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ORIGINAL PAPER

Vitamin E supplementation decreases muscular and oxidative damage but not inflammatory response induced by eccentric contraction

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Abstract The purpose of this study was to investigate the effects of vitamin E supplementation on muscular and oxidative damage, as well as the inflammatory response induced by eccentric exercise (EE) in humans. Twenty-one participants with a mean age of 22.5 ± 4 years, weight of 68.2 ± 4.9 kg, and height of 173 ± 4.3 cm were selected and divided randomly into two groups: supplemented (S) ($n = 11$) and placebo (P) ($n = 10$). Fourteen days after starting supplementation, subjects performed EE (three sets until exhaustion with elbow flexion and extension on the Scott bench, 80% 1 RM). Blood samples were collected on days 0, 2, 4, and 7 after EE. Muscle soreness (MS), lactate dehydrogenase (LDH) activity, lipid peroxidation, protein carbonylation, tumor necrosis factor-α (TNF-α), and interleukin 10 (IL-10) levels were determined. We measured a significant increase in MS, LDH, lipid peroxidation, and carbonylation in both groups on days 2, 4, and 7 after eccentric contractions (EC). Values of the supplement group were lower than those of the placebo group at 4 and 7 days after EC in all parameters. Both groups showed significantly increased TNF-α on the second day and IL-10 concentration on the fourth and seventh days after EE. The results suggest that vitamin E supplementation represents an important factor in the defense against oxidative stress and muscle damage but not against the inflammatory response in humans.

Keywords Vitamin E · Eccentric exercise · Oxidative stress · Inflammation

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Introduction

Eccentric contractions (EC) produce direct muscle injury, and it has been reported that reactive oxygen species (ROS) play a role in both the initiation and the progression of muscle fiber injury after initial mechanical insult [1]. Cannon et al. [2] suggest that mobilization and activation of neutrophils may contribute to increased myocellular enzyme efflux after eccentric exercise (EE), and this can be an important mechanism in the production of ROS. However, other mechanisms are also involved in the generation of ROS during and after high-force EE, such as xanthine and NADPH oxidase production, ischemia reperfusion, prostanoid metabolism, phagocytic respiratory bursts, and disruptions in calcium homeostasis [3].

An alternative approach to determining whether ROS play a critical role in the muscle damage process is to observe the effects of antioxidant supplementation. A number of human studies have been conducted in which dietary antioxidant supplements have been used in an attempt to assess efficacy to attenuate increases in ROS, reduce oxidative damage [1, 4, 5], and confer a "protective" effect against exercise-induced muscle damage [6]. Cannon et al. [7] demonstrated that cytokine secretion is significantly influenced by EE and dietary vitamin E supplementation.

However, studies with vitamin E supplementation still show inconclusive results. For example, Meydani and colleagues [8] reported that vitamin E provides protection against exercise-induced oxidative injury, while Beaton and colleagues [9] concluded that vitamin E supplementation had no effect on muscle damage levels and inflammation.

Vitamin E (α-tocopherol) is considered an antioxidant vitamin because it provides a cellular protection mechanism and acts as a potent scavenger of the peroxyl radical [8, 10]. It is the major hydrophobic chain-breaking antioxidant that prevents the propagation of free radical reactions in the lipid components of membranes, vacuoles, and plasma lipoproteins [11]. However, the effects of vitamin E on inflammation by EE remain unclear.

Whereas several investigations have used aerobic exercise as the physical stressor, few have examined high-intensity anaerobic work [5, 12, 13]. In addition, few studies have used resistance exercise with an eccentric bias as the chosen exercise mode, and these studies have reported isolated findings of oxidative stress or inflammation [12–14].

Thus, the aim of the current study was to examine the effect of vitamin E on several markers to determine muscular and oxidative damage and inflammatory response in serum induced by acute EC in humans. The current study uses supplementation with lipid-soluble vitamin E antioxidant prior to and after ECs to reduce oxidative and muscular damage and inflammation.

Materials and methods

Study design

This study was a 3-week, randomized, double-blind parallel design involving a dietary supplement versus a placebo. The subjects were randomly assigned to receive either the dietary supplement or placebo for 14 days before and 7 days after acute EE. Blood samples were collected at baseline immediately before and after exercise between 10:00 a.m. and 12:00 p.m. The analyses of muscular damages, oxidative damages, and inflammatory markers were accomplished.

Subjects

Twenty-seven male volunteers, who were students at UNESC (Universidade do Extremo Sul Catarinense, Criciuma, Santa Catarina, Brazil), with a mean age of 22.5 ± 4 years, weight of 68.2 ± 4.9 kg, and height of 173 ± 4.3 cm (see data in Table 1) participated in this randomized, double-blind, placebo-controlled study. During the study, six subjects withdrew from the groups due to personal reasons. Personal characteristics did not differ between the two groups (Table 1). After the purposes and risks of the protocol had been explained to each subject, oral and written informed consent was obtained from them. The protocol was approved by the local ethics committee.

Exclusion criteria

All subjects were non-smokers, were not taking vitamin E or any other antioxidant or related supplements, had not participated in resistance training or any other form of structured exercise for at least 6 months, did not have a history of muscular lesions, and were not carriers of any disease that might compromise the results or be aggravated by physical exercise.

Supplementation

The subjects were randomly divided into a supplemented (S) ($n = 11$) and placebo (P) ($n = 10$) groups. The supplementation consisted of capsules containing 800 IU per day of D-α-tocopherol acetate according to Meydani et al. [8], whereas the placebo consisted of a similar capsule containing starch. Volunteers received one capsule per day for a total of 21 days beginning 14 days before the eccentric protocol and continuing throughout the 7-day post-exercise period.

Familiarization with eccentric contraction protocol

Subjects were familiarized and fitted to the Scott bench (the movement of flexion and extension of elbows) 3 days after the start of supplementation (3 sets, 15 repetitions with 2 min rest between sets), with 2 kg in load.

Table 1 Body composition and muscular performance in young university students after muscle lesion induced by eccentric exercise

Groups	*n*	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	1 RM (kg)	Repetitions (mean)
Control	10	22 (19–25)	77.6 (70–89)	179 (170–186)	22.4 (19.4–25.6)	30.4 ± 5.7	13 ± 4
Supplement	11	25 (20–27)	79.2 (72–85)	182 (167–187)	23.3 (20.3–25.3)	31.2 ± 6.1	12 ± 3

1 RM One-repetition maximum

There was no significant difference ($p > 0.05$) between the two groups in the 1 RM test or in the average number of repetitions

Strength testing

The performance test required flexion and extension of elbows, supported by the Scott bench (3 sets with 5 min rest between each set). The subject's one-repetition maximum (1 RM) was assessed [15]. Before testing a standardized warm-up consisting of a 10-min ride on a bicycle ergometer was employed. The weights lifted in the 1 RM test for each group are described in Table 1. After 1 RM testing, participants were instructed to follow their normal dietary pattern and to refrain from strenuous physical exercise during the 21 days of supplementation.

Eccentric contraction protocol

Fourteen days after starting supplementation the subjects performed EC, using the same exercises as the test. EC of short duration and high intensity was performed with elbow flexion and extension on the Scott bench at an intensity of 80% of maximum repetition [15]. The concentric phase of the exercise was performed with manual assistance from the instructor. The eccentric phase was performed for 6–8 s. Three sets of the exercises were performed at 2-min intervals until exhaustion. The average number of repetitions performed by each group is described in Table 1.

Blood collection

Blood samples were collected prior to the exercise and on the second, fourth, and seventh days after the exercise. Blood (10 mL) was obtained from the cubital vein of the right arm and collected in vacutainer tubes without additives. It was then processed, and the serum was separated, aliquoted, and immediately stored in a freezer at −80°C for later analysis.

Muscle soreness

The visual analogue method has been established as a reliable method for assessing soreness [16]. The intensity of the perceived soreness of the biceps muscle was assessed using a 10-cm visual analog scale (VAS), the left and right extremes of which refer to "no muscular soreness" and "maximum muscular soreness," respectively. The VAS is easily and quickly administered and has been used as a reliable measurement for determining the intensity of human pain. Thus, subjects were asked to place a vertical line toward the amount of soreness they perceived, which was quantified as the distance (with a precision of 0.1 cm) between the left extreme of the line and the vertical line carried out by the subject. All the subjects had been previously familiarized with the VAS.

Lactate dehydrogenase

Lactate dehydrogenase (LDH) enzyme activity was used as a marker of cellular damage. A specific kit, supplied by Labtest Diagnóstica SA, was used to determine levels using an enzymatic system with a final point reaction in serum samples. The technical instructions that accompanied the kit were followed.

Lipid peroxidation

As an indicator of lipid peroxidation, the formation of substances that react to the heating of thiobarbituric acid (nmol TBARS/mg protein) were measured spectrophotometrically (532 nm) and expressed as TBARS equivalents [17].

Protein carbonylation

Oxidative damage in proteins was measured by determining the carbonyl groups based on the reaction with dinitrophenylhydrazine. Carbonyl content was determined spectrophotometrically (370 nm) using a coefficient of 22.000 M [18].

Interleukins

Tumor necrosis factor-α (TNF-α) and interleukin 10 (IL-10) were determined by ELISA with commercially available kits (R&D Systems, Minneapolis, MN, USA).

Protein determination

The quantity of proteins in TBARS and carbonyl assays was measured using the technique of Lowry et al. [19].

Statistical treatment and coefficient of variation

Data are expressed in mean and standard error of the mean (SEM) and were analyzed using a two-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. The level of significance established for the test was $p < 0.05$. SPSS version 16.0 was used. In all the assays we performed analyses in duplicate, in two separate runs. Both the intra-assay and the inter-assay coefficients of variation were <8%.

Results

Muscle soreness

We observed a significant increase in muscle soreness (MS) in both subject groups on the second day after EC in relation to the pre-exercise period. The values from the supplement group were lower than those from the placebo group at 4 and 7 days after EC (Table 2).

Table 2 Muscle soreness and lactate dehydrogenase in young university students after muscle lesion induced by eccentric exercise (EE)

Groups	Muscle soreness				LDH (U/dL serum)			
	Pre-EE	2 days	4 days	7 days	Pre-EE	2 days	4 days	7 days
Control	0	6.2 ± 0.5*	2.3 ± 0.7#	1.2 ± 0.3#	224 ± 29	248 ± 15	1,292 ± 161*	1,221 ± 77*
Supplement	0	3.8 ± 0.8*#	1.2 ± 0.5#	0.00	203 ± 17	224 ± 27*#	981 ± 76*#	1,001 ± 45*#

Participants marked their subjective rating of muscle soreness on a scale from 0 (without pain) to 10 (extreme pain). The difference in relation to pre-EE (*) and placebo group (#) was significant at $p < 0.05$

Lactate dehydrogenase

The results show a significant increase in LDH activity in both subject groups at 4 and 7 days after the EC in relation to pre-exercise. Values from the supplement group were lower than those from the placebo group at 4 and 7 days after EC (Table 2).

Lipid peroxidation

As shown in Fig. 1, the TBARS levels in the placebo group increased on the second, fourth, and seventh days after the EC in relation to the pre-exercise period. Values from the supplement group were lower than those from the placebo group at 4 and 7 days after EC.

Protein carbonylation

The results show a significant increase in protein damage in the placebo group on the second, fourth, and seventh days after EC, compared to the pre-exercise period. Values from the supplement group were lower than those from the placebo group at 4 and 7 days after EC (Fig. 2).

Interleukins

To quantify the inflammatory response, we measured TNF-α and IL-10. Both groups showed significantly increased TNF-α on the second day after EC (Fig. 3a) and significantly increased IL-10 concentrations on the fourth and seventh days after EC (Fig. 4) in relation to the pre-exercise period. There was no difference between the groups.

Discussion

We investigated the effects of vitamin E supplementation on biomarkers of muscular and oxidative damage and

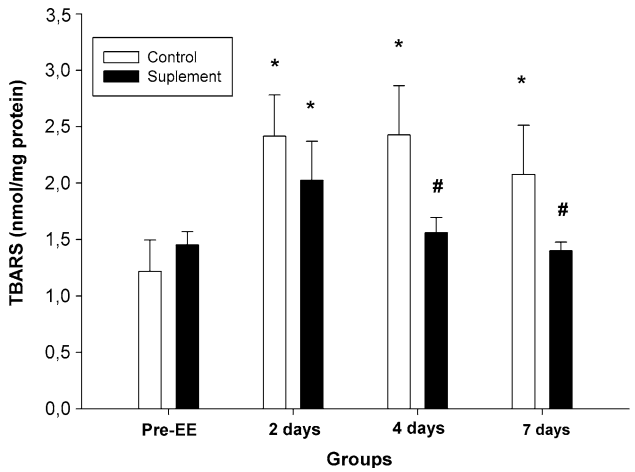

Fig. 1 Serum lipid peroxidation levels in the serum of young university students before (pre-EE) and 2, 4, and 7 days after the eccentric exercise for subjects provided vitamin E supplement or placebo. The values are presented as mean ± SEM and the results expressed in TBARS level (nmol/mg of protein). The difference in relation to pre-EE (*asterisk*) and to placebo group (*hash symbol*) was significant at $p < 0.05$

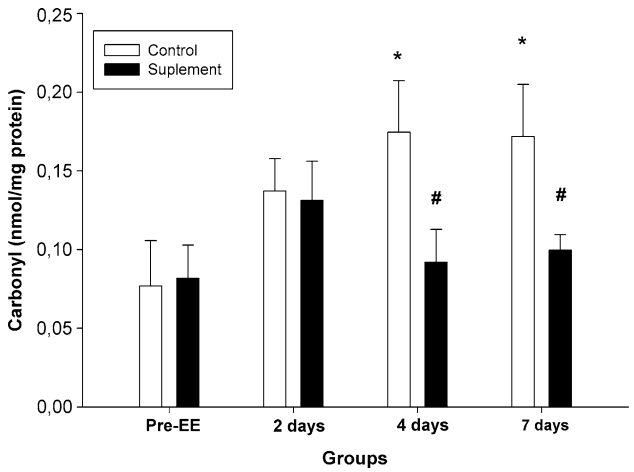

Fig. 2 Serum protein carbonyl levels in the serum of young university students before (pre-EE) and 2, 4, and 7 days after the eccentric exercise for subjects provided vitamin E supplement or placebo. The values are presented as mean ± SEM, and the results expressed in nmol/mg of protein. The difference in relation to pre-EE (*asterisk*) and in relation to placebo group (*hash symbol*) was significant at $p < 0.05$

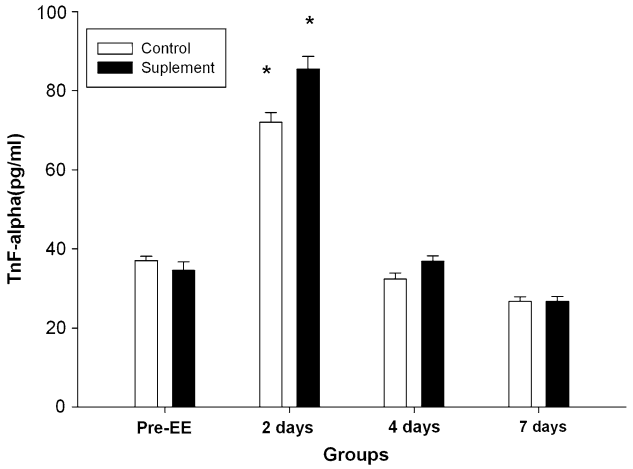

Fig. 3 Serum tumor-necrosis factor-α (TNF-α) levels in the serum of young university students before (pre-EE) and 2, 4, and 7 days after the eccentric exercise for subjects provided vitamin E supplement or placebo. The values are presented as mean ± SEM and the results expressed in pg/ml of serum. The difference in relation to pre-EE (*asterisk*) was significant at $p < 0.05$

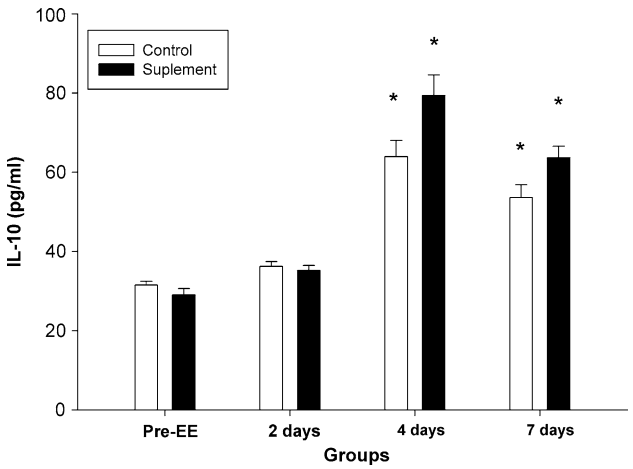

Fig. 4 Interleukin 10 (IL-10) in the serum of young university students before (pre-EE) and 2, 4, and 7 days after the eccentric exercise for subjects provided vitamin E supplement or placebo. The values are presented as mean ± SEM and the results expressed in pg/ml of serum. The difference in relation to pre-EE (*asterisk*) was significant at $p < 0.05$

inflammatory response after EC. In this study, vitamin E supplementation was shown to decrease muscle (LDH and MS) and oxidative [MDA and protein carbonylation (PC)] damage but not the inflammatory response (TNF-α and IL-10) induced by EC.

Several markers have been used to determine the level of muscular lesions induced by EC [9, 14, 20]. LDH enzyme activity [21, 22] and MS [20] were employed as markers of muscular damage. Results show (Table 2) decreased muscular damage in the group supplemented with vitamin E in comparison to the placebo group. The mechanisms that may contribute to skeletal muscle damage include ROS-mediated processes [23, 24]. Specifically, ROS may play a central role in the etiology of skeletal muscle damage via oxidation of ion transport systems, leading to disruption of Ca^{2+} ion homeostasis, impaired mitochondrial respiratory control, distortions in signal transduction pathways, and ultimately cell dysfunction [25]. Sacheck et al. [10] and Itoh et al. [26] demonstrated that vitamin E decreased muscular damage after EE and endurance running in humans. Therefore, we postulate that quenching of ROS by vitamin E could protect against muscle damage caused by EE.

To assess oxidative damage, we measured TBARS levels (Fig. 1) and PC (Fig. 2). The use of TBARS to detect lipoperoxidation in humans has been criticized due to the lack of accuracy and validity in other studies. However, our results are in agreement with those of Mastaloudis et al. [27] and Knez et al. [3] who utilized more sensitive techniques. Data presented herein suggest that vitamin E supplementation reduces lipid peroxidation and PC after EC in humans. Other studies have shown similar results. For example, Meydani et al. [8] demonstrated that vitamin E provides protection against oxidative damage after downhill running. Goldfarb et al. [4] suggested that combinations of antioxidants, including vitamin E, reduce oxidative damage after EC. With regard to the decrease in lipoperoxidation, vitamin E has been reported to protect cellular membranes and other fatty cellular components by donating electrons to free radicals [28, 29].

The formation of PC appears to be related to muscle production of an intermediate resembling the hydroxyl radical [30]. In relation to reducing protein damage, it is possible that vitamin E supplementation accelerates protein turnover. Gene transcription and cell integrity are reduced by the action of reactive species oxygen, which also has the ability to alter the lysosomal system and the proteasomes, two major pathways by which proteins are degraded [31]. The molecular structure of vitamin E enables ROS inactivation [32].

Recent reports described that inflammation induced by EE induces cytokine production [12, 22]. To evaluate this, we measured plasma levels of TNF-α and IL-10. TNF-α is the early response pro-inflammatory cytokine that is most likely synthesized by resident macrophages and local post-capillary vascular endothelium, with synthesis occurring rapidly after the onset of injury or infection [33]. IL-10 is a primary anti-inflammatory cytokine that inhibits pro-inflammatory cytokine production by activating monocytes and macrophages. Our results demonstrated that vitamin E did not decrease inflammation, but

the repair of muscle and oxidative injury is dependent on inflammatory mediators [22], and it is therefore not clear how much a severe inflammatory response must be curbed for better recovery and improved function. Our results are consistent with other studies; for example, Mastaloudis [27] demonstrated that vitamin E and C supplementation prevents oxidative damage but does not decrease the inflammatory response induced by exercise. Beaton et al. [9] suggested that vitamin E had no effect on contraction-induced inflammation as a result of eccentrically biased muscle contractions. In fact, it is rather likely that there are multiple stimuli for cytokine production during exercise [31].

In summary, vitamin E supplementation prevents oxidative and muscle damage but does not decrease the inflammatory response induced by high-intensity EC. Further studies should be carried out to better discuss the relationship between free radicals, antioxidants, and inflammation after EC.

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