No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training

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ABSTRACT

Background: It was recently reported that antioxidant supplementation decreases training efficiency and prevents cellular adaptations to chronic exercise.

Objective: This study aimed to investigate the effects of vitamin C and vitamin E supplementation on muscle performance, blood and muscle redox status biomarkers, and hemolysis in trained and untrained men after acute and chronic exercise. A specific type of exercise was applied (eccentric) to produce long-lasting and extensive changes in redox status biomarkers and to examine more easily the potential effects of antioxidant supplementation.

Design: In a double-blinded fashion, men received either a daily oral supplement of vitamin C and vitamin E (n = 14) or placebo (n = 14) for 11 wk (started 4 wk before the pretraining exercise testing and continued until the posttraining exercise testing). After baseline testing, the subjects performed an eccentric exercise session 2 times/wk for 4 wk. Before and after the chronic eccentric exercise, the subjects underwent one session of acute eccentric exercise, physiologic measurements were performed, and blood samples and muscle biopsy samples (from 4 men) were collected.

Results: The results failed to support any effect of antioxidant supplementation. Eccentric exercise similarly modified muscle damage and performance, blood redox status biomarkers, and hemolysis in both the supplemented and nonsupplemented groups. This occurred despite the fact that eccentric exercise induced marked changes in muscle damage and performance and in redox status after exercise.

Conclusion: The complete lack of any effect on the physiologic and biochemical outcome measures used raises questions about the validity of using oral antioxidant supplementation as a redox modulator of muscle and redox status in healthy humans.

INTRODUCTION

An old and active debate exists in the literature regarding the effect of antioxidant supplementation on the biology of animals and humans. Despite the progress of analytic techniques and the refinement of study designs, striking disagreement exists between studies regarding the influence of antioxidant supplementation on physical performance and redox status. Indeed, several studies have indicated that antioxidant supplementation induces a positive effect (1, 2), a negative effect (3, 4), or no effect (5, 6) on muscle performance. Likewise, several studies have reported that antioxidant supplementation attenuates oxidative stress (7–9), others have reported that it induces a prooxidant effect (10–13), and others have reported that it does not affect redox status (5, 14, 15).

It is worth mentioning, though, that historically there was a shift in the paradigm regarding the effects of antioxidant supplementation on muscle performance and redox status. In fact, back in the 1980s and 1990s, most of the relevant studies reported “positive” effects of antioxidant supplementation on muscle performance, muscle damage, and redox status (16, 17). In contrast, in the past 5 y, an increasing number of well-received studies are pointing toward a negative effect of antioxidant supplementation (3, 18–20). Moreover, many more studies than in the past are now specifically addressing the effects of antioxidant supplementation on the exercise adaptations that take place after chronic exercise. Regarding the latter, it has been reported recently that antioxidant supplementation greatly decreases training efficiency and prevents many cellular adaptations to chronic exercise (3, 18–20). Nevertheless, the debate is still open, and an equal number of recent studies have reported the reverse (ie, positive effects of antioxidant supplementation on exercise adaptations; 8, 21, 22) or virtually no effect of antioxidant supplementation on exercise adaptations (5, 6).

The possible reasons for this divergence regarding the effects of antioxidant supplementation on exercise adaptations and redox status are many and may include differences in the 1) type, mixture, and dose of antioxidants administered; 2) animal species used; 3) training models applied; 4) training status of the participants; 5) oxidative stress biomarkers determined; and 6) biological matrices in which the biomarkers were determined. To take into account some of the above confounding factors, the current study used a valid eccentric exercise model to induce...

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redox status alterations, which is characterized by long (lasting for many days after exercise) and extensive increases in oxidative stress and induce resistance training adaptations (23–26). Moreover, the acute and chronic effects of exercise were measured in both trained and untrained men in a side-by-side comparison. In addition, a battery of oxidative damage biomarkers were determined in different biological matrices (i.e., plasma, erythrocytes and skeletal muscle) to determine the effects of exercise on redox status in more complete dimensions (27).

SUBJECTS AND METHODS

Men were recruited after advertising the study in the local media. Twenty-eight healthy recreationally trained men volunteered to participate in the present study. Men were allocated regarding their age, body mass index, and maximum isometric torque into 2 equal-sized groups: a vitamin-supplemented group and a placebo-supplemented group. During their first visit, body mass was measured to the nearest 0.5 kg (Beam Balance 710; Seca, Birmingham, United Kingdom) while the subjects were lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208; Seca). Percentage body fat was calculated from 7 skinfold-thickness measures (average of 2 measurements of each site) by using a Harpenden caliper (John Bull, St Albans, United Kingdom). The Siri skinfold-thickness equation was used to calculate body fat. Maximum isometric torque of the knee extensor muscles of both legs was measured on an isokinetic dynamometer (Cybex, Ronkonkoma, NY). Written informed consent to participate in the study was provided by all men after the volunteers were informed about all risks, discomforts, and benefits involved in the study. The procedures were in accordance with the 1975 Declaration of Helsinki, as revised in 2000, and approval was received from the institutional review board.

Study design

An overview of the study design is shown in Figure 1. In a double-blinded fashion, men in the vitamin group (n = 14) received oral supplementation with one tablet of 1 g vitamin C (ascorbic acid; Lamberts Health Care Ltd, Kent, United Kingdom) and one tablet of 400 IU vitamin E (d-α toccopherol; Lamberts Health Care Ltd) daily for 11 wk. The control group (n = 14) received placebo tablets (lactose). All men were instructed to take the supplementation once daily before breakfast. Each person received the capsules prepacked in daily doses labeled with the day of consumption. Before supplementation, muscle function measurements were performed, and blood samples were drawn from all men. Muscle biopsy samples were obtained from 8 volunteers (4 from each group). During the next 4 wk, the men were instructed to avoid participation in any physical activity except for their usual program. After this period, at the beginning of week 5, the volunteers performed an eccentric exercise protocol of the knee extensors with both legs in the isokinetic dynamometer. Before and 1, 2, 3, 4 and 5 d after exercise, physiologic measurements were performed and blood samples were drawn at the same time points, except immediately after exercise. Muscle biopsy samples were obtained before exercise and 3 d after exercise from the 8 volunteers. After week 5, the men carried out 4 wk of eccentric training consisting of 2 exercise sessions per week (from weeks 6 to 9). Exercise was performed with both legs under the supervision of the same researcher. On training completion, at week 10, the volunteers abstained from exercise for 1 wk. At week 11, men repeated the eccentric exercise protocol on the isokinetic dynamometer (as performed at week 5). The same physiologic measurements and blood sample and muscle biopsy collections were performed as at week 5.

Acute eccentric exercise

The isokinetic dynamometer was calibrated weekly according to the manufacturer’s instructions. Subjects were seated (120° hip angle) with the lateral femoral condyle aligned with the axis of rotation of the dynamometer and were coupled to the dynamometer by an ankle cuff attached proximal to the lateral malleolus. The position of each subject was recorded and used in follow-up measurements. Each subject’s functional range of motion (ROM) was set electronically between full extension (0°) and 120° of knee flexion to prevent hyperextension and hyperflexion. Gravitational corrections were made to account for the effect of limb weight on torque measurements. Feedback of the intensity and duration of eccentric exercise was provided automatically by the dynamometer. Subjects had to accomplish 5 sets of 15 eccentric maximal voluntary contractions with each leg at an angular velocity of 60°/s in the seated position. A 2-min rest interval was incorporated between sets. Before each exercise session, subjects performed a warm-up consisting of 8 min of cycling on a Monark cycle ergometer (Monark, Vansbro, Sweden) at 70 rpm and 50 W followed by 5 min of ordinary stretching exercises of the major muscle groups of the lower limbs. It is worth mentioning that this protocol of exercise has been used in many recent studies by our group and is capable of inducing severe muscle damage, oxidative stress, and hemolysis (23–26).

![FIGURE 1. Study design. Downward arrows indicate the time of blood sampling, and upward arrows indicate the time of muscle biopsy collection.](https://academic.oup.com/ajcn/article-abstract/93/6/1373/4597849)
Chronic eccentric exercise

The eccentric training was performed on the isokinetic dynamometer twice a week (weeks 6–9) by using the same protocol as that used in the 2 acute bouts of eccentric exercise. The exercise sessions were performed on Mondays and Thursdays or on Tuesdays and Fridays. If a man could not visit the laboratory on the first scheduled day of the week (ie, Monday and Tuesday), he performed the session on the next day, and the following session was done 1 d after the scheduled day. None of the men missed or failed to accomplish a training session.

Muscle function

The isokinetic dynamometer was also used for the measurement of isometric knee extensor peak torque at 90° knee flexion. The average of the 3 best maximal voluntary contractions with the dominant leg was recorded. To ensure that the subjects provided their maximal effort, the measurements were repeated if the difference between the lower and the higher torque values exceeded 10%. There was a 2-min rest between isometric efforts. The test-retest reliability of the isometric peak torque measurement was 0.98. The assessment of pain-free ROM was performed manually. The investigator moved the calf at a very low angular velocity from 0 knee extension to the position where the subject felt any discomfort. The test-retest reliability of the ROM measurement was 0.93. Each man assessed delayed onset muscle soreness (DOMS) during a squat movement (90° knee flexion), and perceived soreness was rated on a scale ranging from 1 (normal) to 10 (very sore). The test-retest reliability of the DOMS measurement was 0.94.

Blood collection and handling

Blood was collected into EDTA-containing tubes and centrifuged immediately at 1370 × g for 10 min at 4°C, and the plasma was collected. The packed erythrocytes were lysed with 1:1 (vol:vol) distilled water, inverted vigorously, and centrifuged at 4000 × g for 15 min at 4°C. Blood samples were stored in multiple aliquots at −80°C and thawed only once before analysis. All blood samples were drawn in the morning after the subjects had fasted overnight and abstained from caffeine and alcohol for 3 d before sampling. On the days of blood sampling, supplements were consumed after the blood was drawn.

Muscle biopsy samples

All muscle biopsy samples were obtained in the morning before supplementation. The samples were taken from the middle portion of the vastus lateralis ≈15 cm from the midpatella by using the needle technique (28) with the application of suction (29). Muscle tissue was immediately frozen in liquid nitrogen and stored at −80°C until further analysis. In preparation for the muscle tissue biochemical analysis, muscle samples were initially ground by using mortar and pestle under liquid nitrogen. One part (g) of muscle powder was then homogenized with 2 parts (mL) of 0.01 mol phosphate-buffer saline/L (pH 7.4; 138 mmol NaCl/L, 2.7 mmol KCl/L, and 1 mmol EDTA/L) and a cocktail of protein inhibitors (1 μmol aprotinin/L, 1 μg leupeptin/mL, and 1 mmol phenylmethylsulfonylfluoride/L). The homogenate was vigorously vortex-mixed and a brief sonication treatment on ice was applied. The homogenate was then centrifuged at 12,000 × g for 30 min at 4°C, and the supernatant fluid was collected.

Assays

Reduced glutathione (GSH), oxidized glutathione (GSSG), thiobarbituric acid–reactive substances (TBARS), protein carbonyls, catalese, and total antioxidant capacity (TAC) were measured as previously described (26). The respective intra- and interassay CVs were 3.5% and 4.2% for GSH, 6.7% and 7.6% for GSSG, 4.3% and 6.6% for TBARS, 3.8% and 6.4% for protein carbonyls, 6.2% and 10.0% for catalase, and 2.5% and 5.7% for TAC. Each assay was performed in duplicate and within 4 mo of the blood collection. All reagents were purchased from Sigma-Aldrich (St Louis, MO). Albumin was determined spectrophotometrically based on the formation of a colored complex with bromocresol green reagent.

Some reservations exist about the validity of the TBARS and protein carbonyls assay in detecting lipid peroxidation and protein oxidation, respectively (30). However, concentrations of TBARS and protein carbonyls have been repeatedly shown to consistently increase after exercise in many studies from our group and from other groups (23, 31, 32). In addition, it has been found that concentrations of TBARS and protein carbonyls follow similar changes to F2-isoprostane concentrations (nowadays considered the reference method) after chronic muscle-damaging exercise (33).

Vitamin C was measured in plasma by using a ferric reducing ascorbate assay kit (K671-100) from BioVision (Mountain View, CA) with the use of spectrophotometry. Vitamin E was measured in plasma with the use of HPLC technique with ultraviolet detector by the method of Talwar et al (34). Creatine kinase (CK), bilirubin, and uric acid were measured in a Cobas Integra Plus 400 chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Plasma hemoglobin was assayed by using a kit from BioAssays System (Hayward, CA) with the use of spectrophotometry.

Dietary analysis

Men were asked to follow and record their diet for 3 d before the first blood sample was drawn at baseline and 3 d before and during the 5 continuous days of the blood sampling at weeks 5 and 11. Each volunteer was provided with a written set of guidelines for monitoring dietary consumption and a record sheet for recording food intake. Diet records were analyzed by using the nutritional analysis system Science Fit Diet 200A (Sciencefit, Athens, Greece).

Statistical analysis

The distribution of all dependent variables was examined by using the Shapiro-Wilk test and was found not to differ significantly from normality. Differences on physical characteristics between the groups at baseline were examined by using an unpaired Student’s t test. A 2-factor analysis of variance (ANOVA) [supplement (placebo and vitamin) × time (before supplementation, at the end of 4 and 10 wk)] with repeated measures on time was used to analyze vitamin C and E concentrations in the blood. The effects of the first 4 wk of supplementation on muscle function, redox status, and hemolysis variables were analyzed by using 2-factor ANOVA [supplement (placebo or vitamin) × time (before supplementation and 4 wk after
supplementation] with repeated measures on time. A three-factor ANOVA [supplement (placebo or vitamin) × training state (trained or untrained) × time (before exercise; 1, 2, 3, 4, and 5 d after exercise; and after training)] with repeated measure on time was used to analyze muscle function, redox status, and hemolysis variables. If a significant interaction was obtained, pairwise comparisons were performed by using the Sidak test method. To examine side-by-side percentage differences between the groups in muscle torque and redox status adaptations after chronic exercise, an unpaired Student’s t test was used. Data are presented as means ± SEMs. The level of significance was set at α = 0.05. SPSS version 15.0 was used for all analyses (SPSS Inc, Chicago, IL).

RESULTS

Physical characteristics and dietary intake

No differences in physical characteristics at baseline between the 2 groups were observed (Table 1). No significant differences were found in daily energy and macronutrient intakes between the 2 groups before supplementation (Table 2), before training, and after training (data not shown).

Vitamin C and E concentrations

A significant supplement × time interaction and main effect of time concerning vitamin C and E concentrations were observed (Figure 2, A and B). Both vitamin C and E concentrations in the vitamin group appeared to be significantly higher at week 4 and week 10 after supplementation than at baseline. No changes vitamin C and E were observed in the placebo group between the end of week 4 and week 10 of supplementation and baseline. As a result, the concentrations of vitamin C and E were higher in the supplemented group than in the placebo group at week 4 and week 10.

Effect of 4 wk of supplementation during rest on muscle function, redox status, and hemolysis

No significant supplement × time interaction or main effect of supplement or time concerning muscle function, redox status, or hemolysis indexes was observed.

Muscle function and performance

A significant training state × time interaction and a main effect of training state and of time on all muscle function indexes were found. Regarding isometric torque, a decline after acute exercise was found only before training. In both groups, all time point values (except on day 1 and day 5 after exercise) were lower before training than before baseline. Isometric torque before training was lower than the respective values after training at all time points (Figure 3A). After 4 wk of eccentric training, baseline isometric torque between pretraining and posttraining values increased by 15% in the placebo group and by 18% in the vitamin group.

ROM decreased only before training, regardless of supplementation. The decrease was noted on days 2 and 3 after exercise in the placebo group and on days 2, 3, and 4 in the vitamin group.

TABLE 1
Anthropometric characteristics of the placebo and vitamin groups

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 14)</th>
<th>Vitamin (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.6 ± 1.2</td>
<td>26.2 ± 1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.7 ± 1.1</td>
<td>175.6 ± 0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.2 ± 2.1</td>
<td>73.8 ± 1.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 0.6</td>
<td>23.9 ± 0.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>11.8 ± 1.4</td>
<td>12.3 ± 1.1</td>
</tr>
<tr>
<td>Torque (Nm)</td>
<td>207 ± 0.7</td>
<td>212 ± 0.12</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. There were no significant differences between the placebo and vitamin groups (unpaired Student’s t test). Nm, Newton meter.

TABLE 2
Analysis of daily energy intake in the placebo and vitamin groups at baseline

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 14)</th>
<th>Vitamin (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2851 ± 133</td>
<td>2912 ± 139</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>56.3 ± 3.8</td>
<td>54.7 ± 3.7</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>27.4 ± 3.6</td>
<td>28.8 ± 2.8</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>16.3 ± 1.4</td>
<td>16.5 ± 1.6</td>
</tr>
<tr>
<td>Vitamin A (mg RE)</td>
<td>1.04 ± 0.19</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>128 ± 12</td>
<td>132 ± 17</td>
</tr>
<tr>
<td>Vitamin E (mg x-)</td>
<td>7.7 ± 0.6</td>
<td>8.9 ± 1.0</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>41.8 ± 3.6</td>
<td>42.2 ± 2.9</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. RE, retinol equivalents; x, α-tocopherol equivalents. There were no significant differences between the placebo and vitamin groups (unpaired Student’s t test).

FIGURE 2. Mean (±SEM) plasma vitamin C (A) and plasma vitamin E (B) concentrations in the placebo (n = 14) and vitamin (n = 14) groups. Two-factor ANOVA (supplement × time) with repeated measurements on time and post hoc pairwise comparisons through the Sidak test method were used. S × T, interaction between supplement × time; S, main effect of supplement; T, main effect of time. *Significantly different from baseline in the same group, P < 0.05. †Significant difference between groups at the same time point, P < 0.05.
compared with the respective baseline values. ROM values before and after training were different on days 2 and 3 in the placebo group and on days 2, 3, and 4 in the vitamin group (Figure 3B).

DOMS increased only before training and remained increased up to day 4 in both groups. DOMS values after exercise were higher before training than after training up to day 4 in both groups. In general, CK activity after exercise was higher before training than after training at all time points in both groups (Figure 3D).

**Blood redox status**

**Glutathione status**

For GSH and GSSG, a significant interaction between training state × time and a main effect of training state and time were found (Figure 4, A and B). Acute eccentric exercise decreased GSH, increased GSSG, and decreased the GSH/GSSG ratio at several time points during recovery compared with baseline only before training in both groups. After 4 wk of eccentric training, resting GSH values were higher than pretraining baseline values in both groups ($P < 0.05$), whereas no changes were observed in GSSG and GSH/GSSG.

**Antioxidant molecules**

For catalase, a significant interaction between training state × time and main effect of time was found (Figure 5A). For uric acid and TAC, a significant interaction between training state × time and a main effect of training state and time were found (Figure 5, B and D respectively). Generally, catalase, uric acid, and TAC increased from baseline at days 2, 3, and 4 only before training in both groups. No changes were observed between the resting values of these antioxidant molecules after eccentric training in either group. No significant interactions or main effects were detected for albumin (Figure 5C).

**Oxidative damage**

For TBARS and protein carbonyls, a significant training status × time interaction and main effect of training state and time were found (Figure 6, A and B). Protein carbonyls increased from baseline at days 3 and 4 in the placebo group and at days 2, 3, and 4.

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**FIGURE 3.** Mean (±SEM) isometric peak torque (A), range of motion (ROM; B), delayed onset muscle soreness (DOMS; C), and creatinine kinase (CK; D) in placebo ($n = 14$; solid line) and vitamin ($n = 14$; dotted line) groups in the untrained and trained states. No significant differences were observed between the placebo and vitamin groups at any time point in either the untrained or trained state. Three-factor ANOVAs with repeated measurements on time and post hoc pairwise comparisons through the Sidak test were used. S, main effect of supplement; TS, main effect of training state; T, main effect of time; Nm, Newton meter. Interactions are shown. *Significantly different from the pre-exercise value in the same group, $P < 0.05$. 

**FIGURE 4.** (A) For GSH and GSSG, a significant interaction between training state × time and a main effect of training state and time were found (Figure 4A). 

**FIGURE 5.** (A) For catalase, a significant interaction between training state × time and main effect of time was found (Figure 5A).

**FIGURE 6.** (A) For TBARS and protein carbonyls, a significant training status × time interaction and main effect of training state and time were found (Figure 6A).
4 in the vitamin group, only before training. TBARS increased only before training at days 2, 3, and 4 and at days 3 and day 4 in the placebo and vitamin groups, respectively. After 4 wk of eccentric training, baseline concentrations of protein carbonyls and TBARS were lower than pretraining baseline values in both groups (P < 0.05 for both variables). The 4 wk of eccentric training induced several changes from baseline in the blood redox status in both groups (Figure 7). However, the magnitude of these changes was similar between the placebo and vitamin groups.

Muscle redox status

The results of the redox status indexes measured in skeletal muscle and obtained from 4 men from each group are presented only as descriptive statistics in Table 3.

Hemolysis

For bilirubin and plasma hemoglobin, a significant training status × time interaction and main effect of training state and time were found (Figure 8, A and B). Bilirubin concentrations increased from baseline only before training at days 2, 3, and 4 in both groups. Generally, plasma hemoglobin values remained increased up to 4 d after exercise, only before training.

DISCUSSION

To our knowledge, this was the first investigation of the effect of chronic eccentric (ie, muscle-damaging) exercise on muscle damage and performance, redox status, and hemolysis. In addition, it was also the first study that investigated the influence of combined vitamin C and E supplementation on redox status, muscle damage, and hemolysis adaptations taking place after chronic eccentric exercise. The current results failed to support any effect of antioxidant supplementation; eccentric exercise similarly modified muscle damage and performance, blood redox status, and hemolysis in both the supplemented and non-supplemented groups. It should be stressed, though, that other adaptive mechanisms (such as heat-shock proteins or redox-sensitive transcription factors) may have responded differently.

Exercise effects

Muscle performance

During the first week of training, the effects of eccentric exercise on muscle damage and performance peaked at 2–4 d and subsided 5 d after exercise (23). However, muscle dysfunction was diminished after the seventh week of training, which indicated that adaptations took place in skeletal muscle after eccentric training.

Blood and muscle redox status

This was the first investigation of the effect of chronic muscle-damaging exercise on the blood (or any other tissue) redox status of animals or humans (32). The current results showed that lengthening contractions uniformly (ie, in a similar pattern through time) modified the concentrations of the selected oxidative stress indexes, which indicated increased oxidative stress.
in the blood. As predicted by the repeated bout effect phenomenon (23), the indirect indexes of muscle damage and performance changed dramatically before training, but much less after training. Accordingly, the marked changes noted in blood biomarkers of oxidative stress before training disappeared after the same bout performed 7 wk later. Regarding the effects of chronic exercise, it was found that the eccentric training modified the concentrations of oxidative stress biomarkers toward a direction indicating enhanced antioxidant potential (ie, increased concentrations of GSH, uric acid, and TAC) and less oxidative damage (ie, decreased concentrations of lipid peroxidation and protein oxidation) either at baseline or after eccentric exercise.

A complete assessment of redox status was performed in skeletal muscle biopsy samples obtained from 4 men. Consequently, the results in skeletal muscle are presented only as descriptive statistics in Table 3 and should be treated with caution. Nevertheless, we believe that it is evident that redox responses took place in muscle and resembled (at least qualitatively) those in the blood. In fact, almost all oxidative stress indexes measured in the muscle changed 3 d after exercise. Additionally, the baseline values of most of the biomarkers in the muscle indicated less oxidative damage and increased antioxidant potential either at baseline or after eccentric exercise.

**Antioxidant effects**

**Muscle performance**

Probably the most interesting finding of the current study was that supplementation with vitamins C and E before and during 11 wk of chronic eccentric exercise did not affect muscle performance and muscle damage either at rest or after an acute eccentric exercise bout. Specifically, although acute eccentric exercise resulted in a marked increase in enzyme leakage from skeletal muscle, redox status perturbations, and impairment of muscle function, prior supplementation with these particular antioxidant vitamins did not attenuate any of these effects relative to placebo supplement. In addition, the muscle adaptations that occurred after the chronic eccentric exercise (ie, increased baseline muscle torque, similar enhanced resistance

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**FIGURE 5.** Mean (±SEM) concentrations of catalase (A), uric acid (B), albumin (C), and total antioxidant capacity (TAC; D) in the placebo (n = 14; ●) and vitamin (n = 14; ◆) groups in the untrained and trained states. No significant differences were observed between the placebo and vitamin groups at any time point in either the untrained or the trained state. Three-factor ANOVAs with repeated measures on time and post hoc pairwise comparisons through the Sidak test were used. S, main effect of supplement; TS, main effect of training state; T, main effect of time; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Hb, hemoglobin. *Significantly different from the pre-exercise value in the same group, P < 0.05.
to muscle damage after training) were comparable between the 2 groups.

A central problem of redox biology is the potential physiologic effect of reactive species. Several articles have indicated that the reactive species produced during exercise are by no means detrimental. Instead, reactive species have been reported to be essential for normal force production in skeletal muscle (35, 36), for the development of training-induced adaptation in endurance performance (3, 19, 20), and for the induction of endogenous defense systems (3, 18). In contrast, other studies have reported negative effects of reactive species production on several aspects of muscle performance and muscle adaptation (1, 2). In addition, many studies have suggested that reactive species do not modify skeletal muscle function in response to exercise (5, 6). The reasons for these striking discrepancies in the relevant literature are hard to identify. However, in the current study, the lack of effect of antioxidant supplementation on muscle function and muscle adaptations fully agrees with the absence of any effect of antioxidant supplementation on blood and muscle redox status.

Blood and muscle redox status

The complete lack of effect of antioxidant supplementation in preventing exercise-induced oxidative stress and modifying redox status casts doubts either on the implication of reactive species as physiologic important agents for the onset of blood and muscle redox status adaptations or on the in vivo efficacy of...
vitamins C and E as redox modulators or on both. There is no doubt that reactive species and redox status play a crucial role in controlling muscle contraction, muscle fatigue, and muscle adaptations (35–38). Nevertheless, the high antioxidant doses administered did not manage to alter the redox status of blood and skeletal muscle, probably hindering any effect of antioxidant supplementation on muscle function and blood responses to be revealed.

**TABLE 3**
Redox status in skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Before exercise</th>
<th>After exercise</th>
<th>Before exercise</th>
<th>After exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(day 0)</td>
<td>(day 3)</td>
<td>(day 0)</td>
<td>(day 3)</td>
</tr>
<tr>
<td><strong>GSH (µmol/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.047 ± 0.011</td>
<td>0.050 ± 0.012</td>
<td>0.036 ± 0.011</td>
<td>0.043 ± 0.018</td>
<td>0.047 ± 0.019</td>
</tr>
<tr>
<td>Vitamin</td>
<td>0.044 ± 0.014</td>
<td>0.043 ± 0.011</td>
<td>0.035 ± 0.017</td>
<td>0.050 ± 0.022</td>
<td>0.048 ± 0.015</td>
</tr>
<tr>
<td><strong>GSSG (nmol/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.81 ± 0.22</td>
<td>1.09 ± 0.34</td>
<td>1.18 ± 0.24</td>
<td>0.80 ± 0.14</td>
<td>0.84 ± 0.24</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1.05 ± 0.31</td>
<td>0.94 ± 0.22</td>
<td>1.19 ± 0.33</td>
<td>1.04 ± 0.13</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td><strong>GSH/GSSG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>43.2 ± 10.9</td>
<td>42.9 ± 13.3</td>
<td>23.6 ± 8.4</td>
<td>44.9 ± 13.4</td>
<td>56.0 ± 8.5</td>
</tr>
<tr>
<td>Vitamin</td>
<td>38.6 ± 5.3</td>
<td>46.4 ± 13.5</td>
<td>30.5 ± 9.5</td>
<td>39.2 ± 8.5</td>
<td>43.9 ± 7.6</td>
</tr>
<tr>
<td><strong>Carbonyls (nmol/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1.59 ± 0.64</td>
<td>1.41 ± 0.55</td>
<td>2.70 ± 0.96</td>
<td>1.54 ± 0.44</td>
<td>1.37 ± 0.35</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1.43 ± 0.53</td>
<td>1.55 ± 0.46</td>
<td>2.34 ± 0.43</td>
<td>1.46 ± 0.65</td>
<td>1.66 ± 0.52</td>
</tr>
<tr>
<td><strong>TBARS (nmol/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>0.72 ± 0.10</td>
<td>0.80 ± 0.13</td>
<td>0.87 ± 0.25</td>
<td>0.69 ± 0.26</td>
<td>0.80 ± 0.23</td>
</tr>
<tr>
<td>Vitamin</td>
<td>0.82 ± 0.39</td>
<td>0.84 ± 0.24</td>
<td>0.94 ± 0.26</td>
<td>0.76 ± 0.14</td>
<td>0.71 ± 0.14</td>
</tr>
<tr>
<td><strong>Catalase (U/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>6.24 ± 1.26</td>
<td>6.93 ± 1.94</td>
<td>8.04 ± 1.48</td>
<td>6.28 ± 0.88</td>
<td>6.75 ± 1.78</td>
</tr>
<tr>
<td>Vitamin</td>
<td>7.16 ± 1.64</td>
<td>7.03 ± 1.37</td>
<td>8.54 ± 1.89</td>
<td>7.69 ± 1.19</td>
<td>7.72 ± 1.47</td>
</tr>
<tr>
<td><strong>TAC (mmol/L DPPH)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>0.105 ± 0.031</td>
<td>0.108 ± 0.034</td>
<td>0.134 ± 0.044</td>
<td>0.110 ± 0.023</td>
<td>0.084 ± 0.013</td>
</tr>
<tr>
<td>Vitamin</td>
<td>0.093 ± 0.032</td>
<td>0.097 ± 0.035</td>
<td>0.113 ± 0.035</td>
<td>0.108 ± 0.024</td>
<td>0.088 ± 0.023</td>
</tr>
<tr>
<td><strong>Albumin (mg/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.34 ± 0.05</td>
<td>0.34 ± 0.03</td>
<td>0.46 ± 0.05</td>
<td>0.37 ± 0.02</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Vitamin</td>
<td>0.30 ± 0.06</td>
<td>0.32 ± 0.06</td>
<td>0.44 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.33 ± 0.05</td>
</tr>
</tbody>
</table>

All values are means ± SEMs; n = 4 for each group. Muscle samples were obtained before vitamin supplementation (baseline), 4 wk after supplementation (before training), and 10 wk after supplementation (after training). GSH, reduced glutathione; GSSG, oxidized glutathione; TAC, total antioxidant capacity; TBARS, thiobarbituric acid–reactive substances; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

vitamins C and E as redox modulators or on both. There is no doubt that reactive species and redox status play a crucial role in controlling muscle contraction, muscle fatigue, and muscle adaptations (35–38). Nevertheless, the high antioxidant doses administered did not manage to alter the redox status of blood and skeletal muscle, probably hindering any effect of antioxidant supplementation on muscle function and blood responses to be revealed.

**FIGURE 8.** Mean (±SEM) bilirubin (A) and plasma hemoglobin (B) concentrations in the placebo (n = 14; •) and vitamin (n = 14; ○) groups in the untrained and trained states. No significant differences were observed between the placebo and vitamin groups at any time point in either the untrained or the trained state. Three-factor ANOVAs with repeated measures on time and post hoc pairwise comparisons through the Sidak test were used. S, main effect of supplement; TS, main effect of training state; T, main effect of time. *Significantly different from the pre-exercise value in the same group, P < 0.05.
Although vitamins C and E are effective antioxidants in vitro, only conflicting evidence regarding the efficacy of these compounds as antioxidants in vivo exists (39). Indeed, many studies have reported that high doses of vitamin E are poorly effective at decreasing levels of lipid peroxidation in humans (40, 41). Similarly, ample evidence indicates that vitamin C also does not modify redox status (42). In fact, many review analyses have concluded that it is imperative that enrolled subjects have hypovitaminosis C and/or E at study entry to ensure the possibility of an effect (39, 43, 44). On the basis of the concentration of vitamins C and E at the study entry, it is clear that none of the subjects in the current study had hypovitaminosis. Moreover, it is worth mentioning that even the in vivo radical scavenging activity of vitamin E is currently strongly debated (45, 46).

Conclusions

The particular supplementation regimen and exercise stressor examined in the current study showed no benefit of 11 wk of mixed antioxidant supplementation with vitamins C and E on muscle performance, blood and muscle redox status, or hemolysis. The complete lack of any effect reported here on the physiologic and biochemical outcome measures used raises questions about the validity of using oral antioxidant supplementation as redox modulators of muscle and redox status in healthy humans. It is critical to appreciate that the lack of effects seen in this and other trials does not necessarily disprove the central role of reactive species in adaptations taking place in exercise. It is still possible that reactive species play a role in exercise adaptations, but other more specific antioxidants may be needed to reveal their existence. In addition, it is probable that the use of another type of exercise stimulus (eg, aerobic exercise) may have differently affected the redox responses to antioxidant supplementation. Similarly, it is also possible that other adaptive mechanisms (such as heat-shock proteins or redox-sensitive transcription factors) may have exhibited a response different from that of redox biomarkers. Future studies should attempt to modify redox status by using specific redox modulators (such as mitoQ) and/or select subjects with diseases or lifestyle factors relating to oxidative stress disturbances (such as obesity and smoking).

The authors’ responsibilities were as follows: AAT: performed the biochemical determinations, the physiologic measurements, and training intervention and co-wrote the manuscript; AZJ, MGN, and IGF: conceived the study, and co-wrote the manuscript; VP: set up and performed the statistical analysis and co-wrote the manuscript; and YK: co-wrote the manuscript and supervised the entire study. The authors had no personal or financial conflicts of interest.

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