Antioxidants Did Not Prevent Muscle Damage in Response to an Ultramarathon Run

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ABSTRACT

MASTALOUDIS, A., M. G. TRABER, K. CARSTENSEN, and J. J. WIDRICK. Antioxidants Did Not Prevent Muscle Damage in Response to an Ultramarathon Run. Med. Sci. Sports Exerc., Vol. 38, No. 1, pp. 72–80, 2006. Purpose: This study was conducted to determine if 6 wk of supplementation with vitamins E and C could alleviate exercise-induced muscle damage. We studied 22 runners during a 50-km ultramarathon. Methods: Subjects were randomly assigned to one of two groups: (a) placebos (PL) or (b) antioxidants (AO) (1000 mg vitamin C and 300 mg RRR-α-tocopherol acetate). Blood samples were obtained before supplementation (baseline), 24 h pre-, 12 h pre-, and 1 h prerace; midrace, postrace, 2 h postrace, and for 6 d postrace. Plasma α-tocopherol (α-TOH) and ascorbic acid (AA), and muscle damage markers (creatine kinase (CK) and lactate dehydrogenase (LDH)), as well as maximal voluntary contraction (MVC) of the hamstring and quadriceps were assessed. Results: With supplementation, plasma α-TOH and AA increased in the AO but not the PL group. LDH and CK increased in response to the race; LDH peaked at postrace and CK reached maximal values 2 h and 1 d postrace; neither was affected by treatment. Adjusting for between-subject differences in baseline CK values revealed that men had higher levels of CK than did women throughout the study. Correcting CK values for lean body mass (kg) eliminated sex differences, but not changes over time. CK was significantly correlated (R = 0.52, P < 0.0001) with C-reactive protein, an acute phase response marker. MVC decreased 14–26% in all groups in response to the run. Eccentric hamstring (EH) torque and concentric quadriceps (CQ) power exhibited the largest deficits, 26 and 24%, respectively, with no effect of treatment. CQ recovered at a faster rate in women than in men. Conclusion: Antioxidants appeared to have no effect on exercise-induced increases in muscle damage or recovery, but important sex differences were observed. Key Words: VITAMIN E, VITAMIN C, EXERCISE, OXIDATIVE STRESS, ENDURANCE EXERCISE

Endurance running can cause damage to the active muscle, as indicated by ultrastructural disruption of the sarcomere (20), increased release of muscular enzymes into the plasma (20), and substantial impairment in maximal torque production (23). The practical implications of this damage have been reviewed (34) and include decreased joint range of motion, increased fatigability, decreased shortening velocity, and prolonged strength loss. A loss in torque production of the knee extensors as large as 20–30% has been reported following endurance running (22,23). Although these effects may be the result of ultrastructural damage to the sarcomere, impaired excitation–contraction uncoupling (34), or central fatigue (23), evidence that oxidative damage by reactive oxygen species (ROS) mediates skeletal muscle damage is accumulating (20).

It is generally accepted that at rest the body produces ROS continuously. In healthy individuals at rest, these ROS are produced at levels well within the capacity of the body’s antioxidant defense system. In response to endurance exercise, oxygen (O2) consumption increases 10- to 20-fold systemically and as much as 100- to 200-fold at the level of the skeletal muscle (8), resulting in substantially increased mitochondrial electron flux (4). ROS leaking from the mitochondria during exercise are considered a main source of oxidative stress (8) as oxidant production outpaces antioxidant defenses. Other potential sources of sources of ROS during exercise include enhanced oxidation of purines, prostanoid metabolism, damage to iron-containing proteins, disruption of Ca2+ homeostasis, neutrophil activation, and autoxidation of catecholamines (10).

Vigorous exercise results in oxidative stress as evidenced by increased lipid peroxidation (17), DNA damage (19), and protein oxidation (29). Damage to skeletal muscle cell membranes by ROS, specifically lipid peroxidation, can impair cell viability and lead to necrosis and an acute phase inflammatory response (8). Specifically, ROS may play a central role in the etiology of skeletal muscle damage via oxidation of ion transport systems, leading to disruption Ca2+-ion homeostasis, impaired mitochondrial respiratory control, distortions in signal transduction pathways, and ultimately cell dysfunction (14).

Therefore, quenching of ROS by antioxidants could protect against muscle damage caused by exercise. The present study was conducted to determine if prior supplementation with the antioxidant vitamins E and C could attenuate exercise-induced muscle damage, accelerate the rate of recovery from damaging exercise, or both.
METHODS

Human Subjects

A description of the study design has been published previously (18,19). Briefly, the protocol for this study was approved by the Oregon State University institutional review board for the protection of human subjects and written informed consent was obtained from each subject. Runners (11 women and 11 men) were recruited from the pool of participants in a 50-km (32-mile) ultramarathon trail run.

Baseline Testing

Before enrollment into the study, all subjects completed a submaximal oxygen consumption (VO\textsubscript{2}) test, a standard blood chemistry screening, a body composition assessment, a 3-d diet record, and a general health screening questionnaire. For the VO\textsubscript{2max} test, the Bruce Treadmill Protocol was used (16). Maximal oxygen consumption (VO\textsubscript{2max}) was then extrapolated from the VO\textsubscript{2} data based on age predicted maximal heart rate as described previously (18,19). Body density was estimated with an air displacement densitometry plethysmograph (BOD POD, Life Measurement Instruments, Concord, CA) using the Siri equation (30). The BOD POD has been established as a reliable and valid instrument for evaluating body composition in a wide range of subjects (7). The validity and reliability of this method to accurately assess body composition in both lean women (15) and men (33) has been demonstrated previously. The intraclass correlation (ICC) for this technique at our institution is 0.92 (99% CI = 0.85–0.96) (15). A 3-d diet record (two weekdays and one weekend day) was used to estimate subjects’ average daily intake of vitamins E and C before the beginning of the study. Records were analyzed using Esha Food Processor Program (Salem, OR).

Subject Participation Criteria

Inclusion criteria for participation in the study included nonsmoking status, age 18–60 yr, and a VO\textsubscript{2max} classified as excellent fitness by Powers and Howley (26). Potential participants were excluded based on antioxidant supplement use (e.g., vitamin C, vitamin E, selenium, or carotenoids), abnormal cholesterol (≥7.8 mmol·L\textsuperscript{-1}; 300 mg·dL\textsuperscript{-1}), triglyceride (≥3.8 mmol·L\textsuperscript{-1}; 300 mg·dL\textsuperscript{-1}), or fasting blood glucose levels (≥7.8 mmol·L\textsuperscript{-1}; 140 mg·dL\textsuperscript{-1}), or other supplement use (performance-enhancing, or herbal type products), vegetarian or other restrictive dietary requirements, pregnancy or suspected pregnancy, and chronic upper respiratory infections.

Subject Characteristics

The physical characteristics of the subjects have been reported previously (18,19). Average age was 39 ± 2.5 yr, VO\textsubscript{2max} 58 ± 1 mL·kg\textsuperscript{-1}·min\textsuperscript{-1}, and self-reported weekly training distance 43 ± 3 km; no differences existed between treatment groups or sexes for these characteristics. Men were taller, weighed more, and had lower percent body fat than women. Women in the AO group weighed slightly less than women in the PL group (P < 0.05), whereas men in the AO group were slightly taller than men in the PL group (P < 0.05). Within each sex, VO\textsubscript{2max} and percent body fat did not differ among treatment groups. During training, estimated average daily nutrient intakes were 2653 ± 201 kcal, 142 ± 26 mg vitamin C, and 14 ± 3 mg vitamin E; no differences existed between men and women in energy or dietary vitamin E or C intakes.

Study Design

Randomization to treatment group. Subjects were randomly assigned in a double-blind fashion to one of two treatment groups: 1) PL (300 mg soybean oil and 1000 mg citric acid (500 mg twice daily), or 2) AO (300 mg RRR-\textalpha-tocopheryl acetate [2,5,7,8-tetramethyl-2R-(4’R,8’R,12-trimethyltridecyl)-6-chromanol] in soybean oil and 1000 mg ascorbic acid (500 mg twice daily).

Muscle function assessment. Maximal voluntary contraction (MVC) of the knee extensors and flexors (quadriceps and hamstrings) was measured using an isokinetic dynamometer (Kin-Com 500H; Kin-Com, Chattex Corp., Chattanooga, TN). For all measurements, strength values were corrected for the effect of gravity on the limb in the horizontal position. The ability of this instrument to provide reliable and valid measurements of muscle strength has been established (6); the coefficient of variation (CV) for muscle strength assessments in our laboratory has been reported previously (CV < 4% for lower limbs) (35,36). Subjects were tested at baseline (before supplementation), the day before the race (1 d prerace), 2 h after the race (2 h postrace), and for 6 d following the race for a total of nine testing sessions.

After a demonstration of the procedures, subjects were seated and secured in the rigid seat of the dynamometer with hip and knee joints at 90°. Seat and shoulder belt and Velcro straps were used to secure subjects and to isolate the left knee extensor and flexor muscle groups. Subjects were instructed to perform approximately 10 contractions well below their maximum as a warm-up. After the warm-up, subjects performed three maximal isokinetic concentric knee extensions and flexions followed by three maximal eccentric knee extensions and flexions at a velocity of 60°·s\textsuperscript{-1}; peak voluntary torque (N·m) and total power (W) were recorded for each contraction. A 1-min rest was provided between the concentric and eccentric measurements. Subjects were verbally encouraged throughout the testing. For consistency, the same investigator conducted all of the tests. The left leg was used for all sessions.

For each test, torque (N·m) was recorded by the dynamometer over time throughout the 90° of flexion and extension. A representative torque response of a single contraction is depicted in Figure 1. From the individual torque records, we measured peak torque (N·m; Fig. 1), as an index of the muscles’ maximal ability to generate torque and total power (calculated as area under the curve (AUC) and expressed in newton-meters per second or watts, Fig. 1), and
as an index of the muscles’ ability to sustain torque across the contraction.

In the present study, the average of the three maximal torques and the three total power measurements were analyzed for each of four contraction modes (eccentric hamstring, eccentric quadriceps, concentric hamstring, concentric quadriceps). To examine changes in performance after the race, seven rates of change were calculated for both torque and power: prerace to postrace (rate 1), postrace to post 1 d (rate 2), post 1–2 d (rate 3), post 2–3 d (rate 4), post 3–4 d (rate 5), post 4–5 d (rate 6), and post 5–6 d (rate 7). Differences in rates of recovery were then assessed by comparing with zero (no change) and between sexes using analysis of variance (see statistics section).

Blood samples. Blood samples were obtained before antioxidant or placebo supplementation (baseline), the day before the race (24 h prerace), 1 h before the race (prerace (0 h)), in the middle of the race at kilometer 27 (midrace (5 h)), immediately postrace, 2 h after race end (2 h postrace (10 h)), and for 6 d after the race (post 1–6 d (24–144 h)), for a total of 12 time points. All samples were fasting morning blood draws except mid-, post-, and 2-h postrace.

Analytical Methods

Muscle damage markers. Plasma creatine kinase and lactate dehydrogenase were measured by standard clinical assays (Sigma, St. Louis, MO).

C-reactive protein. Plasma C-reactive protein (CRP) was measured as reported previously (18).

Plasma antioxidants. Ascorbic acid and α-tocopherol were determined by paired-ion, reversed-phase HPLC coupled with electrochemical detection, as described previously (18,19).

Statistical Analyses

Values are adjusted means (±SE) from the ANOVA, ANCOVA, or both for 22 subjects. ANOVA and ANCOVA for repeated measures were used to detect statistically significant between- and within-subject effects as described previously (18). To adjust for preexisting differences between individuals before supplementation, baseline concentrations of the following markers were used as covariates in the corresponding statistical model. Baseline covariates included CK (P < 0.003); LDH (P < 0.0001); MVC peak torque corrected for lean body mass (LBM; N·m·kg⁻¹) eccentric hamstring (EH) (P < 0.0001), concentric hamstring (CH) (P < 0.0001), eccentric quadriceps (EQ) (P < 0.0001), and concentric quadriceps (CQ) (P < 0.0002); and MVC work output (power) corrected for LBM (W·kg⁻¹) eccentric hamstring (EH) (P < 0.0001), concentric hamstring (CH) (P < 0.0001), eccentric quadriceps (EQ) (P < 0.0001), and concentric quadriceps (P < 0.0001). An unpaired t-test was used to analyze differences between sexes with regard to subject characteristics (i.e., age, height, weight). Statistics were calculated using The SAS System (SAS Institute Inc, Cary, NC).

RESULTS

Race Results

Race results have been reported in detail previously (18,19). Briefly, all 22 subjects completed the race; previously only 21 were reported because of spurious findings for the comet assay in one subject (19). Run time averaged 423 ± 11 min at a pace of 13.7 ± 0.4 min per mile and an intensity of 71 ± 2% VO₂max; no statistically significant differences were reported in run time, pace, or %VO₂max between the sexes or the treatment groups. Energy expenditure was approximately 2000 kcal greater for men than women, and energy intake was greater for men than for women (energy intake (kcal) 2530 ± 325 (AO); 2468 ± 279 (PL) for men compared with 1844 ± 137 (AO); 2040 ± 221 (PL) for women); neither parameter was different between AO and PL groups within each sex. Vitamin E and C intakes during the run were nominal: <5 mg and <50 mg, respectively; intakes did not differ between treatment groups or sexes.

Plasma Antioxidants in Response to Supplementation

In response to 6 wk of supplementation, as reported previously (19), plasma α-tocopherol increased in the AO supplement group (28 ± 2 vs 46 ± 3 μM, P < 0.05), but was unchanged in the PL group (24 ± 2 vs 26 ± 2 μM). Similarly, ascorbic acid increased in the AO group (113 ± 14 to 127 ± 12 μM, P < 0.05), but was unchanged in the PL group (93 ± 11 vs 73 ± 12 μM).

Plasma Muscle Damage Markers

Levels of LDH increased in response to the race (time main effect, P < 0.005, Fig. 2A) and was unaffected by sex or treatment. Compared with prerace, LDH was increased at midrace, peaked at postrace (P < 0.0001), and remained elevated through day 4 of the recovery period. Similarly, CK increased in response to the race (time main effect, P < 0.0001, Fig. 2B). Compared with prerace, CK was elevated at midrace, continued to increase reaching maximal values 2 h and 1 d postrace (P < 0.0002), and remained elevated through day 5 of the recovery period.
Adjusting for between-subject differences in baseline CK values (baseline covariate $P < 0.01$) revealed sex differences (sex main effect $P < 0.03$) such that men had higher levels of CK than women throughout the study period. CK increased similarly in men and women in response to the race (time main effect, $P < 0.0001$ Fig. 3A). Correcting CK values for lean body mass (kg) eliminated sex differences, but not changes over time (Fig. 3B).

Adjusting for between-subject differences in baseline LDH values (baseline covariate $P < 0.0001$) revealed no treatment or sex differences. LDH increased in response to the race (time main effect, $P < 0.002$; Fig. 4A). Compared with prerace, LDH was elevated at midrace, continued to increase, peaking at postrace ($P < 0.01$), and remained elevated through day 5 of the recovery period. Correcting LDH values for lean body mass (kg) revealed a nonsignificant trend for higher levels in women (Fig. 4B).

Over the course of the study, muscle damage markers CK and LDH were significantly correlated (Pearson product moment correlation $R = 0.64$, $P < 0.0001$) (Table 1). Furthermore, CK was significantly correlated with C-reactive protein (CRP) (Pearson product moment correlation $R = 0.52$, $P < 0.0001$). During the period of maximal

FILEFIGURE 3—Creatine kinase adjusted for baseline covariance. A. Adjusting for between-subject differences in baseline CK values revealed that men had higher levels of CK than women throughout the study period. CK increased similarly in men and women in response to the race. Compared with prerace, CK was elevated at midrace, continued to increase, reaching maximal values 2 h and 1 d postrace, and remained elevated through day 5 of the recovery period. B. Correcting CK values for lean body mass (kg) eliminated sex differences, but not changes over time. * Compared with prerace; &, maximal concentration.

FILEFIGURE 4—Lactate dehydrogenase adjusted for baseline covariance. A. Adjusting for between-subject differences in baseline LDH values revealed no treatment or sex differences. Compared with prerace, LDH was increased at midrace, peaked at postrace, and remained elevated through day 5 of the recovery period. B. Correcting LDH values for lean body mass (kg) revealed a nonsignificant trend for higher levels in women. * Compared with prerace; &, maximal concentration.
muscle damage marker levels (midrace to post 1 d), similar correlations were observed (CK with LDH, Pearson product moment correlation R = 0.64, P < 0.0001; and CK with CRP, Pearson product moment correlation R = 0.63, P < 0.0001).

Maximal Voluntary Contraction: Muscle Damage and Recovery

Muscle torque and power deficits. One subject declined to participate in the MVC testing and another subject did not complete the testing; therefore, results presented are for N = 20 (AO = 10; PL = 10). MVC, expressed either as mean peak torque (N·m) or as total power output (W), was higher in men than in women for each of the three contractions at baseline (Table 2). Adjusting peak torque and total power output for subjects’ lean body mass completely eliminated sex differences. Therefore, maximal peak torque (N·m) and power output (W) for each of the three maximal contractions of each test was corrected for subjects’ lean body mass (kg) and expressed as newton-meters per kilogram and watts per kilogram, respectively. These values were then used to determine torque or power deficit and recovery in response to the run.

The MVC decreased similarly in all groups in response to the run (P < 0.0001) (Figs. 5 and 6). The largest torque deficit, 26%, was observed for EH (Fig. 5A). In the three other muscle contractions, torque was decreased similarly at postrace: CH 17%, EQ 19%, and CQ 16% (Fig. 5B-D). In addition to exhibiting the largest decline in MVC, EH torque was compromised for the duration of the study (Fig. 5A). In contrast, CH and EQ, had recovered to prerace values by day 3 postrace (Fig. 5B–C). Only CQ exhibited any indication of sex differences (sex × time interaction P = 0.0516), but this trend did not reach statistical significance and subjects had recovered by day 3 postrace. No differences between treatment groups were observed for any parameter.

Deficits in power (Fig. 6) were similar to torque deficits. One exception was that CQ exhibited a larger power deficit than torque deficit (Fig. 6D), 24% compared with 16%. Differences between torque and power with regard to recovery, EH recovered power faster, reaching prerace levels by day 5 postrace and CQ power took longer to recover, not reaching prerace levels until day 6 postrace.

Rate of change of muscle torque and power. The rate of muscle torque and power deficits were calculated for each contraction as described in the Methods section. EH, CH, EQ, and CQ all exhibited main effects for time (P < 0.0001, P < 0.005, P < 0.01, and P < 0.0001, respectively; Fig. 7A–D). A substantial decrease in torque production from prerace to postrace was observed for all contractions (rate 1; EH P < 0.0001, CH P < 0.0001, EQ P < 0.01, and CQ P < 0.005). In addition to a main effect for time, CQ exhibited a sex × time interaction (P < 0.003; Fig. 7D). For both men and women, a decline in torque was observed in the prerace to postrace period. In women, this decrease was followed closely by a recovery of torque in the period from postrace to 1 d postrace (rate 2; P < 0.05). In men, recovery appeared to be delayed, not regaining torque until the period from 1 to 3 d postrace (rates 3 and 4; P < 0.01 and P < 0.02, respectively).

Overall, evidence of differences between the sexes was more apparent when examining the rate of muscle power change compared with the rate of muscle torque change. For example, EH, in addition to a time main effect (P < 0.0001), exhibited a sex × time interaction (P < 0.05; Fig. 8A). In both men and women, a significant decrease in power was noted from prerace to postrace (rate 1; P < 0.0001) followed by a rapid recovery in the period from postrace to 1 d postrace (rate 2; P < 0.02). Rate of recovery was faster in men than in women in the postrace period (rate 2; P < 0.002), whereas men showed greater recovery in the period 1–3 d postrace (rates 3 and 4; P < 0.01 and P < 0.04 respectively).

Overall, evidence of differences between the sexes was more apparent when examining the rate of muscle power change compared with the rate of muscle torque change. For example, EH, in addition to a time main effect (P < 0.0001), exhibited a sex × time interaction (P < 0.05; Fig. 8A). In both men and women, a significant decrease in power was noted from prerace to postrace (rate 1; P < 0.0001) followed by a rapid recovery in the period from postrace to 1 d postrace (rate 2; P < 0.02). Rate of recovery was faster in men than in women in the 1–2 d postrace period (rate 3; P < 0.01), whereas men showed less improvement than women in the period 3–4 d postrace (rate 5; P < 0.01). CH exhibited a sex main effect (P < 0.04) in addition to a main effect for time (P < 0.0001; Fig. 8B). In both men and women, a substantial decrease in power from prerace to postrace was observed (rate 1; P < 0.006) followed by a delayed recovery of power in the 1–2 d postrace period (rate 3; P < 0.04). EQ also demonstrated a sex main effect (P < 0.01) as well as a time main effect (P < 0.004; Fig. 8C). In both men and women, a considerable power deficit was observed from prerace to postrace (rate 1; P < 0.01), but no other significant changes were observed. Only changes in rates of CQ power resembled those of torque. EQ revealed both a time main effect (P < 0.0001) and a sex × time interaction (P < 0.01) (Fig. 8D). For both men and women, a decline in power was observed in the prerace to postrace period (rate 1; P < 0.0001). Women recovered at a faster rate than men during the postrace to 1 d postrace period (rate 2; P < 0.04), whereas men showed greater recovery in the period 1–3

<table>
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<tr>
<th>Characteristic</th>
<th>CK</th>
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<tbody>
<tr>
<td>LDH</td>
<td>R = 0.64</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>R = 0.52</td>
<td>P &lt; 0.0001</td>
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TABLE 1. Pearson product correlation coefficients for CK.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak torque (N·m)*</td>
<td>213 ± 13</td>
<td>302 ± 30</td>
</tr>
<tr>
<td>Peak torque (N·m·kg⁻¹)</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Power (W)*</td>
<td>193 ± 14</td>
<td>273 ± 24</td>
</tr>
<tr>
<td>Power/LBM (W·kg⁻¹)</td>
<td>4.1 ± 0.3</td>
<td>4.1 ± 0.3</td>
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TABLE 2. Baseline MVC.

Values are means ± SE. LBM, lean body mass.

* Male vs Females, P < 0.05
d postrace (rates 3 and 4; \( P < 0.002 \) and \( P < 0.05 \), respectively).

**DISCUSSION**

The ultramarathon resulted in substantial increases in muscle damage markers and deficits in maximal voluntary contractions of the knee extensors and flexors. Prior supplementation with the antioxidant vitamins E and C appeared to have no effect on these parameters of muscle damage and recovery, but important sex differences were observed, including an enhanced rate of recovery in women for some measures.

In response to the run, a significant reduction occurred in muscle peak torque production and total power, ranging from 14 to 26%, depending on the mode of contraction (Figs. 5 and 6). This loss of strength and performance capability was similar, although somewhat less, than that reported for a 65-km ultramarathon in which a 30% torque deficit was observed postrace (22). The present study was of a shorter distance (50 km) and muscle testing was isokinetic as opposed to isometric, both of which could explain the smaller attenuation in voluntary torque reported here. In a recent review, Millet and Lepers (21) described greater strength loss in isometric contractions than in concentric contractions following distance running. As would be expected, the contraction mode exhibiting the largest torque deficit postrace, the EH contraction, took the longest to recover (Fig. 5). Similarly, the contractions exhibiting the greatest power deficits postrace, the EH and CQ contractions, also demonstrated the slowest recovery (Fig. 6). No evidence of antioxidant protection against muscle torque or power production impairment was observed for any of the contractions tested.

The inability of antioxidant supplementation to attenuate the exercise-induced reduction in muscle performance or rise in markers of damage is striking in that antioxidants prevented increases in lipid peroxidation in these same
subjects during this race (18). This suggests that muscle performance and enzyme efflux during and after prolonged endurance exercise may be independent of oxidative damage. Instead, CK was significantly correlated with the acute phase inflammatory marker C-reactive protein, suggesting a possible relationship between sarcolemmal integrity and inflammation following the race. Previously, we reported that, although antioxidants abrogated the increase in lipid peroxidation in response to ultramarathon running, they had no effect on the inflammatory markers including TNF, CRP, IL-1, and IL-6 (18).

Millet et al. (22) reported that the 30% decrease in isometric MVC of the knee extensors following a 65-km ultramarathon was caused primarily by a decrease in maximal voluntary activation, offering evidence that the decrease in torque production following endurance exercise may be more the result of central fatigue than damage at the level of the skeletal muscle. Although ROS are likely involved in the etiology of muscle damage via oxidation of ion transport systems, disruption of Ca\(^{2+}\)-ion homeostasis, impaired mitochondrial respiratory control, and distortions in signal transduction pathways (14), it is unknown if ROS are involved in central fatigue. Our findings suggest that loss of torque and power generating capacity following endurance running is unaffected by antioxidant supplementation and, therefore, ROS may not be a cause of the impairment in muscle performance.

Alternatively, the ultramarathon run may have been so strenuous that the oxidative stress overwhelmed the protective effects of the antioxidants. Previously, vitamin C was found to be protective in a more moderate exercise protocol, 60 min of box-stepping exercise (11). One week of prior supplementation with 400 mg vitamin C enhanced the rate of recovery of MVC torque deficit (11). In this same study,
supplementation with 400 mg vitamin E had no effect on recovery; however, plasma vitamin E was not substantially increased in the short supplementation period (3 wk before and 1 wk postexercise) (11). When vitamin E was supplemented for a longer time period at a higher dose (1200 IU for 3 wk) and plasma vitamin E levels were more than doubled, supplementation still had no effect on exercise-induced concentric torque deficits following a short anaerobic muscle-damaging exercise protocol (3).

Creatine kinase is the most commonly used marker of muscle damage; it is well documented that CK increases in response to marathon running, reaching peak levels 24 h postexercise in trained runners (2). In the present study, unadjusted CK concentrations were elevated at postrace, but did not reach maximal concentrations until 24 h postexercise, which is consistent with previous reports (2). Study of the response of CK to antioxidant supplementation has yielded equivocal results. α-Tocopherol supplementation (1200 IU·d⁻¹ for 4 wk) attenuated CK increases following six successive days of running (9), but no effect of 13.5 mg·d⁻¹ for 3 wk before a marathon run on the plasma CK response was observed (12). Vitamin C supplementation as a single 1000-mg dose 1 h before a 4-h run did not attenuate CK increases (28), but supplementation with 1000 mg·d⁻¹ vitamin C for 1 wk before a 90-km run exacerbated the CK and CRP response to exercise, suggesting an adverse effect of supplementation (24). Our observation that combined supplementation with 300 mg vitamin E and 1000 mg vitamin C had no effect on exercise-related increases in CK is in agreement with the study of Petersen et al. (25). They reported no influence of 2 wk of supplementation with 400 mg vitamin E and 500 mg vitamin C on increases in CK following a 90-min treadmill run (25). Our findings are in contrast, however, to those of Rokitzki et al. (27), who reported that 4.5 wk of supplementation with 400 IU vitamin E and 200 mg vitamin C attenuated increases in CK following a 90-km ultramarathon. Thus, results appear to be influenced by amount and duration of dose and the type of antioxidant supplemented, as well as intensity, duration, and type of exercise.

Adjusting for between-subject differences in baseline CK values (baseline covariate) revealed that men had higher levels of CK than women throughout the study period, including baseline. This finding is consistent with previous assertions of sex differences in sarcolemmal stability, and in exercise-induced muscle damage and inflammatory responses (32). Specifically, it has been proposed that men have higher resting CK levels and a higher CK response to aerobic exercise than do women (5). In the present study, no differences were found between men and women with regard to training regimen, VO₂max, or race time; therefore, none of these variables could explain the difference in CK concentrations. A common explanation for lower CK in women is that higher levels of estrogen in women may increase sarcolemmal stability, reducing CK efflux from the muscle cell (32). In the present study, however, the CK sex difference could be completely accounted for by adjusting CK values for subjects’ lean body mass (Fig. 3). Apple and Rhodes (1) reported higher levels of CK in men than in women after adjusting for differences in total body weight and body surface area, but they did not adjust for differences in lean body mass. In our study, we adjusted for lean mass because a major component of lean body mass is muscle, and muscle is the source of CK. Our findings suggest that the higher CK levels in men versus women can be explained simply by the higher absolute muscle mass of the men. Based on our findings that lean body mass accounted for sex differences in CK response, we propose that future studies investigating muscle damage should adjust for lean body mass when analyzing the CK response.

A second, less common plasma marker of muscle damage, LDH, is known to increase in response to marathon running (13). Few studies have investigated the ability of antioxidant supplementation to modulate LDH responses to marathon running. Presently, we report that supplementation with vitamins E and C had no effect on LDH increases following distance running. This finding differs from the report of Itoh et al. (9) that supplementation with 1200 IU·d⁻¹ α-tocopherol for 4 wk attenuated LDH increases following six successive days of running, suggesting that a larger dose of vitamin E is required to attenuate the increase in muscle damage resulting from endurance exercise. Clearly, further research is needed to confirm this hypothesis.

Although no apparent effect of antioxidant supplementation on muscle damage and recovery occurred, an interesting finding merits further research was the sex differences in the rate of recovery of muscle function for some contractions. With regard to the CQ contraction, women exhibited faster rates of recovery of torque and power generating capacity in the postrace to 1 d postrace period, whereas a significant increase in the rate of recovery was not observed in men until the 1–3 d postrace period (Figs. 7 and 8).

Sex differences with regard to MVC did not correspond with sex differences in plasma muscle damage markers or inflammatory markers. Stupka et al. (31) reported in untrained subjects that the CK response was not representative of the extent of muscle damage assessed using histologic techniques. Furthermore, they reported higher levels of the inflammatory marker bcl-2-positive cells per square millimeter of tissue in men than in women following an eccentric exercise protocol (31). Although we did not observe any differences in plasma markers of inflammation, muscle biopsies were not collected in the present study, and therefore bcl-2-positive cells were not measured. The use of muscle biopsy techniques in addition to plasma markers may offer a more comprehensive picture of the exercise-induced muscle damage response in both men and women.

Important sex differences were observed for the muscle damage marker CK and for the recovery of muscle function and performance. Future studies may take advantage of muscle biopsy techniques to examine the effects of antioxidant supplementation directly at the level of the skeletal muscle. Although the present study yielded no effect of antioxidants on exercise-induced increases in muscle damage or recovery from adverse consequences resulting from a marathon run, previous studies using higher doses of vitamin E, 1200 IU·d⁻¹, did see protection. Therefore, future studies may investigate higher levels of vitamin E supplements in addition to further investigating the interesting sex differences observed here.
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