

Vitamin C Supplementation Reduces Exercise-Induced Oxidative Stress and Increases Peak Muscular Force

Levi W. Evans, Fan Zhang, Stanley T. Omaye

Agriculture, Nutrition and Veterinary Sciences Department, University of Nevada, Reno, USA
Email: levie@nevada.unr.edu

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Abstract

Vitamin C is a popular supplement in exercise and sport for its chemical properties *i.e.* antioxidant capabilities. However, no clear role has been established for vitamin C supplementation (VCS) within these areas despite nearly a century's worth of ongoing research. This study examined peak muscular pushing force (PMF) before and after a VCS intervention, 250 mg every 12 hrs for 28 days, in nine participants whom were naive to VCS and resistance exercise (RE). A dynamometer was used to perform two RE bouts, pre- and post-intervention, that quantified PMF during a state of exercise-induced oxidative stress (EI-OS). Saliva was collected for EIOS analysis from each participant before and after each RE bout; salivary vitamin C (VC) and free salivary malondialdehyde (MDA) were the examined biomarkers. PMF increased significantly post-intervention ($405.48 \pm 92.75 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$) from baseline ($368.31 \pm 76.36 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$, $p < 0.05$). MDA elevated significantly after the pre-intervention RE bout ($0.385 \pm 0.017 \mu\text{g/mL}$) and the post-intervention RE bout ($0.373 \pm 0.014 \mu\text{g/mL}$) compared to the baseline measures ($0.373 \pm 0.014 \mu\text{g/mL}$, $0.359 \pm 0.008 \mu\text{g/mL}$; $p < 0.01$); a significant reduction in MDA was observed post-intervention, confirming vitamin C's ability to reduce oxidative stress ($p < 0.05$). VC increased post-intervention ($1.22 \pm 0.73 \mu\text{g/mL}$) from baseline ($0.47 \pm 0.51 \mu\text{g/mL}$, $p < 0.001$). The results from this study suggest VCS is capable of increasing PMF by reducing EIOS in untrained individuals, and can be possibly used for enhancing resistance-exercise performance.

Keywords

Untrained Persons, Free Salivary Malondialdehyde, Isokinetic Exercise

1. Introduction

A redox reaction imbalance, in which oxidized compounds are in abundance

compared to reducing agents, is known as oxidative stress, specifically in regards to biological systems. Oxidative stress can be detrimental to biological systems as it can be damaging on a cellular and molecular level [1]. A variety of exercises, including resistance/anaerobic exercise [2] [3], at various levels of intensities will produce reactive species and induce a state of oxidative stress [4] [5]. Reactive species interact with Ca^{2+} at the sarcoplasmic reticulum (SR) site of release and on the Troponin protein complex binding site; this environment's redox state plays a role in the overall outcome of the muscle contraction [6]. Oxidative stress can restrict the muscle's ability to contract and produce force [7], factors pivotal to exercise and sport performance. Reactive species and free radicals, however, play important roles in cellular processes and exercise adaptations [8]. A person whom is naive to exercise might observe negative effects [9], depending on the intensity level; adaptation to the activity and EIOS, however, might allow for beneficial outcomes as the person becomes more experienced and exposed to the exercise.

Reducing agents, also known as antioxidants, are available either by endogenous production (such as superoxide dismutase, glutathione peroxidase, and catalase) or by exogenous food/supplement consumption (such as vitamin C and vitamin E). Vitamin C is an essential nutrient for humans, and other animals, that acts as a cofactor, a co-substrate, and as an antioxidant [10]. It is involved in hydroxylation reactions for the synthesis of cartilage, cortisol, and epinephrine; tyrosine and fatty acid metabolism require vitamin C, as well. It is capable of recycling oxidized antioxidants, such as vitamin E, and reducing oxidative stress. Active locations of vitamin C can be found throughout the body's tissues; these include the lungs, adrenal glands, plasma, leukocytes, and saliva [11]. Foods containing vitamin C include peppers, kale, and strawberries. Supplemental doses greater than 500 mg of vitamin C per day will mostly be excreted [12]; smaller doses spread throughout the day will fully saturate the body and maximize its bioavailability, this technique was implemented in the present study.

Antioxidants may be beneficial in physical exercise with their ability to reduce reactive species, theoretically countering the detriments of oxidative stress e.g. muscular force inhibition and fatigue. This has led to a trend of antioxidant supplementation, with vitamin C being one of the more popular choices [13] [14]. Vitamin C's role in sport and exercise was examined as early as the 1930's [15] [16] and has since been a spotlight. VCS can reduce EIOS [17] [18]; however, evidence confirming this as beneficial [18] [19], detrimental [20], or indifferent [21] is unclear due to confounding results. The variance amongst study methods and instrumentation may be reasons for the overall inconclusiveness. Despite this, VCS is still commonly implemented in sport and exercise nutrition.

VCS has been historically restricted to studies focused on aerobic exercise as it can be actively located in the lungs, theoretically reducing free radicals produced from increased electron transport chain activity. Research examining VCS in RE is limited compared to research in aerobic exercise. A reduction in muscle soreness induced via RE has been witnessed after acute VCS at 3 g/day and 400 mg/

day [17] [18]. VCS has also had no effect on RE-induced muscle soreness [22]. RE performance is often assessed via strength and force; common methods for this assessment are maximal weight achieved via free weight exercises and muscular force measured via dynamometry [23]. VCS has had no effect on [17] and has inhibited [22] such RE performance measures at doses of 3 g and 1 g/day, respectively.

Both antioxidants as well as reactive species, and their products, should be assessed when monitoring oxidative stress [8]. Salivary analysis has been proposed for EIOS assessment [24] and has since been used successfully [25]. Relevant biomarkers that can be found in saliva are lipid peroxidation products, such as thiobarbituric acid reactive substances (TBARS) and MDA, as well as vitamin C. Methods and instrumentation are important factors when using saliva, as it has been unsuccessful at observing EIOS that was otherwise present in plasma [2]. The reason behind these confounding results might be utilizing the TBARS assay; which, despite its popularity, lacks sensitivity and specificity [8]. Free MDA, analyzed via high performance liquid chromatography (HPLC), is a better biomarker as it can address such issues. The present study utilized VC and MDA for EIOS assessment.

The aim of the present study was to investigate if VCS would improve PMF by reducing EIOS in persons naive to RE and VCS. Saliva was utilized as the biospecimen for examining the following biomarkers: VC and MDA. All methods and procedures were approved by the University of Nevada, Reno's Institutional Review Board (IRB) prior to conducting the study. Each person signed a consent form prior to participation.

2. Methods

Nine persons ($n = 9$) naive to RE and VCS, that is whom did not associate with either, participated in this pre-post intervention designed study; seven males and two females between the ages 22 and 34 y/o participated. No other descriptive data from participants were controlled. Participants had to be in good health to be included in the study; common colds, illnesses, smoking, kidney disorders, and iron-metabolism disorders excluded participants from the study as each can alter systemic vitamin C and salivary results [26] [27]. Participants were asked to maintain regular diet and exercise patterns throughout the duration of the study. The intervention was VCS for 28 days. One RE bout was performed pre-VCS and one was performed post-VCS; PMF measures were quantified during each RE bout.

The Biodex System 3 Isokinetic Dynamometer (Biodex) was used with the closed kinetic chain attachment (CKC) to quantify PMF via isokinetic push-pull repetitions. Three days prior to the initial start date, participants were familiarized with the Biodex. Instructions from the Biodex manual were followed for the CKC setup. Individual setup measures for each participant were taken during this familiarization, which consisted of: attachment height; seat placement; hand position; CKC tilt; shoulder angle; handle-extension travel; and handle-

retraction travel. Neutral hand, shoulder angle, and tilt positions were used. The neutral shoulder angle for the apparatus was 25°. Full handle-retraction was set for the participant's elbow to create a 90° angle at his/her side at the end of travel. Full handle-extension was set for the arm to fully extend at the end of travel, creating a straight arm. Height was measured for the hand to be directly in-line with the shoulder at full extension. These parameters were used for both RE bouts.

The two RE bouts for each participant were performed at the same time of day. RE bouts consisted of a warmup set of bodyweight pushups, three working sets (WS) of 10 isokinetic contracting push-pull repetitions, and one maximal effort set (ME) of five isokinetic contracting push-pull repetitions. First, participants were asked to complete as many pushups as they could while using any style. Immediately following the pushups, participants performed the WS on the Biodex at a velocity of 120°; 10 sec rest periods were given between each set as this was standard. A 90 sec rest period was given after the WS before performing the ME set; the Biodex was set at 60° for the ME set. Researchers did not cheer during either RE bout. Whole saliva samples were collected before and immediately after each RE bout; samples were frozen in a -80° freezer (Revco) for storage until analyzed. The highest quantified pushing force from the ME was recorded as the ME-PMF. The highest pushing force from each of the three performed sets during WS were also averaged and recorded as the WS-PMF.

Participants consumed 250 mg of an over-the-counter vitamin C supplement every 12 hrs (500 mg/day) for a total of 28 days. The over-the-counter supplement was 96% potent when tested against a laboratory-grade L-ascorbic acid (Sigma-Aldrich). The United States Department of Health and Human Services' (HHS) Automated Self-Administered 24-hour Dietary Recall System (ASA24) evaluated each participant's dietary vitamin C intake. Participants were asked to complete three 24-hour dietary recalls, two from days during the week and one from a Saturday or a Sunday; the three intakes were averaged and recorded.

Methods previously described [11] were used to determine VC. Briefly, vitamin C was preserved in trichloroacetic acid in the standards and saliva (saliva was preserved immediately after collection); oxidized with cupric sulfate pentahydrate to form dehydroascorbic acid; and then reacted with 2,4-dinitrophenylhydrazine prior to incubating for three hrs at 37° Celsius. All solutions were then reacted with 65% sulfuric acid for 30 min to form bis-2,4-dinitrophenylhydrazone. Absorbances of samples and standards were read in triplicates via a microtiter plate reader (Synergy HT microplate reader from Bio Tek Instruments, Inc.) at wavelength 520 nm.

Methods previously described were used to determine MDA [28]. Saliva was preserved in 5% ethylenediaminetetraacetic acid (EDTA) and butylated hydroxytoluene (BHT). To free malondialdehyde from proteins, 0.05 mL of each saliva sample was added to 0.25 mL 0.1 M perchloric acid (HClO₄) and 0.7 mL deionized water. Standards were prepared from 1,1,3,3-tetraethoxypropane (TEP). Peak heights from the standards were used to create the calibration curve via a

Hewlett-Packard Series 1100 HPLC with the following features: C 18 reversed-phase column at ambient temperature (25 cm, 4.6 mm i.d.; 5- μ m particles); 30 mM KH_2PO_4 -methanol (65 + 35 v/v%) for the single mobile phase; and the flow rate set at 1.5 mL/min. Chromatograms were monitored at 254 nm and injection volume was 0.002 mL. Retention time was 1.88 - 1.91 min.

All data was put into Graph Pad Prism 5 for statistical analysis. A repeated samples t-test was used to analyze statistically-significant differences in PMF measures, VC, and MDA; **Figure 1** represents when each measure of the study was taken. The level of significance was set at $p < 0.05$.

3. Results

Nine subjects participated in a pre-post 28-day VCS intervention study; RE bouts were performed pre- and post-VCS to quantify PMF. MS-PMF experienced a significant increase post-VCS compared to pre-VCS MS-PMF and is presented in **Figure 2** ($p = 0.016$). MS-PMF pre-VCS measures averaged $368.31 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$ (SD = $76.36 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$); post-VCS measures averaged $405.50 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$ (SD = $92.75 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$). WS-PMF experienced a significant increase post-VCS compared to pre-VCS and is presented in **Figure 3** ($p = 0.026$). Pre-VCS WS-PMF measures averaged $247.01 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$ (SD = $56.30 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$); post-VCS measures averaged $286.82 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$ (SD = $68.91 \text{ m}\cdot\text{kg}\cdot\text{s}^{-2}$).

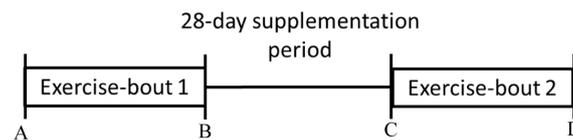


Figure 1. The study design was a pre-post intervention 28-day VCS period exercise-bouts 1 and 2 represent the RE bouts performed pre- and post-VCS. A, B, C, and D represent the time points salivary samples were taken, before and after each RE bout.

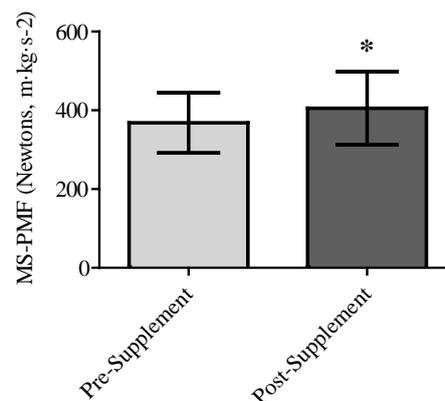


Figure 2. Peak muscular pushing force from the maximal-effort set before and after the VCS period. MS-PMP experienced a significant increase post-VCS ($*p < 0.05$). MS-PMF = maximal-effort peak muscular pushing force; VCS = vitamin C supplementation.

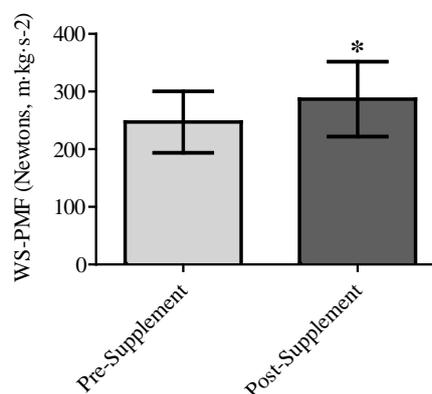


Figure 3. Peak muscular pushing force averaged from the three working sets, before and after the VCS period. WS-PMF experienced a significant increase post-VCS (* $p < 0.05$). WS-PMF = average peak muscular pushing force from the three working sets; VCS = vitamin C supplementation.

MDA experienced a significant increase after each respective RE bout and is presented in **Figure 4** ($p < 0.01$). Pre-VCS and post-VCS baseline measures averaged $0.37 \mu\text{g/mL}$ ($\text{SD} = 0.014$) and $0.36 \mu\text{g/mL}$ ($\text{SD} = 0.008$), respectively; MDA averaged $0.39 \mu\text{g/mL}$ ($\text{SD} = 0.017$) pre-VCS and $0.37 \mu\text{g/mL}$ ($\text{SD} = 0.014$) post-VCS after each respective RE bout. MDA experienced a significant decrease post-VCS; this was observed before and after the post-VCS RE bout ($p = 0.01$; $p = 0.03$) as well as overall ($p = 0.0007$).

VC experienced a significant increase post-VCS compared to pre-VCS and is presented in **Figure 5** ($p < 0.0001$). Pre-VCS measures averaged $0.47 \mu\text{g/mL}$ ($\text{SD} = 0.51 \mu\text{g/mL}$); post-VCS measures averaged $1.22 \mu\text{g/mL}$ ($\text{SD} = 0.73$). The average dietary vitamin C intake amongst the participants was 76 mg/day , with the highest being 257 mg/day and the lowest being 18 mg/day . No significant differences in PMF measures were calculated amongst the participants in relation to dietary vitamin C intakes.

4. Discussion

This study reports that VCS of 250 mg every 12 hrs for 28 days can reduce oxidative stress and increase muscular force in persons whom were naive to RE and VCS. VCS can also substantially increase systemic vitamin C as observed via VC. The elevation of MDA after each RE bout suggests that oxidative stress was induced. MDA measures taken post-VCS decreased; this was observed before and after the respective RE bout, as well as overall, suggesting EIOS reduction. Plasma MDA after acute exercise has previously been reduced post-VCS [29]; this study confirms VCS's ability to reduce EIOS at 500 mg/day . The increased MS-PMF and WS-PMF post-VCS suggests reducing oxidative stress can affect muscular force positively in the involved population.

Few researchers have studied vitamin C in resistance/anaerobic exercise. The Biodex with the CKC attachment at 60° and 120° speeds was utilized in this

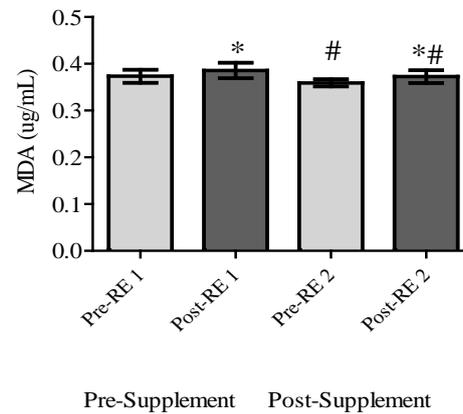


Figure 4. Free salivary malondialdehyde measures from the four separate saliva collections. MDA experienced significant increases after both RE bouts (* $p < 0.01$). Significant reductions were experienced post-VCS compared to the same time points pre-VCS (# $p < 0.01$). MDA = free salivary malondialdehyde; RE = resistance exercise; VCS = vitamin C supplementation.

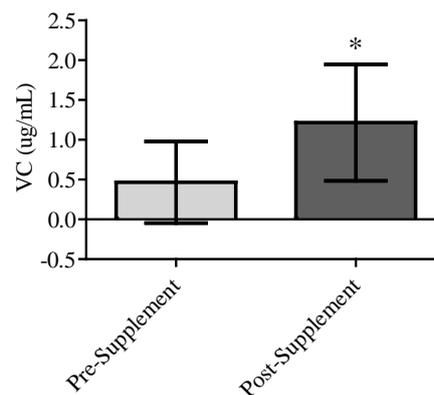


Figure 5. Salivary vitamin C pre- and post-vitamin C supplementation for 28 days. VC experienced a significant increase post-VCS compared to pre-VCS (* $p < 0.0001$). VC = salivary vitamin C; VCS = vitamin C supplementation.

study; methods and speeds have previously been researched in test-retest environments (pre-post studies without intervention) with no significant changes in PMF [30] [31]. The increases in PMF suggest the VCS intervention as the major contributing factor. VCS at doses (400 mg/day) similar to this study's dose (500 mg/day) have also been able to demonstrate a positive effect on muscular force/function via dynamometry [18] [32]. Higher doses (1 g/day, 3 g/day), however, have had a negative effect or no effect at all on muscular force/function [17] [22]. In these respective studies, results of RE-induced muscle soreness differed as one was not reduced while the other was. The exercises also differed; both differed from this study's RE bouts, however, as muscle soreness was not examined. Other resistance/anaerobic exercise studies have observed no effect on RE-induced muscle soreness after VCS at low and high doses [33] [34] suggesting dose

might not contribute to the outcome.

Force produced by the muscle, specifically the myofilament portion, is heavily influenced by sarcoplasmic reticulum Ca^{2+} . Reactive/nonreactive species can diminish myofilaments' sensitivity to Ca^{2+} or can cause Ca^{2+} to leak and not attach during the muscle contraction process, which will ultimately reduce muscular force [35]. This might be due to reactive species' ability to nitrosylate ryanodine receptor 1 proteins (RyR1) [36]. Vitamin C has been shown to denitrosylate the sarcoplasmic reticulum RyR1 proteins that are S-nitrosylated [37]; it has also been shown to attenuate S-nitrosylation [38]. RyR1 proteins are more so affected by hydrogen peroxide, a compound that does not necessarily react physiologically with vitamin C [39]. It could be possible; however, that vitamin C indirectly reacts with hydrogen peroxide products to which could affect such redox sensitive pathways.

This study did not have a control group, which should be included in future studies. A study with a group supplementing 3 g of vitamin C for 14 days observed no difference in muscle function compared to a control group [17]; 70 eccentric contractions were performed as the exercise to induce oxidative stress. This study and the current study used untrained persons. It is possible that the exercise performed previously was more damaging as this study had subjects perform a total of 30 concentric contractions; eccentric contractions might be more damaging. Future studies should consider different intensity levels with a lower VCS dose, as doses less than and equal to 500 mg/day tend to have positive results. RE experience should also be controlled. Furthermore, the muscle contraction pathway (sarcoplasmic reticulum and RyR1) should be further examined with VCS.

In conclusion, this study reports that 250 mg of VCS every 12 hrs for 28 days reduced EIOS in conjunction with increased muscular force in persons naïve to RE and VCS. No other RE bouts were performed during the VCS intervention. The salivary biomarkers, MDA and VC, were influenced in a way that has previously been witnessed in plasma, the biospecimen more often used in research. VCS increased VC and reduced MDA. VCS was set at a dose known to limit excretion and was supplemented in persons thought to experience more detrimental effects in regards to EIOS. Results suggest VCS could be potentially useful in RE for individuals beginning to weight train and take vitamin C supplements.

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