

## Effect of vitamin C on delayed onset muscle soreness indexes

Shahram Foroohi <sup>1\*</sup>

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- (1) Department of Exercise physiology, Shiraz branch,  
Islamic Azad University, Shiraz, Iran.  
(\* ) MS in Exercise Physiology, Department of Exercise  
Physiology, Shiraz branch, Islamic Azad University,  
Shiraz, Iran.  
E-mail: foroohi.sh@yahoo.com

### Abstract

*Introduction:* Delayed onset muscle soreness (DOMS) refers to the skeletal muscle pain that is experienced following eccentric exercise. The aim of the present study was to examine the effect of vitamin C on DOMS after an eccentric exercise.

*Material & Methods:* Twenty healthy male age 21 to 24 years participated as subjects in this study. The subjects were assigned to either an experimental (3500 mg of vitamin C; n=10) or a placebo group (Same dosage of juice powder; n=10) using a double-blind research design. Knee range of motion (ROM), perceived pain and serum activity of the enzyme creatine kinase (CK), Lactate dehydrogenase (LDH) and C reactive protein (CRP) were taken before, immediately and 48 hours after the eccentric exercise.

*Results:* Lactate dehydrogenase, creatine kinase, CRP and pain increased ( $P < 0.05$ ) and knee ROM decreased ( $P < 0.05$ ) in the both groups immediately after the eccentric exercise. The vitamin C supplementation decreased the pain and CRP levels compare to the placebo group at 48 hours after the eccentric exercise ( $P < 0.05$ ) but it has not effective to improve LDH, CK and knee ROM.

*Conclusion:* In conclusion, further studies are needed to examine the effects of vitamin C supplementation on DOMS induced by eccentric exercise.

**Key words:** DOMS, LDH, CK, CRP, Range of motion, Vitamin C

## 1. Introduction

DOMS is the sensation of muscular discomfort and pain during active contractions that occurs in a delayed fashion after strenuous exercise (1). Subjects with DOMS have painful, tender, and swollen muscles with reduced range of motion of adjacent joints especially after unaccustomed exercise (2, 3). In addition to muscle tenderness with palpation, prolonged strength loss and a reduced range of motion are observed (4). These symptoms develop 24 to 48 hours after exercise, and they disappear within 5 to 7 days (2, 3). The pathophysiology of DOMS remains still undetermined, but it has been reported that after strenuous exercise muscle cell damage and inflammatory cells are observed in damaged muscle (5).

Although DOMS is not a serious condition and can be prevented by prior training (6), it may discourage further participation in exercise, and it is possible that muscle injury may result if heavy exercise is performed during the period of muscle weakness. No simple effective treatment is as yet available for DOMS. There is some evidence that DOMS may result from an inflammatory process which occurs in the muscle after exercise (7). If this is the case, then it is possible that antioxidant vitamins such as vitamin C may be effective in reducing DOMS and muscle damage. By our knowledge, the effects of vitamin C

on DOMS are not well known. Close et al (2006) indicated that vitamin C supplementation improves knee range of motion but it has not effective to improve the pain (8). Connolly et al. (2006) also demonstrated that vitamin C supplementation has not effective to improve the eccentric exercise induced- DOMS (9). The aim of the study was to test the effects of vitamin C supplementation on muscle soreness, muscle damage inferred from plasma enzyme activity changes and pain.

## 2. Material & Methods

### *Subjects*

Twenty healthy male with a mean ( $\pm$ SD) age of  $22.7\pm 0.7$  year, volunteered to participate in this study. The subjects were given both verbal and written instructions outlining the experimental procedure, and written informed consent was obtained. The study was approved by the Marvdasht branch, Islamic Azad University Ethics Committee. The subjects were assigned to either an experimental (3500 mg of vitamin C;  $n=10$ ) or a placebo group (Same dosage of juice powder;  $n=10$ ) using a double-blind research design. The subjects took 500 mg vitamin C or placebo in each day for a week before the eccentric exercise.

### *Anthropometric and body composition*

Height, weight and lower leg length were measured, and body mass index (BMI) was calculated by dividing weight (kg) by height ( $m^2$ ). Waist circumference was determined by obtaining the minimum circumference (narrowest part of the torso, above the umbilicus) and the maximum hip circumference while standing with their heels together. The waist to hip ratio (WHR) was calculated by dividing waist by hip circumference (cm).

### *Range of motion (ROM)*

Knee ROM was determined in the supine position, with the knee in full extension by using a Jamar goniometer. The stationary arm of the goniometer was aligned with the lateral midline of the thigh, using the greater trochanter as a reference point. The fulcrum was placed over the lateral epicondyle of the femur. The moving arm was aligned with the lateral of the fibula, using the lateral malleolus as a reference point.

Knee flexion was recorded as the movement of the lower leg from the neutral position to a position in which the lower leg and heel are maximally drawn toward the buttocks (10).

#### *Eccentric exercise training*

Subjects stepped up on a bench set at 110% of their lower leg length. Exercise was continued for 10 minutes at a rate of one step per second. The order of steps was right leg up, left leg up, left leg down, right leg down. This regimen causes greatest soreness in the right thigh and left calf. If the subject failed to complete the exercise test, this was recorded.

#### *Nutrition*

Before the beginning of the study, each subject was supervised to continue his normal sport nutrition program. On the testing day the subjects were supervised not to use any sport or dietary supplements. They were supervised also to keep food diaries for seven days in the 2-week period for what they were provided with specific verbal and written instructions and procedures for reporting detailed dietary intake, including how to record portions by using household measures, exact brand names and preparation techniques.

#### *Blood sampling*

Blood samples were taken from an antecubital vein in the sitting position. Ten milliliters blood from a vein was taken pre- and post-exercise, and at 48 hours after the eccentric exercise. Serum was separated and frozen at  $-20^{\circ}\text{C}$  prior to analysis. CK, LDH and CRP were measured using an enzyme-linked immunosorbent assay (ELISA) kits.

#### *Score of muscle soreness and pain*

After the bench stepping exercise, each subject was given an outcome form, which they were asked to complete at specified times, immediately and 48 hours after exercise. The outcome form consisted of a Likert scale as described by High et al (Figure 1; 11). The outcome was the mean score of soreness and pain over the three period.

Please tick the sentence below that best describes your level of muscle soreness over the past 12 hours.

A complete absence of soreness

A light pain felt only when touched/a vague ache

A moderate pain felt only when touched/a slight persistent pain

A light pain when walking up or down stairs

A light pain when walking on a flat surface/painful

A moderate pain, stiffness or weakness when walking/very painful

A severe pain that limits my ability to move

**Figure 1.** Likert scale of muscle soreness (taken from High et al. 1989).

### *Statistical analysis*

Results were expressed as the mean  $\pm$ SD and distributions of all variables were assessed for normality.  $2 \times 3$  repeated measures of ANOVA test was used to evaluate time-course change in variables. Post hoc analyses (Bonferroni) were then performed when warranted. The level of significance in all statistical analyses was set at  $P \leq 0.05$ . Data analysis was performed using SPSS software for windows (version 17, SPSS, Inc., Chicago, IL).

### **3. Results**

Physical and physiological characteristics of the subjects are presented in Table 1. No significant differences in any of variables were observed among the two groups.

**Table 1.** Physical and physiological characteristics (mean  $\pm$  SD) of the subjects

	Vitamin C	Placebo
Age (yr)	22.8 $\pm$ 0.9	22.7 $\pm$ 4.4
Body weight (kg)	71.2 $\pm$ 17.2	73.2 $\pm$ 18.2
BMI (kg/m <sup>2</sup> )	22.5 $\pm$ 5.0	22.8 $\pm$ 5.3
WHR	0.86 $\pm$ 0.05	0.86 $\pm$ 0.08

The knee ROM and the mean score of muscle soreness and pain before and after the eccentric exercise were presented in Table 2 and Table 3.

**Table 2.** knee ROM (mean  $\pm$  SD) of the subjects

		Pre-exercise	Post-exercise	48 h after exercise	
Knee ROM(o)	Placebo	Right leg	131.8 $\pm$ 7.3	130.3 $\pm$ 9.6	130.3 $\pm$ 8.7
		Left leg	132.3 $\pm$ 6.9	132.2 $\pm$ 8.5	130.4 $\pm$ 8.0
	Vitamin C	Right leg	128.8 $\pm$ 11.8	126.6 $\pm$ 11.7	127.4 $\pm$ 11.5
		Left leg	129.3 $\pm$ 11.4	126.5 $\pm$ 11.6	128.3 $\pm$ 11.3

The results showed that knee ROM has not significant change before and after the eccentric exercise and no significant differences were observed among the two groups. The results, also, showed that the mean score of muscle soreness and pain increase after the exercise in both group and the pain decrease in the supplementation group in compare to the placebo group ( $P < 0.05$ ).

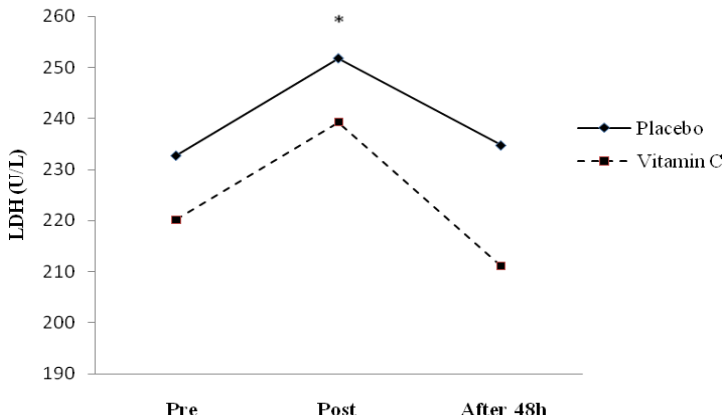
**Table 3.** Muscle soreness and pain (mean  $\pm$  SD) of the subjects

	Pre-exercise	Post-exercise	48 h after exercise
Placebo	3.2 $\pm$ 2.4	4.6 $\pm$ 2.1*	4.7 $\pm$ 1.7*
Vitamin C	1.2 $\pm$ 1.3	2.0 $\pm$ 1.8*†	1.6 $\pm$ 1.5

\*  $P < 0.05$ , pre exercise *vs.* post and 48h after exercise values

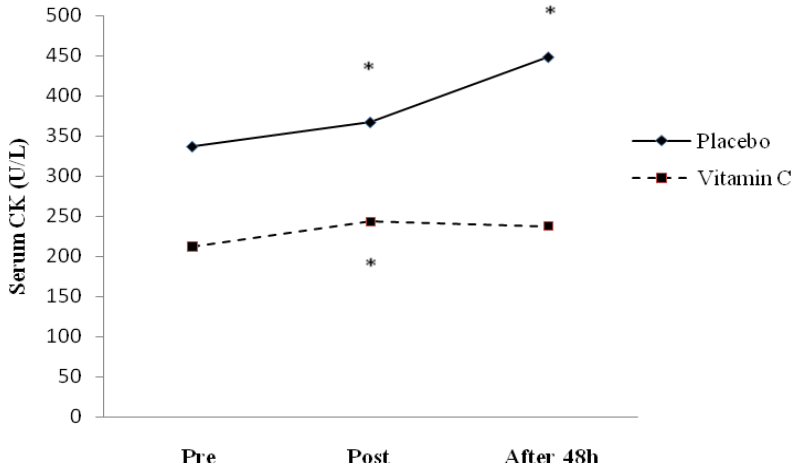
†  $P < 0.05$  for between-group differences

LDH changes during the intervention are presented in Figure 2. The results demonstrated that LDL concentration increase immediately after the eccentric exercise only in the placebo group.



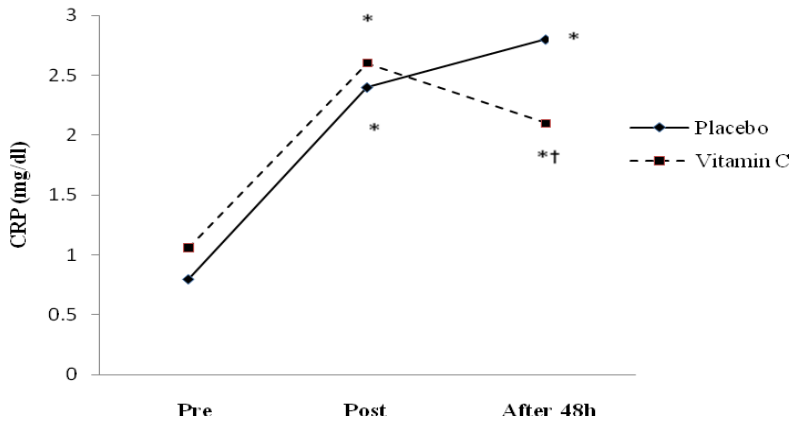
**Figure 2.** LDH levels pre- and post-exercise and 48 hours after the eccentric exercise. (\*  $P < 0.05$ , pre exercise *vs.* post and 48h after exercise values)

The results showed that the serum CK and CRP levels increased in the both groups immediately after the eccentric exercise and increased to maximum at 48 hours after the eccentric exercise ( $P < 0.05$ ; Figure 3,4).



**Figure 3.** Serum CK levels pre- and post-exercise and 48 hours after the eccentric exercise. (\*  $P < 0.05$ , pre exercise *vs.* post and 48h after exercise values)

The vitamin C supplementation decreased ( $P < 0.05$ ) the serum CK and CRP levels compare to the placebo group 48 hours after the eccentric exercise.



**Figure 4.** CRP levels pre- and post-exercise and 48 hours after the eccentric exercise.

\*  $P < 0.05$ , pre exercise *vs.* post and 48h after exercise values

†  $P < 0.05$  for between-group differences

#### 4. Discussion

Exercise that results in the development of soreness is associated with the rapid destruction of muscle tissue (12). DOMS is a familiar experience for the elite or novice athlete. Eccentric activities induce micro-injury at a greater frequency and severity than other types of muscle actions (1). Up to six hypothesized theories have been proposed for the mechanism of DOMS, namely: lactic acid, muscle spasm, connective tissue damage, muscle damage, enzyme efflux and the inflammation theories (5). The inflammation mechanism is not as well understood, but free radical initiated damage or release of pro-inflammatory substances has been implicated. The tissue injury appears to initiate an inflammatory response resulting in a release of cytokines, localized edema due to the migration of monocytes, macrophages, PGE2, histamines, etc., and increased blood flow and tissue permeability. The increase in edema and release of the prostaglandins and histamine may contribute to the pain sensation. The next series of events is the formation of proteases, phospholipases, and free radicals, which may lead to additional muscle tissue breakdown and pain. The last stage of events may result in a protective remodeling of the muscle to prevent muscle soreness with repeated or subsequent exercise (13-15). Therefore, the inflammatory response may be responsible for the initiation, amplification, and/or resolution of the skeletal muscle injury.

A number of treatment strategies have been introduced to help alleviate the severity of DOMS and to restore the maximal function of the muscles as rapidly as possible. There have been some reports that mixed antioxidant supplementation, including vitamin C offer some benefits in terms of lipid peroxidation and muscle damage after exercise (16). Vitamin C is hydrophilic, accumulates in the cytosol and extra-cellular fluid that it is able to protect different cellular compartment.

LDH, CK and CRP have commonly been used as an indicator of muscle injury (17). The results levels increased in the both groups immediately after the eccentric exercise and increased to maximum at 48 hours after the eccentric exercise ( $P < 0.05$ ; Figure 3,4). The vitamin C supplementation decreased the serum CK and CRP levels compare to the placebo group 48 hours after the eccentric exercise ( $P < 0.05$ ). The



results demonstrated that LDL concentration increase immediately after the eccentric exercise only in the placebo group. Other studies also, thought have shown positive effects of vitamin C supplementation on DOMS (18). However, Rahmani-nia et al. (2008) indicated that supplementation for 3 days with vitamin C had no effect on CK (19). It has been suggested that the best measure of muscle damage is not biochemical marker such as CK (20). Differences in the type and duration of exercise performed, duration and kind of vitamin consumed and the fitness of the subjects used maybe important factors in explaining these contrasting results.

Raphael et al. (2007) examined the effect of antioxidant supplementation and repeated bouts of moderate intensity endurance exercise on CK and 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 (21). Bloomer et al. (2004) determined the effects of antioxidant therapy on the indirect markers of muscle damage following eccentric exercise in 18 women. Plasma CK activity, muscle soreness, maximal isometric force, and range of motion 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 muscle soreness, with little impact on maximal isometric force and range of motion loss (22). 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 (22). 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.

On the other hand, the mean score of muscle soreness and pain decreased ( $P < 0.05$ ) in the experimental group compare to the placebo group at 24 and 48 hours after the eccentric exercise. Thus it seems that the aspirin supplementation may effective in decrease the serum CK levels and muscle soreness and pain.

## 5. Conclusion

Eccentric exercise has been shown to produce muscle cellular damage and decrements in motor performance as well. These findings suggested that vitamin C supplementation may effective treatment for DOMS and exercise induced muscle soreness.

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