Elastic resistance training is more effective than vitamin D3 supplementation in reducing oxidative stress and strengthen antioxidant enzymes in healthy men

Fardin Kalvandi1, Mohammad Ali Azarbayjani2, Ronak Azizbeigi3 and Kamal Azizbeigi4,5

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INTRODUCTION

Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are generated in all cells by mitochondrial and enzymatic sources [1]. In aerobic organisms in a resting state, incomplete reduction of mitochondria oxygen can lead to ROS [2] and left unchecked, these reactive species can cause oxidative damage to DNA, proteins, and membrane lipids [1]. Also, ROS can cause cell necrosis by cell membrane lipid peroxidation, the oxidation of carboxylated proteins, the decrease of glutathione content, and suppress the antioxidant enzyme such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC). In aerobic organisms, ROS, such as superoxide and hydrogen peroxide, are generated in all cells by mitochondrial and enzymatic sources [1]. In aerobic organisms in a resting state, incomplete reduction of mitochondria oxygen can lead to ROS [2] and left unchecked, these reactive species can cause oxidative damage to DNA, proteins, and membrane lipids [1]. Also, ROS can cause cell necrosis by cell membrane lipid peroxidation, the oxidation of carboxylated proteins, the decrease of glutathione content, and suppress the antioxidant enzyme such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC), and all of these finally lead to low cellular lifespan [3].

SOD catalyzes the dismutation of superoxide anion free radical (O2−) into molecular oxygen and hydrogen peroxide (H2O2) and decreases O2− a level that damages the cells at excessive concentration [4], while GPX reduces H2O2 to water to limit its harmful effects [5]. Both of these mechanisms will reduce oxidative stress and Malondialdehyde (MDA) concentration as a lipid peroxidation factor. Oxidative stress is associated with the ageing process and ageing-related functional disorders, and it has been reported to cause many diseases including cardiovascular disease (CVD), diabetes, chronic obstructive pulmonary disease (COPD), chronic kidney disease, cognitive impairment, and dementia, cancer, and sarcopenia [6].

Various approaches purpose to strengthen the antioxidant system and reduce oxidative stress, and many studies have examined the effect of the different types of exercise training such as aerobic training (AT), which increases antioxidant capacity and attenuates oxidative stress [7, 8]. We previously showed that AT at load progressive mode could decrease oxidative stress and Malondialdehyde (MDA) concentration as a lipid peroxidation factor. Oxidative stress is associated with the ageing process and ageing-related functional disorders, and it has been reported to cause many diseases including cardiovascular disease (CVD), diabetes, chronic obstructive pulmonary disease (COPD), chronic kidney disease, cognitive impairment, and dementia, cancer, and sarcopenia [6].

OBJECTIVE: Today, reducing oxidative stress and improving the antioxidant system with antioxidant supplements along with exercise training has received a lot of attention. Vitamin D plays a very important role in general health and reducing oxidative stress. The aim of this study was to examine the effect of vitamin D3 supplements during elastic-band resistance training (EBT) on oxidative stress and antioxidant indices in healthy men.

METHODS: Forty healthy men (Serum: 20 ≤ 25 (OH) D ≤ 25 ng/mL) voluntarily participated in the current study and randomly were assigned to EBT-vitamin D3 (ED, n = 10), EBT-placebo (EP, n = 10), vitamin D3 (VD, n = 10), and control (Con, n = 10). EBT was performed three times per week on non-consecutive days for eight weeks, in seven exercises. The subjects in the ED, VD, and EP consumed 50,000 IU vitamin D3 or placebo once every 2 weeks. Ten ccs blood samples were collected before and after exercise training and the total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPX), and creatine kinase (CK) activities were measured in the plasma. Malondialdehyde (MDA), as the lipid peroxidation index, and 25(OH) D were measured in the plasma.

RESULTS: We found that there was a significant difference between ED with VD (p = 0.011) and Con (p = 0.022) for MDA. A significant difference was also seen for SOD in ED with VD (p = 0.024) and Con (p = 0.038) and TAC in ED with VD (p = 0.020) and Con (p = 0.030), and GPX in ED with VD (p = 0.040) and Con (p = 0.010). While there were no significant differences between ED and EP in all mentioned variables (p > 0.05).

CONCLUSION: Finally, it can be said that elastic resistance training improved antioxidant defence. However, vitamin D3 supplementation during resistance elastic training has no synergistic effect on attenuating oxidative stress indices.

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1Department of Physical Education and Sport Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran. 2Department Exercise Physiology, Tehran Central Branch, Islamic Azad University, Tehran, Iran. 3Department of Basic Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran. 4Department of Exercise Physiology, Tehran Central Branch, Islamic Azad University, Tehran, Iran. 5Email: kazizbeigi@gmail.com

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other hand, conventional RT with barbell and dumbbell and also RT machines are expensive. Access to machines requires both facilities and financial resources. Besides these disadvantages, RT with conventional machines and free weight may pose a higher risk of injury or overuse, especially to older people [12]. Also, in some special conditions, such as the COVID-19 pandemic situation, individuals may not be able to attend clubs and public places to use these machines. For this reason, the need to replace RT with weight machines bulk with other less costly, simple training methods is another type of exercise called elastic RT [13]. Elastic resistance devices such as elastic bands are being increasingly used for muscular conditioning for different aims and population types; they are more affordable and more accessible than weight machines [14, 15]. It has been shown that elastic RT is able to promote similar strength gains to conventional RT, in different population profiles and using diverse protocols [16].

Furthermore, research shows that antioxidant supplements with exercise training serve to increase the effects of synergies of exercise training on the antioxidant capacity system and attenuate oxidative stress [17]. Vitamin D is a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects such as antioxidant and anti-inflammatory [18]. Although Vitamin D is a membrane antioxidant with inhibited lipid peroxidation, a few studies were conducted on the effect of exercise training with vitamin D3 supplementation on oxidative stress and antioxidant capacity.

Therefore, the present study was carried out with the aim of investigating the effect of vitamin D3 supplementation during elastic-band resistance training (EBT) on enzyme antioxidant activities and oxidative stress in healthy males.

MATERIALS AND METHODS

Subjects

Forty healthy males with no experience of RT at least in the one year before the current study volunteered to participate in the study and were randomly assigned to EBT-vitamin D3 (ED; n = 10), EBT-placebo (EP; n = 10), vitamin D (VD; n = 10), and control (Con; n = 10) using the online software randomisation [19]. According to the feature of the present study and p ≤ 0.05 as the significance level, also based on the results of previous studies (standard deviation, sample size of similar studies with similar aims and method), 10 subjects was considered as the sample size in each group. The subjects were asked to refrain from resistance and cardiovascula training during the study. Also, we asked them to minimise recreational physical activity such as swimming, climbing and walking. The participants revealed their medical history, obtained written approval from their physicians, and signed an informed consent form to participate in the study. None of the subjects was taking exogenous anabolic-androgenic steroids, drugs, medication, or dietary supplements with potential redox or inflammatory responses. To reduce the interference of sunlight in the results, the study was done in December, January, and February (2016–2017). The investigation was approved by the committee on the use of human research subjects at the Islamic Azad University of Sanandaj Branch. The subjects attended an information and familiarisation session in which all details of the research procedures were explained. A flow chart of subject enrolment and allocation to the study was presented in Fig. 1.

Primary assessment

In a session before any intervention, 10 ccs peripheral blood was gathered from the antecubital vein of the left arm in a seated position after 5 min of rest. After that, in the second session, the anthropometric characteristics such as the subjects’ height and weight (Seca, Mod 220, Germany) were measured according to the current instruction. The body fat percentage (BF%) was estimated by Lange skinfold caliper (Beta Technology Incorporated, Cambridge, Massachusetts, USA) in three parts—chest, abdomen, and thigh in standing position—using the equation of Jackson and Pollock [20], and was repeated after the exercise training period.

Then, in order to assess the effect of the elastic-band RT on improving strength and to evaluate the efficacy of the exercise training programme, functional capacity tests were done.

A push-up test was applied to access muscular endurance chest and shoulders. This test was done continuously without rest and asked the subjects to do more push up as many as possible. The squat test (without loading) was also used to monitor the subjects’ development of lower extremity function. The test requires the subjects to complete as many squats as possible with no rest. The subjects performed a specific warm-up for 10–15 min, with the push-up test always performed before the squat test, and with a minimum recovery period of 10 min between the 2 tests [21]. These tests were done before intervention (pretest) and were repeated after 8 weeks of elastic training (posttest). The descriptive and functional characteristics of the subjects are presented in Table 1.

Elastic resistance band training

Subjects were instructed in all exercise techniques by an expert technician. Then the subject was trained in the techniques used for each exercise and then, performed several sets at minimum resistance by an elastic band, and necessary feedback was given to the subjects (Thera-Band®, The Hygenic Corporation, OH, USA; colours—Tan) to ensure that the exercise was being performed correctly. The participants also learned how to control the intensity of the exercises through the scale OMNI-RES.

The elastic RT was performed three times a week on non-consecutive days for eight weeks. Each exercise session consisted of a general warm-up

![Fig. 1 Flow chart of subject enrollment, allocation, dropout, intervention, and final analysis, n = 4 excluded from the study because they were not eligible. n = 2 were excluded due to not meeting inclusion criteria. n = 1 was excluded because he did not unable to cooperate until the end of the study. Finally, 40 subjects were analyzed in the end of the study.](image)
of 10 min at the running fashion and dynamic stretching, and finally with 10–15 min of stretching exercises for a cool down at the end of the exercise session [22]. The movements involved were upper body and lower body exercises. The exercises included: Knee flexion (unilateral exercise performed in the prone position), Knee extension (unilateral exercise performed in the prone position), Squat (Standing status), The upper limb exercises consisted of bench flies (Standing status), Overhead press (Standing status), elbow flexion and extension (Standing status), Lat pulldown (Standing status), and Trunk Curl-up (performed in the prone position). All training sessions were performed under the supervision of a qualified and experienced instructor. The subjects were asked to perform exercises at the full range of motion at moderate to high velocity as possible [23]. To control the intensity of training, the OMNI-RES was always visible during each training session for all subjects. Following an adaptation phase of 2 weeks using low resistance (yellow Thera-Band®, 3 sets of 10–15 repetitions per exercise) exercise intensity was progressively increased by adapting the resistance of the elastic band (based on the Thera-Band® force-elongation table) [13], from yellow to green, silver, or even and further to gold colour. The rate of progression was based on individual improvements, band colour was changed if the active muscle scale (0 extremely easy to 10 extremely hard) [21]. It should be noted that 10–15 repetitions were maintained for the 8-week elastic RT, with three sets during the 8 weeks. The control group was asked to refrain from any RT and maintain their normal physical activity and nutrition behaviours for the study period. Exercise training details were present in Table 2.

**Table 2.** Exercise training protocol.

| Week | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|------|-----|-----|-----|-----|-----|-----|-----|-----|
| Session/week | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   |
| Sets      | 3   | 3   | 3   | 3   | 3   | 3   | 4   | 4   |
| Repetition (no. of reps) | 15  | 15  | 10  | 10  | 10  | 10  | 10  | 10  |
| OMNI-RES  | 1–7 | 8–7 | 8–9 | 7–9 |
| Rest interval between sets (s) | 60  | 60  | 90–120 | 90–120 | 90–120 | 90–120 | 90–120 |

OMNI-RES OMNI-resistance exercise scale.

**Table 3.** Nutrient analysis of the dietary records before and after the training and supplementation.

| Vitamin          | ED       | EP       | VD       | Control | P       |
|------------------|----------|----------|----------|---------|---------|
| Vitamin C (mg/day) | Pre 64.1 ± 23 | 61.4 ± 6.7 | 67.6 ± 9 | 72.6 ± 5.51 | 0.318 |
|                   | Post 73 ± 12 | 64.5 ± 8.8 | 74 ± 6.5 | 73.12 ± 7.3 | 0.229 |
| α-tocopherol (mg/day) | Pre 6.3 ± 1.1 | 5.1 ± 0.8 | 6.2 ± 0.9 | 5.89 ± 1.1 | 0.203 |
|                   | Post 6.6 ± 1.7 | 5.2 ± 0.9 | 6.5 ± 1.2 | 6.1 ± 1.2 | 0.06 |
| β-carotene (µg/day) | Pre 500.3 ± 393 | 615.2 ± 399 | 620.2 ± 391 | 625.8 ± 248 | 0.455 |
|                   | Post 560.3 ± 987.2 | 643 ± 410 | 640.9 ± 210 | 634 ± 238 | 0.116 |
| Selenium (mg/day) | Pre 61.7 ± 15.1 | 56.3 ± 9.2 | 53.1 ± 7.9 | 55.2 ± 7.7 | 0.118 |
|                   | Post 62.9 ± 13 | 55.3 ± 8.3 | 54.1 ± 9.9 | 54.7 ± 6.1 | 0.146 |
| Vitamin D (µg/day) | Pre 5.2 ± 6.4 | 5.9 ± 5.8 | 5.2 ± 4.9 | 5.6 ± 5.5 | 0.512 |
|                   | Post 5.4 ± 5.8 | 5.5 ± 5.1 | 5.8 ± 5.2 | 5.9 ± 6 | 0.724 |

Data expressed as Mean ± SD.

Blood sampling and biochemical assays

Before any intervention, a 10 ccs blood sample was obtained from the antecubital vein of the left hand. The blood sampling was repeated 72 h after the last session of the training. The blood samples were centrifuged at 2000–2500 rpm for 10 min and plasma and the surface layer were separated from haematocrits. The plasma samples were used for the measurement of TAC, MDA, SOD, and GPX and Creatine Kinase (CK), and 25(OH)D.

The SOD and GPX activity was measured by using a commercially available kit (Randox Laboratories Ltd., Crumlin, Country Antrim, UK), and were expressed in U/L. Similarly, a commercially available kit (Cat. No. NX 2332, Randox, Crumlin, UK) was used for the measurement of the antioxidant capacity in the plasma (TAC). The plasma MDA was measured based on the method of Buege and Aust [24]. The plasma CK activity was tested using a standard kit (Pars Azmoon, Iran) and enzyme activity was expressed as U/L.

**Statistical analyses**

All values were reported as mean ± SD. First, the normal distribution of data was confirmed with the Kolmogorov–Smirnov test. The homogeneity of the physical characteristics of the subjects at the beginning of the study was tested with one-way ANOVA. Also, one-way ANOVA was used to compare the results of the dietary survey. The data regarding the biochemical parameters were compared using repeated measures (pre- and post-training) ANOVA to investigate the influence of time and the training intervention. Where a significant main effect and/or interaction was observed, the Tukey post hoc test was applied to determine the source of significance. Partial eta-squared (ηp2) was used to determine the effect size of training, vitamin D3 and training, vitamin D3 interaction.
RESULTS

The results showed no significant difference in total antioxidants between the four groups \((p > 0.05)\). The antioxidant analyses of the dietary records of the subjects before and after the elastic RT and supplementation period are presented in Table 3.

We found that elastic training increased push up tests in ED \([\text{pre: 18 vs. post 29 reps; } (p \leq 0.001)]\) and EP \([\text{pre: 17 vs. 27 reps; } (p \leq 0.001)]\) by 61% and 58.8%, respectively.

Also, the squat test was increased significantly in ED \([\text{pre: 27 vs 38 reps; } (p \leq 0.001)]\) and EP \([\text{pre: 26 vs 39 reps; } (p \leq 0.001)]\) by 40.7% and 50%, respectively. However, there is no significant difference between ED and EP for the mentioned variables. Also, push up and squat test changes in the VD and control groups was insignificant \((p > 0.05)\).

Body fat percentage (BF%) was significantly decreased in the ED \([-10.5%; \ p = 0.003]\), EP \([-10.4%; \ p = 0.001; \ (0.28)]\), and VD \([-26.2%; \ p = 0.024]\), while there was a significantly in the ED and EP than VD and Con \((p < 0.05)\).

The results showed that there is a significant difference in the main effect of elastic RT, and vitamin D3 supplementation for MDA \((p = 0.001, \ np^2 = 0.437)\), CK \((p = 0.005, \ np^2 = 0.334)\), SOD \((p = 0.049, \ np^2 = 0.112)\), GPX \((p = 0.001, \ np^2 = 0.125)\), and TAC \((p = 0.010, \ np^2 = 0.185)\) after training or supplementation.

We found that the MDA significantly decrease in the ED \((-43.98%; \ p = 0.001, \ d = 0.713)\) and EP \((-37.75%; \ p = 0.001, \ d = 0.701)\). The SOD \((13.71%, \ p = 0.023, \ d = 0.454)\), GPX \((44%, \ p = 0.002, \ d = 0.654)\), and TAC \((28.91%, \ p = 0.022, \ d = 0.501)\) significantly increased in ED in the post-test. Also, in the EP, SOD \((p = 0.003, \ d = 0.622)\), GPX \((p = 0.002, \ d = 0.379)\) and TAC \((p = 0.034, \ d = 0.324)\) significantly increased by 15.89%, 30.24%, and 13.33%, respectively.

Also, the time x groups interaction was significant in MDA \((p = 0.006, \ np^2 = 0.313)\), SOD \((p = 0.007, \ np^2 = 0.299)\), GPX \((p = 0.001, \ np^2 = 0.113)\), and TAC \((p = 0.048, \ np^2 = 0.211)\). We found that there were significant differences between ED and VD \((p = 0.040)\), and ED with Con \((p = 0.019)\) for MDA.

Furthermore, there is a significant difference in SOD \([\text{ED with VD } (p = 0.003), \text{ and ED with Con } (p = 0.007)]; \text{ EP with VD } (p = 0.023)]; \text{ EP with VD } (p = 0.028), \text{ and ED with Con } (p = 0.040)], \text{ and GPX } [\text{ED with VD } (p = 0.006), \text{ and ED with Con } (p = 0.003); \text{ EP with VD } (p = 0.002), \text{ EP with Con } (p = 0.001)].\]

It was observed that supplementation with vitamin D3 increased the 25(OH) D in the plasma by \((123% \ p = 0.001, \ d = 0.969)\) and \((96.4% \ p = 0.001, \ d = 0.960)\) in the ED and VD, respectively.

There is a significant difference between groups \([\text{ED with EP and control } (p = 0.001), \text{ VD with Con } (p = 0.001)]\) see Table 4.

DISCUSSION

The aim of the present study was to investigate the effect of vitamin D3 supplementation during elastic training, as a simple and inexpensive training method, on MDA and antioxidant enzymes in healthy men.

Overall, we found that although vitamin D3 supplementation had a positive effect in attenuating oxidative stress, the effect of elastic training is greater and more effective in this issue.

The results showed that the push-up and squat tests improved significantly in ED and EP. It implies the elastic RT programme was effective in functional and physiological features. Ageing, inactivity, and disuse are associated with a decline in muscle mass, structure, and strength [25]. It has been reported that elastic RT is able to promote similar strength gains to conventional RT, in different population profiles and using diverse protocols [16]. Therefore, it seems that elastic RT could be used as a rehabilitation strategy in the elderly and clinical conditions.

The prevalence of obesity has increased worldwide reaching pandemic levels [26]. We found that body fat percent (BF%) was affected by elastic training. On the other hand; adipose tissue may increase oxidative stress [27]. Therefore, BF% decrement may have had a positive effect on oxidative stress and antioxidant variables behaviour in the current study. Thus, today elastic RT could be used as an inexpensive and simple approach to reduce body fat and body weight management.

The results showed that SOD and GPX activity increased significantly in ED and EP compared to the VD and the control group. It can be said that the influence of elastic training was greater and more crucial in increasing the antioxidant enzyme activities. It is known that exercise-induced adaptation of antioxidants is dependent on several variables, which include tissue, age, duration, intensity, and specific exercise mode [28].

### Table 4. Changes variable before and after training and supplementation.

| Variables       | ED       | EP       | VD       | Control | Time x group \((p)\) |
|-----------------|----------|----------|----------|---------|----------------------|
| MDA (nmol/ml)   | Pre: 1.91 ± 0.53 | 2.04 ± 0.45 | 1.96 ± 0.6 | 1.92 ± 0.52 | 0.004 |
|                 | Post: 1.07 ± 0.30 | 1.27 ± 0.36 | 1.75 ± 0.54 | 1.90 ± 0.46 |         |
| SOD (U/l)       | Pre: 188.2 ± 25.3 | 180.6 ± 20.5 | 176.6 ± 24.5 | 188.7 ± 17.3 | 0.001 |
|                 | Post: 214 ± 21.4 | 209.3 ± 17.3 | 179.2 ± 22.6 | 171.5 ± 19.9 |         |
| TAC (mmol/l)    | Pre: 1.28 ± 0.15 | 1.35 ± 0.24 | 1.26 ± 0.17 | 1.32 ± 0.17 | 0.02 |
|                 | Post: 1.65 ± 0.38 | 1.53 ± 0.19 | 1.37 ± 0.25 | 1.28 ± 0.15 |         |
| GPX(U/l)        | Pre: 4299.1 ± 1017.9 | 4585.2 ± 1151.1 | 4225.9 ± 889.1 | 4291.2 ± 1854.1 | 0.001 |
|                 | Post: 6194 ± 1472.4 | 5971.8 ± 1070 | 4404.6 ± 926.9 | 4209.5 ± 588.4 |         |
| CK(U/l)         | Pre: 119.1 ± 14.9 | 122 ± 25.1 | 121 ± 25.1 | 123.8 ± 19.4 | 0.7 |
|                 | Post: 135.8 ± 33.5 | 137.16 ± 13.2 | 123.1 ± 24.6 | 125.9 ± 11.1 |         |
| 25(OH)D (ng/mL) | Pre: 20.4 ± 4.5 | 21.2 ± 4.5 | 22.5 ± 3.4 | 21.2 ± 4.9 | 0.001 |
|                 | Post: 45.51 ± 6.9 | 22.4 ± 7.1 | 44.2 ± 9.4 | 22.7 ± 6.1 |         |

The data are presented as Mean ± SD. MDA malondialdehyde, SOD superoxide dismutase, TAC total antioxidant capacity, GPX glutathione peroxidase, CK creatine kinase, 25(OH)D 25-hydroxy vitamin D.
In the present study, we used low to moderate intensity, while most studies have investigated the effects of moderate to high-intensity RT [9, 29]. It seems that the present protocol of elastic RT with low intensity could challenge the physiological haemostasis to increase antioxidant activity such as SOD and GPX because it has been reported that resistance exercise at 30% 1RM (similar intensity to the present study) can increase free radical generation to stimulate antioxidant adaptation [30].

Also, we found that the combination of vitamin D3 with elastic training has low effects of synergies. It has been reported that training has low effects of synergies. It has been reported that RT could decrease MDA concentration in the present study, it has been reported that RT could decrease MDA concentration following RT [29]. It seems that TAC, SOD, and GPX improvement could decrease MDA concentration in the present study. However, vitamin D3 supplementation could not affect lipid peroxidation. Recently, consuming antioxidants such as vitamin D have increased due to reports suggesting that vitamin D deficiency exists in some athletes, particularly those who participate in indoor sports such as bodybuilding [36]. However, we found that vitamin D3 supplementation had no effect on oxidative stress markers. Our subjects were healthy men with normal body fat percentages. It seems that oxidative stress and existing free radicals may interfere in this issue because oxidative stress results in the oxidative catabolism of 25 (OH) D [37]. On the other hand, low vitamin D3 dose may affect present results. We used 50,000 IU vitamin D3 for each subject, once every two weeks, which means 3600 IU vitamin D3 per day, approximately. Before starting the supplementation, we found that level 25 (OH) D basal was low and it reached 45 ng/mL after the supplementation. Some authors recommend much higher initial dosages if 25(OH)D levels are deficient, ranging from 2000 to 200,000 IU to maintain optimal 25(OH)D status [37]. Also, the consumption instruction of vitamin D (daily vs. weekly) may interfere with the function of this vitamin in attenuating oxidative stress. It has been shown that a daily dose of the vitamin may be superior in raising 25(OH)D levels when compared to a weekly dose [38].

CONCLUSION
We found that vitamin D3 supplementation during resistance elastic training had no synergistic effect on attenuating oxidative stress indices. We observed that the effect of elastic RT was greater and more prominent in decreasing oxidative stress and in the improvement of the defence antioxidant system.

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FK designed the research and collected the data. KA designed the research, supervised the work, and wrote the article. MAA analysed data. RA contributed to the design and implementation of the research.

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ADDITIONAL INFORMATION
Correspondence and requests for materials should be addressed to Kamal Azizbeigi.

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