The Effect of Vitamin C and E Supplementation on Muscle Damage and Oxidative Stress in Female Athletes: A Clinical Trial

Maryam Taghiyar[1,2], Leila Darvishi[1,2], Gholamrez Askari[1,2], Awat Feizi[3], Mitra Hariri[1,2], Nafiseh Shokri Mashhadi[4], Reza Ghiasvand[1,2]

[1]Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Biostatistics and Epidemiology, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Dr. Reza Ghiasvand,
Department of Community Nutrition,
School of Nutrition and Food Sciences,
Isfahan University of Medical Sciences,
Isfahan, Iran.
E-mail: ghiasvand@hlth.mui.ac.ir

Date of Submission: Feb 29, 2012

Date of Acceptance: Apr 10, 2012

How to cite this article: Taghiyar M, Darvishi L, Askari G, Feizi A, Hariri M, Mashhadi NS, *et al*. The effect of vitamin C and E supplementation on muscle damage and oxidative stress in female athletes: A clinical trial. Int J Prev Med 2013; Supplement 1: S16-23.

Original Article

ABSTRACT

Background: The need for energy in strenuous exercises necessitates an increase in oxygen consumption and production of reactive oxygen species. It seems that supplementation of vitamins C and E reduces exercise-induced oxidative stress. Therefore, this study aims to investigate the effects of vitamin C and E supplementation on muscle damage and oxidative stress in female athletes.

Methods: The study was a four-week randomized, double-blind clinical trial, conducted on 64 trained female athletes recruited in the Isfahan sports club. They were randomly assigned to one of the following four groups: (a) vitamin C (250 mg/day), (b) vitamin E (400 IU), (c) vitamin C + vitamin E, and the control (placebo). Aspartate transaminase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) for assessing muscle damage, and malondialdehyde, were measured before and after the intervention.

Results: In the between-groups comparison, only creatine kinase significantly changed at the end of the period ($P = 0.03$). However, in the intergroup comparison creatine kinase was significantly decreased in group 1 ($P = 0.002$). As for Aspartate aminotransferase, no significant difference was spotted in any of the comparisons. Lactate dehydrogenase was significantly decreased in group 2 ($P = 0.02$). Finally, this study revealed a significant decrease in oxidative stress markers in groups 1, 3, and 4 ($P < 0.05$).

Conclusions: It is induced from the results that vitamin C and E supplementation plays a role in reducing muscle damage markers of aerobic exercises.

Keywords: Vitamin E, female athlete, oxidative stress, muscle damage, vitamin C

INTRODUCTION

Strenuous exercises inflict metabolic and mechanical stresses on the human body to some degree. This leads to inflammation and oxidative stress.[1] The need for energy for doing strenuous exercises increases oxygen consumption in the tissues.[2] Therefore, oxygen consumption increases 10- to 20-fold systemically[3] and equally and 100- to 200-fold at the level of the skeletal muscles.[4] Increased oxygen consumption leads to a spike in the mitochondrial electron flow, and thus, may cause more leakage of the reactive oxygen species (ROS) in the mitochondria, ultimately increasing the production of the reactive oxygen species.[4] Other potential sources for the production of ROS are disruption of calcium ion homeostasis, damage in iron-containing proteins,[5-6] and production of xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The amount of ROS production is proportional to the antioxidant capacity of a normal body, in the position rest, but an increase in ROS production will also cause homeostasis imbalance in an oxidant–antioxidant.[7] The increase in ROS production increases the antioxidant defense system activity, and therefore, decreases the antioxidant reserves. This increases the susceptibility of the tissues to oxidative stress.[8] Oxidative stress occurs in case the body does not have enough capacity to defend itself against free radicals. ROS is the main source of oxidative stress and plays a major role in the initiation and progression of damage to the muscle fibers after exercise.[9] Strenuous exercises increase the production of free radicals, which leads to increased lipid peroxidation. There is less DNA damage and protein oxidation, which means that cell damage will lead to oxidative stress.[10] Oxidation of lipids produces products such as malondialdehyde, which is an indicator of lipid oxidation.[8] Thus, strenuous exercises are not necessarily beneficial, but harmful at times.[7,11,12]

The effect of intense exercise training might necessitate using antioxidant supplements to prevent oxidative stress[13] and muscle damage.[6,14] Several antioxidants have been introduced to protect the cells from free radicals such as vitamins C and E, carotenoids, and flavonoids.[15,16] Of course, antioxidant vitamins C and E are among the most commonly used antioxidants, as sports supplements.[17] According to a study conducted in the field of the effect of different resistance exercise protocols on nitric oxide, lipid peroxidation, and creatine kinase activity in sedentary males, there is a relationship between lipid peroxidation and creatine kinase (marker of muscle damage).[10] This study reports a positive correlation between these two indices of physical activity.[11]

The use of antioxidant supplements can delay oxidative stress that is caused by the effect of exercise on blood and skeletal muscle.[18,19] A similar study reveals that pretreatment with vitamin C can reduce muscle soreness, delay CK increase, and prevent blood glutathione oxidation, with little influence on muscle function loss.[20] However, in another study, it has been concluded that vitamin C and E supplementation (500 or 1000 mg or IU per day) for four weeks does not reduce either the biochemical or ultra-structural indices of muscle damage in experienced runners, after a half marathon.[21] It seems that the research results are influenced by the level of exercise, frequency, duration, and type of antioxidant supplement consumption.[22] There are many different factors involved in the cell damage caused by free radicals produced in exercises, including the intensity of exercise, diet, and type of exercise.

According to conflicting results of different studies on the usefulness of supplements as well as most studies in this field being on men,[21,23-26] and also because female athletes are the most known groups to be at risk, and ultimately due to the large physiological differences between men and women, more research is needed in this area. In that light this study aims to investigate the effects of vitamin C and E supplementation on muscle damage and oxidative stress in female athletes.

METHODS

Subject

The study was a four-week randomized, double-blind clinical trial on 64 trained (The best or most skilled members who had been doing aerobics for at least three years) female athletes, having the inclusion criteria, who were randomly recruited from the Isfahan sports club. The study was approved by the Ethics Committee of the Isfahan University of Medical Sciences. This study was registered in the Iranian Registry of Clinical Trials (www.irct.ir) on October 7, 2012, with IRCT registration number: IRCT201111095062N2. Considering Malondialdehyde (MDA) as the main variable in the study sample and at the significance level of 5% and statistical power of 80%, to detect a measurable amount of 1.2, 12 subjects were assigned to each of the groups. The subjects were randomized using the permuted block size of 4, by another person, to one of the four groups: A: Vitamin C (250 mg/day), B: Vitamin E (400 IU), C: Vitamin C + vitamin E, and control (placebo). They were initially willing to participate in the study and already had three to six years of experience in sports. They had not taken antioxidant supplements in the previous month, were in complete physical health, and were free of disorders related to pregnancy and lactation. During the study, one person was excluded from the sample due to a fracture in the leg. Moreover, one person was excluded due to digestive problems and weight changes and four subjects withdrew from the groups due to personal reasons.

Methods

After explaining the purpose and details of the sample, written informed consent was obtained from each subject. Each participant's weight and height were measured and recorded using medical scales Seca (made in Germany) with an accuracy of 100 grams and stadiometer with an accuracy of 0.5 cm. The athletes consumed a vitamin pill daily, with their meals, vitamin C (Iran, Osveh) or vitamin E (Iran, Zahravi). Vitamin placebos were prepared by the same company. In the beginning, each participant took supplements for four weeks and by counting the total number of pills remaining in the study, it was figured out if the participants had followed the research code or not consumed the pills. They were asked to keep doing their daily routine physical activity, without changing it. Using two-day food records, information about their diet was collected at the outset and in the last week of intervention and analyzed with the Nutritionist software, version 4. Their physical activity was also assessed using a physical activity questionnaire and described according to the metabolic equivalent — minutes-a-week standard

Blood collection

Blood biochemical evaluation was performed at the baseline and at the end of the fourth week before giving supplements to participants. Blood samples of 10 ml were taken from the brachial vein. To separate the serum and blood samples, the samples were stored at room temperature for one hour and then centrifuged at 2000 rpm for 15 minutes. After that, the serum samples were stored at -70°C before they were analyzed. Aspartate aminotransferase (AST) was measured using the enzymatic photometric method (IFCC), Creatine kinase (CK) was determined using the colorimetric method, Lactate dehydrogenase (LDH) was measured using the colorimetric enzymatic assay (DGKC) (Pars test, Tehran, Iran) and Malondialdehyde (MDA) was determined using the spectrophotometer assay.

Statistical analysis

Analyses were performed with the SPSS software version 16 statistical package. The results were presented as mean ± standard error. Due to the lack of normal distribution of variables, they were log transformed for normalization. The method of multivariate analysis of variance was used to examine the differences (percentage changes before and after the intervention) of variables among the groups. The Paired T-test was used to compare changes within the groups of variables and the Bonferroni test was used to compare the changes between the groups, as a method for their *post hoc* comparisons.

RESULTS

Descriptive information about the sample is shown in Table 1. The age of the participants in the study was significantly different among the groups ($P < 0.05$), but the body mass index (BMI) and physical activity were not, between the groups [Table 1].

Their dietary intake was assessed using a two-day food record when using the Nutritionist Software version 4, and it was revealed that there was no significant difference between and within the groups [Table 2]. The aim was to compare the intake of calories, macronutrients, saturated fatty acids, and cholesterol between the groups. The analysis showed that antioxidant supplementation had a significant effect on some markers of muscle damage ($P = 0.02$, f = 1.7, value = 0.5). On comparison between groups, only creatine kinase had a significant change at the end of the period ($P = 0.03$). However, in the intergroup comparison it was significantly decreased in group 1 ($P = 0.002$). Aspartate aminotransferase did not significantly change in any of the comparisons. Lactate dehydrogenase significantly decreased in group 2 ($P = 0.02$). Moreover, a significant decrease in oxidative stress markers in groups 1, 3, and 4 ($P < 0.05$) was observed and in group 2, it decreased to a slightly significant level [Table 3]. We show the schematic diagram of this study in Figure 1.

DISCUSSION

The purpose of this study was to investigate the effects of vitamin C and E supplementation on muscle damage and oxidative stress in female athletes. The results showed that taking vitamin C and vitamin E reduced the muscle damage markers through a significant reduction in creatine kinase. The oxidative stress markers reported a significant decrease in groups 1, 3, and 4, although, there was no significant difference between the groups. This partly reflected the effect of supplementation with vitamins C and E, and vitamin C alone.

In a similar study, Silva *et al.*, investigated the effects of vitamin E (800 IU) supplementation on muscular and oxidative damage, as well as the inflammatory response induced by eccentric exercise (EE) on 21 participants. Fourteen days after starting the supplementation, the subjects performed EE. Blood samples were obtained on days 0, 2, 4, and 7 after EE. Both groups showed significantly increased TNF-alpha on the second day and IL-10 concentration on the fourth and seventh days after EE. The results suggested that vitamin E supplementation represented an important factor in the defense against oxidative stress and muscle damage, but not against the inflammatory response in humans.[23] In this field Roohi *et al.*, investigated the effects of vitamin C (1000 mg) supplementation on exercise-induced lipid peroxidation, muscle damage, and inflammation, in 16 healthy, untrained men. Blood samples were collected at the baseline, two hours after supplementation (immediately pre-exercise), post exercise (two and 24 hours after the exercise). The results indicated a significant increase in MDA two hours after the exercise in the placebo group. Also, CK showed a significant increase 24 hours after the exercise in the placebo group. They revealed that vitamin C supplementation prevented exercise-induced lipid peroxidation and muscle damage, but had no effect on the inflammation markers. Raphael *et al.* examined the effect of antioxidant supplementation and repeated bouts of moderate intensity endurance exercise on markers of muscle damage (creatine kinase-CK) and systemic inflammation (C-reactive protein-CRP) in 20 healthy, young, sedentary men. They revealed that antioxidant supplementation might reduce muscle damage if caused by prolonged moderate intensity endurance exercises, but had no effect on the systemic inflammatory response.[25] Bloomer *et al.* determined the effects of antioxidant therapy on the indirect markers of muscle damage following eccentric exercise (EE) in 18 women. Plasma creatine kinase (CK) activity, muscle soreness (MS), maximal isometric force (MIF), and range of motion (ROM) were assessed before and during the 14 days post exercise. They showed that antioxidant supplementation was helpful in reducing the elevations in plasma CK activity and MS, with little impact on MIF and ROM loss.[9] Some researchers reported different results. Mastaloudis *et al.* examined the effect of vitamin supplements E (300 RRR-a-tocopheryl

Table 1: Descriptive information

Variables	Groups	Number	Mean±SE	*P*
Age (year)	1	16	31.3±1.8	0.007***
	2	13	38.5±1.6	
	3	14	33.9±1.5	
	4	15	38.1±1.4	
	Total	58	35.3±0.9	
BMI (kg/m²)	1	16	26.7±1.1	0.8
	2	13	25.4±1.09	
	3	14	25.8±1.2	
	4	15	25.7±0.8	
	Total	58	25.9±0.5	
Physical activity (Met. minutes/ week)	1	16	636.1±93.2	0.1
	2	13	676.1±137.1	
	3	14	997.3±160.6	
	4	15	659.6±145.9	
	Total	58	736.3±68.38	

***$P<0.01$

Table 2: Mean±SE dietary intake

Groups	Kcal	Protein	Carbohydrate	Fat	Cholestrol	SATF
Group 1	1607.8±180.2	72.9±11.1	231.2±21.8	61.58±12.4	202±47.7	19.68±5.3
Group 2	1683.57±138.7	58.1±5.02	256.4±27.9	50.68±6.3	147.1±22.1	15.6±1.9
Group 3	1596.96±61.41	62.9±3.09	247.2±17.3	42.92±3.5	122.9±26.01	14.06±0.9
Group 4	1402.55±66.42	50.1±4.7	210.5±15.01	43.04±3.7	109.5±21.8	12.5±0.9
P	0.2	0.1	0.4	0.2	0.1	0.3

Table 3: Mean±SE indicators of muscle damage and oxidative stress compared between and within the groups

Groups	AST (U/L)	LDH (U/L)	CK (U/L)	MDA (ng/ml)
Group 1				
Pre	21.6±1.7	336.1±16.2	83.4±10.2	5.6±0.6
Post	20.1±1.9	335.4±1.5	60.8±6.05	1.9±0.1
Changes¹	-1.5	-0.68	-17.28	-3.77
P¹	0.2	0.9	0.002***	0***
Group 2				
Pre	20.3±1.08	332.1±17.1	84.7±16.8	5.1±0.9
Post	18.8±0.97	295.8±13.6	60.8±5.5	1.7±0.1
Changes¹	-1.5	-36.3	-23.9	-3.1
P¹	0.2	0.02**	0.06*	0.06*
Group 3				
Pre	19±1.1	325.2±14.8	116.7±22.2	4.3±0.6
Post	19.7±1.47	320±11.9	95.07±13.7	1.6±0.05
Changes¹	0.7	-5.3	-21.65	-2.7
P¹	0.6*	0.7	0.1	0.01**
Group 4				
Pre	22.8±1.6	321.7±13.4	86.05±6.6	5.1±0.4
Post	23.06±1.5	316±14.4	97.7±16.04	1.6±0.05
Changes¹	0.26	-5.73	11.7	-3.4
P¹	0.7	0.6	0.8	0***
P value²	0.2	0.2	0.034**	0.4

P¹ intergroup comparisons, P² between-group comparison, ¹The mean difference between the beginning and end, ****P*<0.01, ***P*<0.05, **P*<0. 1, AST=Aspartate transaminase, LDH=Lactate dehydrogenase, CK=Creatine kinase, MDA=Malondialdehyde

acetate IU) and C (1000 mg) in 22, over 50 km ultra-marathon runners. Blood samples were obtained before supplementation (baseline), 24 hours pre-, 12 hours pre-, and one hour pre-race; midrace, and for post-race, two hours post-race and six hours post-race. They showed that antioxidants appeared to have no effect on exercise-induced increases in muscle damage or recovery, but important sex differences were observed.[26] Gaeini *et al.*, investigated the effect of vitamin E supplements on oxidative markers in 20 male student athletes. The supplement group received 450 mg of alpha-tocopherol daily for eight weeks. They then evaluated the CK, MDA, protein carbonyl levels, and endurance performance. The results showed that E supplementation had no significant effect on these variables.[27] Dowson *et al.*, investigated whether four weeks of daily supplementation with 500 or 1000 mg of Vitamin C and 500 or 1000 IU of Vitamin E could modify the biochemical and ultrastructural indices of muscle damage following a 21 km run. They examined the indicators of creatine kinase, myoglobin, and malondialdehyde in the male runners after the endurance exercises. In both groups, there was a significant increase in creatine kinase and myoglobin, but the study did not report any significant changes in these three variables.[21]

According to other studies, ROS plays a major role in the etiology of muscle damage through oxidation of the ion transport systems, and that the suppressing of ROS by taking vitamin E can protect versus muscle damage caused by exercise.[28] Also, vitamin E has been reported to protect cellular velum and other fatty cellular parts by gifting electrons to the free radicals, and in this way it helps to reduce muscle damage.[29,30] Lipid peroxidation may lead to velum penetrance and the release of muscle constituents such as CK,[32,33] and Vitamin C inhibits lipid peroxidation on muscle damage effectively.[24] Based on the data obtained from the study conducted by Roohi *et al.*, Vitamin C reduces the consumer group vitamins and this is the reason for removing excessive material from the plasma through the urine. Therefore, it seems that long-term intake of vitamins is not necessary to prevent the production of free radicals.[34-36]

Also, Vitamin C in large amounts is a redox factor; as such, it has been found to operate as a pro-oxidant in some conditions and even leads to radical production.[31] Thus, high-dose antioxidant supplements have the opposite effect, and maybe for this reason, this has not been seen in the group combination effect of supplementation on the markers of muscle damage.

Although, antioxidant supplementation is useful to some extent, even for individuals it can be dangerous to use high doses for long periods of time, and its intake, especially with Vitamin E, makes it a fat-soluble vitamin.

Possible reasons for the significant reduction of the MDA levels in the placebo group may be the reduced intensity of exercises in this group, which result in decreased oxygen intake and oxidative stress and also the placebo effect.

This study could also be designed to examine the separate vitamins E, C, and the combined model of these vitamins, with the same study population of female athletes.

However, there was a limit in the number of samples in this study, as more samples would yield

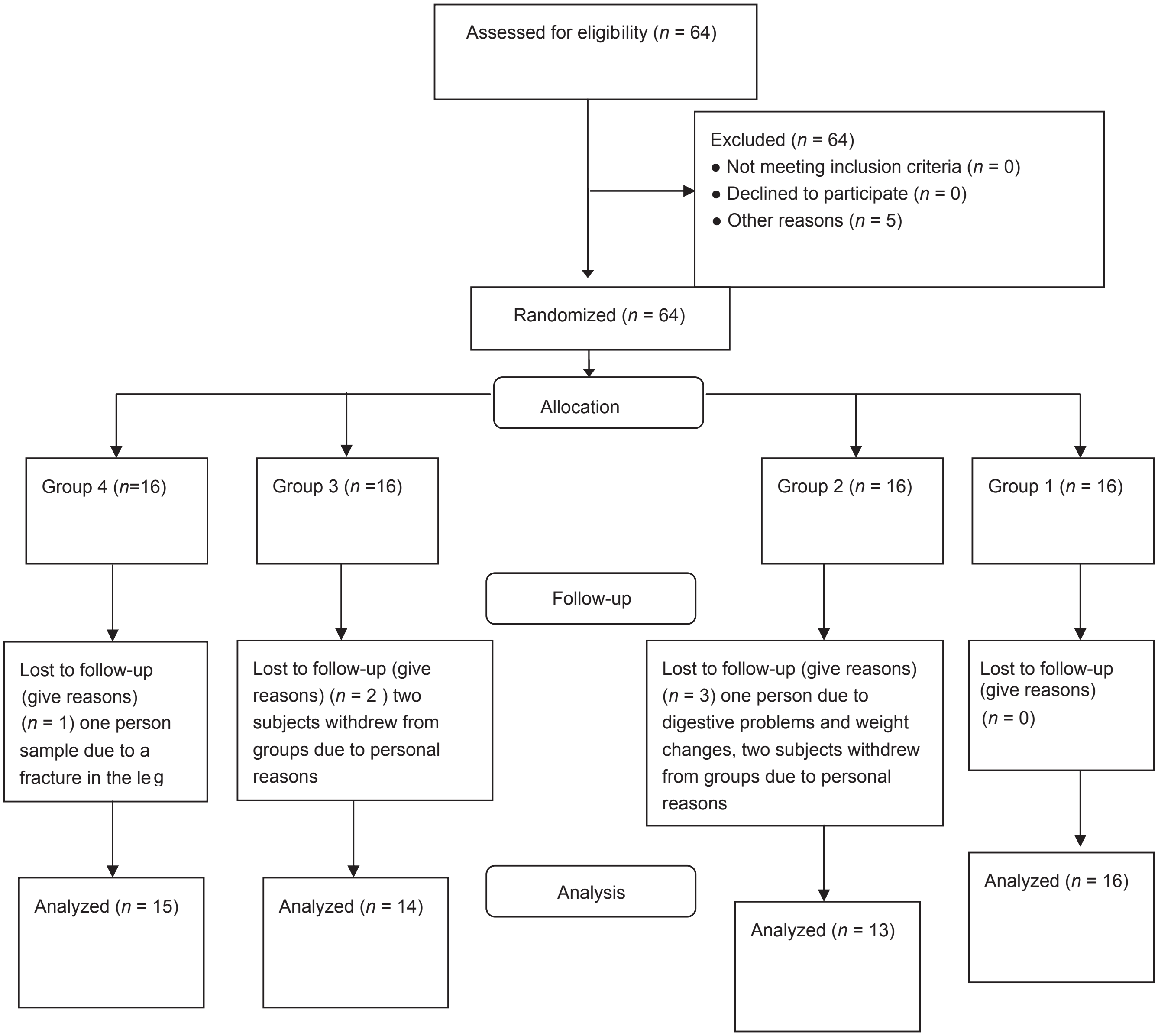

Figure 1: The schematic diagram of the study

more reliable results, so this point was suggested to be considered in further related researches. Future studies were suggested to be conducted with a larger sample size and on other athletes, using different antioxidant supplements.

Generally speaking, the results of this study showed that supplementation with vitamins C and E can only help to reduce muscle damage markers induced by exercise, during aerobics, in women, and considering the fact that the women who participated in this study were in the childbearing age, it seems that the use of supplements for athletes in prolonged and intense exercises are especially useful for female athletes. According to the results, the supplements are recommended for this group of athletes.

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Source of Support: This study was conducted as a thesis funded by Isfahan University of Medical Sciences, Isfahan, Iran, **Conflict of Interest:** None declared.