

Effects of four weeks supplementation of vitamin C on total antioxidant capacity and malondialdehyde among inactive men after an eccentric exercise

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ABSTRACT

Purpose: The aim of this study was to investigate the effect of four weeks supplementation of vitamin C on serum levels of total antioxidant capacity and malondialdehyde among inactive men after an eccentric exercise.

Materials and Methods: Twenty non-athletic healthy volunteer men (24 ± 1.6 years old, 22.59 ± 2.62 body fat percentage and 48.96 ± 3.58 mL/kg/min maximum oxygen uptake) were divided into two randomized groups, i.e. vitamin C (1000mg/day) and placebo groups. Four participants left the study before completion. After four weeks of supplementation, all participants participated in an intermittent aerobic exercise on a treadmill with 80% oxygen consumption (the negative slope of 10 degrees) and ran for 45 minutes. The first blood sample was taken before supplementation, a second blood sample after the end of supplementation period and the third sample immediately after the exercise. Normalized data were analyzed using repeated measures by Bonferroni t-test. Analysis was done using for (SPSS) software version 21.

Results: The capacity for total antioxidant ($P = .001$) and malondialdehyde ($P = .001$) in sera were significant. Four weeks of vitamin C supplementation also significantly increased the basic total antioxidant capacity. In addition, vitamin C was able to increase the antioxidant capacity after exercise.

Conclusion: Supplementation of vitamin C can increase the basic total antioxidant capacity. Adverse changes in oxidative stress-induced activities can reduce damages by aerobic exercises in non-athletic men.

Keywords: antioxidant capacity; lipid oxidation; vitamin C; eccentric exercises; inactive men.

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INTRODUCTION

Nowadays, researchers believe that regular participation in vigorous aerobic activity can be quite effective in improving the health and aerobic capacity for athletes or even people who are suffering from cardiovascular diseases.^{1,2} Doing these activities may release more free bases. Reduction of endogenous resources undermines the endogenous antioxidant capacity and increases oxidative damage to biological macromolecules such as lipids

membrane, malondialdehyde, proteins and nucleic acids.^{3,4} An eccentric muscle contraction, on the other hand, is the stretching of a muscle in response to an opposing force on that muscle, in which the opposing force (weight being lifted) is greater than its current force production.⁵

Herzog and colleagues have shown that when the filaments of a muscle fiber are stretched while contracting (i.e. doing an eccentric contraction), there may be a decreased rate of cross-bridge detachments (thus an

increased percentage of cross-bridges remain attached), leading to greater force production on the eccentric bout. In addition, they have stated there is an increase in the stiffness of the titin protein during the eccentric contraction. Titin adds a passive (i.e. a tautness) force enhancement to the muscle's force productions while being lengthened (under load). Herzog and colleagues speculated that other, not fully elucidated, metabolic force enhancement changes in the sarcomere also occur during eccentric muscle actions. Examples of eccentric muscle contractions are walking down a hill, or resisting the force of gravity while lowering a weight or object. Eccentric actions place a stretch on the sarcomere to the point at which the filaments may experience sarcomere strain, or damage called exercise-induced-delayed-onset muscle soreness.⁶

A study on Thirty and six animals were divided in control; eccentric exercise (EE); EE + saline gel 0.9%; EE + Thermoplastic polyurethane (TPU) 0.8 W/cm²; EE + Dimethyl sulfoxide (DMSO) gel; EE + TPU + DMSO gel and submitted to one 90-min downhill run (1.0 km h⁻¹). TPU was used 2, 12, 24, 46 h after exercise session and 48 h after the animals were killed and the gastrocnemius muscles were surgically removed. Production of superoxide anion, creatine kinase (CK) levels, lipoperoxidation, carbonylation, and antioxidants enzymes were analyzed. Showed that TPU and gel-DMSO improved muscle healing. Moreover, superoxide anion production, TBARS level and protein carbonyls levels, superoxide dismutase and catalase activity were all decreased in the group TPU plus gel-DMSO. Our results show that DMSO is effective in the reduction of the muscular lesion and in the oxidative stress after eccentric exercise only when used with TPU.⁷

This study describes in detail two standardised protocols for the treadmill exercise of mdx mice and profiles changes in molecular and cellular events after a single 30 min treadmill session (Protocol A) or after 4 weeks of (twice weekly) treadmill exercise (Protocol B). Both treadmill protocols increased multiple markers of muscle damage. We conclude that a single 30 min treadmill exercise session is a sufficient and conveniently fast screening test and could be used in 'proof-of-concept' studies to evaluate the benefits of pre-clinical drugs in vivo. Myofibre necrosis, blood serum CK and oxidative stress (specifically the ratio of oxidised to reduced protein thiols) are reliable markers of muscle damage after exercise; many parameters demonstrated high biological variation including changes in mRNA levels for key inflammatory cytokines in muscle. The sampling

(sacrifice and tissue collection) time after exercise for these parameters is critical. A more precise understanding of the changes in dystrophic muscle after exercise aims to identify biomarkers and new potential therapeutic drug targets for Duchenne Muscular Dystrophy.⁸

On the other hand, a way to cope with the adverse effects of oxidative stress caused by intense exercise is the heavy use of natural supplements and oral antioxidation.^{5,9,10} In this respect the beneficial effects of vitamin C can be an oral antioxidant, eliminating free base hydroxyl foundation.^{11,12} Thus, consuming 1,000 milligrams of vitamin C and 400 international units of vitamin E for four weeks increases antioxidant capacity in the base case and can prevent antioxidant capacity loss because of exercise.¹³ In another study, 12 weeks of vitamin E consumption did not change malondialdehyde level after 45 minutes of treadmill running at 75% maximum oxygen consumption with a negative slope of 16.⁵

Therefore, given the conflicting results of antioxidants supplementation, the present study was carried out to investigate the effects of four weeks acute vitamin C supplementation on total antioxidant capacity and malondialdehyde of non-athletic men after an eccentric exercise.

MATERIALS AND METHODS

This was a quasi-experimental research with the two groups (experimental and control) by repeated measures (three-phase) which was conducted double-blind. The population consisted of healthy non-smoking, non-athletic male students of Tabriz University. None of the participants did regular exercises and had not used any additives and drugs in the past six months before participating in the study. From among 50 volunteers, 20 were eligible to participate in the research. Coordinating volunteers explained the objectives of the study to all participants and all participants signed an informed consent form. They also filled a health questionnaire and a 24-hour dietary recall form. Medical examinations were also done. Two weeks before the study, the antropometric indicators (body clock), height, weight and body fat percentage of participants were measured using a Japanese Mikusha skin caliper thickness gauge (Skinfold Calipers) and three-point formula of American College of Sports Medicine (brachial triceps skin folds, abdominal and iliac above right). The thickness of the skin pin and measuring three points of the body were placed in the following formula.¹⁴

Percentage of fat = (0.39287)* (all three parts) - 0.00105* (all three parts)² + [0.15772*(age)] - 5.18845

Maximum oxygen consumption test was determined by Bruce on a treadmill (Teknojim, Italy) using the following formula.¹⁴

$$VO_2\text{max (mL/kg/min)} = 14.76 - (1.379 \times (\text{time})) + (0.451 \times (\text{time})^2) - (0.012 \times (\text{time})^3)$$

Then, the 20 participants with a mean age of 24 ± 1.6 years old, $22.59\% \pm 2.62\%$ body fat and 48.96 ± 3.58 mL/kg/min maximum oxygen consumption were randomly divided into two equal groups. One group received vitamin C (1000 mg daily to 500 mg effervescent tablets twice daily) and the other placebo.¹⁵ Vitamin C was prepared from Pharmaceutical Co., Tehran products with Sath License No. 10103/11. Also, for reminding the participants of consuming vitamin C a dietary survey questionnaire was used during the 24-hour study period.¹⁶ Initial blood samples were collected at baseline until 24 hours before the start of supplementation from the elbow of the right arm in the morning in our center.

After a four-week period of vitamin C consumption, a second blood sample was taken 12 hours after the last supplementation at 8-9 am in our center and before the eccentric workout. All the participants ran a distance for 30-minute after the general warm-up, stretching and exercise using a treadmill. Then they ran in nine 5-minute periods with 2 minutes rest between each period. The slope was set at 10 degrees in running periods and zero degrees in resting times. They had 80% of maximum oxygen consumption during running and three blood samples were taken after immediately the exercise.⁵

Sampling

Five milliliters of blood was taken in the sitting position and from the right elbow from a vein in tubes without spilled anticoagulant substance. Serum samples were incubated for 10 min at room temperature and the head of the clot was prepared by centrifugation at 3000 rpm for 10 minutes. The serum was separated from clot. Samples were stored in the freezer until tests in -20 degrees. All the samples were taken in the same conditions (8-9 am, $26-28^\circ\text{C}$ temperature and 50% humidity). In addition, the participants did not do heavy physical activity and had the same meals 48 hours before the study.

Laboratory measurements

Serum total antioxidant capacity was measured using Randox assay kits (Randox Diagnostics, UK) with an automated analyzer with product number MX2332 apparatus (Model Alcyon 300 Abbott, America and Germany jointly) at a wave length of 600 nm. Reaction with malondialdehyde serum levels based on thiobarbituric acid and 532 nm was determined using spectrophotometry.¹⁴ This method was based on the attack of free lipids, i.e. a variety of aldehydes, including malondialdehyde due to thiobarbituric acid at acidic pH and high temperature reaction. The maximum complex absorption was pink at 532 nm.

Statistical analysis

Normal distribution of the data was investigated using the Kolmogorov-Smirnov test. Since the distribution of data was normal, the data were described by mean and standard deviation. Then any change of the studied parameters during different stages of the repeated analysis of variance (ANOVA) and post hoc Bonferroni test were analyzed. Differences between the groups were determined using independent t-test. All statistical analyses were done using statistical package for social sciences (SPSS) software version 21 and Excel 2013.

RESULTS

A number of 20 participants participated in this study (two 10-participant groups). Four participants were excluded from the study because of their illness, leaving eight participants for each group. The mean and standard deviation of individual characteristics shown in the table below.

The two groups were very similar in terms of physiological and anthropometric indices. (**Table 1**)

The total antioxidant capacity for the base state in the group receiving the vitamin C after four weeks significantly increased ($P = 0.001$), while significant changes did not occur in other variables ($P = 1.000$) (**Table 2**). In addition, total antioxidant capacity decreased in the vitamin C group after eccentric activity which was significantly lower than the placebo group ($P = .04$). However, increase of malondialdehyde level was significantly lower in the vitamin C group than the

Table 1. The mean and standard deviation of individual characteristics.

group	Age (old)	Weight (kg)	Height (cm)	Body Mass Index (kg/m ²)	Body Fat (point)	Aerobic Capacity (mmol/kg/min)
Vitamin C	23.63 ± 1.3	72.12 ± 4.51	179.25 ± 6.71	22.48 ± 1.63	22.67 ± 7.4	48.03 ± 3.16
Placebo	24.38 ± 1.92	71.75 ± 8.25	170.5 ± 3.29	24.7 ± 3.03	22.46 ± 7.29	49.89 ± 3.94

Table 2. Mean and standard deviation for each group and measured indicators.

Blood levels Indicators	Groups	Basic Steps	Placed before Exercise	Step into the Sport
Malondialdehyde (nmol/L)	Vitamin C	19.1 ± 0.22	99.1 ± 0.12	91.2 ± 0.15 ^{†*}
	Placebo	76.1 ± 0.23	87.1 ± 0.22	36.2 ± 0.2 [*]
Antioxidant capacity (mmol/L)	Vitamin C	1.96 ± 0.08	2.41 ± 0.16 ^{**}	2.59 ± 0.43 ^{†*}
	Placebo	1.91 ± 0.22	1.99 ± 0.12	2.19 ± 0.15 [*]

*Significantly ($P < .05$)

**Significantly ($P < .001$)

[†]Significant supplement group compared to placebo ($P < .05$).

placebo group ($P = .01$). Analysis of variance (ANOVA) suggested that both oxidative vitamin C supplementation loading and eccentric activities affect malondialdehyde changes (**Table 3**).

Bonferroni test results showed that weekly supplementation of vitamin C in the base state for four weeks significantly increased total antioxidant capacity (**Table 4**). Also, decreasing amplitude of total antioxidant capacity in each groups receiving vitamin C after a 45 minute eccentric exercise was greater than the placebo group (**Table 2**). On the other hand, vitamin C supplementation for four weeks has more effect on total antioxidant capacity than the blood serum.

Also, the Bonferroni test showed that vitamin C supplementation has no significant effect on basal malondialdehyde. Thus, vitamin C supplements can cause significant changes in blood malondialdehyde in the base state. However, the increase in malondialdehyde was significant after 45 minutes of an eccentric training in both groups (**Table 4**). Still, changes in the scope of malondialdehyde in the group receiving vitamin C after 45 minutes of an eccentric training was significantly lower than the placebo group (**Table 2**). In other words, its effect on blood malondialdehyde changes is more than its differences between the studied groups.

Table 3. Results of ANOVA for repeated antioxidant capacity and malondialdehyde changes in vitamin C and placebo groups.

Indicators	Source Change	Sum of Squares of Deviation from the Mean	degrees of freedom (df)	Mean Square Deviation	F	P Value	Of Chi Eta [*]
Antioxidant capacity (nmol/L)	Process of measuring	1.80 [*]	2	0.90	29.35	0.001	0.67
	Of group differences	0.48 [*]	1	0.48	4.94	0.04	0.26
	the measurement of the mass difference	0.26 [*]	2	0.13	4.36	0.02	0.23
	Error of the group	0.86	28	0.03			
	Error of the group	1.35	14	0.09			
Malondialdehyde (nmol/L)	Process of measuring	6.59 [*]	2	3.29	169.7	0.001	0.92
	Of group differences	0.99 [*]	1	0.99	7.7	0.01	0.35
	the measurement of the mass difference	0.18 [*]	2	0.09	4.71	0.01	0.25
	Error of the group	0.54	28	0.01			
	Error of the group	1.8	14	0.12			

*Indicates significant at 5% level.

**The coefficient for the variable efficacy of our shows.

Table 4. Bonferroni hoc test results for the group of Malondialdehyde and total antioxidant capacity.

Indicators	Group	Steps	Steps	The Difference between the two-step	Deviation Error	P Value
Antioxidant capacity (nmol/L)	Vitamin C	Basic Steps	Before Exercise	0.18 [*]	0.04	0.01
		Before Exercise	After Exercise	0.45 [*]	0.11	0.01
	Placebo	Basic Steps	Before Exercise	-0.07	0.04	0.79
		Before Exercise	After Exercise	-0.2 [*]	0.05	0.03
Malondialdehyde (nmol/L)	Vitamin	Basic Steps	Before Exercise	-0.02	0.03	1
		Before Exercise	After Exercise	-0.64 [*]	0.1	0.001
	Placebo	Basic Steps	Before Exercise	-0.11	0.01	0.001
		Before Exercise	After Exercise	-0.85 [*]	0.04	0.001

*Indicates significant at 5% level.

DISCUSSION

Our findings suggest that four weeks of vitamin C supplementation significantly increases the total antioxidant capacity in the base case. In addition, vitamin C was able to increase the antioxidant after exercise. These results are in consistence with some other studies.^{13,15,17-20} According to Oberbach and colleagues, consuming 1,000 milligrams of vitamin C and 400 international units of vitamin E for four weeks, can prevent decrease of antioxidant capacity in the base case.¹³ Effects of vitamin C supplementation in relation to the proposed mechanism for the increase in total antioxidant capacity is due to the superoxide and hydroxyl radicals which vitamin C directly reacts with. Besides, it converts vitamin E radicals into vitamin E and it changes into dehydroascorbate radical toxicity.¹⁸

Ascorbic acid is the dominant antioxidant in plasma. It removes free bases of the plasma and prevents them from entering into the low density lipoprotein protein.¹⁹ However, this study was to evaluate the effect of exercise of two different cycling intensities on oxidative stress and antioxidant response in trained males. Twenty male trained cyclists participated in this study. The maximal exercise test consisted of an incremental cycling test until voluntary exhaustion, and the submaximal test was a steady state at 75% $\text{VO}_{2\text{max}}$ for 30 min on a cycloergometer. In maximal exercise test (16 ± 4 min of cycling), the results showed short time of high intensity cycling leads to oxidative stress increasing plasma and decreasing erythrocyte vitamin C levels.²¹ Meanwhile, Kang and colleagues showed that in patients with cardiovascular disease, consuming vitamin C (500 mg), vitamin E (402 mg) and beta carotene (20 mg) daily had the best performance in reducing stress oxidative.¹⁹ However, further research shows that vitamin C can increase the incidence of antioxidant and prevents oxidative stress related symptoms such as inflammation.²² Our study confirmed that vitamin C supplementation for four weeks can be effective on serum antioxidant capacity in non-athletic men after eccentric exercise.

Furthermore, this study showed that four weeks of vitamin C supplementation significantly decreased malondialdehyde after a session of eccentric activity in the vitamin C group than in the placebo group. These results are in consistence with some other studies.²³⁻²⁵ However, Block and colleagues showed Nonsmokers (n = 396) Subjects were randomized to 1000 mg/day vitamin C, 800 IU/day vitamin E, or placebo, for 2 months. Treatment effect was examined in multiple regression analyses using an intention-to-treat approach. Vitamin C and vitamin E reduced plasma F2-isoprostanes. In the overall sample, changes from baseline were + 6.8, -10.6, and -3.9% for

placebo, vitamin C, and vitamin E groups, respectively. However, a significant interaction with baseline F2-isoprostane was found. When baseline F2-isoprostane was >50 µg/mL, vitamin C reduced F2-isoprostane by 22%. Vitamin E reduced it by 9.8% (P=0.46). Below that cut point, neither treatment produced further reductions. F2-isoprostane >50 µg/mL was strongly associated with obesity, and was present in 42% of the sample. Change in malondialdehyde concentration was minimal. These findings suggest a role for vitamin C in reducing lipid peroxidation.²⁷ These findings are inconsistent with some other studies.^{5,13,26-30} Bloomer and colleagues have noted that using 1,000 milligrams of vitamin C per day for two weeks after 2.5 hours on the bike with an intensity of 60% maximum oxygen consumption has significant changes in malondialdehyde level. In other words, both groups saw an increase but the difference might be because of duration of vitamin C loading time.³⁰ Another study showed that after 45 minutes of treadmill running with 75% oxygen uptake in the negative slope of 16 after 12 weeks of vitamin E consumption, does not change the amount of malondialdehyde.³⁰ This difference could be due to the type of supplement. Thus, four weeks vitamin C supplementation can increase malondialdehyde levels of non-athletic men after eccentric exercise and can increase amplitude changes of serum malondialdehyde (indicator of oxidative damage).

CONCLUSION

Four-week supplementation with vitamin C can enhance the antioxidant base case of unfavorable changes in oxidative stress and inflammatory markers after eccentric exercise to prevent extreme consequences. Hence, taking into account aspects of care can be offered to non-athletes to prevent the loss of antioxidative capacity and the incidence of severe eccentric exercise-induced oxidative stress resulting in inflammatory activity of vitamin C supplementation use.

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