON) Inclusion criteria included restriction from use of vitamin D in the last six months, not drinking alcohol, no smoking, and a maximum oxygen consumption above 40 mL/kg/min.

3.1. Data Collection

In the first session, measurement of demographic variables including height, weight, body mass index, and body fat percentage was performed after fully explaining the objectives of the measurement using the tools and the International Society for the Advancement of Kinanthropometry' (ISAK) anthropometric criteria (20). The 8-week course of interventions included administered by vitamin D3 supplementation (intramuscular injection of 50,000 units) to the EXS and SUP groups. -ESE performed resistance exercises (chest press, shoulder pulley stretching exercises with pulley, forearm and triceps)

with barbells to strengthen the upper limbs and lower body (front and back squats) three sessions per week with an intensity of 75 - 60% of one repetition-maximum (Table 1)(21). The SUP received only the Vitamin D supplement and performed no exercise while the CON groups performed no exercise and received not supplement. Blood sampling was done before and after the intervention period. At both times, the serum was immediately separated by centrifugation of blood samples at 3000 rpm for 15 minutes and stored at -70°C. Serum levels of 1.25 (OH) 2D were then measured using the enzyme-linked immunosorbent assay' (ELISA) immunoassay protocol (Kit 25OH Vitamin D - VIDAS-Biomerieux).

Maximal oxygen consumption (VO_{2max}) was estimated using the Bruce treadmill test before and after the training and supplementation period. Subjects were assessed and monitored for vitamin D intake at the beginning and during the course of study using a three-day diet registration questionnaire (22). In the first session, subjects received written instructions and oral explanations on how to record their diet. It should be noted that the subject's normal diet did not provide sufficient amounts of vitamin D, but they were asked to follow their normal dietary guidelines without any changes. The analysis was conducted with Nutritionist IV Windows software program (version 4.0, N-Squared Computing, First Databank Division, was used for analysis. The Hearst Corporation, San Bruno, CA) to determine the amount of energy received by the athletes in a day (23). All dietary analyses were performed by the same laboratory technician.

3.2. Ethical Considerations

Subjects of the present study were healthy except for vitamin D deficiency and the amount of vitamin D obtained could be cited according to the opinion of the relevant expert. Other sources also included the amount of physical activity according to expert's comments and the unique level of physical fitness of the subjects.

Criteria of the ISAK Advancement Association was used by a bodybuilding technician to measure body composition and anthropometric indicators using the Kit with Slim Guide. Brzycki's formula was also used to estimate the strength of a repetition maximum (24).

$$1RM = \frac{\text{repetition weight (kg)}}{1.0278 - (\text{number of repetition}) \times 0.0278} \quad (1)$$

The method for measurement of cardiorespiratory fitness was the standard Bruce protocol: Subjects warmed up for 10 to 15 minutes in a self-selected manner before performing the protocol. The treadmill protocol started with at a speed of 2.74 km/h and a 10% slope. At each 3-minute interval, treadmill speed and slope were increased with a constant ratio until the subjects reached

the phase of terminal fatigue. Subjects cooled down for 8 minutes after completion of the protocol. Test run time was utilized in the Foster formula (22) by each subject included 3 times $[VO_{2max}]$ (mL/kg/min) = (3 (time) 0-2/012 (time) 0.451 + (time) × (time) × 1.379-14.76.

3.3. Statistical Analysis

Descriptive statistics (mean ± SD) were used to analyze and categorize demographic characteristics data. The Shapiro-Wilk test and Levene's test were used to assess the normal distribution of data and the homogeneity of variance, respectively. A paired *t*-test was used to examine the within group differences. Differences between groups were analyzed by one-way analysis of variance (ANOVA) and LSD post hoc test. Data were analyzed using IBM SPSS statistical software (version 20) with the P-value = 0.050 for significance.

4. Results

Descriptive statistics (mean ± SD) for demographic characteristics of the subjects are reported in Table 2.

The results of paired *t*-test showed that muscle mass index (biceps, quadriceps and calf) in EX and EXS groups had a significant increase between the two stages, but no significant increase was observed in the SUP and CON groups ($P < 0.05$). These results showed that muscle strength index (in both upper and lower body muscles) showed a significant increase in EX, EXS, and SUP from pre-test to post-test. No significant increase was observed in CON from pre-test to post-test. There was no significant difference between the two stages in the cardiorespiratory fitness index, which was determined by measuring the maximal oxygen consumption, in any of the groups. Results also showed there was a significant increase in vitamin D index in both SUP and EXS groups. While the EX group improved significantly, the observed increase was much smaller than for EXS and SUP that took high doses of vitamin D supplementation (Table 3).

The results of one-way analysis of variance test showed a significant difference in all of measured indicators across the 4 groups except for cardiac and respiratory readiness variables (Table 3). LSD post hoc follow up showed that there was no significant difference in the indices of biceps, quadriceps and calf muscle mass between EX and EXS, but these groups were significant different from the SUP group. The increase in muscle size can be attributed to resistance training after eight weeks of intervention since this increase was not significant from the SUP group. It appears that receiving injections of a vitamin D supplement for 8 weeks had no effect on increasing muscle size of young futsal players. LSD post hoc test indicated a significantly greater gain in upper body muscle strength index in EX than in EXS and SUP groups. LSD post hoc

Table 1. Resistance Training Program in 8 Weeks

Variables	Week							
	1	2	3	4	5	6	7	8
Intensity (%1RM)	60	65	65	70	70	75	75	75
Repetition sets	14	12	12	10	10	8	8	8
Rest between set (s)	3	3	3	3	3	3	4	4
	60	80	80	100	100	120	120	120

Table 2. Mean and Standard Deviation for Anthropometric Variables in Different Research Groups^a

Variables	Groups			
	Exercise	Exercise-Supplement	Supplement	Control
Age (y)	25.5 ± 4.40	25.2 ± 1.78	26.2 ± 2.82	26.9 ± 2.92
Height (cm)	176.2 ± 4.896	176.4 ± 5.496	178.3 ± 5.018	176.4 ± 4.716
Weight (kg)	68.9 ± 5.8.	68.85 ± 4.59	70.20 ± 5.80	71.10 ± 5.195
BMI (kg/m ²)	21.83 ± 1.31	22.10 ± 1.1	21.6 ± 3.25	22.83 ± 1.03
Body fat (%)	13.07 ± 3.6	13.51 ± 2.4	12.65 ± 2.95	14.02 ± 3.44

^a Values are expressed as mean ± standard deviation.

test for lower body muscle strength showed that after 8 weeks of resistance exercise intervention and vitamin D supplementation the EXS group had a significantly greater performance than the other groups, which did not differ significantly.

5. Discussion

The objective of this study was to investigate the effect of vitamin D supplement on performance improvement in young futsal players with vitamin D deficiency following resistance training. The results showed that resistance training during 8 weeks leads to a significant increase in muscle size and strength. Two factors of contractile elements which could contribute to this phenomenon might be increases in sarcomeres and myofibrils involved in cellular mechanisms (25).

The results also showed that vitamin D supplementation intake did not increase the effect of resistance training on the measured indicators, considering that the increase observed in the EXS group was not significant different from the REX group. It seems that a significant increase in the EXS group was more related to the effect of resistance training. Saremi et al. (14) concluded that vitamin D injection and resistance training simultaneously may have beneficial effects beyond what has been observed for resistance training alone. Hayes et al. (26) found that combining physical activity with vitamin D supplementation can have positive effects on muscle function. However, as noted above, vitamin D

supplementation alone does not increase muscle size. Lopez-Torres Hidalgo (27) found no significant effect on muscle volume following vitamin D supplementation, similar to the current findings. These discrepancies may be related in part to differences in size vitamin D-baseline status, size of supplement dose, age, sex, and level of fitness of the subjects. Additional research may be needed to target specific supplementation doses and frequencies when combined with exercise.

The results of the present study showed that resistance exercise significantly increased the upper and lower body muscles strength in post-test compared to the pre-test. These results were consistent with the findings of Amirsasan et al. (28). Vitamin D supplementation intake alone also significantly increased upper and lower body muscle strength. Diamond et al. (29) found that daily intake of 200 to 500 units of vitamin D was associated with increased muscle strength in middle-aged people with vitamin D deficiency. Vitamin D receptors in skeletal muscle tissue cells are a main factor explaining the molecular mechanisms involved in increasing potency of vitamin D supplementation intake (11, 30). Nuclear receptors activate the slow response pathway associated with changes in gene expression and membrane receptors that mediate rapid responses independent of genetic changes with respect to their place of presence. In fact, vitamin D rapid responses that cannot be justified by the genetic pathway have strengthened the evidence for these receptors (11, 30). On the other hand, nuclear receptors are activated by binding a biologically active

Table 3. Comparison of the Mean of Variables, Before and After Exercise and Supplementation Intervention in Different Research Groups ^a

Statistics**/Groups	Intergroup Differences ***	Phases of Exercise		P-Value
		Pre-stest	Post-test	
Muscle mass (cm)				
Biceps (cm)				
Exercise	a	2.94 ± 37.70	2.66 ± 33	0.0001 *
Exercise +supplement	a	2.63 ± 38.40	1.72 ± 32.9	0.001
Supplement	b	1.79 ± 34.50	1.76 ± 34	0.610
Control	b	2.53 ± 33	2.78 ± 32.66	0.654
Quadriceps				
Exercise	a	5.03 ± 58.40	5.18 ± 54	0.0001 *
Exercise +supplement	a	2.6 ± 60.90	2.55 ± 55.10	0.0001 *
Supplement	b	4.09 ± 56.1	4.17 ± 55.1	0.523
Control	b	3.81 ± 55.9	3.97 ± 54	0.702
Calf				
Exercise	a	3.92 ± 40.9	3.34 ± 36.10	0.0001 *
Exercise +supplement	a	2.78 ± 42	1.82 ± 37.30	0.0001 *
Supplement	b	5.12 ± 37.30	3.41 ± 36.10	0.391
Control	b	3.25 ± 2\37.2	3.26 ± 36	00.339
Muscle strength (kg)				
Upper body				
Exercise	a	6.81 ± 69.17	5.39 ± 48.70	0.0001 *
Exercise +supplement	b	11.89 ± 64.2	8.51 ± 47.17	0.0001 *
Supplement	c	5.73 ± 55.26	7.80 ± 49.64	0.021 *
Control	d	6.47 ± 48.7	6.46 ± 47.88	0.223
Lower body				
Exercise	a	15.78 ± 159.92	17.43 ± 135.75	0.0001 *
Exercise +supplement	a	9.81 ± 162.73	10.65 ± 138.19	0.0001 *
Supplement	b	12.10 ± 149.88	18.75 ± 138.14	0.006 *
Control	c	14.33 ± 141.13	11.82 ± 134.87	0.987
VO _{2max} (mL/kg/min)				
Exercise	a	8.43 ± 62.16	5.35 ± 57.61	0.082
Exercise +supplement	a	6.18 ± 63.38	5.45 ± 59.35	0.353
Supplement	a	5.09 ± 61.81	6.26 ± 57.43	0.091
Control	a	5.07 ± 59.63	5.15 ± 57.05	0.926
25(OH)D (ng/mL)				
Exercise	a	9.65 ± 21.40	4.57 ± 18.07	0.08
Exercise + supplement	b	8.97 ± 56.53	4.74 ± 19.92	0.0001 *
Supplement	b	10.8 ± 58.47	5.61 ± 21.01	0.0001 *
Control	c	4.24 ± 19.22	5.69 ± 18.96	0.795

^a * Significance at 5% alpha error level ($P < 0.05$) (intragroup *t*-test results). ** *t*-tests and one-way analysis of variance. *** Different Latin letters indicate a significant difference between groups in each variable (results of one-way analysis of variance between groups).

form of vitamin D, (ie, 2D (OH) 1.25). The final activation occurred following activated VDR heterodimerization with a steroid receptor of retinoic acid. It alters and facilitates mRNA transcription and expression of genes encoding some proteins. Ultimately, this pathway changes muscle calcium transport and phospholipid metabolism (11, 31). The non-genomic pathway is activated by binding 1.25 (OH) 2D to membrane receptors. This linkage activates several signaling pathways of the secondary messenger, which leads to the rapid regulation of calcium within muscle cells in one of the most important functions affecting calcium transport (11, 32). Increased calcium transport in both slow and fast pathways mediated by the active form of vitamin D can be an effective factor in stronger muscle contractions (33).

On the other hand, simultaneous training and complementary interventions did not caused a double increase in muscular strength in both upper and lower body muscle groups, with the effect of sport activity being more than supplementation activity. Skeletal muscle biopsy in adults with vitamin D deficiency often indicates type II muscle fiber atrophy. In other words, vitamin D deficiency appears to selectively affect fast-twitch muscle fibers (9, 11, 27). The response and adaptations created follow more resistance training towards the expansion of oxidative potential among muscle fibers and an increase the oxidative capacity of slow-twitch fibers (34). According to research results, after applying treatment courses with vitamin D supplementation, the relative composition of muscle fibers seems to have changed, so that the size and percentage of type IIa muscle fibers have significantly increased after treatment in three months to two years, in such a way that 1.25 (OH) 2D activates the signaling pathways of mitogen-activated protein kinases (MAPK) that lead to Myogenesis. The 1.25 (OH) 2D activates extracellular signal-regulated kinases. Finally, it stimulates muscle cells growth and differentiation, especially in fast-twitch muscle fibers (11), while neuromuscular adaptations after resistance training affect slow-twitch fibers. For example, the factor myocyte enhancer factor-2 (MEF2), which regulates myogenic factors, is associated with increased oxidative potential in fast-twitch muscle fibers that have a high tendency toward slow-twitch fibers and increases the expression of myosin heavy chain in type IIa fibers (34). Perhaps such interactions of 1.25 (OH) 2D and resistance training did not increase the interactive and combined effect of the two interventions for increasing strength.

The results of the present study showed that 8 weeks of resistance training had no significant effect on VO_{2max} which supports previous research noting that resistance training with the conventional methods used alone was not a sufficient stimulus to improve VO_{2max} (35). Simultaneous use of vitamin D supplementation

and resistance training did not have a significant compounding effect on improving the cardiorespiratory fitness index. Marawan et al. (12) and Ardestani et al. (36) stated that there is a positive and reasonable correlation between serum levels of vitamin D and VO_{2max} , which is independent of age, sex, race, body mass index, hypertension, diabetes, and smoking (12, 36). According to Ardestani et al. (36), an interactive effect was observed between vitamin D levels and physical activity, so that in people with lower levels of physical activity, a stronger relationship was shown between vitamin D and VO_{2max} . A strong positive association has been noted between VO_{2max} and vitamin D in people who had not previously experienced regular and strenuous exercise (12, 36). The biological pathways responsible for this relationship have not yet been identified, but in a general explanation, researchers have concluded that VO_{2max} depends on cardiac output, arterial oxygen content, blood flow toward active muscles, and extraction of oxygen by muscles. On the other hand, energy production in the mitochondria of ventricular myocytes is low when vitamin D levels are low, so that vitamin D deficiency is associated with decreased cardiac contractility, increased blood pressure, and endothelial dysfunction. As a result, low levels of vitamin D can reduce cardiac output and increase vascular resistance, thereby affecting VO_{2max} (12, 26). One limitation of the study was low number of participants and short duration of exercise protocol and supplementation which should be taken into consideration in future studies.

5.1. Conclusions

Concomitant use of vitamin D supplementation with eight weeks of resistance training had no ergogenic effect on improving cardiovascular endurance and strength in futsal players. One of the possible reasons for the positive effects of vitamin D in some studies seems to be over-reliance on the cross-sectional studies used, rather than randomized controlled trials.

Footnotes

Authors' Contribution: It was not declared by the authors.

Conflict of Interests: Maghsoud Nabilpour, the Associate Editor, and Roghayeh Afroundeh, the Reviewer, of the International Journal of Sport Studies for Health, were excluded from the peer-review process of this article. There are no other conflicts of interest.

Data Reproducibility: It was not declared by the authors.

Ethical Approval: None declared.

Funding/Support: This research received no external funding.

Informed Consent: Informed consent was obtained.

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