Vitamin C Consumption Does Not Impair Training-Induced Improvements in Exercise Performance

Llion A. Roberts, Kris Beattie, Graeme L. Close, and James P. Morton

Purpose: To test the hypothesis that antioxidants can attenuate high-intensity interval training-induced improvements in exercise performance. Methods: Two groups of recreationally active males performed a high-intensity interval running protocol, four times per week for 4 wk. Group 1 (n = 8) consumed 1 g of vitamin C daily throughout the training period, whereas Group 2 (n = 7) consumed a visually identical placebo. Pre- and posttraining, subjects were assessed for VO₂max, 10 km time trial, running economy at 12 km/h and distance run on the YoYo intermittent recovery tests level 1 and 2 (YoYolRT1/2). Subjects also performed a 60 min run before and after training at a running velocity of 65% of pretraining VO₂max so as to assess training-induced changes in substrate oxidation rates. Results: Training improved (P < .0005) VO₂max, 10 km time trial, running economy, YoYoIRT1 and YoYoIRT2 in both groups, although there was no difference (P = .31, 0.29, 0.24, 0.76 and 0.59) between groups in the magnitude of training-induced improvements in any of the aforementioned parameters. Similarly, training also decreased (P < .0005) mean carbohydrate and increased mean fat oxidation rates during submaximal exercise in both groups, although no differences (P = .98 and 0.94) existed between training conditions. Conclusions: Daily oral consumption of 1 g of vitamin C during a 4 wk high-intensity interval training period does not impair training-induced improvements in the exercise performance of recreationally active males.

Keywords: antioxidants, high-intensity interval exercise, training adaptation

Acute exercise of sufficient intensity (ie, moderate to high) causes an increased production of reactive oxygen species (ROS) within adult skeletal muscle. The parent radical produced is superoxide (O₂•⁻), which can be rapidly dismutated to hydrogen peroxide (H₂O₂) via the antioxidant enzyme superoxide dismutase (SOD). Hydrogen peroxide can be further reduced to water by catalase (CAT) or glutathione peroxidize (GPx), or alternatively to the hydroxyl radical (·OH) in the presence of copper or iron. Potential sources of exercise-generated ROS within skeletal muscle include both intracellular and extracellular sources. For example,
the mitochondria, xanthine oxidase (located in the endothelium), and both a plasma membrane and sarcoplasmic reticulum oxidoreductase system have been identified as potential sources of contractile-derived ROS.\textsuperscript{3} Since the first report demonstrating direct evidence for contraction-induced ROS production,\textsuperscript{1} the majority of studies conducted throughout the 1980s and 1990s focused on the role of ROS as damaging agents, given their capacity to cause damage to lipids, proteins and DNA.\textsuperscript{4}

In recent years, however, a paradox has emerged surrounding the exact role of exercise-induced ROS given their ability to activate a number of signaling pathways associated with skeletal muscle adaptation to exercise.\textsuperscript{5} Indeed, the transcription factors nuclear factor kappa B (NFkB), activator protein 1 (AP1) and heat shock transcription factor 1 (HSF1) are all known to be redox sensitive.\textsuperscript{6} Furthermore, the protein kinases AMPK, ERK1 and p38MAPK can also be activated by ROS, which, in turn, has implication for some of their downstream targets including the proposed master regulator of mitochondrial biogenesis, PGC1-\textalpha.\textsuperscript{7–9} In this regard, Gomez-Cabrera et al observed that daily supplementation of vitamin C for 6 wk inhibited the training-induced up-regulation of PGC1-\textalpha and cytochrome C protein content as well as the gene expression of MnSOD, GPx, Tfam and NRF1 in rodent skeletal muscle.\textsuperscript{10} More recently, Ristow et al also demonstrated in human skeletal muscle that the training-induced up-regulation of mRNA content of PGC1\textalpha, SOD and GPx is attenuated with a combination of vitamin C and vitamin E supplementation.\textsuperscript{11} Collectively, such research suggests that exercise-induced ROS production is an important signaling mechanism involved in stimulating the beneficial muscular adaptations associated with exercise training. This research is particularly important given that many athletes consume dietary antioxidants in the belief they will reduce cell damage and attenuate fatigue during daily training.\textsuperscript{12} However, undertaking such practices may actually attenuate muscle adaptations to exercise training and impair the training-induced improvements in endurance performance, although this remains to be tested in humans. Nevertheless, despite the sound theory supporting this notion, some evidence also suggests that antioxidant supplementation during training enhances aspects of exercise performance.\textsuperscript{13}

The aim of the present study was therefore further examine the uncertainty surrounding this issue by specifically testing the hypothesis that antioxidant supplementation impairs training-induced improvements in exercise performance. To this end, two groups of recreationally active males performed a 4 wk high-intensity interval training protocol that has previously been shown in our laboratory to induce oxidative adaptations of the vastus lateralis and gastrocnemius muscles.\textsuperscript{14} Each group consumed daily either 1 g of vitamin C or a placebo supplement. Participants completed a battery of exercise performance tests before and after training consisting of VO\textsubscript{2}max, 10 km time trial performance, distance ran on a Yo-Yo Intermittent Recovery Test 1 and 2 as well as completing a submaximal exercise protocol to assess training-induced changes in substrate oxidation.

**Methods**

**Subjects**

Eighteen recreationally active males volunteered to participate in the study after providing informed written consent. Two subjects withdrew from the investigation
before data collection. The remaining sixteen participants were randomly assigned to two training groups and consumed either vitamin C (VITC) or placebo (PLA) supplementation. Upon commencement of training, a third subject withdrew, thus resulting in a VITC and PLA training groups of eight and seven participants, respectively. Baseline characteristics of each training group are displayed in Table 1. All subjects refrained from the consumption of alcohol and caffeine for at least 24 h before any of the testing or training sessions. The study was approved by the Ethics Committee of Liverpool John Moores University.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PLA</th>
<th>VIT C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23 ± 2</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 ± 0.04</td>
<td>1.76 ± 0.07</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>73.9 ± 7.6</td>
<td>75.8 ± 1.3</td>
</tr>
</tbody>
</table>

**Experimental Design**

A double blind placebo matched pairs design was implemented where both experimental groups completed a series of baseline performance tests on a Monday (VO2max and running economy), Tuesday (10 km time trial), Wednesday (60 min run at 65% of VO2max to assess substrate oxidation rates during submaximal exercise), Thursday (YoYoIRT1) and Friday (YoYoIRT2) before the completion of 16 high-intensity interval training sessions (see the subsection High-Intensity Interval Training Protocol) performed over a 4 wk training period. More details on the nature of these tests are provided in subsequent sections. Following completion of training, the performance tests were completed once more in the same order so as to assess training-induced changes in exercise performance. All performance tests (pre- and posttraining) and training sessions were undertaken at the same time of day to avoid diurnal variation in performance.15 Each of the five tests were performed on a different day from Monday to Friday (separated by at least 24 h) and all tests were performed in the same order and using the same model of equipment pre- and posttraining. For the two days (Saturday and Sunday) preceding the pretesting week and for the subsequent five testing days, subjects were instructed to consume a habitual diet which was recorded, photocopied and given back following training to be replicated for posttests. Throughout the testing and training period, subjects restricted their physical activity outside of the study requirements to that consisting of essential day-to-day activities such as walking to and from their place of employment.

**Assessment of Maximal Oxygen Uptake and Running Economy.** On their first visit to the laboratory, all subjects were assessed for VO2max during an incremental treadmill test to volitional exhaustion on a motorized treadmill (HP Cosmos, Nussdorf, Germany). Respiratory measures were measured continuously by an on-line gas analysis system (Metamax I, Cortex, Germany). The test commenced
with a 4 min stage at a treadmill velocity of 8 km·h⁻¹ followed by subsequent 4 min stages at velocities of 9, 10, 11, 12, 13 and 14 km·h⁻¹ respectively. Upon completion of the 14 km·h⁻¹ stage, velocity increased to 16 km·h⁻¹ for 2 min. If necessary, upon completion of the 16 km·h⁻¹ stage, the velocity was maintained although inclined at a gradient of 2% every 2 min until volitional exhaustion was reached. The VO₂max was taken as the highest attained within any 10 s period and on the fulfillment of the following criteria (1) VO₂ plateau, (2) respiratory exchange ratio (RER) of ≥ 1.1, (3) heart rate (HR) within 10 bpm of age-predicted maximum heart rate (HRmax). Heart rate was measured continuously during exercise (Polar S610, Kempele, Finland). Running economy was calculated as the average VO₂ during the last minute of the 12 km·h⁻¹ stage.

10 km Time Trial. Subjects performed a 10 km running time trial (TT) on a motorized treadmill (HP Cosmos, Nussdorf, Germany). Subjects were free to increase/decrease the velocity of the treadmill at will, although their running velocity was not visible to them or the investigator. The subjects were simply instructed to “complete the TT as fast as you can.” Heart rate was measured continuously during exercise (Polar S610, Kempele, Finland) and subjects were permitted to consume 5 mL·kg⁻¹ of water during exercise with the pattern replicated for the posttraining test.

Criterion Exercise. In order to assess training-induced alterations in substrate metabolism, subjects performed a 60 min continuous running protocol on a motorized treadmill (HP Cosmos, Nussdorf, Germany) before and after training at the same absolute exercise intensity, corresponding to 65% of pretraining VO₂max. This exercise intensity was chosen as it has been shown to correspond to peak fat oxidation rates. Oxygen uptake (Metamax I, Cortex, Germany) and heart rate (Polar S610, Kempele, Finland) were measured continuously during exercise. Carbohydrate (CHO) and fat oxidation rates (g·min⁻¹) were calculated according to Jeukendrup and Wallis assuming negligible contribution of protein oxidation.

YoYo Intermittent Recovery Test Level 1. Subjects were assessed for intermittent exercise performance maximizing the use of the aerobic energy system using the YoYo intermittent recovery test level 1 (YoYoIRT1). The test consists of a series of 20 m shuttle runs at progressive velocities controlled by an audio beep from a CD player. A standardized 10 s recovery period exists between each bout. The test was terminated when a subject failed to keep up with the audio beep in reaching the finish line on two separate occasions, with the final distance covered recorded as the test result. The test was performed on an indoor running surface, marked out with cones. The test-retest coefficient of variation for this test in our laboratory is 9.3% (Roberts et al unpublished observations).

YoYo Intermittent Recovery Test Level 2. Subjects were assessed for intermittent exercise performance a second time using the YoYo intermittent recovery test level 2 (YoYoIRT2), on this occasion testing the subjects’ ability to recover from intense exercise. This test follows the same principles at the YoYoIRT1 but commences with higher initial running velocities and was performed in the same manner. The test-retest coefficient of variation for this test in our laboratory is 8.8%.

High-Intensity Interval Training Protocol. The training protocol consisted of high-intensity interval running and was performed on a motorized treadmill (HP Cosmos, Nussdorf, Germany) four times per week (Monday, Tuesday, Thursday...
and Friday) for 4 wk. The protocol commenced with a 10 min warm-up at a running velocity corresponding to 70% of VO₂max followed by five 3 min bouts at a running velocity corresponding to 90% VO₂max. The high-intensity efforts were separated by 3 min active recovery periods (1.5 min at a velocity corresponding to 25% VO₂max followed by 1.5 min at velocity corresponding to 50% VO₂max). Following the interval and recovery periods, subjects then performed a 10-min cool-down period at a running velocity corresponding to 70% of VO₂max. The exercise protocol therefore gave a total of 15 min of interval exercise and 15 min of active recovery time thus giving a total time for the intermittent training protocol of 30 min. When including the warm-up and cool down times, the total duration of the exercise protocol was 50 min. Training intensities were increased by 5% of the original VO₂max following 2 wk of training.

**Supplementation.** With the exception of weekend days, subjects reported to the exercise physiology laboratories at Liverpool John Moores University between 7 and 8:30 AM to consume either 1 g of a commercially available vitamin C supplement (Quest vitamins Ltd Vitamin C 1 g, Birmingham, England) or a visually identical placebo supplement (1 g dextrose monohydrate; Thornton and Ross Ltd, Huddersfield, England). All supplements were consumed in view of the investigators throughout the training period so as to verify 100% compliance on weekdays. On days of training, supplements were therefore consumed approximately 1.5 to 2 h before each training session. Following Friday’s training session, subjects were provided with the supplements to consume on the Saturday and Sunday and were instructed to consume the supplements at similar times. All subjects reported compliance to this procedure.

**Statistical Analysis.** All statistical analyses were conducted using the Statistical Package for Social Sciences program (SPSS V.16.0.0, Chicago, Illinois). Changes in exercise performance (VO₂max, running economy, 10 km time trial performance, YoYoIRT1 and YoYoIRT2) and substrate oxidation rates during submaximal exercise were assessed using a two-way mixed design repeated measures general linear model (GLM), where the within factor was time (pre- and posttraining) and the between-factor was supplementation (VITC versus PLA). The level of statistical significance was set at 0.05 with exact P values cited (P values of “0.000” have been recorded as <0.0005). Data are presented in text, tables and figures as means (SD), as suggested by current guidelines for the publication of physiological data sets.19

**Results**

Exercise performance of each group pre- and posttraining is shown in Table 2. Training induced significant improvements in VO₂max (% change: PLA, 7 ± 3; VITC, 4 ± 4; P = .000), running economy (% change: PLA, 5 ± 2; VITC, 7 ± 4; P = .000), 10 km time trial (% change: PLA, 11 ± 5; VITC, 13 ± 6; P = .000) and distance run on the YoYoIRT1 (% change: PLA, 16 ± 10; VITC, 22 ± 21; P = .000) and YoYoIRT2 (% change: PLA, 10 ± 4; VITC, 5 ± 5; P = .001). There was, however, no significant difference between groups in the magnitude of training-induced improvements in performance in any of the aforementioned variables (P = .313, 0.236, 0.293, 0.760 and 0.586, respectively).
Table 2  Performance measures of VO₂max, running economy, 10 km time trial, YoYoIRT1 and YoYoIRT2 pre- and posttraining

<table>
<thead>
<tr>
<th>Performance Variable</th>
<th>PLA</th>
<th>VITC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Posttraining</td>
</tr>
<tr>
<td>VO₂max (mL·kg⁻¹·min⁻¹)</td>
<td>58.2 ± 3.6</td>
<td>62.1 ± 2.6 *</td>
</tr>
<tr>
<td>Running Economy (mL·kg⁻¹·min⁻¹)</td>
<td>47.0 ±3.4</td>
<td>44.8 ± 2.7 *</td>
</tr>
<tr>
<td>10 km Time Trial (min:s)</td>
<td>50:46 ± 5:11</td>
<td>45:08 ± 3:01 *</td>
</tr>
<tr>
<td>YoYoIRT 1 (m)</td>
<td>1114 ± 327</td>
<td>1297 ± 415 *</td>
</tr>
<tr>
<td>YoYoIRT 2 (m)</td>
<td>337 ± 107</td>
<td>371 ± 99 *</td>
</tr>
</tbody>
</table>

*Denotes significant main effect of training, \( P < 0.05 \).
Figure 1 — (A) Average CHO oxidation, (B) average fat oxidation, and (C) average RER values during the submaximal criterion exercise session pre- and posttraining. *Denotes significant main effect of training, $P < .05$. 
Mean CHO and fat oxidation rates and RER during the submaximal exercise test are shown in Figure 1. Training reduced CHO oxidation (PLA: pretraining, 2.60 ± 0.23; posttraining, 2.15 ± 0.36; VITC: pretraining, 2.58 ± 0.37; posttraining, 2.17 ± 0.49; P = .000) and increased lipid oxidation rates (PLA: pretraining, 0.33 ± 0.11; posttraining, 0.45 ± 0.17; VITC: pretraining, 0.35 ± 0.16; posttraining, 0.45 ± 0.16; P = .000), though there was no difference in the magnitude of changes in substrate oxidation rates between training conditions (P = .979 (CHO) and 0.935 (lipid oxidation) respectively). Similarly, training reduced mean RER during exercise (PLA: pretraining, 0.93 ± 0.02; posttraining, 0.90 ± 0.03; VITC: pretraining, 0.93 ± 0.02; posttraining, 0.91 ± 0.05; P = .016) with no difference between groups (P = .610).

Discussion

The aim of the present study was to test the hypothesis that antioxidant supplementation impairs training-induced improvements in exercise performance. Our rationale for undertaking this experimental work was based on findings from both rodents and humans demonstrating that antioxidant supplement attenuates exercise-induced oxidative adaptations of skeletal muscle. It therefore seemed logical to deduce that antioxidant supplementation during a period of high-intensity exercise training may impair training-induced improvements in exercise performance. However, our data suggest that oral consumption of 1 g of vitamin C does not impair training-induced improvements in exercise performance in recreationally active males, as demonstrated by similar improvements in VO2max, running economy, 10 km time trial and distance ran on a YoYoIRT1 and 2 compared with placebo-supplemented subjects.

We chose a training protocol consisting of high-intensity interval exercise due to recent developments demonstrating that this form of training is superior to conventional continuous training approaches. Furthermore, this training model has been previously shown in our laboratory to induce oxidative adaptations of both the vastus lateralis and gastrocnemius muscles in similar populations, as demonstrated by increased protein content of PGC1-α, MnSOD, COXIV and activity of succinate dehydrogenase. We also chose this duration and intensity of training so as to mimic intense training periods (such as preseason) for those athletes involved in sports characterized by intermittent activity profiles such as soccer. Our rationale for the choice and dosage of our antioxidant supplement protocol was based on previous data indicating that vitamin C supplementation attenuates training-induced increases in protein content of NRF1, mTFA, PGC1-α and cytochrome C in rodent skeletal muscle. Furthermore, although we acknowledge the lack of redox measurements in muscle tissue or plasma vitamin C status as a limitation to our study, previous data from our laboratory has shown that this dosage is known to raise plasma vitamin C levels 3-fold within 2 h of oral consumption.

In contrast to a vitamin C-induced attenuation of training-induced improvements in endurance capacity in rodents, our data suggest that short-term supplementation of vitamin C does not impair training-induced improvements in exercise performance in recreationally active humans. It is difficult to compare between studies due to species differences. Nevertheless, a recent study in a similar sample of males employed here has also demonstrated that combined antioxidant supplementation of vitamin C (500 mg per day) and E (400 IU per day) does not impair
training-induced improvements in VO₂max or lactate threshold, as induced by 12 wk of cycling training. Our data confirm and extend these findings by utilizing a differing mode of exercise training and adopting additional performance tests such as 10 km time trial and distance ran on the YoYoIRT1 and 2 tests. As such, our data are therefore of relevance to other sports where running is the exercise mode, especially high-intensity invasive team sports. Given the potential of antioxidant supplementation to attenuate mitochondrial related adaptations of skeletal muscle, we also subjected our participants to a submaximal exercise test performed at 65% of pretraining VO₂max so as to assess training-induced alterations in substrate metabolism. However, consistent with our performance data, we also provide novel data by demonstrating that vitamin C supplementation does not attenuate the down-regulation of CHO oxidation and up-regulation of fat oxidation rates during submaximal exercise, as calculated from changes in oxygen uptake and carbon dioxide production following training.

It is, of course, possible that antioxidants do attenuate training adaptations but that our chosen performance tests may not have been sensitive enough to detect subtle changes at the molecular and cellular level of skeletal muscle. At present, data examining this level of organization are conflicting. Indeed, whereas Ristow et al. observed attenuated adaptations of mRNA expression of PGC1-α, MnSOD, CuZnSOD and GPx; Yfanti et al. observed vitamin C and E supplementation (albeit at a lower absolute dose) to have no detrimental effect on training-induced up-regulation of citrate synthase or β-hydroxyacyl-CoA dehydrogenase activity as well as MnSOD content of the vastus lateralis muscle.

The data presented here may also be specific to the population, training mode and duration studied. We deliberately chose a high-intensity interval exercise protocol and recreationally active subjects in an attempt to overwhelm antioxidant defenses and induce adaptation. Indeed, contractile-induced ROS production is known to be intensity dependent and it is also known that endurance training can attenuate ROS production during acute customary exercise. However, despite being classified as recreationally active it should be noted that our sample of participants were aerobically fitter than the sample studied by Ristow et al., where attenuated skeletal muscle adaptations were observed following vitamin C and E supplementation. It is therefore possible that antioxidants may only attenuate skeletal muscle and exercise performance adaptations in subjects who are largely sedentary and who would experience large changes in ROS production upon the commencement of an exercise training program. Support for this hypothesis is also provided by Ristow et al., as they observed a more pronounced antioxidant-induced attenuation of exercise-induced CuZnSOD and GPx gene expression in previously untrained subjects compared with subjects with prior training history. As such, when participants become more aerobically trained they may require more intense and longer periods of training so as to stimulate ROS sensitive pathways that may translate to molecular, cellular and physiological adaptations. Furthermore, although the magnitude of training-induced improvements in performance was similar to that observed previously by us and others when examining comparable subject populations and training interventions, utilizing a sedentary population would likely have induced greater absolute magnitudes of improvements which, in turn, may allow for a greater range of increase for which to detect statistically significant differences between groups. Further studies investigating the effects
of antioxidant supplementation in differing populations and indeed, more intense training periods are therefore warranted.

In addition to subject population, it is also possible that the effects of antioxidant supplementation on training adaptations are heavily dependent on the choice and dosage of specific antioxidant(s) treatment. For example, Ristow et al\textsuperscript{11} utilized a combination of vitamin C (1 g per day) and E (400 IU per day), whereas Yfanti et al\textsuperscript{24} observed no detrimental effects of supplementation when providing a similar antioxidant cocktail at a lower absolute dose of 500 mg and 400 IU per day, respectively. Furthermore, several researchers have observed that administering allopurinol (an inhibitor of xanthine oxidase, the enzyme thought to be responsible for superoxide production in endothelial cells) inhibits the exercise-induced activation of p38MAPK, ERK1 and ERK 2 as well as attenuating the up-regulation of PGC1-\( \alpha \) protein content in rodent skeletal muscle.\textsuperscript{9,26} These data therefore suggest that non-muscle cell-derived ROS are produced during exhaustive exercise and have an important signaling role. Clearly, definitive conclusions on the role of ROS on stimulating training adaptations can only be made when the dominant source(s) of ROS production under the particular contractile conditions is known and subsequent inhibition studies using the specific antioxidant for the appropriate ROS source are undertaken.

Conclusion and Practical Applications

In summary, the present data demonstrate that daily oral consumption of 1 g of vitamin C supplementation does not impair high-intensity interval training-induced improvements in exercise performance in recreationally active males. These findings are of practical relevance as they may provide some support to those athletes and coaches who currently advocate the use of antioxidant supplementation during intense training periods (in the belief they will reduce exercise-induced muscle damage and subsequently promote training adaptation and recovery) given that there appears to be no detrimental effect on training-induced improvements in performance. However, further research using differing subject populations, a variety of antioxidant supplement protocols, and both a longer duration of training and supplementation is warranted. Such research should also be supported by sensitive measurements ranging from the molecular and cellular level of organization through to whole body physiological adaptations.

Acknowledgments

The authors thank all of the subjects who participated in this study for their outstanding efforts during an intensive training and testing period. We also acknowledge the support of the laboratory technicians for ensuring the supplements were administered in a double blind fashion. The results of this present study do not constitute endorsement on the behalf of the authors of any product, manufacturer, or distributor discussed herein. This work was not supported by any form of external financial grant.

References


