

# Protective Role of Vitamin C Intake on Muscle Damage in Male Adolescents Performing Strenuous Physical Activity

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## Abstract

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**BACKGROUND:** Strenuous non-regular exercise increases reactive oxygen species ROS level leading to an impaired balance between the endogenous antioxidant defence system and the free radicals production. Antioxidants intake can detoxify the peroxides produced during exercise, attenuating the inflammatory responses and therefore may prevent exercise-induced muscle damage.

**AIM:** The purpose of this study was to determine the role of vitamin C intake in attenuating markers of muscle damage, oxidative stress and inflammatory responses in male adolescents performing the non-regular strenuous exercise.

**MATERIAL AND METHODS:** Twenty recreationally active male adolescents were assigned to participate in the study. Eligible subjects performed strenuous recreational exercise (2-3 times per week) were randomly divided into two groups: The vitamin C (VC) group that consumed 500 mg of capsulated vitamin C after breakfast for a period of 90 days and the placebo (PL) group that consumed identical capsules in form and aspect that contained 500 mg of maltodextrin for the same period. Aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) were assessed for muscle damage. Malondialdehyde (MDA) was evaluated as a marker of lipid peroxidation. Plasma creatinine, uric acid and urea were determined to monitor kidney function. C-reactive protein, a marker of systemic inflammation was also measured.

**RESULTS:** In comparison between PL and VC groups, the plasma concentrations of muscle damage markers, oxidative stress markers, kidney function and inflammatory markers showed no significant difference in their baseline values ( $P > 0.05$ ). The plasma concentrations of CK, LDH, MDA, urea, uric acid and CRP were significantly decreased in the VC group ( $P < 0.05$ ) as compared to their values before the intake of vitamin C.

**CONCLUSION:** The present results support the intake of vitamin C as an antioxidant for attenuating exercise-induced muscle damage, oxidative stress and inflammatory markers in male adolescents performing the strenuous physical activity.

## Introduction

A growing amount of evidence indicates that regular and chronic exercises produce physiological adaptations and enhance the endogenous antioxidant system, minimise the oxidative stress and therefore protect the body against adverse effects of oxidative damage that may occur after an acute bout of exercise [1]. However, strenuous non-regular exercise increases reactive oxygen species ROS level leading to an impaired balance between the endogenous antioxidant defence system and the free radicals production [2]. This disturbance in cellular homeostasis induces oxidation of cellular

macromolecules such as proteins, lipids and DNA in the contracting muscles, and then lipid peroxidation occurs leading to muscle damage [3]. It has been postulated that the generation of reactive oxygen species occurs mainly by contracting skeletal muscles, inflammatory processes and increased the release of catecholamine [4]. The antioxidant vitamin supplementation effect on detoxifying peroxides produced during exercise has been given a special focus in recent years. It has been suggested that the endogenous antioxidant systems within tissue cells in subjects under strenuous activities are not able to maintain optimal tissue levels of antioxidant vitamins, even if they consume the recommended daily allowances in their diet [5]. Antioxidants

supplementation can detoxify the peroxides produced during exercise as they are capable of scavenging peroxy radicals and attenuating the inflammatory responses to exercise and may, therefore, prevent exercise-induced muscle damage [6].

The purpose of the present study is to determine the effectiveness of the vitamin C intake in attenuating muscle damage, oxidative stress and inflammatory markers, in male adolescents performing the non-regular strenuous exercise. Our hypothesis is that muscle damage, and oxidative stress is likely to be worse following strenuous exercise in the placebo group. The extent of these changes could be reduced for the group receiving vitamin C.

## Materials and Methods

Twenty recreationally active male adolescents, at a mean age of  $19 \pm 0.5$  years,  $68.9 \pm 7.3$  kg weight,  $178.9 \pm 4.6$  cm height and body mass index (BMI) of  $24.3 \pm 0.6$ , were assigned to participate in the study. Eligible subjects performed the strenuous recreational exercise (2-3 times per week) and considered healthy without a history of the disease or medication use. They reported that they had not engaged in any intense training, including eccentric contractions in the 3 months before the experiment. They completed a medical history, diet and supplementation history, and a physical activity questionnaire to determine eligibility. All subjects were non-vegetarian, non-smokers, nor did they use anti-inflammatory drugs, or antioxidant supplements three months before the study period. Subjects were informed about all procedures and possible risks involved, and they wrote consent to participate in the study at the National Research Centre. The study was carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt. All participants were instructed to maintain their normal diet during the study period.

The 20 subjects were randomly divided into two groups: Vitamin C (VC) group ( $n = 10$ , age:  $19.0 \pm 0.2$  years, height:  $179.6 \pm 6.2$  cm, weight:  $66.7 \pm 7.1$  kg), and placebo (PL) group ( $n = 10$ , age:  $19.3 \pm 0.5$  years, height:  $178.6 \pm 3.9$  cm, weight:  $67.3 \pm 7.9$  kg). The VC group consumed 500 mg of vitamin C packed in the hard gelatinous capsule (C-Retard, slow-release ascorbic acid Hekma Pharma, Egypt) once a day after breakfast for 90 days. The PL group consumed identical capsules in the form and aspect that contained 500mg of maltodextrin for the same period.

Venous blood samples were collected from all subjects at morning in heparinised tubes, at baseline and after 90 days of vitamin C intake after performing strenuous exercise. Blood was centrifuged for 15

minutes at 3000rpm, and plasma was stored at  $-70^{\circ}\text{C}$  until assays were conducted. Aspartate aminotransferase (AST) was determined according to the method of Reitman and Frankel [7], Creatine kinase (CK) was determined using the colourimetric method [8], and Lactate dehydrogenase (LDH) was measured using the colourimetric enzymatic assay [9]. Malondialdehyde (MDA) was determined as an indicator of lipid peroxidation by the thiobarbituric acid (TBA) method [10]. Plasma creatinine [11], uric acid [12] and urea [13] were determined to monitor kidney function. C-reactive protein, a marker of systemic inflammation was determined by the method of Saxtad et al., [14].

The data was analysed statistically using Statistical Package for the Social Sciences SPSS software for Windows (SPSS Inc., Chicago, IL, version 17.0) was used for the statistical analysis. Results are expressed as mean  $\pm$  SEM. The changes in VC and PL groups before and after the intake were tested by using the Student's t-test.  $P$ -Value  $< 0.05$  indicated a statistically significant difference for all tests.

## Results

The changes in plasma markers of muscle damage and oxidative stress of male adolescents in response to vitamin C intake are shown in Table 1. In comparison between PL and VC groups, the plasma concentrations of muscle damage markers, oxidative stress markers, kidney function and inflammatory markers showed no significant difference in their baseline values ( $P > 0.05$ ). No significant change was shown in the plasma concentrations of AST, CK, LDH and plasma MDA in the PL group ( $P > 0.05$ ). In VC group, the plasma concentrations of CK and LDH were significantly decreased by 34.6 % and 16.25% respectively ( $P < 0.05$ ) as compared to their values before the intake of vitamin C. Plasma concentration of AST showed no significant change ( $P > 0.05$ ). A significant decrease in plasma MDA was observed in the VC group by 50.9% as compared to its value before the intake of vitamin C ( $P < 0.05$ ).

**Table 1: Indicators of muscle damage and oxidative stress**

	Groups							
	PL group			P value	VC group			
	Before-intake	After-intake	% change		Before-intake	After-intake	% change	P value
AST (U/L)	24.2 $\pm$ 0.6	23.9 $\pm$ 1.3	-1.2	0.858	26.3 $\pm$ 0.7	22.5 $\pm$ 1.3	-14.4	0.026
LDH (U/L)	204.9 $\pm$ 5.2	217.4 $\pm$ 12.1	6.1	0.382	202.4 $\pm$ 5.6	169.5 $\pm$ 9.4*	-16.3	0.013
CK (U/L)	227 $\pm$ 5.8	234.8 $\pm$ 13.1	3.4	0.611	212.3 $\pm$ 11.8	138.8 $\pm$ 7.7**	-34.6	0.001
MDA ( $\mu$ ML)	5.4 $\pm$ 0.1	5.8 $\pm$ 0.3	7.4	0.224	5.7 $\pm$ 0.1	2.8 $\pm$ 0.2**	-50.9	0.001

Values are expressed as Mean  $\pm$  S.E.M. \*significantly different within VC group ( $p < 0.05$ ), \*\* high significant difference within VC group ( $p < 0.01$ ). AST = Aspartate transaminase, LDH= Lactate dehydrogenase, CK= Creatine kinase, MDA= Malondialdehyde.

As shown in Table 2, the plasma concentrations of creatinine, urea and uric acid

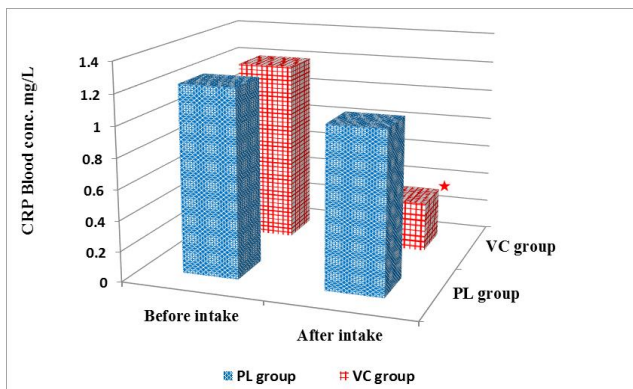
showed no significant difference in their baseline values ( $P > 0.05$ ) in the comparison between PL and VC groups. In the PL group, no significant difference was shown in the plasma concentrations of creatinine, urea and uric acid ( $P > 0.05$ ). In VC group, the plasma concentrations of urea and uric acid were significantly decreased by 18.5% and 8.8% ( $P < 0.05$ ) respectively as compared to their values before the intake of vitamin C, while plasma concentration of plasma creatinine showed no significant change ( $P > 0.05$ ).

**Table 2: Effect of vitamin C intake on kidney function**

Groups	Creatinine	Urea	Uric acid
PL group			
Before-intake	1.1 ± 0.0	30.5 ± 0.8	5.3 ± 0.1
After- intake	1.0 ± 0.1	31.6 ± 1.8	5.6 ± 0.1
% change	-9.1	3.6	5.7
P value	0.361	0.573	0.059
VC group			
Before-intake	1.1 ± 0.0	27.5 ± 2.1	5.7 ± 0.2
After- intake	0.9 ± 0.1	22.4 ± 0.9*	5.2 ± 0.1*
% change	-18.2	-18.5	8.8
P value	0.087	0.016	0.013

Values are expressed as Mean + S.E.M. \* significantly different within the VC group ( $p < 0.05$ ).

As shown in Figure 1, the plasma concentration of CRP showed no significant difference in their baseline values ( $P > 0.05$ ) in the comparison between the PL and VC groups. In the VC group, there was a significant decrease in CRP plasma concentration by 62.6% ( $P < 0.05$ ) as compared to its value before the intake of vitamin C.



**Figure 1: C-reactive protein concentration in PL group and VC group following strenuous exercise in male adolescents. \*Significantly different within VC group ( $p < 0.05$ )**

## Discussion

The purpose of this study was to investigate the effect of vitamin C intake on exercise-induced muscle damage, oxidative stress and inflammatory markers in male adolescents after performing the strenuous physical activity. Initially, a bout of vigorous non-regular exercise results in muscle damage through an inflammatory response mediated by phagocytosis and a respiratory burst of neutrophils

leading to the release of ROS [15]. Other studies have shown that ROS are incriminated in the aetiology of muscle damage by causing oxidation of the ion transporting systems [16]. Furthermore, Lipid peroxidation in which free radicals "steal" electrons from the lipids in cell membranes may lead to the release of muscle components such as CK and LDH [17]. Myofibrillar proteins such as CK and LDH are often used as indicators of muscle damage [18] as plasma CK levels alone may not provide a fully accurate reflection of structural damage to muscle cells [19]. Many studies have used plasma malonaldehyde as a measure of oxidative stress caused by exercise. In a previous study, serum MDA has been reported to be increased following a bout of intense exercise [20]. There is a confirming role of exercise in the production of free radicals and subsequent oxidative stress by the significant increase in serum MDA levels about both acute and regular exercise [21].

In the present study, plasma MDA was measured as a marker of lipid peroxidation, as well as the plasma AST, CK and LDH levels were measured as muscle damage markers. Strenuous exercise induced an increase in post-exercise plasma CK, LDH and MDA at baseline values in PL and VC groups, which indicate some degree of myofibre damage [22]. Such results also showed that taking vitamin C reduced the oxidative stress and muscle damage markers through a significant reduction in post-exercise plasma, CK, LDH and MDA in the VC group as it inhibits lipid peroxidation. The potential reason for this phenomenon may be due to the decrease of membrane permeability and reducing the escape of constituents such as CK and LDH through inhibition of lipid peroxidation. This could be due to the antioxidant supplementation [23]. Vitamin C is a potent reducing agent, due to its facility in donating electrons, and has an important antioxidant property [24]. Vitamin C intake significantly blunted the post-exercise high plasma MDA level as it reduces the production of ROS and oxidative stress inflicted by exercise-induced muscle damage. Vitamin C inactivates a variety of reactive species and minimises the damage of body tissues [25].

CRP was measured in blood as an inflammatory marker. In the placebo group, no significant change was shown in post-exercise CRP level, and it was within the normal range. Some studies have shown a slight increase in CRP level after strenuous exercise [26], while in other studies, a decrease in CRP level was observed after prolonged training [27]. Our results are inconsistent with the findings of these previous studies. Differences in serum CRP level after training may be due to the involvement of different mechanisms in the regulation of acute phase responses that may differ from one condition to another [28]. The present study showed that vitamin C intake demonstrated to be efficient in attenuating CRP level. However, in another study of

six weeks, vitamin C intake prevented endurance exercise-induced lipid peroxidation but did not affect inflammatory markers [29].

Furthermore, our study showed that vitamin C intake showed a significant reduction in plasma uric acid levels. A previous study has described mechanisms by which vitamin C reduces serum uric acid SUA and suggested that vitamin C has uricosuric properties, therefore lowering SUA level by inhibiting uric acid synthesis [30]. Vitamin C may act specifically at uric acid reabsorption sites in the apical brush border of the proximal tubule. It is also possible that vitamin C increases the dilatation of afferent arterioles, reduces glomerular microvascular ischemia, leading to an increase in the glomerular filtration rate [31]. Vitamin C as a strong antioxidant decreases free radical-induced damage to the body cells [32] leading to a reduction in the SUA production [33].

In conclusion, the present study revealed that vitamin C intake prevented exercise-induced lipid peroxidation which if uncontrolled, it increases the membrane permeability and may lead to the escape of muscle constituents as CK. Vitamin C intake showed an effect on the systemic inflammatory response by reducing the level of inflammatory marker CRP, and a remarkable reduction in plasma uric acid level. Our findings could, therefore, support the use of antioxidants like vitamin C on attenuating exercise-induced muscle damage markers, oxidative stress and inflammatory markers in male adolescents performing the strenuous physical activity.

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