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The Effect of Vitamin D3 Supplementation on Delayed Onset of Muscle Soreness of Active Boys with Vitamin D Deficiency

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ABSTRACT

Aim: This study aimed to investigate the effect of two weeks of vitamin D3 supplementation on some indices of Delayed Onset of Muscle Soreness (DOMS) after eccentric exercise.

Method and Materials: In this semi experimental study, 20 nonathletic male collegiate students (19.75±1.29 years) participated in the study voluntarily and were divided randomly into two groups of experimental (n=11) and placebo (n=9). The experimental group consumed vitamin D3 (Cholecalciferol) containing 50000 IU while the placebo group took apparently similar capsules containing starch like, two capsules for two weeks. To induce DOMS, participants performed a step protocol that included four sets (5 minutes for each set), with a 1-minute of rest interval between each set using a 46 cm-step. Range of Motion (ROM) of low extremity joints and circumferences, and Visual Analog Scale (VAS were measured before, immediately after (Time 0), and 24 hours (Time 1) and 48 hours (Time 2) after eccentric exercises.

Findings: This study showed that thigh Thigh circumference increased following eccentric exercise in the placebo group, while it did not change significantly in the experimental group ($p>0.05$). There was no significant difference between the two study groups regarding other variables ($P>0.05$). Vitamin D3 supplementation with the dosage and duration used in the present study reduced the limited symptoms of DOMS. Future studies of longer duration or higher dosage of vitamin D supplementation are recommended.

Conclusions: According to the finding of this study, vitamin D3 supplementation could not reduce the limited symptoms significantly. However, future studies are suggested to evaluate the effect of higher dose or longer duration of vitamin D supplementation on DOMS

Keywords: Delayed Onset of Muscle Soreness, Step Test, Vitamin D3.

Introduction

Any type of physical activity that causes unaccustomed and high loads on muscles may lead to Delayed-Onset Muscle Soreness (DOMS) [1]. Delayed- Onset Muscle Soreness occurs 8 to 24 hours after strenuous exercise and reaches its maximum level at 24 to 48 hours after exercises [2]. Amateur and professional athletes are concerned about muscular discomfort and pain phenomena because it can limit further exercise and training activity [3].

Exhaustive eccentric exercise is usually accompanied by inducing DOMS. During an eccentric contraction, the muscles must be contracted while stretched concomitantly. For instances, eccentric muscular contractions in downhill running, hopping, plyometric exercising, squatting, and the lowering phase of lifting weights occurred frequently and makes muscles prone to DOMS [2].

The mechanisms underlying the cause of DOMS are not fully understood; however, it is generally accepted that DOMS is associated with muscle and/or connective tissue damage and/or subsequent inflammatory responses [4]. The muscle microscopic injury is induced by a mechanical disruption to sarcomeres, swelling results from the movement of immune cells and fluid from the bloodstream into the interstitial

spaces which are accompanied by inflammation and pain [5]. Following the muscle injury, enzymatic reactions, and inflammatory mediators such as thromboxanes, prostaglandins, and leukotrienes from the cyclooxygenase and lipoxygenase pathways increase which is associated with enhancing vascular permeability. Pain perception can increase by stimulating type III and IV afferent nerve fibers to both chemical and mechanical stimuli [2, 6].

Multiple preventive or treatment methods and strategies have been introduced to alleviate the severity of DOMS and to restore the maximal function of the muscles as rapidly as possible. Some interventions like cryotherapy [7] had some positive effects on muscle soreness or other DOMS symptoms while some other methods like stretching [8, 9], demonstrated no effect on the alleviation of DOMS. Massage [10], vibration [11] ultrasound and electrical current modalities [4, 12] have shown controversial effects. In addition, exercises are among the most effective means to alleviate pain in DOMS; however, the analgesic effect is temporary [13]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have demonstrated dosage-dependent effects that may also be influenced by the time of administration [14]. Apart from medical drugs, various food supplementations are widely used including cratin [15], anti-oxidants [16, 17] ,and protein or carbohydrate beverages [18] which were found to have some positive effects as well. Recently, vitamin D was shown to potentially improve athletic performance and recovery, myopathy and fatigue in vitamin D-deficient athletes [19-21]. It may improve muscle strength through a highly specific nuclear receptor in muscle tissues [19]. While in healthy endurance-trained runners the inverse association between 25(OH)D and TNF-a concentrations have been shown [22]. One study reported no significant changes in TNF-a and IL-6 levels in a high dose of vitamin D supplementation following a 12-week progressive resistance exercise training [23]. However, the physiological effects of vitamin D on DOMS in vivo has not been proved yet.

Therefore, we hypothesized that vitamin D3 could be beneficial for preventing skeletal muscles damages from high-intensity exercise. Because the modulation of inflammation process via the MAPK-NF-jB signaling involved the vitamin D receptor and also there is strong evidence that Vitamin D3 has anti-inflammatory effects [2], however, little is known about the effects of Vitamin D3 on DOMS.

The purpose of this study was to investigate the effect of two weeks of Vitamin D3 supplementation on some indices of DOMS including the Range of Motion (ROM) of the knee joint, and thigh circumference.

Methods and Materials

This is a semi-experimental and double-blinded study. Based on a call at Shiraz University, 35 students volunteered to participate in the research. Blood testing indicated that 29 of the volunteers were vitamin D deficient. However, because 4 students were excluded due to supplement, herbal, or drug use, 25 students were selected according to inclusion criteria. Of these students, 5 students were excluded (2 students for not interested in continuing the cooperation and 3 students for probable effective disease such as cold). Finally, the subjects of the study included 20 active men (20.09±1.22 years) with vitamin D deficiency who were selected according to inclusion and exclusion criteria.

Inclusion criteria were having no regular training, no other vitamin or herbal supplementation, no inflammatory drug use or muscular injection 10 days before and during the study. Exclusion criteria

were existing any pain, acute trauma or inflammation, bleeding disorders, infection, severe ischemia or poor thermal regulation, diabetes, and immune system disorders. Subjects were randomly assigned to two experimental (E) and placebo (P) group swhich experimental group (n= 11) received vitamin D3 tablets and the placebo group (n=9) received a matched placebo capsule (Fig. 1). All subjects were informed of the experimental procedures and signed written consent form. The experimental protocol was approved by the local ethics committee of Shiraz University. The study was conducted according to the Declaration of Helsinki to research in humans.

Vitamin D_3 supplementation

The subjects in the experimental and placebo groups were asked to take one supplementary capsule per week, for a 2- week period. The experimental group took vitamin D3 (Cholecalciferol), containing 50000 IU. The placebo group took a matched placebo capsule. Supplements were provided by Daana Pharma Company, Iran. The study was double-blinded.

Eccentric exercise Protocol

Step test was used as an eccentric exercise to produce muscle soreness. Subjects performed a 20 minutes of bench stepping exercise containing climbing up and down on bench with the height of 46 cm and 3.5-meter length. The protocol was done in 4 sets of a 5-minute exercise with 1-minute rest be-

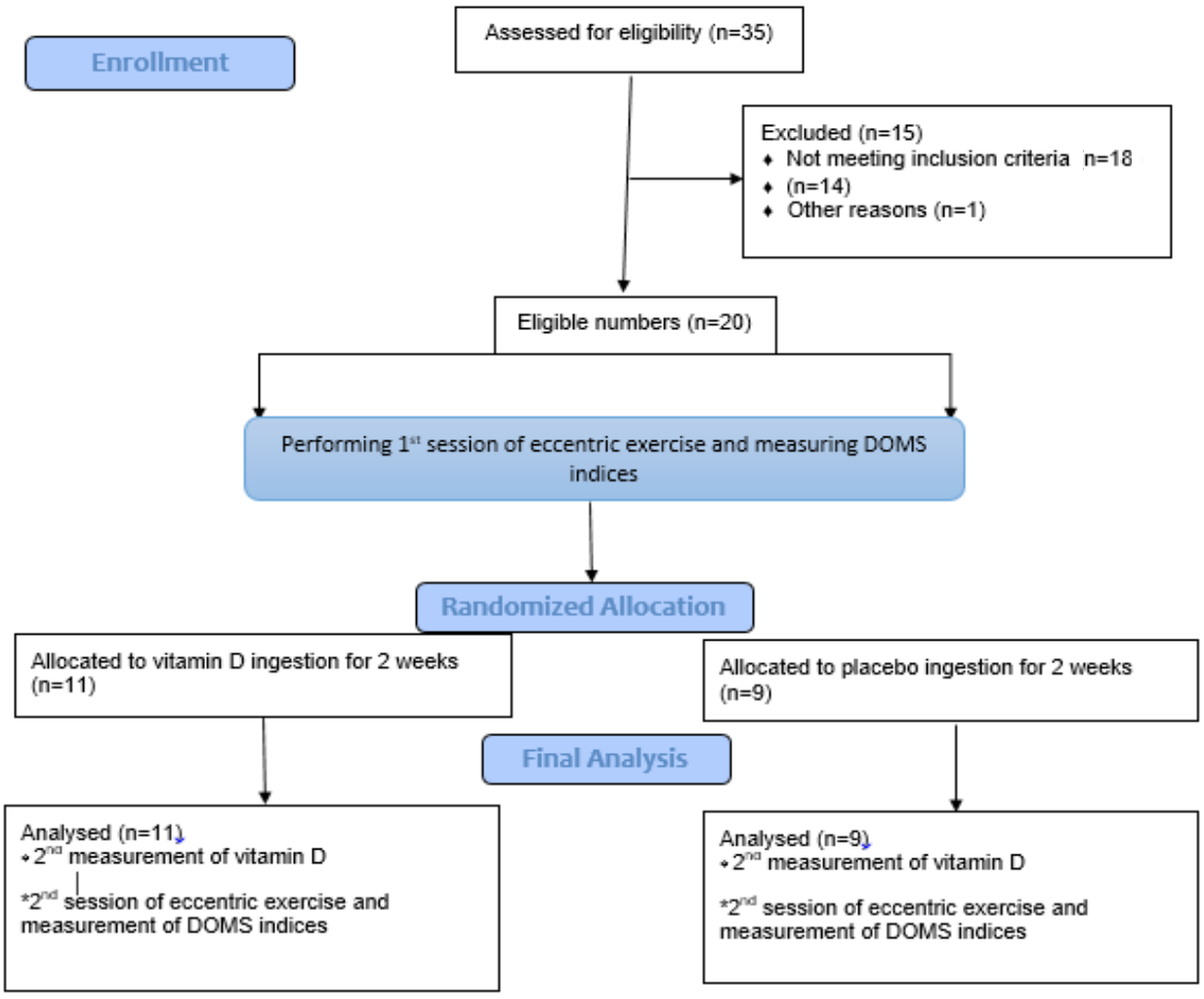

Figure 1) A summary of study process

tween each set (passive recovery). The protocol was performed at the rate of 15 steps per minute which was regulated by metronome (a mobile application). Subjects began using the right leg as the leading leg and changed the leading leg every 10 minutes. The eccentric protocol was done a week before and after the period of vitamin D or placebo ingestion period.

Measurements

Vitamin D deficiency test was done pretest and after 2 weeks in posttest to measure any changes of total serum 25-hydroxyvitamin D [25(OH)D] and below 24 ng/mL was considered as deficiency level. For vitamin D measurement, blood samples were obtained and centrifuged immediately to separate the serum portion and were analyzed either within 30 min of collection. HPLC method was used for Vitamin D3 measurement. Functional factors of DOMS, perceived pain, thigh circumference, and knee ROM, were also measured before, immediately (time 0), 24 hours (time 1), and 48 hours (time 2) after the eccentric exercise protocol. Every measurement was performed before and after the intervention (vitamin D or placebo consumption) in both groups.

Perceived Pain

Muscle soreness was evaluated during stretching the hamstring and quadriceps muscles in a standing position. Each subject was asked to evaluate his perceived pain level via a Visual Analogue Scale (VAS) which was a 10 cm line that was scaled from 0 to 10. In this scale, score 0 exhibited no pain, and the score 10 indicated the maximal perceived pain.

Thigh circumference

Thigh circumference was measured using a flexible tape in the middle of the femur (largest) while standing, and the leg did not support the weight. The same site was signed and measured before and after exercise. The test was performed 3 times for each leg and the average number was recorded.

Knee range of motion (ROM)

The knee active flexion ROM was determined by using a handheld goniometer. The center of the goniometer was placed on the lateral epicondyle (joint center of rotation) and the goniometer's movable limbs were lined along the femur and tibia alignments. Subjects were instructed to flex their knee as much as possible while they were sitting on a table and knee was in 90-degree of flexion. Measurements were done 3 times and the average was used for further evaluations [24].

Data were analyzed using SPSS software (version 16). Descriptive statistics including means and standard deviation (SD) calculated for each parameter. Shapiro-Wilk test was used to test the data normal distribution. Regarding the normal distribution findings, the parametric test of Mixed ANOVA was used for between and within-group comparisons and a t-test was used for between-group comparisons. Repeated measure test was used for within-group comparisons. In the case of non-normal distribution (VAS scales), non-parametric tests of Mann-Whitney U was used to compare two groups. The level of significance was set as $P \leq 0.05$.

Findings

Descriptive data of the two groups are shown in Table 1. There were no significant differences in age, height, and weight between the two groups ($P > 0.05$). Between-group comparisons showed no significant difference in basal variables of vitamin D in experimental and control groups. Blood vitamin D level increased significantly compared to the control group after two weeks of vitamin D supplementation ($P<0.05$). There was no significant differ-

Table 1) Descriptive characteristics of the studied participants

Variable Group	Age (y) M(SD)	Weight (Kg) M(SD)	Height (cm) M(SD)	Vitamin D M(SD)
Experimental (n=11)	20.09(1.22)	71.18(13.86)	173.81(8.14)	15.33 (2.34)
Placebo (n=9)	19.33(1.32)	68.38(12.99)	175.11(6.60)	19.88(1.87)

*Mean (Standard Deviation)

ance in study variables before the interventions between the study groups (vitamin D supplementation or placebo group).

Range of motion (ROM)

Findings of mixed ANOVA indicated that after vitamin D3 intake, there was no significant difference in right knee ROM (P= 0.313), but the left knee showed significant differences (P=0.020). In experimental group, within-group comparison indicated significant differences (P= 0.006, F (3,30) =5.005, Λ= 0.522), that these differences existed between pre protocol data with time 0, (P=0.018), time 1 (P= 0.020) and time 2 (0.031) data. In the placebo group no within-group differences was seen (P= 0.286, F (3, 24) =1.338, Λ=0.618). However, t-test results for between-group comparisons indicated that this difference were only significant in pre-exercise test (p= 0.015, t (18) = 2.674) (figure 2). In other words, vitamin D supplementation increased the left knee range of motion before eccentric exercise compared to placebo group, while following eccentric exercise the range of motion reduced and there was no significant difference was found between the experimental and placebo groups.

Thigh Circumference

As it is shown in Figure 3 no significant difference was found between the study groups regarding right or left thigh circumference for each measurement before eccentric exercise, time 0, time 1, and time 2 after two weeks of vitamin D or placebo consumption (P > 0.05). Moreover, within group differences in the right thigh of the experimental group were not significant (P = 0.208, F (3, 24) = 1.737, Λ= 0.524) but in the right thigh of placebo group significantly changed over time (P = 0.000, F (3, 24) = 15.124, Λ= 0.104). The significant increase of thigh circumference was found in time 1 (p= 0.001) and time 2 (P= 0.001) compared to before exercise. Furthermore, differences between time 0 and time 1 (p=0.040) and time 2 (p= 0.022) were also significant. Within-group comparison in the left thigh showed no significant differences (P > 0.05). In other words, in vitamin D supplementation, the thigh circumference (as an index of inflammation) did not change over time following eccentric exercise, while in the placebo group, the right thigh circumference increased significantly over time.

Findings of mixed ANOVA indicated that after vitamin D3 intake, there was no significant difference in right knee ROM (p= 0.313), but the left knee showed significant differences (p=0.020). In experimental group, within-group comparison indicated significant differences (P= 0.006, F (3,30) =5.005, Λ= 0.522). These differences existed between pre protocol data with time 0, (P=0.018), time 1 (P= 0.020) and time 2 (0.031) data. In the placebo group no within-group differences were seen (P= 0.286, F (3, 24) =1.338, Λ=0.618). However, t-test results for between-group comparisons indicated that these differences were only significant in pre-exercise test (p= 0.015, t (18) = 2.674). In other words, vitamin D supplementation

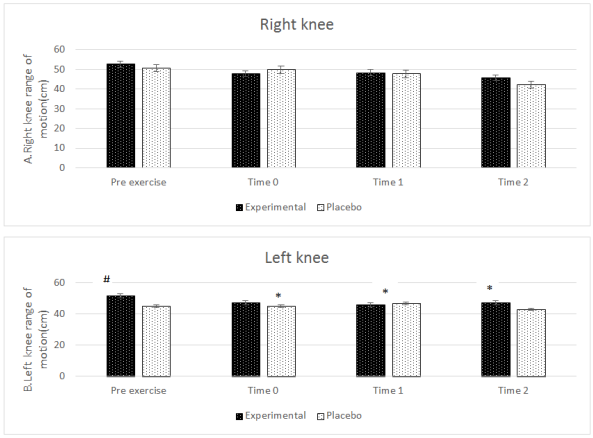

Figure 2) ROM over time in right (A) and left (B) knees. Comparison of experimental and placebo groups. *: significantly different from pre exercise, P≤ 0.05, # : significantly different compared to placebo group, P≤ 0.05.

increased the left knee range of motion before eccentric exercise compared to the placebo group while following eccentric exercise the range of motion reduced and there was no significant difference was found between the experimental and placebo groups.

Perceived pain (VAS scale)

Considering non-normal distribution of findings, Mann-Whitney U test was used for between group comparisons. The

Figure 3) Left (A) and right (B) thigh circumferences over time. Comparison of experimental and placebo groups,*: significantly different than pre, P≤ 0.05.

findings indicated that no significant difference was observed in perceived pain between groups before protocol, time 0, time 1 and time 2 after supplementation (P > 0.05)(figure 4).

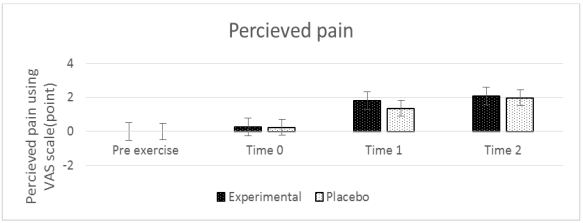

Figure 4) Left (A) and right (B) thigh circumferences over time. Comparison of experimental and placebo groups,*: significantly different than pre, P≤ 0.05.

Discussion

Results of this study indicated that vitamin D supplementation increased the left knee range of motion before eccentric exercise compared to the placebo group while following eccentric exercise the range of motion was reduced and no significant difference was found between the experimental and placebo groups. No study was found regarding the effect of vitamin D on the range of motion. However, considering the other supplements, the finding of the present study was similar to findings of Goldfarb et al (2006), Lenn et all (2002), Ston et al (2009), Tokmakidis et al (2003), and Barlas (2000) [25-29] who showed no improvement in ROM after supplementation. However, Tatibian et al (2009), found a positive effect of omega-3 on improving ROM 48 hours after exercises. The application of omega-3 for supplementation instead of vitamin D3 may be the cause of this difference. as well as Memarbashi study (2013) who used Cinnamon supplementation showed an increase in ROM in 48 and 72 hours after exercise [30]. Tanabe (2018) compared the effects of Curcumin supplementation and 7 days before and after exercise and showed that before exercise supplementation could reduce inflammation while after exercise supplementation improved the ROM and decreased muscle soreness [31]. Therefore it may be time of supplementation play a role in positive effects of supplementation and if we used the supplementation after exercise

more effects might have been detected. Decreasing ROM induced by DOMS can be considered as an indicator of muscle stiffness that possibly happens due to inflammatory process after severe exercises [28, 32, 33]. Thus, according to our findings it seems that vitamin D3 supplementation could not prevent decreasing ROM or muscle damage in DOMS which is induced by exercise.

Moreover, in vitamin D supplementation, the thigh circumference did not change over time following eccentric exercise, while in the placebo group, the right thigh circumference increased significantly over time. Thus, it can be concluded that vitamin D especially the form of 25(OH)D has ameliorating effects on exercise-induced inflammatory process and neurological function [34, 35]. Limb circumference is an indicator of muscle swelling or inflammation after exercise [33, 36]. This result is partly similar to Tanabe (2018) while they used Curcumin supplementation [31]. Furthermore, Tartibian et al. showed a significant decrease of 24 and 48 hours after exercise following omega-3 supplementation, however, it could not prevent inflammation immediately after eccentric exercise. Similarly, Jouris (2011), Tartibian (2011), Lemberk (2014) indicated the anti-inflammatory effects of omega3 or fish oil supplementation [37-40]. On the other hand, some other researchers found no effects of vitamin D2 [41] on muscle damage, ibuprofen [42] ,and fish oil [26, 43] on inflammation.

Vitamin D3 is known to have anti-inflammatory effects. Although, there is not much research on the effect of vitamin D3 on humans but some researches on rats have shown the positive effects of vitamin D3 on muscle damages during intensive exercise [44, 45]. Thereby, regarding our results which indicated and increased range of motion in the vitamin D supplemented group before exercise, it is likely that vitamin D3 supplementation could attenuate the inflammatory process and decrease the oxidative damage and increase flexibility. However, regarding the non-significant effect of vitamin D supplementation following exercise it seems that the dosage or duration of supplementation was not enough to be effective. As can be seen in the results of blood vitamin D, although the amount of vitamin D had increased, it had not yet reached sufficient levels. There was no significant effect of vitamin D supplementation that may be another indicator but it is not sufficient to induce a significant effect.

Conclusion

According to the finding of this study, vitamin D3 supplementation with the method used in the present study reduced the limited symptoms, but this effect was not significant. However, future studies are suggested to evaluate the effect of higher dose or longer duration of vitamin D supplementation on DOMS. Findings of the present study revealed that short duration of vitamin D supplementation without normalizing serum vitamin D cannot reduce most of the muscle soreness indices following a session of eccentric exercise. Short-term vitamin D intake reduce limited symptoms of the delayed onset muscle soreness following eccentric exercise in active boys with vitamin D deficiency.

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Authors' contribution

All the authors contributed in the study design, AAM performed the study. NK provided the original manuscript. MKJ contributed in the study data analysis. MKJ and NK provided the original manuscript MH contributed in the study design. All

authors reviewed and confirmed the final manuscript.

Confilict of Interest

There was no conflicts of interest to be declared.

Ethical Permission: Graduate commitee of Shiraz University approved the study proposal and procedures.

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