

Effect of High Dose Vitamin C Supplementation on Muscle Soreness, Damage, Function, and Oxidative Stress to Eccentric Exercise

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This study investigated if vitamin C supplementation before and after eccentric exercise could reduce muscle soreness (MS), oxidative stress, and muscle function. Eighteen healthy men randomly assigned to either a placebo (P) or vitamin C (VC) (3 g/d) treatment group took pills for 2 wk prior and 4 d after performing 70 eccentric elbow extensions with their non-dominant arm. MS increased in both groups with significantly reduced MS for the first 24 h with VC. Range of motion was reduced equally in both groups after the exercise ($P \geq 0.05$). Muscle force declined equally and was unaffected by treatment. VC attenuated the creatine kinase (CK) increase at 48 h after exercise with similar CK after this time. Glutathione ratio (oxidized glutathione/total glutathione) was significantly increased at 4 and 24 h with P but VC prevented this change. These data suggest that vitamin C pretreatment can reduce MS, delay CK increase, and prevent blood glutathione oxidation with little influence on muscle function loss.

Key Words: glutathione, creatine kinase, range of motion

Eccentric exercise or unaccustomed exercise of sufficient intensity and duration can induce muscle damage as evidenced by disruption of sarcomere integrity, loss of muscle proteins, and leakage of muscle proteins out of the cell and can also demonstrate micro trauma to the contractile elements and connective tissue within skeletal muscle (10, 20). Eccentric resistance-type exercise can damage muscle with a resultant force decrement after the exercise (4) which is often accompanied by a loss of range of motion (12). Typically, fewer motor units are recruited for the forces applied to the muscle(s) with eccentric actions which results in a greater strain per fiber. It is believed that eccentric exercise-induced muscle forces increase the strain on the fiber, contributing to muscle damage. Various modes of exercise such as box stepping (15), shuttle running (28, 29, 30), downhill running (31, 34, 35), lowering weights (16), and isokinetic machines with eccentric actions (4, 17) have reported muscle soreness and oxidative stress.

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The mechanisms underlying this damage are not yet fully elucidated, but it appears that the greater forces on the contractile and connective components within the muscle during eccentric exercise are a major factor. As a consequence of these initial events, inflammatory and oxidative stress processes are subsequently involved (24). Inflammatory mediated processes are activated with lengthening contractions that induce damage and appear to contribute to muscle restructuring (3, 23). A number of the inflammatory mediated processes also involve reactive oxygen/nitrogen species (RONS) that involve phagocytic leukocytes (32). Neutrophils and monocytes produce RONS during their "oxidative burst" activity that can alter muscle structure (5, 6). RONS and the inflammatory processes are implicated in the secondary damage associated with eccentric muscle damage and delayed onset muscle soreness (DOMS) (14, 19).

Strategies that have been purported to reduce or prevent the loss of muscle function and DOMS associated with eccentric muscle actions have used numerous nutritional treatments (11, 14). Numerous treatments have used antioxidants either as a single antioxidant (22, 25) or combined antioxidants (4, 33, 35). The theory behind usage of supplementing with antioxidants is to bolster the defense mechanisms prior to the eccentric exercise so as to reduce RONS and perhaps the inflammatory processes. Neutrophils use vitamin C during activation and it appears that vitamin C is an important factor in maintenance of immune action (5).

Vitamin C was reported to have potent antioxidant properties *in vitro* especially in blood plasma within the aqueous compartment (9). Vitamin C (ascorbate) can also help to regenerate oxidized vitamin E back to its reduced form (21). Vitamin C was reported to reduce soreness after previous bouts of sit-ups (26). A delay in the onset of muscle stiffness with patients taking ascorbic acid was also noted (27). Vitamin C (400 mg/d) for 2 wk prior to an intermittent shuttle run was able to show a modest attenuation in soreness with IL-6 and plasma malondialdehyde (MDA) increases also modestly reduced compared to placebo (29).

In contrast, vitamin C (200 mg) given after a similar shuttle run was not effective in reducing muscle soreness, leakage of creatine kinase into the serum, or recovery of muscle function compared to a placebo (30). Vitamin C (400 mg/d) for 2 wk given prior to a 30 min downhill run (-18% grade) at 60% $\text{VO}_{2\text{max}}$ resulted in similar soreness, plasma CK, and IL-6 levels compared to placebo (31). It is unclear why the same dose of vitamin C (400 mg/d) for 2 wk prior to the exercise was able to reduce soreness in one study but not the other. In addition, acute treatment with vitamin C had little influence on soreness and oxidative stress (1, 28). Vitamin C supplementation (12.5 mg/kg) with N-acetyl-cysteine (10 mg/kg) taken for 7 d after eccentric activity enhanced muscle protein leakage, oxidative stress, and inflammatory mediated process (7). However, a high dose of vitamin C (3 g/d) taken for 3 d prior to an eccentric exercise and continued for four more days after the exercise was reported to reduce muscle soreness (16). This study suggested that a 3 g/d dose of vitamin C prior to and continuing after the exercise could be more effective than the lower doses that reported mixed results. Unfortunately, the study by Kaminski and Boal only assessed DOMS (16). We decided to supplement the subjects for 2 wk prior to the eccentric exercise similar to the lower dose vitamin C and continue the supplementation for 4 d after the eccentric exercise similar to the Kaminski and Boal study (16). In addition, our research with a combined antioxidant formula for 2 wk prior to eccentric exercise (isokinetic machine) and

continued for 3 d after demonstrated reduced DOMS, attenuated CK (4), and reduction in oxidative stress (12).

Therefore, the purpose of this investigation was to determine if this high dose vitamin C (3 g/d) pretreatment could reduce muscle soreness, alter the loss in ROM, muscle forces and muscle damage as indicated by CK. In addition, glutathione status was determined to ascertain if the vitamin C supplementation or the eccentric exercise influenced this marker of oxidative stress.

Methods

Eighteen healthy young untrained males that did not resistance train for the previous 6 months were randomly assigned to either a placebo (P) ($n = 8$) or vitamin C (VC) ($n = 10$) group. The P was starch and the VC was supplied by Natural Alternatives International (San Marcos, CA) with all pills similar in size and shape. All subjects were prescreened, in good health, abstained from vitamin or herbal supplementation for at least 6 months and were free of any muscular injuries. Subjects signed a consent form prior to participation which had been approved by the institutional review board for human subjects. All subjects were verbally notified of potential benefits and risks associated with this study and instructed to maintain their normal activity and eating patterns throughout the study. Subjects were to refrain from unaccustomed exercise during the course of the study.

All subjects had their height and weight determined on a scale (Seca) and body composition determined using the three site skin-fold technique (13) to characterize the subjects. Each subject was placed into the dynamometer (Biodex System 3 Isokinetic Dynamometer, Biodex Medical Systems, Shirley, NY), adjusted the settings to each individual (height, arm length) and the settings recorded. Subjects performed three concentric maximum voluntary contractions (MVC) and the forces were recorded for both their dominant and non-dominant arms through a full range of motion at a speed of 1.75 rad/s. The highest force obtained with no movement of the lever arm was accepted as the maximum isometric force (MIF).

Subjects were given a numbered container and instructed to begin taking the supplement 2 wk prior to the eccentric trial. They were instructed to take three pills per day, one in the morning, afternoon, and evening. The spacing of the pills was to help assist in absorption as too high a dose would inhibit absorption (18). The subjects continued to take the pills during the 4 d after the eccentric exercise. Compliance was determined by counting the number of pills returned versus those given. Subjects were also instructed on how to fill out a 3 d food record. They turned this record in at the time of the eccentric exercise and at the end of the 3 d after the eccentric exercise. The food records were used to monitor total calories and antioxidants in their diet to determine if this influenced the outcome variables. Nutritional data were analyzed using a NutriQuest program.

The eccentric exercise sessions took place between 8:00 to 10:00 AM with subjects in a post-absorptive state (overnight fast). Subjects rested at least 15 min upon arrival at the laboratory and then a resting blood sample was obtained by Vacutainer (14 mL). A portion of the blood was immediately processed for glutathione status with the remainder centrifuged to obtain plasma. Muscle soreness (MS) was assessed by means of a linear scale from 1 to 10 (8) in a rested position and in response to palpation. Range of motion (ROM) was assessed on both arms

using a goniometer placed on markings from the medial aspect of the elbow of the humerus.

Seventy eccentric actions using the elbow flexors from 5° from full extension to 5° from full extension were performed with the non-dominant arm. Ten seconds were allowed between actions with the bar replaced by the tester between eccentric actions. Subjects were encouraged to perform maximum voluntary effort against the lever bar for all repetitions. The bar would not move without force placed against it and the forces recorded.

Immediately after the eccentric actions (< 5 min) a blood sample was taken from the antecubital region of the elbow of the non-dominant arm. MS and ROM were assessed and then the MIF was obtained on both arms. Subjects were instructed to return at 4 h post exercise as well as at 24, 48, 72, and 96 h to obtain another blood sample and have MS, ROM, and MIF determined. Subjects were reminded to take their pills during this time.

Creatine kinase (CK) was determined from plasma using the Sigma Diagnostics kit (procedure 520, Sigma-Aldrich, St. Louis, MO). The absorbance of the samples were determined on a Shimadzu spectrophotometer (model 1601, Shimadzu Corp., Nakagyo-ku, Japan) at 520 nm and compared to standards. All samples were performed in duplicate.

Total glutathione (TGSH) and oxidized glutathione (GSSG) were determined by the methods of Anderson et al. (2) as previously described (17). Whole blood was treated with 10% 5-sufosalicylic acid containing 1 mM bathophenanthroline-disulfonic acid (BPDS) and mixed vigorously. The mixture was then centrifuged at 10,000 rpm at 4 °C for 30 min and the supernatant collected in polypropylene microcentrifuge tubes and then centrifuged at 12,000 rpm for 15 min. The supernatant was stored at -80 °C until analyzed. Total TGSH was determined by recycling GSSG back to the reduced form with 2-vinylpyridine. The amount of reduced glutathione (GSH) was calculated by subtracting out the GSSG/2 from the TGSH. All samples were performed in duplicate.

Statistical analysis of the data used the SPSS statistical data analysis software package (SPSS, Inc., Chicago, IL). Descriptive and nutritional data were analyzed using independent *t*-tests between groups. All other variables were analyzed using a 2 × 7 repeated measures ANOVA with two groups compared across time. Post hoc Tukey HSD analysis was used to isolate differences in time where appropriate. The level of significance was set at a $P \leq 0.05$.

Results

Eighteen healthy males volunteered and completed this study. Their descriptive data are presented in Table 1. There were no differences in age, height, weight, or percent body fat between the two groups. There was no difference in total dietary caloric intake between the placebo group (P) and vitamin C group (VC) over the 6 d monitored ($P = 2305 \pm 340$ kcals, $VC = 2143 \pm 328$ kcals) based on the nutrition records. In addition, dietary vitamin C and E intake were not different between the groups ($P = 174 \pm 60$ mg and 2.19 ± 0.7 mg with $VC = 164 \pm 69$ mg and 5.78 ± 3.4 mg), respectively. In addition, the dietary intakes were no different prior to and after the exercise. Compliance for the P was 99.3% and for VC was 98.8%.

Table 1 Descriptive Characteristics of Subjects

| Variable | Placebo group | Vitamin C group |
|-------------|---------------|-----------------|
| Age (y) | 24.4 ± 1.7 | 21.4 ± 0.8 |
| Height (cm) | 177.5 ± 2.2 | 177.0 ± 2.6 |
| Weight (kg) | 74.6 ± 3.6 | 73.9 ± 2.3 |
| % body fat | 10.0 ± 2.5 | 14.1 ± 1.8 |

Data are means ± standard error. $N = 8$ for the Placebo group, $N = 10$ for the Vitamin C group.

MS prior to the eccentric exercise was at baseline for both dominant and non-dominant arms and did not differ between arms. The dominant arm remained at baseline soreness throughout the study in both the P and VC groups. The MS ratings in the non-dominant arm to palpation increased over time ($P = 0.001$ for both groups) and are presented in Figure 1. The MS was significantly higher in the P group across time compared for all time points. The VC group reported a significant increase in MS after 24 h. In addition, the P group showed significantly higher MS to the VC group ($P = 0.023$) over time. The magnitude of MS was attenuated at each time point immediately after through 24 h after the exercise in the VC group. Thereafter the pattern of MS was similar for both groups.

A similar pattern of MS was demonstrated with the arm at rest with both groups.

MIF was reduced significantly in both groups after the eccentric protocol with the patterns being similar. There was no difference in the extent of loss of MIF between the two groups. The MIF recovered over time but was slightly below normal at the end of the study (Figure 2).

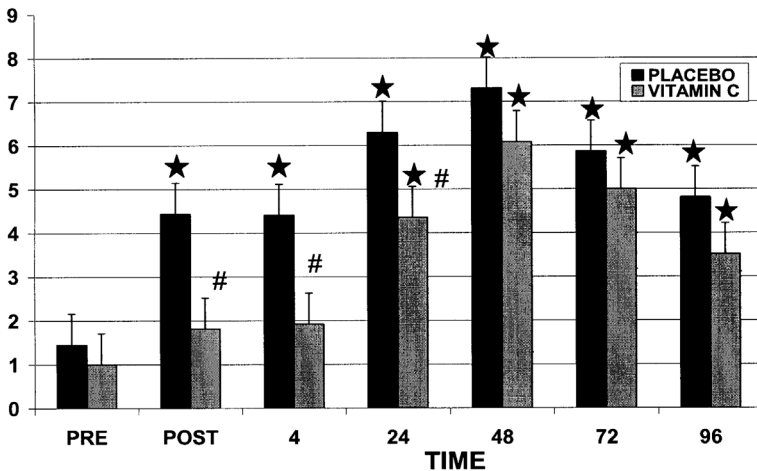


Figure 1 — Soreness over time in non-dominant arm. Comparison of placebo and vitamin C treatments. $N = 8$ for Placebo and $N = 10$ for Vitamin C group.

* = significantly different than pre, $P < 0.05$; # = significantly different than placebo, $P < 0.05$.

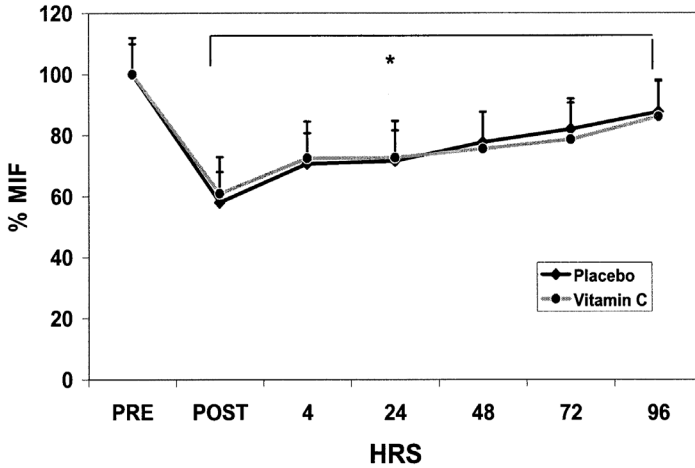


Figure 2 — Maximal isometric force after eccentric exercise.
 * = significantly different than pre, $P < 0.05$ independent of group; MIF, maximal isometric force.

ROM over time in the non-dominant arm that did the eccentric actions was significantly decreased over time in both groups (Figure 3). The decrease in ROM was similar for both groups and started to return towards normal by 96 h after the exercise. There was no change in ROM in the dominant arm (data not shown).

Plasma creatine kinase activity (CK) increased significantly from 48 through 96 h after the eccentric exercise independent of treatment (Figure 4). There was a tendency for a treatment effect over time but this did not reach significance ($P < 0.06$).

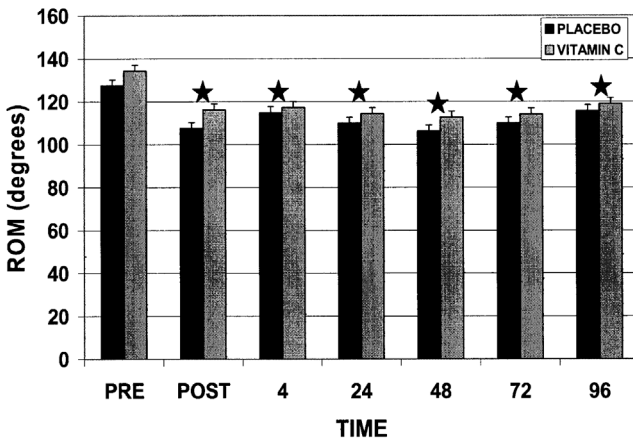


Figure 3 — Range of motion in non-dominant arm.
 * = significantly different than pre, $P < 0.05$ for both groups.

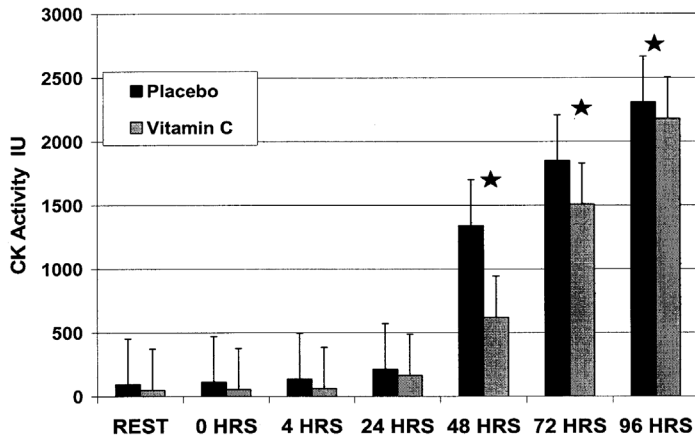


Figure 4 — Plasma creatine kinase activity over time.

* = significantly different than pre, $P < 0.05$ for both groups; CK, creatine kinase.

The glutathione ratio of oxidized glutathione (GSSG) to total glutathione (TGSH) is presented in Figure 5. Whole blood total glutathione was unchanged over time in both groups (data not shown). There was a significant increase in the GSSG/TGSH ratio at 4 and 24 h only in the P group. The VC group had a significantly lower GSSG/TGSH ratio compared to P at 4 and 24 h. There were no differences at all other times.

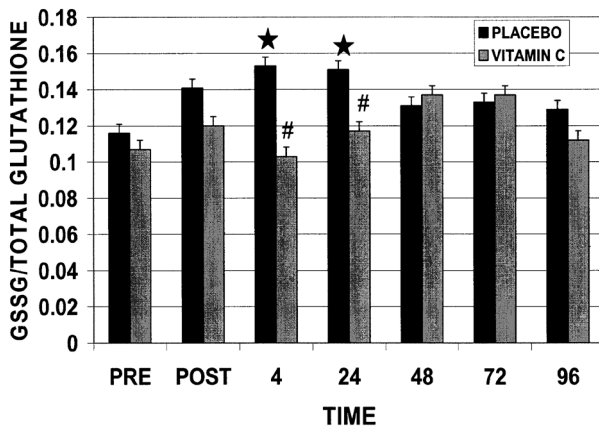


Figure 5 — Blood glutathione ratio in response to eccentric exercise.

* = significantly different than pre, $P < 0.05$; # = significantly different than placebo, $P < 0.05$

Discussion

This study reports that a high dose of vitamin C (3 g/d) taken 2 wk before and for 4 d after 70 eccentric actions of the elbow flexors can attenuate muscle soreness and prevent the increase in whole blood GSSG/TGSH with no effect on ROM, or MIF. In addition, there was no apparent protection on leakage of muscle proteins from the damaged muscles as indicated by CK within the circulation. This study confirms a previous study that muscle soreness can be reduced by this 3 g/d dose of vitamin C (16). The present study also suggests that this vitamin C dosage does not prevent the loss of ROM and muscle force but can reduce the amount of oxidized glutathione within the blood directly after the eccentric exercise.

The potential benefit of taking vitamin C prior to and after the eccentric exercise is controversial, in part, because various doses were used and the length of time of supplementation and timing of supplementation relative to the exercise has varied. In addition, not all of the exercises were similar. Some studies exercised the elbow flexors while other studies used shuttle runs or downhill running. Finally, the extent of muscle damage may have differed within the studies.

Several previous studies have examined the effectiveness of vitamin C as a pretreatment prophylactic compound given to prevent MS and reported varied results (1, 16, 28, 29, 30, 31). In addition, one study continued to supplement the subjects after the eccentric exercise (16). This study reported that muscle soreness was significantly attenuated with a 3 g/d dose of vitamin C. That study reported less soreness in both the rested or stretched positions. This is similar to the present study, as we now report significantly reduced MS in both the rested and palpated conditions over time. The extent of soreness in the palpated condition in the present study was similar to the soreness in the Kaminski and Boal study with their stretch condition (16). It is interesting to note that the attenuation in MS for the present investigation using the same dose of vitamin C (3 g/d) and a similar pain scale was analogous to that in the Kaminski and Boal study (16).

Despite a decrease in muscle soreness there did not appear to be any influence on recovery rates of ROM or MIF. ROM and MIF were also reported not to be influenced by a combined antioxidant pretreatment to eccentric exercise (4). In contrast, the combined treatment helped to reduce CK appearance in the blood. Vitamin E pretreatment was shown to attenuate CK appearance without any influence on muscle damage (34). Therefore it is likely that the vitamin E with the combined antioxidant treatment helped attenuate the leakage of CK but probably did not alter muscle function loss. However, it was reported that 400 mg of vitamin C given for 21 d prior and for 7 d after box stepping could improve recovery from soreness (14). This is in contrast to studies using a vitamin C dose (400 mg/d) for 2 wk and indicating there was similar soreness compared to placebo (29, 30, 31). The present study supports the notion that the vitamin C pretreatment (3 g/d) and continued supplementation can reduce some of the soreness but does not by itself aid in the recovery from muscle-damaging exercise nor prevent the loss of muscle function.

In the present study we noted only a very small protection with the 3 g/d vitamin C on glutathione ratios in the blood. It appears that the slight protection in the reduction in the amount of glutathione in the oxidized form at 4 h and 24 h after the exercise might be related to the soreness protection however, this needs to be

substantiated. Vitamin C is an aqueous antioxidant and could have protected the oxidation of glutathione within the red blood cells. This protection within the blood may not translate to protection within the muscles as it was recently reported that antioxidant pretreatment can influence muscle differently than blood (35).

It is unclear why there was less soreness with the vitamin C pretreatment at the early times after the exercise but this could be related to several pain-producing substances. It is suggested that future studies examine vitamin C pretreatment and factors related to pain such as prostanoids. It should be noted that the time frame for the lower soreness was similar to the time course for the lower glutathione ratio for the vitamin C treated group. This suggests that some of the attenuation in pain may be related to oxidative stress. In contrast, creatine kinase levels were significantly elevated in both groups after this time with soreness being similar. This suggests that the extent of damage within the muscles probably was not attenuated with this 3 g/d dose of vitamin C. Vitamin E studies have shown an attenuated CK response with no difference in exercise-induced muscle injury (34). It was also reported that a combined antioxidant treatment can attenuate CK response but had little influence on forces or ROM (4.) Therefore, the CK response may be a better indicator of membrane integrity loss rather than a good indicator of muscle damage or loss of muscle function. The study with vitamin C (1 g/d) and vitamin E (400 IU/d) showed similar findings with the forces and ROM (4) and also reported a reduction in protein carbonyls and MDA (12). It is possible that the reduction in oxidative stress in the present study and the study with the combined antioxidants could influence muscle pain processes and inflammatory mediated processes such as prostanoids.

In conclusion, this study reports that high dose vitamin C (3 g/d) for 2 wk prior to and continuing for 4 d after 70 eccentric contractions can attenuate muscle soreness with little influence on loss of range of motion, or maximal isometric force and CK response with only a modest protection in blood glutathione status. This dosage of vitamin C does not appear to enhance recovery rate of muscle soreness or functional parameters. It is suggested that future studies examine the mechanism(s) for the reduced muscle soreness with this dose of vitamin C.

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