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Exercise-Induced Oxidative Stress Before and After Vitamin C Supplementation

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Vitamin C (ascorbic acid) was supplemented (1 g/day) for 1 day and 2 weeks in the same subjects. Plasma thiobarbituric acid reacting substances (TBARS) and oxygen radical absorbance capacity (ORAC) before and after 30 min submaximal exercise were measured. Different vitamin C supplementations did not affect resting TBARS or ORAC. Following 30 min exercise, values for TBARS were 12.6 and 33% above rest with 1 day and 2 weeks of vitamin C supplementation, respectively, compared to 46% higher with placebo. ORAC did not significantly change (11%) after exercise with a placebo, nor when subjects were given vitamin C supplements for 1 day or 2 weeks (4.9% and 5.73%, respectively). TBARS:ORAC, a ratio representing oxidative stress, increased 32% ($p < .05$) with placebo compared to 5.8 and 25.8% with vitamin C supplements for 1 day and 2 weeks, respectively. It was concluded that exercise-induced oxidative stress was highest when subjects did not supplement with vitamin C compared to either 1 day or 2 weeks of vitamin C supplementation.

Key Words: antioxidants, ascorbic acid, physical activity

Dietary supplementation with vitamin C (ascorbic acid) has occurred at least since the 18th century, when British sailors added vitamin C–rich foods to their diets to prevent scurvy. In the late 20th century, vitamin C supplementation in vitamin C–deficient people is believed to enhance health (7) and improve performance (8, 19). The proposed mechanisms by which vitamin C prevents disease and enhances health in vitamin C–deficient people vary, but one theory that has received recent attention has focused on vitamin C's contribution to antioxidant chain reactions. Antioxidant activity of vitamin C continues to be investigated to determine how it interacts with reactive oxygen species (ROS) (9). Vitamin C directly reduces peroxyl radicals and indirectly reduces other free radicals by regenerating alpha tocopherol (vitamin E) (Figure 1) after it is oxidized by either peroxyl or hydroxyl radicals. The antioxidant benefits of vitamin C are of particular interest because of the widespread use of vitamin C supplements and the ability of most humans to tolerate high supplementation without toxic side effects (3). Tolerance of vitamin

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C supplements may in part be due to its water solubility and relatively fast digestion and excretion. Nevertheless, there is both indirect and direct evidence that prolonged megadosing of vitamin C can contribute to iron-overload toxicity (12), especially if one depends upon supplements to meet the recommended dietary allowance of vitamin C. Herbert (12) explained that when taken as a supplement, vitamin C is totally in the reduced form and can contribute to prooxidant activity. Yet, most Americans ingest 20% more vitamin C in food than is required (11), and vitamin C in food is comprised of equal portions of reduced and oxidized forms.

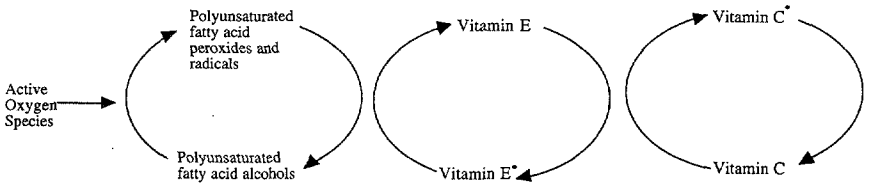

Figure 1 — Interaction among active oxygen species, vitamin C, and vitamin E.

Over 95% of oxygen consumption ($\dot{V}O_2$) during normal respiration is reduced in the mitochondria, forming energy and water. The remainder is converted univalently into superoxide (O_2-•), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), collectively referred to as ROS. Although the percentage of oxygen that is converted to these intermediates is less than 5%, cell components in the vicinity of O_2-•, •OH, and H_2O_2 are nevertheless at risk for a short-lived yet virulent attack. Acute exercise raises $\dot{V}O_2$ at least tenfold in active muscle tissue (22). Due to the greater total amount of oxygen consumed during exercise, more oxygen may undergo univalent reduction, thus increasing net cellular levels of prooxidants and ROS. Antioxidant enzymes and nonenzyme antioxidants, including vitamin C, respond to ROS and are often consumed or oxidized in the process of defending the cell. If antioxidants are not replenished and prooxidant reactions persist, then cell damage may occur as membrane fats become oxidized, membrane-bound enzymes change shape (10), and DNA bases undergo mutations (5).

Although much is known about the biological functions of vitamin C, such as hormone and neurotransmitter synthesis and regenerating reduced alpha-tocopherol, the role vitamin C has in defending cells against oxidative stress during exercise is not clear. Exercise studies have primarily focused on the effects of vitamin C on exercise performance (8, 19). One study reported that 3 weeks of vitamin C supplementation (400 mg/day) resulted in a 13% increase in total antioxidant activity following eccentric muscle contractions (18). The balance of prooxidant to total antioxidant activity during aerobic exercise has not been examined in subjects supplemented with vitamin C. If the balance shifts toward prooxidant activity, then the risk for oxidation and damage to cell components may increase. The purpose of this study was to describe how vitamin C supplementation (1 day or 2 weeks) affects oxidative stress balance in human blood by assessing a prooxidant biomarker, thiobarbituric acid reactive substances (TBARS), and a total antioxidant activity biomarker, oxygen radical absorbance capacity (ORAC), before and after 30 min of running exercise at 80% of $\dot{V}O_2$max.

Methods

Nine male subjects signed written consent forms prior to participating in the study. Subjects were young, fit, and highly motivated individuals who were interested in exercise and health but did not compete in sports. Maximum oxygen consumption ($\dot{V}O_2max$) tests were administered 1 week prior to the first trial. All subjects reported to the laboratory in a postabsorptive state (8–10 a.m.) with no food or drink for at least 10 hr prior to determinations. Subjects exercised on a motorized treadmill (Quinton Q-65) in a thermally controlled room (22 ± 2 °C, 40–60% relative humidity). Heart rate was monitored from electrocardiograph tracings (Quinton Q-3000). Ventilation and expired gases were monitored on an on-line system. Oxygen and carbon dioxide content was determined by Ametek analyzers that were coupled to an IBM computer. The analyzers were calibrated to known gases prior to each test. Subjects were given a warm-up to bring their heart rates to approximately 140–150 beats · min^{-1}, and then the treadmill grade and slope increased every 2 min. The subject breathed through a one-way valve that was coupled to a mixing chamber, with the line connected to the gas analyzers. Criteria for reaching $\dot{V}O_2max$ included volitional exhaustion or two or more of the following: less than a 100 ml oxygen · min^{-1} increase when workload was increased, respiratory quotient exceeding 1.1, and a decreased heart rate despite an increased workload. In the exercise condition, each person ran on a motorized treadmill at a submaximal intensity equal to 80% of his $\dot{V}O_2max$.

During the study, subjects were asked not to engage in any extra activities and to follow their regular diets, taking no additional vitamin or mineral supplements except those provided by the investigators. Subjects completed a 3-day food record, which was given to the researchers prior to the first trial. Based upon the 3-day food record, typical vitamin C intake for the subjects was estimated to be less than 100 mg per day. Upon a subsequent visit to the laboratory, copies of the 3-day food record were returned to the subjects so that they would be reminded to eat similar foods throughout the study. All subjects reported that they were not taking supplements for at least 6 months prior to the study. Each subject participated in all three phases of the investigation in a randomized order: submaximal exercise with placebo (sugar tablet) for 1 day, one day of 1 g vitamin C supplement, and two weeks of 1 g · day^{-1} vitamin C supplement. One gram of vitamin C was chosen because of the numerous studies that have used between 500 and 1,000 mg of vitamin C supplements with no side effects. Supplements were taken in two doses of 500 mg each, once in the morning and once in the evening, with meals. The last time of dosing prior to the exercise test was 10 hr prior to the test. There was a 2-week period of no supplements between each condition.

Based on the number of tablets supplied and subjects' reports, 100% compliance was achieved. We attribute the perfect compliance to the relatively short time period for the vitamin C supplementation. Blood was drawn from an antecubital vein before and after 30 min of exercise. The blood was collected in an evacuated tube, placed on ice, and centrifuged at 3,000 rpm for 5 min at 4 °C. Plasma was removed and stored at –70 °C.

Lipid peroxidation was measured in plasma using a fluorescent TBARS assay (21) with malonaldehyde as a standard. Blood samples were treated with sodium dodecylsulfate, acetic acid, and thiobarbituric acid, then heated at 100 °C for 1 hr

and cooled. Butanol:pyridine (15:1) was added, mixed, and centrifuged, and then the top layer of the butanol:pyridine was read using a fluorometer at 515 excitation and 553 emission.

Antioxidant activity was indicated by the oxygen radical absorbance capacity in plasma according to an assay previously described (4). In the ORAC assay system, a small aliquot of blood plasma (1–10 μl) was used for analysis. The blood plasma was not deproteinized. β-phycoerythrin (β-PE) was used as a target for free radical attack. 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) was used as a free radical (peroxyl radical) generator. Serum, β-PE, and AAPH were mixed together, and the fluorescence of β-PE was monitored (excitation = 540 nm, emission 575 nm) every 5 min until the emission of β-PE was zero (approximately 45–60 min). The control standard was 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analog. Results are expressed as ORAC units/ml plasma, where 1 ORAC unit equals the net protection produced by 1 μM Trolox per milliliter plasma.

A two-way ANOVA with repeated measures was used to compare ORAC and TBARS in subjects before and after supplementation and before and after exercise. Comparison-contrast tests were used in post hoc analysis when appropriate. The probability level was set at $p < .05$.

Results

Subjects were generally highly fit (mean $\dot{V}O_2max = 56.24 \pm 2.5$ ml · kg^{-1} · min^{-1}) and motivated young men (mean age = 33 ± 2.6 years). Prooxidant reactions in blood were estimated by measuring plasma TBARS; the data are shown in Table 1. Following an acute bout of exercise at 80% of $\dot{V}O_2max$, TBARS levels were an average 12.6% above resting levels when the subjects had been supplemented with vitamin C for 1 day and 33% above resting levels when supplemented for 2 weeks compared to an average 46% increase in the same subjects when they received a placebo. The change in TBARS before and after exercise reached significance only when subjects were not supplemented. No significant increase in TBARS occurred in the same subjects who were supplemented with vitamin C for either 1 day or 2 weeks.

ORAC, a biomarker of total antioxidant activity in plasma, tended to rise 12.6%, 4.9%, and 5.7% above resting levels after submaximal exercise for the placebo treatment, for 1 day of vitamin C supplementation, and for 2 weeks of vitamin C supplementation, respectively. Nevertheless, ORAC levels did not significantly change following exercise regardless of vitamin C supplementation (Table 2).

Figure 2 shows the ratio of prooxidant to antioxidant activity in subjects under the three different conditions. The prooxidant:antioxidant balance, as indicated by the TBARS:ORAC ratio, was tilted most toward oxidative stress when the subjects had no vitamin C supplements (32.5%). The only significant difference was between the placebo condition compared to 1 day of vitamin C supplementation (5.8%). Two weeks of vitamin C supplementation resulted in a 25.5% change in TBARS:ORAC after exercise, which was not significantly different than either of the two other conditions following exercise.

Table 1 Thiobarbituric Acid Reactive Substances[a] Before and After Exercise for Each Condition

	Rest		Exercise	
	M	*SD*	*M*	*SD*
Placebo	0.895	0.12	1.314[b]	0.10
1 Day vitamin C supplementation	1.006	0.09	1.133	0.08
2 Weeks vitamin C supplementation	0.850	0.09	1.131	0.19

[a]Units are nmol malonaldehyde · ml^{-1} plasma. [b]Significantly different between rest and exercise.

Table 2 Oxygen Radical Absorbance Capacity Before and After Exercise for Each Condition

	Rest		Exercise		Percentage change
	M	*SD*	*M*	*SD*	
Placebo	1,368	85	1,515	70	10.7
1 Day vitamin C supplementation	1,366	37	1,434	77	4.97
2 Weeks vitamin C supplementation	1,291	31	1,365	72	5.73

[a]Units are μM Trolox, an analogue of vitamin E, per milliliter plasma.

Discussion

Acute bouts of exercise influence oxidative stress depending on intensity and muscle tissue type (1, 2). Chronic exposure to oxidative stress associated with regular exercise has been shown to change the expression of different antioxidant enzymes in vitro (14, 15, 19), with some increasing in activity and others decreasing. The body's antioxidant defense system consists of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase; small molecule antioxidants, like vitamin E, vitamin C, beta-carotene, bilirubin, and urate; and macromolecule antioxidants, such as albumin, transferrin, and ceruloplasmin. Plasma and other extracellular fluids usually have very low antioxidant activities from antioxidant enzymes (6, 10). It is clear that there is redundancy in the actions of many antioxidants and that a decrease in one may be compensated for by an

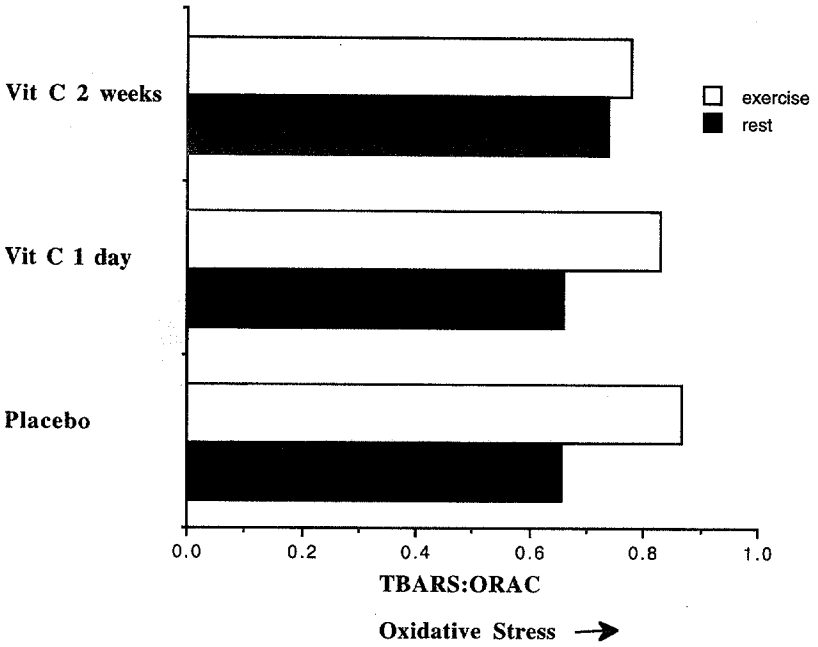

Figure 2 — Oxidative stress before and after exercise with no vitamin C supplementation and with 1 day and 2 weeks of vitamin C supplementation.

increase in another, resulting in a negligible shift in total antioxidant protection. For example, when GSH levels are reduced, vitamin C may compensate for the change in this antioxidant. When animals are GSH deficient, usually there is a reduction in vitamin C and an increase in dehydroascorbate concentration. Dehydroascorbate can be recycled back to vitamin C by reduced nicatinamide adeninedinucleotide (NADH) and glutathione peroxidase within erythrocytes. This pathway helps to stabilize the antioxidant capabilities in the aqueous compartment of the body (21).

Vitamin C appears to be an effective antioxidant, capable of preventing oxidation of molecules in humans (6) and animals (19). Vitamin C is water soluble and can be flushed from the system rapidly. Nevertheless, some of our unpublished data show that vitamin C levels are increased 50% above presupplementation levels when 1.25 g is ingested by adult women. There are interactions among different antioxidants, which is why we used the ORAC assay in this study. Vitamin C can reproduce vitamin E, for example, and vitamin E can be stored in the body for a long time. Therefore, the antioxidant action of vitamin C could have a lasting impact. Conversely, there is some evidence of vitamin C toxicity with prolonged, high doses (12). The 1 g of vitamin C supplement used in this study is not considered to be a high dose capable of inducing prooxidant reactions.

In the present study, resting plasma TBARS did not differ in the subjects independent of vitamin C treatment. At rest, the prooxidant–antioxidant balance was approximately the same regardless of supplementation. Unless nutrition is severely compromised, oxidative stress under resting conditions would be expected

to be minimal and would not be discernible in the same healthy subjects before and after 1 day or 2 weeks of vitamin C supplementation. This observation raises a number of possibilities when studying oxidative stress in healthy compared to diseased models. It is possible, for example, that indirect methods to assess oxidative stress or antioxidant activity may not be sensitive enough to distinguish short-term changes in healthy subjects under resting conditions. If a stress is used to induce an antioxidant response, then the prooxidant stimulus needs to be intense and continued for a prolonged period of time. Compared to healthy subjects, diseased models may show different response patterns to oxidative stresses, including exercise. In the present study, only under exercise-induced oxidative stress were the effects of vitamin C supplementation observable.

No significant differences of resting plasma ORAC were observed in this study when the subjects ingested a placebo, supplemental vitamin C for 1 day, and supplemental vitamin C for 2 weeks. This may be explained by the small contribution of absorbed vitamin C to the total antioxidant activity of plasma, especially without an oxidative stress challenge. Following acute exercise, ORAC increased 10.7% above resting levels with no vitamin C supplementation; however, this change did not reach the .05 level of significance. There was no significant change in plasma ORAC following exercise with vitamin C supplementation (either 1 day or 2 weeks). This result indicated that exercise-induced oxidative stress stimulated the antioxidant defense system to the extent that total plasma antioxidant activity was slightly but not statistically elevated above resting levels. Another study (18) reported little change in ORAC following submaximal exercise with and without N-acetylcysteine supplementation. Other studies have reported similar results, that is, a slight but statistically insignificant increase in total antioxidant capacity, following eccentric exercise that caused muscle soreness (13, 18).

When expressed as a ratio of oxidative stress, resting TBARS:ORAC equaled .654 with no vitamin C supplements. This ratio increased 32%, to .867, following exercise but fell short of statistical significance ($p = .059$). When subjects were given 1 day of vitamin C supplementation, TBARS:ORAC remained relatively unchanged (.736 vs. .770) following exercise. When subjects were given 2 weeks of vitamin C supplementation, TBARS:ORAC was .658 at rest and .828 following exercise. The oxidative stress ratio indicated that oxidative stress was highest (32%) following 30 min of running exercise when a placebo was given instead of vitamin C supplementation (5.8% after 1 day of vitamin C supplementation and 25.8% after 2 weeks of supplementation). None of the increased oxidative stress ratios reached statistical significance.

After an acute bout of exercise-induced oxidative stress when oxygen consumption is increased tenfold or more, there may very well be a noticeable shift in prooxidant and/or antioxidant activity that shifts the oxidative stress balance toward prooxidant activity. It is difficult to determine the threshold for an increased oxidative stress that has physiological significance. In the present study, biomarkers for oxidative stress were more likely to tilt the oxidative stress balance toward prooxidant rather than antioxidant activity in the placebo compared to the vitamin C supplement treatments. The direction and extent of the shift in oxidative stress balance probably depend on the health, fitness, and nutritional status of the subjects as well as the degree of acute oxidative stress to which the subjects are exposed.

Conclusion

One day or 2 weeks of vitamin C supplementation (1 g · day^{-1}) in healthy young males was associated with little or no difference in TBARS or ORAC at rest. Following exercise, the shift in exercise-induced oxidative stress was greater when the subjects were not supplemented with vitamin C. Based on the shifts away from prooxidant and toward antioxidant activity, vitamin C supplementation was associated with slightly more antioxidant protection compared to a placebo. This result suggests that (a) exercise can cause oxidative stress, which was evidenced by the elevated plasma TBARS after exercise; (b) exercise can also enhance the antioxidant defense system as an adaptive response to increased oxidative stress; and (c) vitamin C supplementation can partly reduce exercise-induced oxidative stress.

In conclusion, a submaximal exercise–induced oxidative stress was observed in human subjects in this study. This oxidative stress led to formation of oxidative by-products in plasma and also to an adaptive enhancement in the antioxidant defense system. Vitamin C supplementation attenuated the exercise-induced oxidative stress when taken for 1 day and did not significantly change the TBARS:ORAC ratio after 2 weeks. Short-term vitamin C supplementation may act by regenerating existing oxidized stores of vitamin E. Two weeks or more of extra vitamin C may act in a similar way or may increase ascorbate levels to the extent that some may become prooxidant. Further research may elucidate the ways in which vitamin C contributes to prooxidant and antioxidant activities and the impact of vitamin C supplementation and exercise on health and disease.

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