



Effect of Elastic Resistance Training and Vitamin D on Systemic Inflammation Indices in Untrained Men: A Clinical Trial

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ARTICLE INFO	ABSTRACT
<p>Article type: <i>Original Article</i></p> <p>Bullet points:</p> <ul style="list-style-type: none">• <i>Resistance training exercises reduces inflammation.</i>• <i>In spite of anti-inflammatory effect of vitamin D, adding it to resistance training does not have more benefits in reducing inflammation.</i> <p>Article history: Received: 10 Mar 2017 Accepted: 23 May 2017 Available online: 1 Oct 2017 CJNS 2017; 3 (11): 196-205 DOI: 10.29252/nirp.cjns.3.11.196</p>	<p>Background: Recent studies demonstrate the ability of vitamin D to reduce inflammation and oxidative stress.</p> <p>Objectives: The current study aimed at examining the effect of vitamin D supplementation on systemic inflammation markers during an eight-week elastic resistance training (ERT) on untrained men.</p> <p>Materials and Methods: Forty healthy males with no experience in resistance training were assigned to elastic training—vitamin D (ED, n=10), elastic resistance training—placebo (EP, n=10), vitamin D (VD, n=10), and control (Con, n=10) groups. ERT was performed with eight exercises three times a week on non-consecutive days for eight weeks. Also, the subjects in the ED, VD, and EP groups consumed 50,000 IU vitamin D or placebo once every two weeks. Blood samples were collected before and 72 hours after the last session of eight weeks of ERT for measuring concentration of tumor necrosis factor-alpha (TNF-alpha), Interleukin-6 (IL-6), and C reactive protein (CRP).</p> <p>Results: It is found that ED elicited a significant reduction in IL6 than VD ($p<0.05$) and control ($p<0.05$). Also, there is a significant difference between EP group and VD ($p<0.05$) and control ones ($p<0.05$). However, there is no significant difference between ED and EP in the variables mentioned ($p<0.05$). Also, it was observed that there was no statistical significant alteration in CRP concentration between pre- and post-training ($p>0.05$).</p> <p>Conclusion: Finally, we can claim that the combination of elastic resistance training with vitamin D did not have extra advantages in attenuation of systemic inflammatory indices when compared with the training alone.</p> <p>Keywords: Vitamin D; Resistance Training; Interleukin-6</p>
<p>*Corresponding author: Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran</p> <p>Email: hasanmatinhomae@gmail.com</p>	<p>➤ Please cite this paper as: Zobairy M, Matinhomae H, Hatamian H, Azizbeigi K, Azarbayjani M. Effect of Elastic Resistance Training and Vitamin D on Systemic Inflammation Indices in Untrained Men: A Clinical Trial. <i>Caspian J Neurol Sci</i> 2017; 3(11): 196-205.</p> <ol style="list-style-type: none">1. PhD Candidate in Exercise Physiology, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran2. Assistant Professor of Exercise Physiology, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran3. Neurology Department, Poursina Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran4. Assistant Professor of Exercise Physiology, Department of Physical Education and Sports Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran5. Professor of Exercise Physiology, Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Introduction

It is well known that exercise training with a well-balanced diet is an important factor in promoting health and increasing an athlete's performance. However, high-intensity or exhaustive exercise training may increase susceptibility to injuries, promote chronic fatigue and overtraining, partially due to the high synthesis of free radicals and oxidative stress (1). Free radicals quickly react with unsaturated fatty acid, proteins and other molecular structures and can cause cell necrosis and damage (2). Free radicals can activate the nuclear factor kappa B (NF- κ B), which can activate genes expression pro-inflammatory cytokine, such as TNF- α and IL-6 (3). Therefore, many studies have investigated the effects of exercise training, especially resistance training, on systemic inflammation indices adaptation (4). Recently, Hagstrom *et al.* demonstrated that resistance training (RT) has a beneficial effect on the natural killer cell and natural killer T-cell cell expression of TNF- α , indicating that resistance training may be beneficial in improving the inflammatory profile in breast cancer survivors(5). Azizbeigi *et al.* (2015) reported that moderate to high intensity resistance training did not affect IL-6 and TNF- α in healthy men (4). Also, Libardy *et al.* (2012) reported that endurance, resistance and concurrent training for 16 weeks did not affect the above mentioned variables (6). Therefore, some authors have used antioxidant supplement during resistance training to attenuate oxidative stress and inflammation (7, 8).

Vitamin D is a membrane antioxidant which inhibits iron-dependent liposomal lipid peroxidation (9). It has been reported that there is an association between inflammatory diseases and low serum 25-hydroxyvitamin D

(10). Recently, elastic resistance training is being increasingly used for muscular support; because it is more affordable and more accessible than weight machines (11). The additional advantage of exercise training with elastic resistance bands includes high acceptance among older adults, its simplicity, versatility, portability, minimal space requirements and the comparatively low costs (12). Despite many advantages of elastic resistance training, there is no study that has investigated elastic resistance training with vitamin D to attenuate inflammation. Therefore, the current study was carried out with the aim of investigating the effect of vitamin D supplement with elastic resistance training on systemic inflammation indices in untrained males.

Materials and Methods

Subjects

The research was of the quasi-experimental design type. Statistical population was Islamic Azad University (Sanadaj Branch) students in 2016-2017 year that they were 9500 people. Forty untrained males students with no experience in resistance training at least in a year before the study volunteered to participate in the study and randomly were assigned to elastic training with vitamin D (ED, n=10), elastic resistance training with placebo (EP, n=10), vitamin D (VD, n=10), and control (Con, n=10) groups. It should be mentioned that according to the feature of the research and the results of previous studies, the significance level of 0.05 was considered for the present study. Therefore, the number of subjects in each group was determined by the following formula six subjects. Although, 10 subjects were used to increase validity in each groups.

$$n = \left(\frac{Z^2 \cdot S^2}{d^2} \right)$$

Subjects were considered untrained based on the fact that none had participated in a resistance training program for the last 12 months. Inclusion criteria for participation in the present study were lack of smoking, cardiovascular, pulmonary, or neuromuscular diseases, and lower body musculoskeletal injury in the previous 6 months. Subjects were considered untrained based on the fact that none had participated in a resistance training program for the last 12 months. The subjects were asked to refrain from resistance and cardiovascular exercise training during the study except for the main sessions of the exercise training in the current research. Subjects completed a medical history, obtained written approval from a physician, and signed the informed consent form. The investigation was approved by the committee on the use of human research subjects at Kurdistan Medical University (KMU), all exercise training sessions and biochemical experiments were done in KMU. Subjects attended an information and familiarization session in which all detailed research procedures were explained. The criteria of exclusion were missing more than three sessions of exercise training, flu and Infectious diseases, and hormonal abnormalities. None of the subjects was taking exogenous anabolic-androgenic steroids, and medication or dietary supplements with the potential of redox and inflammatory responses.

Physiological measurements

Before the intervention period, all subjects under study visited the laboratory three days at baseline. In the first day, blood sample was taken (pretest). Also, subjects' height and

weight (Seca, Mod 220, Germany) were measured, and their body fat percentage (BF%) was estimated by measuring the skin fold thickness (Lafayette, Mod 01127, USA) in three parts of abdomen, suprailiac and triceps using the equation of Jackson and Pollock (1985) (13). Then, in order to assess the effect of the elastic training program on strength and efficacy of the training program, one repetition maximum (1RM) on bench press and leg press were measured by Heeger Sport equipment. Also, 1RM was measured at the end of the training, and was not done in the same days that subject had main training program sessions.

Subjects were required to perform 10 repetitions at 50% of 1RM estimated according to each participant's capacity. After 2–3 minutes, subsequent trial was performed for 1RM with progressively heavier weights until the 1RM was determined within three attempts, with 3–5 minutes of rest between trials.

Controlled diet and vitamin D supplementation

During the eight-week period, all subjects in the ED group consumed vitamin D capsules (50,000 IU, Sobhan, Iran). A capsule was taken every two weeks by ED, VD, and EP (500 mg sucrose, after breakfast) groups. Capsules (vitamin D and placebo) were identical in appearance. We asked the subjects to record the amount and type of food they consume during the day before and after supplementation and training. Diet was analyzed using Food Processor software with regard to antioxidant and macronutrient content.

Elastic resistance training

In familiarization and training sessions, the subjects were given instructions on eight

different exercises used in the program. Each subject was trained in the techniques used for each exercise and then performed several sets at minimum resistance to ensure that these were performed correctly. Elastic resistance training was performed three times a week on non-consecutive days for eight weeks. The

movements involved were upper body and lower body exercises: Chest press, later alpulldown, leg extension, flexion, biceps and triceps curl, squat, and sit-ups. The elastic resistance training protocol has been presented in table 1.

Table 1. Elastic training protocol during eight weeks

Week	1	2	3	4	5	6	7	8
Repetition	16	16	14	14	12	12	10	10
Sets	3	3	3	3	3	4	4	4
Rest interval between sets (Sec)	60	60	80	80	90	90	90	90

All training sessions were performed at the same time of the day (5–7 pm) and took place at the laboratory under the supervision of a qualified and experienced instructor.

Blood analysis

Before any intervention, a 10 ml blood sample was obtained from the antecubital vein of the left arm of each participant in a seated position after a five-minute rest. The blood sampling was repeated 72 hours after the last session of the elastic training. Firstly, 150 microliters of ethylene diamine tetra acetic acid (EDTA) (Merck, Germany) was added to the blood samples and the mixture was centrifuged at 2500-2700 rpm for 7-10 minutes. Then, plasma was separated from haematocrits. The plasma samples were used for the measurement of CRP, IL-6 and TNF-alpha. Concentrations of TNF- α and IL-6 were determined by an enzyme-linked immunosorbent assay (ELISA) according to the specifications of the manufacturer (Bender Med System). Cytokines are presented in values of picograms per milliliter ($\text{pg}\cdot\text{mL}^{-1}$). Also, CRP was measured in plasma and by ELISA. CRP is presented in milligram per liter ($\text{mg}\cdot\text{L}^{-1}$).

Statistical analyses

All values were reported as mean \pm SD. First, the normal distribution of all dependent variables was confirmed by the Kolmogorov-Smirnov test. The homogeneity of physical characteristics of subjects at the start of the study was tested with one-way ANOVA. Also, one-way ANOVA was used to compare results of the dietary survey. Data regarding biochemical parameters were compared using analysis of covariance (ANCOVA) followed by a Bonferroni multiple comparison test. Data analysis was performed by using SPSS for WINDOWS software, version 19. A $p\leq 0.05$ level of significance was used.

Results

Firstly, it should be mentioned that after of resistance training and supplementation and during research, none of the subjects left the research. All of the subjects regularly took vitamins D capsules. Therefore, the number of subjects remained unchanged.

Physical characteristics of subjects at the start of the study are presented in table 2.

Table 2. Physiological and functional variable before starting study

	ED	EP	VD	Control
Age(yr.)	21.4±1.1	21.55±2.65	22.8±2.96	22.3±2.5
Wight (Kg)	72.87±3.86	70.81±2.79	75.56±5.8	71.37±3.27
Height (cm)	172±3.77	178±3.67	178.11±5.5	176±6.68
BF (body fat) (%)	22.87±6.43	23.43±3.24	23.3±2.87	22.54±5.52
1RM Chest press (Kg)	32.5±5.2	30.77±5.93	32.41±4.97	31.47±7.2

ED: elastic training with vitamin D. EP: elastic resistance training with placebo. VD: vitamin D and control groups

The results showed that there is no significant difference in antioxidant micronutrients among groups measured by food processor software. Antioxidant analysis

results of the dietary records of all groups before and after the study period are presented in table 3.

Table 3. Antioxidants micronutrients analysis of the dietary records before and after the training period

Variables		ED	EP	VD	Control	F	P
Vitamin C (mg/day)	Pre	63.2±11	60.5±6.5	64.7±9	59.1±7.1	1.51	0.138
	post	74.1±15.8	65.6±8.6	71±7.8	60.1±8.8	2.12	0.119
α- tocopherol (mg/day)	post	5.3±2.1	4.1±0.9	5.2±1.1	4.9±1.5	1.94	0.113
	post	5.6±2	4.2±1	5.5±1.5	5.1±1.1	3.14	0.059
Vitamin A (µg/day)	pre	517.9±148.1	511±128	512.1±120.2	530.1±88.4	1.46	0.251
	post	556.7±130.7	544.5±115.7	457.9±127.9	547.9±33.3	3.05	0.064
β- carotene (µg/day)	pre	639.3±393	647.2±499	637.2±391	629.7±199	0.248	0.755
	post	698.3±987.2	677.6±400	651.9±210	639.7±211	0.338	0.716

Data expressed as Mean ±SD. ED: elastic training with vitamin D. EP: elastic resistance training with placebo. VD: vitamin D and control groups. $p \leq 0.05$ is significant difference.

According to the results, the strength was increased significantly in both upper body and lower body exercises ($p \leq 0.05$). It is found that chest press increased by 30.1 percent and 29.7 percent in ED and EP respectively, while

the squats were increased by 23.7 and 26.9 percent in ED and EP respectively. However, body fat percent had no significant change after the training period ($p \leq 0.05$), see table 4.

Table 4. Functional variable before and after training and supplementation

		ED	EP	VD	Control
BF (%)	Pre	20.4 ± 3.82	22.6 ± 3	22. ± 3.2	21.4 ±3.4
	Post	19.2 ± 3.8	22.6 ± 3.10	22.6± 3.5	21.8 ± 3.3
Chest press(Kg)	Pre	34.2 ± 3.9	35.6 ± 4.7	37.3± 4.6	36.9 ± 4.9
	Post	44.5± 9.2	46.2 ± 9.43	38.1± 3.9	37.8 ± 4.9
Squat(Kg)	Pre	48.9 ± 6.7	49.3 ± 5.4	49.5 ± 6.9	48.2 ± 4.4
	Post	60.5 ± 5.5	62.6 ± 4.5	50.1 ± 7.5	49.9 ± 4.3

ED: elastic training with vitamin D. EP: elastic resistance training with placebo. VD: vitamin D and control groups. Data were expressed by Mean±SD

The results showed that mean concentration of IL-6 and TNF-alpha significantly decreased from pre- to post-

training ($p=0.01$). Also, there was a statistically significant difference in IL-6 ($p=0.01$) and TNF-alpha ($p=0.01$) concentration

changes among the four groups after eight weeks of training. Bonferroni multiple comparison test showed that there was significant difference between ED with VD ($p<0.05$) and control ($p<0.05$) in IL6 and TNF-alpha. Also, Bonferroni test showed there is a significant difference in EP compared to VD ($p<0.05$) and control ($p<0.05$) in mentioned variables. However, it

was observed that there is no significant difference between ED and EP in the abovementioned variables ($p>0.05$). Moreover, the results showed that there is no statistical significance in CRP concentration between pre- and post-training ($p>0.05$), leading to no statistically significant difference in CRP concentration ($p>0.05$). See figure 1.

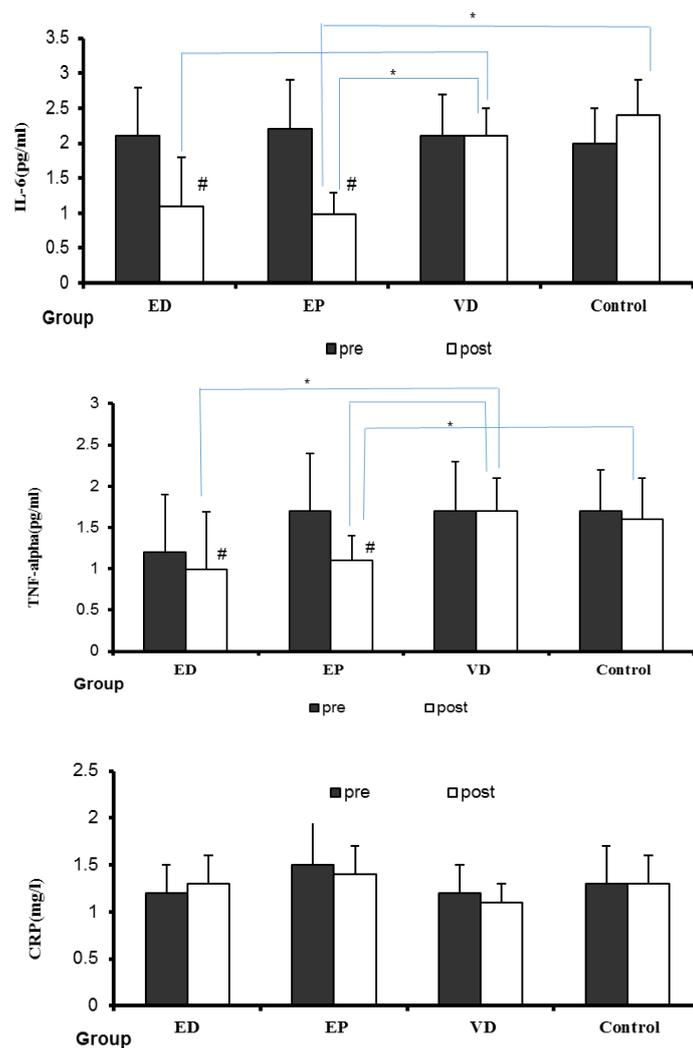


Figure 1. Interleukine-6, TNF-alpha, and CRP concentration changes before and after supplementation and elastic resistance training. # Significant difference from pre-training. Also, *shows significant difference between post test among groups. $p\leq 0.05$. ED: elastic training with vitamin D. EP: elastic resistance training with placebo. VD: vitamin D and control groups.

Discussion

This study is the first one to investigate the effects of vitamin D supplementation on biomarkers of systemic inflammation during elastic resistance training in untrained males. The results show that vitamin D supplementation with elastic resistance training selectively decreased some plasma systemic inflammation markers. However, vitamin D intervention did not affect inflammatory markers, including BF%.

It is shown that the eight-week elastic resistance training alone or with vitamin D supplementation could not affect BF%. Fat tissue and its changes have an important role in cytokine resting levels (14) because some cytokines such as TNF- α and IL-6 that were released from fat tissue could be an expression of CRP genes (14). However, it has been reported that individuals with CRP concentrations less than 1 mg.L⁻¹ are considered to be at low risk for cardiovascular disease, whereas people with values from 1–3 mg.L⁻¹ and greater than 3 mg.L⁻¹ are at moderate and high-risk for cardiovascular disease respectively (15). Therefore, CRP concentration of the subjects in the current study is between 1 and 2 mg.L⁻¹, and they are at moderate risk for cardiovascular disease. However, in the current study, neither elastic resistance training nor vitamin D supplementation has an effect on CRP. It seems that this issue may attribute lack of changes to BF%. The relation to body fat and its distribution is well known in the studies (16, 17).

The results showed that the concentration of IL-6 and TNF- α was decreased in ED and EP compared to VD and control, although there is no significant difference between in ED and EP. This means that our protocol training as elastic resistance training

with vitamin D did not have a synergistic effect. Several authors have studied the cytokine response to physical exercise in recent years. Recently, Azizbeigi *et al.* reported that eight weeks of moderate to high intensity resistance training could not reduce the systemic inflammation in untrained men (4). On the other hand, recently Phillips *et al.* reported that 10 weeks of moderate to high intensity resistance training reduced systemic inflammatory milieu in sedentary elderly women (18). These contradictions may be explained by the difference in intervention durations and related ability to adapt. Situations may exert influence upon inflammatory response, given subjects' gender and age. Gender, thanks to the effect of hormones, may result in different cytokines' response to exercise (19) or age that is associated with increase in basal CRP and TNF- α levels (20). In the current study, elastic training is applied to attenuate indices of systemic inflammation, a novel method of training. It seems that this may affect the results. It has been reported that the exercise training anti-inflammatory effect seems to be dependent on the intensity and duration of the exercise bout (21).

It is found that combination of elastic resistance training with vitamin D had a selectively synergistic effect to attenuate systemic inflammatory indices compared to the VD and control groups. Recently, some antioxidants such as vitamin D had increased due to reports suggesting that vitamin D deficiency exists in some athletes, particularly those who participate in indoor sports such as body building (22, 23).

It is observed that vitamin D supplementation alone could not affect inflammatory markers, while several studies have reported that vitamin D may reduce the

inflammatory response and improve insulin sensitivity (24).

In the current study, untrained men with moderate to high basal inflammatory markers with low 25 (OH) D concentrations were tested. The relationship between vitamin D and inflammation has been controversial. Some hypothesize that inflammation reduces 25 (OH) D concentrations (25), while others say that increasing vitamin D status reduces inflammation (26). However, neither vitamin supplementation nor combination elastic training with vitamin D could affect CRP. In the current study, vitamin D and elastic training decreased IL-6 and TNF-alpha, although there is a direct relationship between IL-6 and TNF-alpha with CRP, and baseline, CRP was moderately correlated with IL-6, highlighting the proposed relationship between systemic IL-6 release and systemic CRP concentration (27). In contrary to our results; some authors have reported that 1 year of moderate resistance training could decrease CRP, with no change in IL-6 (28).

It is found that vitamin D supplementation could not affect inflammatory markers. The subjects were untrained men with body fat percent greater than normal. It has been reported that oxidative stress is more than a normal subject (29). It seems that oxidative stress and free radical existing may interfere with this issue because inflammation can lower serum 25 (OH) D via oxidative stress resulting in oxidative catabolism of 25 (OH) D (30).

It is shown that the combination of elastic resistance training with vitamin D has a synergistic effect in attenuating inflammation. When the groups are compared (ED vs. control and VD; and EP vs. and control), and with regard to the fact that there is no significant difference between ED with EP, it

can be concluded that elastic resistance training has a key role in decreasing inflammatory indices than vitamin D.

Unlike the current results, it has been reported that vitamin D (4,000 IU/day) supplementation in healthy, overweight, and obese adults participating in a resistance training intervention did not augment exercise-induced changes in inflammatory (IL-6, TNF-alpha and CRP) biomarkers (31). This contradiction can be explained by differences in methodology, such as vitamin dosage, used.

Conclusion

In this study, the effect of vitamin D supplementation during elastic resistance training in untrained men was investigated. Finally, we can claim that the combination of vitamin D with elastic resistance training did not have extra advantages in attenuation of systemic inflammatory indices when compared with the training alone.

Conflict of Interest

The authors have no conflict of interest.

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