Free Radical Biology & Medicine, Vol. 36, No. 10, pp. 1329–1341, 2004

0891-5849/$-see front matter

ELSEVIER

doi:10.1016/j.freeradbiomed.2004.02.069

Original Contribution

ANTIOXIDANT SUPPLEMENTATION PREVENTS EXERCISE-INDUCED LIPID PEROXIDATION, BUT NOT INFLAMMATION, IN ULTRAMARATHON RUNNERS

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(*Received* 26 *January* 2004; *Accepted* 19 *February* 2004)

Abstract—To determine if 6 weeks of supplementation with vitamins E and C could alleviate exercise-induced lipid peroxidation and inflammation, we studied 22 runners during a 50 km ultramarathon. Subjects were randomly assigned to one of two groups: (1) placebos (PL) or (2) antioxidants (AO: 1000 mg vitamin C and 300 mg *RRR*-α-tocopheryl acetate). Blood samples were obtained prior to supplementation (baseline), after 3 weeks of supplementation, 1 h pre-, mid-, and postrace, 2 h postrace and for 6 days postrace. Plasma levels of α-tocopherol (α-TOH), ascorbic acid (AA), uric acid (UA), F_2-isoprostanes (F_2-IsoPs), tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) were measured. With supplementation, plasma α-TOH and AA increased in the AO but not the PL group. Although F_2-IsoP levels were similar between groups at baseline, 28 ± 2 (PL) and 27 ± 3 pg/ml (AO), F_2-IsoPs increased during the run *only* in the PL group (41 ± 3 pg/ml). In PL women, F_2-IsoPs were elevated postrace ($p < .01$), but returned to prerace concentrations by 2 h postrace. In PL men, F_2-IsoP concentrations were higher postrace, 2 h postrace, and 1, 2, 3, 4, and 6 days postrace (PL vs. AO group, each $p < .03$). Markers of inflammation were increased dramatically in response to the run regardless of treatment group. Thus, AO supplementation prevented endurance exercise-induced lipid peroxidation but had no effect on inflammatory markers.

Keywords—α-Tocopherol, Ascorbic acid, Ultramarathon, Free radicals, Oxidative stress, Vitamin E, Vitamin C, Running, Exercise, Inflammation, Cytokines, Antioxidants

INTRODUCTION

Strenuous exercise causes oxidative stress, resulting in lipid peroxidation [1–7] and DNA damage [8]. Evidence of protein oxidation resulting from exercise-induced oxidative stress is less definitive [1,9]. In response to endurance exercise, oxygen (O_2) consumption increases 10- to 20-fold systemically [10] and as much as 100- to 200-fold at the level of the skeletal muscle [11], resulting in substantially increased mitochondrial electron flux. Reactive oxygen species (ROS) ''leaking'' from the mitochondria during exercise are considered a main source of oxidative stress [11]. Other potential sources of ROS during exercise include enhanced purine oxidation, damage to iron-containing proteins, disruption of Ca^{2+} homeostasis [12], and NADPH oxidase [4]. These exercise-induced ROS are also thought to modulate acute-phase inflammatory responses [13].

Previously we demonstrated a near-2-fold increase in F_2-isoprostanes (F_2-IsoPs) and accelerated disappearance of deuterium-labeled α-tocopherol immediately after a 50 km ultramarathon. Therefore, our rationale for the present study is based on this observation of increased lipid peroxidation in endurance runners. Hypothetically, supplementation with the antioxidant vitamins E and C

This work was presented in part at the Annual Meeting of the Oxygen Society in Seattle, WA, USA on November 21, 2003 and was published in abstract form (*Free Radic. Biol. Med.,* 35:S42;2003).

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could alleviate exercise-induced lipid peroxidation. However, results from previous studies investigating the protective effects of supplementation with vitamins E and/or C have been inconclusive: inhibition of lipid peroxidation [14–20], no effect [21–25], and even increased lipid peroxidation [17,26]. Possible reasons for these inconsistencies include: differences in modes, duration, and intensity of exercise as well as variation in the methodologies used to assess lipid peroxidation. A lack of consensus regarding the effectiveness of antioxidants in inhibiting exercise-induced lipid peroxidation and the popularity of antioxidant supplements in the physically active community make this an important area for research [27].

Endurance and/or damaging exercise elicits a stress response analogous to the acute-phase immune response [13]. Exercise-induced tissue damage and/or increased reactive oxygen species (ROS) production stimulate cytokine production, upregulating the inflammatory cascade [13,28,29]. Initially, pro-inflammatory cytokines, tumor necrosis factor α (TNF-α) and interleukin (IL)-1β are produced, stimulating IL-6 production [28]. IL-6, the primary mediator of the acute-phase reaction, stimulates production of acute-phase proteins, including C-reactive protein (CRP), and restricts the extent of the inflammatory response by enhancing production of anti-inflammatory cytokines [29,30]. Neutrophils, monocytes, and lymphocytes recruited to the site of inflammation produce ROS and proteolytic enzymes to clear and repair damaged tissue [13,28,30,31].

It has been postulated that, in response to exercise, ROS stimulate cytokine production from various cell types including skeletal muscle [32]. Thus, antioxidant supplementation may attenuate this stress response to exercise [32]. The few studies examining the effects of antioxidant supplementation on exercise-stimulated cytokine production have generated mixed results. Vitamin C supplementation has been demonstrated to attenuate increases in cortisol [22,33] but to stimulate increases in CRP [33]. Most studies found no effect of vitamin E or C on the cytokine response to exercise [33–36], but Cannon et al. [37] reported an attenuation of the increase in IL-1β with vitamin E supplementation and Vassilakopoulos et al. [32] reported that increases in TNF-α, IL-1β, and IL-6 were all prevented with an antioxidant cocktail. However, only two studies [22,26] included a marker of lipid peroxidation; therefore, the link between ROS and cytokine production remains unresolved.

The purpose of the present study was to determine whether exercise-induced lipid peroxidation and inflammation could be alleviated by 6 weeks of prior supplementation with vitamins E and C in recreationally trained women and men participating in an ultramarathon run. We believe that this is the first study to investigate the effects of both vitamin E and vitamin C on exercise-induced lipid peroxidation using F_2-IsoPs in addition to measuring inflammatory markers.

EXPERIMENTAL PROCEDURES

Human subjects

A description of the study design has been published previously [38]. Briefly, the protocol for this study was approved by the Oregon State University Institutional Review Board for the Protection of Human Subjects. Runners (11 women and 11 men) were recruited from the pool of participants in a 50 km (32 mile) ultramarathon trail run. All subjects were physically fit, noncompetitive athletes. Prior to enrollment into the study, all subjects completed a submaximal oxygen consumption ($VO_{2\ submax}$) test, body composition assessment, standard blood chemistry screening, 3-day diet record, and general health-screening questionnaire. For the $VO_{2\ submax}$ test the Bruce Treadmill Protocol was used [39]. Subjects ran on a treadmill beginning at a moderate pace; every 3 min the grade and intensity were increased until subjects achieved 85% of their age-predicted maximum heart rate (HR). Age-predicted maximum HR was calculated with the equation: predicted heart rate (bpm) = 220 − age (years) [40]. Oxygen consumption and carbon dioxide expiration were measured throughout the test using a SensorMedics Metabolic cart (SensorMedics, Yorba Linda, CA, USA). Heart rate was recorded using a Polar HR monitor (Polar Electro Inc. Woodbury, NY, USA). For body composition assessment, a Bod Pod was used to determine percentage body fat. Three-day diet records (2 week days and 1 weekend day) were used to estimate subjects' average daily intake of vitamins E and C prior to the beginning of the study. Records were analyzed using Esha Food Processor Program (Salem, OR, USA).

The physical characteristics of the subjects have been reported previously [38]. Average age was 39 ± 2.5 years, $VO_{2\ max}$ 58 ± 1 ml/kg/min, and estimated weekly training distance 43 ± 3 km; there were no differences between treatment groups or genders with respect to these characteristics. Men were taller, weighed more, and had lower percentage body fat than women. Antioxidant (AO) group women weighed slightly less than placebo (PL) group women ($p < .05$), whereas AO men were slightly taller than PL men ($p < .05$). Within each gender, VO_{2max} and % body fat did not differ among treatment groups. Estimated average daily nutrient intakes did not differ between men and women: 142 ± 26 mg vitamin C and 14 ± 3 mg vitamin E.

Criteria for subject participation. Inclusion criteria for participation in the study included nonsmoking status, age 18–60 years, and a $VO_{2\ max}$ classified as excellent fitness

by Powers and Howley [40]. Potential participants were excluded based on antioxidant supplement use (e.g., vitamin C, vitamin E, selenium, or carotenoids); abnormal cholesterol (≥7.8 mmol/l (300 mg/dl)), triglyceride (≥3.8 mmol/l (300 mg/dl)), or fasting blood glucose (≥7.8 mmol/L (140 mg/dl)) levels; other supplement use (performance-enhancing or herbal-type products); vegetarian or other restrictive dietary requirements; pregnancy or suspected pregnancy; and chronic upper respiratory infections.

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*-α-tocopheryl acetate and 1000 mg ascorbic acid (500 mg twice daily)).

Blood samples. Blood samples were obtained prior to supplementation (baseline), after 3 weeks of supplementation (compliance), 1 h prior to 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 daily for 6 days after the race (post 1–6 days, 24–144 h), for a total of 12 time points. All samples were fasting morning blood draws except those midrace, postrace, and 2 h postrace.

Blood was drawn into two 5 cc green-top Vacutainer tubes (containing 143 USP units sodium heparin) and one 5 cc purple-top Vacutainer tube (containing 1 mg/ml EDTA). Blood was centrifuged at 2500*g* for 10 min; plasma was then aliquoted to cryotubes for various assays. Samples were flash frozen in liquid nitrogen and stored at −80°C until time of analysis.

For vitamin C analysis, freshly drawn plasma (50 μl) was mixed with an equal volume of chilled 5% (w/v) metaphosphoric acid in 1 mM diethylenetriaminepentaacetic acid (made fresh daily) and centrifuged to remove the precipitated proteins. A portion of the supernatant was frozen at −80°C until day of analysis.

Diet. Subjects were instructed to consume a restricted diet low in vitamins E and C for the 6 weeks prior to the race and the 6 days after the race (in conjunction with the supplements or placebos) and then a controlled diet for 2 days: 1 day prior to the race and race day. Subjects were provided with a list of foods high in vitamins E and C to be avoided and they were instructed on how to read food labels to identify foods fortified with these nutrients.

All foods for the 2 days of the controlled diet were prepared and provided to the participants in the Metabolic Feeding Unit in the Human Nutrition Research Laboratory at Oregon State University. Subjects were then instructed to resume the restricted vitamin C and E diet for 6 days postrace. Both diets consisted of 10–15% protein, 55–65% carbohydrate, and 25–30% fat; the macronutrient breakdown recommended for optimal athletic performance [40,41]. For the entirety of the study, subjects were provided with energy bars, carbohydrate pastes, and carbohydrate drinks known to be low in vitamins E and C, to be used for fuel during their training and during the race.

Activity log. For the 6 weeks prior to the ultramarathon, subjects recorded daily the amount, type, duration, and intensity of exercise performed each day. This information was used to identify intersubject differences in training regimen.

Nonsteroidal anti-inflammatory drugs (NSAIDs). Subjects were asked to refrain from using NSAIDs, including naproxen sodium and ibuprofen, throughout the 7 weeks of the study. As an alternative to these drugs, subjects were allowed to use acetaminophen.

Race schedule. Subjects were weighed immediately before and after the race to estimate losses in body water. Selected foods and beverages were provided to the subjects at check points along the race course, allowing for quantification of the caloric and nutrient intake of the subjects, and as a means to control vitamin E and vitamin C intake before and during the race. Subjects wore a Polar heart rate monitor (Polar Electro Inc., Woodbury, NY, USA) during the run; average heart rate in conjunction with oxygen consumption levels, measured previously in the laboratory, were then used to quantify individual energy expenditure during the race. Subjects were asked to refrain from physical activity for the 6 days following the ultramarathon to investigate the effects of AO supplementation on recovery.

Energy expenditure calculation. Energy expenditure was calculated for each individual based on average heart rate during the run and the corresponding oxygen consumption (VO_2) multiplied by the time it took each subject to finish the race:

$$\text{average HR (bpm)} - \text{VO}_2\text{(l/min)} \times 5 = \text{kcal/min} \times \text{race time (min)} = \text{total kcal.}$$

Assessment of lipid peroxidation. Plasma F_2-IsoPs were measured by stable isotope dilution gas chromatography negative ion chemical ionization mass spectrometry as described previously [42].

Plasma antioxidants. Ascorbic and uric acids were determined by paired-ion reversed-phase HPLC coupled with electrochemical detection using a modification of

the method described by Kutnink et al. [43]. α-Tocopherol was measured by HPLC coupled with electrochemical detection using a modified version of the method of Podda et al. [44]. Plasma triglycerides and cholesterol were measured by standard clinical assays (Sigma kit). Plasma α-tocopherol was standardized for changes in lipoproteins using plasma lipid levels (cholesterol + triglycerides).

Inflammatory markers. Plasma cytokines and CRP were measured as reported previously: IL-1β and TNF-α [45], IL-6 and CRP [46].

Statistical analyses

Data are expressed as the means ± SE of 22 subjects. Analysis of covariance for repeated measures was used to detect statistically significant between- and within-subject effects [47]. To adjust for preexisting differences between individuals prior to supplementation, baseline concentrations of the following markers were used as covariates in the corresponding statistical model. Baseline covariates included: ascorbic acid ($p < .01$), α-tocopherol ($p < .003$), α-tocopherol/lipid ($p < .0001$), F_2-IsoPs ($p < .001$), IL-6 ($p < .002$), TNF-α ($p < .02$), and CRP ($p < .02$). An unpaired *t*- test was used to analyze differences between genders with respect to subject characteristics (i.e., age, height, weight). Statistics were calculated using the SAS System (SAS Institute Inc, Cary, NC, USA).

RESULTS

Race results

Race results have been reported [38]. Briefly, all 22 subjects completed the race (Table 1); previously only 21 were reported due to spurious findings for the comet assay in one subject [38]. Run time averaged 423 ± 11 min at a pace of 13.7 ± 0.4 min/mile and an intensity of 71 ± 2% $VO_{2\ max}$; there were no statistically significant differences in run time, pace, or $\%Vo_{2max}$ between the genders or the treatment groups. Energy *expenditure* was approximately 2000 kcal greater for men than women, and energy *intake* was greater for men than women (energy intake in kcal: 2530 ± 325 (AO) and 2468 ± 279 (PL) for men compared with 1844 ± 137 (AO) and 2040 ± 221 (PL) for women); neither parameter was different between AO and PL groups within each gender. Carbohydrate intakes were higher in men than women, but percentage of total calories from carbohydrate did not differ between genders or treatment groups (Table 1). Intakes of vitamins E and C from food during the run were nominal, < 5 mg and < 50 mg, respectively, and did not differ between treatment groups or genders.

Table 1. Race Results[a]

Parameter	Females		Males	
	AO ($N=6$)	PL ($N=5$)	AO ($N=6$)	PL ($N=5$)
Run time (min)	422 ± 17	436 ± 35	408 ± 22	430 ± 31
% VO_{2max}	74 ± 5	67 ± 3	71 ± 6	70 ± 7
CHO intake (kcal)[b]	1626 ± 99	1636 ± 180	2030 ± 290	1895 ± 247
% CHO	78 ± 3	81 ± 4	80 ± 1	76 ± 3

[a] Values are means ± SE. HR, heart rate; VO_{2max}, maximal oxygen consumption; CHO, carbohydrate.
[b] Males versus females, $p < .05$.

Plasma antioxidants in response to supplementation

In response to 6 weeks of supplementation [38], plasma α-tocopherol increased in the AO group (28 ± 2 vs. 46 ± 3 μM, $p < .05$), 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, $p < .05$) but was unchanged in the PL group (93 ± 11 vs. 73 ± 12 μM).

Plasma antioxidants in response to the ultramarathon run

Ascorbic acid concentrations were significantly higher in the AO group compared with the PL group at all time points except baseline (Fig. 1) (treatment main effect, $p < .0007$). In response to the 50 km ultramarathon run, plasma ascorbic acid *increased* similarly in both treatment groups (time main effect, $p < .002$), with significant increases compared with prerace at midrace and postrace ($p < .01$), returning to prerace values by 2 h postrace. Men and women had similar ascorbic acid concentrations.

Concentrations of uric acid, a plasma water-soluble antioxidant [11], were not different between treatment groups, but were higher in men than in women at all time points (gender main effect, $p < .01$). Adjustment for differences in presupplementation uric acid concentrations (baseline covariate, $p < .0002$) eliminated these gender differences; therefore, uncorrected uric acid concentrations are presented. Uric acid concentrations increased in response to the run in both men and women (Fig. 2) (time main effect, $p < .001$). Compared with prerace, plasma uric acid concentrations were elevated at midrace, postrace, 2 h postrace, and 1 day postrace ($p < .02$), not returning to prerace until 2 days postrace.

Similar to ascorbic acid, α-tocopherol concentrations were significantly higher in the AO group compared with the PL group at all time points except baseline (Fig. 3A) (treatment main effect, $p < .0001$). α-Tocopherol concentrations changed differently in the treatment groups over

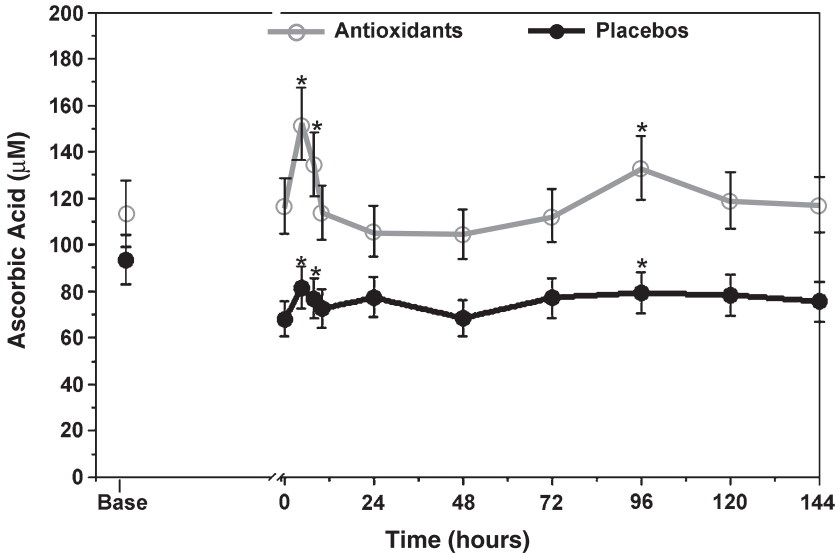

Fig. 1. Plasma ascorbic acid concentrations increased in response to supplementation and to the ultramarathon run. Ascorbic acid concentrations (means ± SE) were significantly higher in the AO than in the PL group at all time points except baseline (treatment main effect, $p < .0007$). In response to the run, plasma ascorbic acid increased in both treatment groups (time main effect, $p < .002$). Ascorbic acid concentrations were increased compared with prerace at midrace and postrace ($p < .01$), returning to prerace values by 2 h postrace; there were no differences between men and women. Uncorrected baseline (Base) values used as the covariate in the model are also presented. Time 0 h = prerace. *Compared with prerace.

time (treatment × time interaction, $p < .007$). In the AO group, compared with prerace, α-tocopherol concentrations increased at midrace ($p < .003$), returned to prerace values by postrace, and fell to below prerace concentrations for the duration of the study ($p < .01$). In the PL group, no changes in α-tocopherol concentrations were observed during the race, but levels declined to below prerace values 2 and 5 days postrace.

Lipid-corrected α-tocopherol concentrations (per lipids = α-tocopherol/cholesterol + triglycerides) were also significantly higher in the AO group than the PL group at all time points except baseline (Fig. 3B) (treatment main

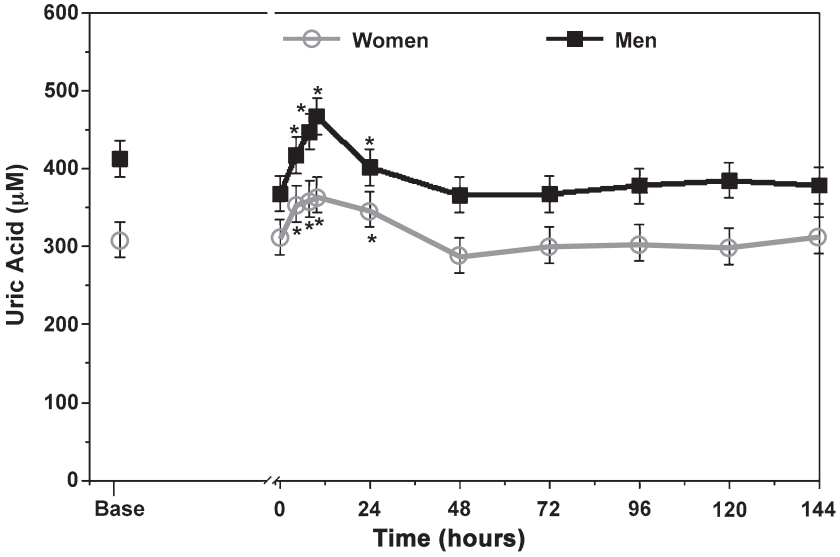

Fig. 2. Plasma uric acid concentrations increased in response to the ultramarathon run. Uric acid concentrations (means ± SE) were significantly higher in men than in women at all time points (gender main effect, $p < .01$), and increased in response to the run in both genders (time main effect, $p < .001$). Compared with prerace, plasma uric acid concentrations were elevated midrace, postrace, 2 h postrace and 1 day postrace ($p < .02$), not returning to prerace until 2 days postrace. Uric acid concentrations were not different between treatment groups. Time 0 h = prerace. Values shown are not corrected for differences between the genders at baseline (Base). *Compared with prerace.

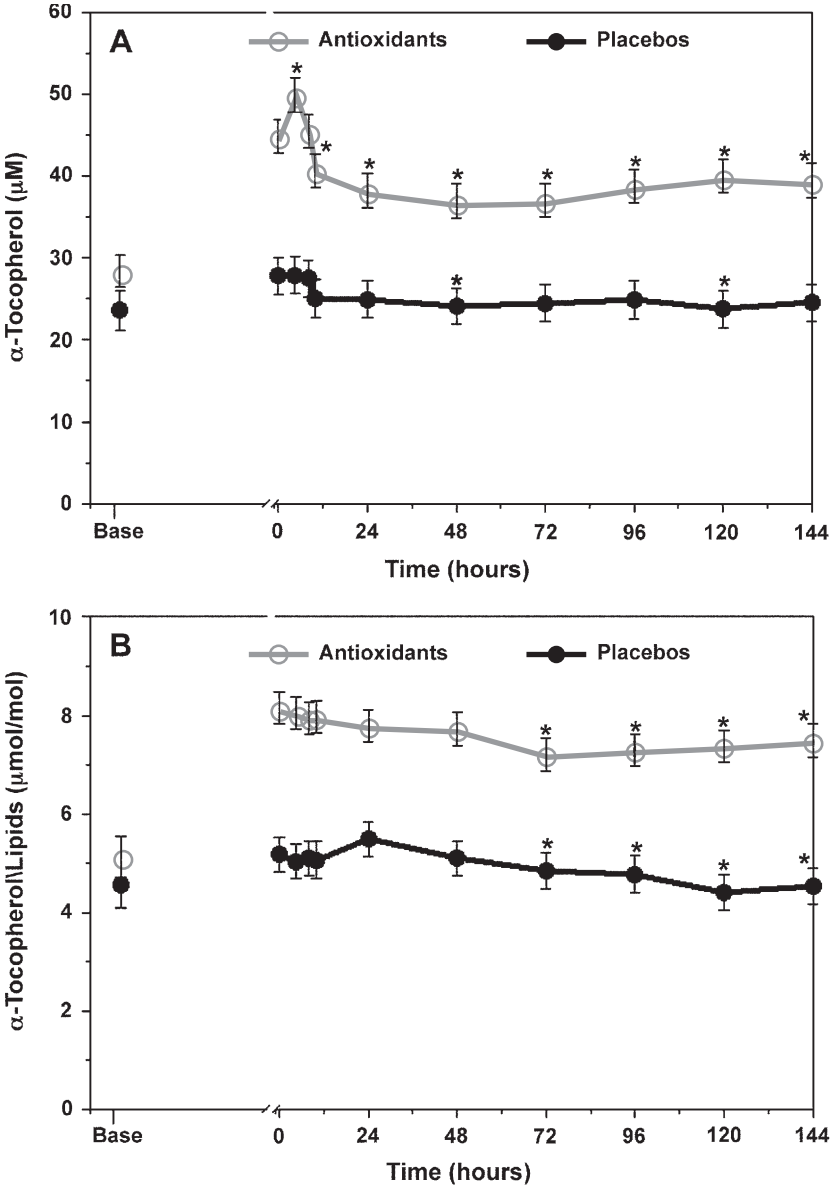

Fig. 3. Plasma α-tocopherol but not α-tocopherol/lipid concentrations increased in response to the ultramarathon run. (A) α-Tocopherol concentrations (means ± SE) were significantly higher in the AO than in the PL group at all time points, except baseline (treatment main effect, $p < .0001$). α-Tocopherol concentrations changed differently in the two treatment groups over time (treatment × time interaction, $p < .007$). In the AO group, α-tocopherol concentrations increased at midrace (compared with prerace, $p < .003$), returned to prerace values by postrace, and decreased below prerace concentrations for the duration of the study (compared with prerace, $p < .01$). α-Tocopherol concentrations were unchanged in the PL group during the race, but declined to below prerace values 2 and 5 days postrace. (B) α-Tocopherol/lipid ratios (means ± SE) were significantly higher in the AO group compared with the PL group at all time points except baseline (treatment main effect, $p < .0001$). α-Tocopherol/lipid concentrations decreased similarly in both treatment groups at 3, 4, 5, and 6 days postrace (compared with prerace, $p < .01$; time main effect, $p < .003$). Uncorrected baseline (Base) values used as the covariate in the model are also presented. Time 0 h = prerace. *Compared with prerace.

effect, $p < .0001$). Compared with pre-race, α-tocopherol per lipids was decreased 3, 4, 5, and 6 days postrace in all subjects (Fig. 3B)(time main effect, $p < .003$).

Lipid peroxidation

At baseline (prior to supplementation), plasma F_2-IsoP concentrations were negatively correlated with $VO_{2\ max}$ ($R = -0.55$, $p < .01$). No statistically significant differences were detected between genders or treatment groups in F_2-IsoP concentrations at baseline or after 3 and 6 weeks of daily supplementation with AO or PL (Fig. 4).

F_2-IsoP concentrations increased significantly at postrace compared with prerace *only* in the in the PL group (28 ± 2 to 41 ± 3 pg/ml, $p < .0001$) (Fig. 4). Moreover,

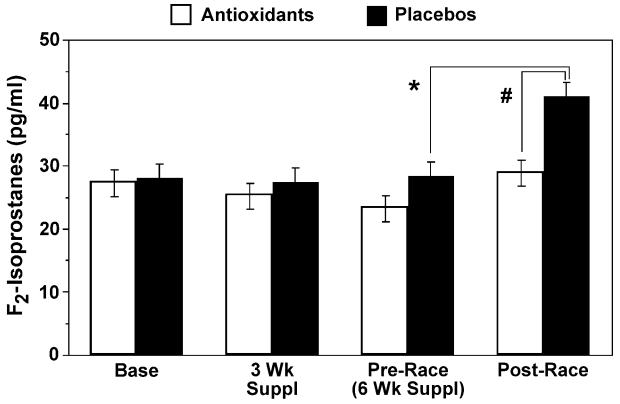

Fig. 4. Antioxidant supplementation prevented increases in plasma F_2-IsoP concentrations following a 50 km ultramarathon. No statistically significant differences were detected between genders or treatment groups in F_2-IsoP concentrations (mean ± SE) at baseline or after 3 and 6 weeks daily supplementation with vitamin E and vitamin C or placebos. At postrace F_2-IsoP concentrations were elevated in the PL group (compared with prerace, $p < .0001$), but not in the AO group, and were significantly higher in the PL group compared with the AO treatment group (AO vs. PL group, $p < .001$). No statistically significant differences were detected between genders prior to or during the race (ANOVA main effect for time, $p < .0001$; main effect for treatment, $p < .04$; and significant treatment × time interaction, $p < 0.01$). *Compared with prerace; #AO group versus PL group.

at postrace, F_2-IsoP concentrations in the PL group were significantly higher than in the AO group ($p < .001$). No statistically significant differences were detected between genders during the run (ANOVA, main effect for time, $p < .0001$; main effect for treatment, $p < .04$; and treatment × time interaction, $p < .01$).

Although there were no differences in F_2-IsoP concentrations between genders prior to or during the race, men and women did respond differently to the treatments *after* the ultramarathon (gender × treatment interaction, $p < .03$). In women, F_2-IsoP concentrations were elevated in the PL compared with the AO group at postrace ($p < .01$), but returned to prerace levels by 2 h postrace and did not differ from those of the AO women for the duration of the study (Fig. 5A). By contrast, F_2-IsoP concentrations in men were higher in those taking PL than in those taking AO postrace, 2 h postrace, and 1, 2, 3, 4, and 6 days postrace (PL vs. AO, $p < .03$) (Fig. 5B).

By use of paired data from each subject at every time point, plasma F_2-IsoP concentrations were negatively correlated with α-tocopherol/lipids: $R = -0.40$, $p < .0001$). Postrace, when F_2-IsoP concentrations were maximal, concentrations were negatively correlated both with α-tocopherol/lipids ($R = -0.61$, $p < .003$) and with ascorbic acid ($R = -0.41$, $p = .05$).

Inflammatory markers

Inflammation may be mediated by ROS [13,32]; therefore, we also measured markers of inflammation. The ultramarathon run elicited dramatic increases in most of these markers, but AO supplementation did not appear to have an effect on any of the selected parameters. For example, IL-6 concentrations increased during the race similarly in both AO and PL groups (Fig. 6) (time main effect, $p < .0001$); but by 1 day postrace, plasma IL-6 concentrations returned to prerace values. Interestingly,

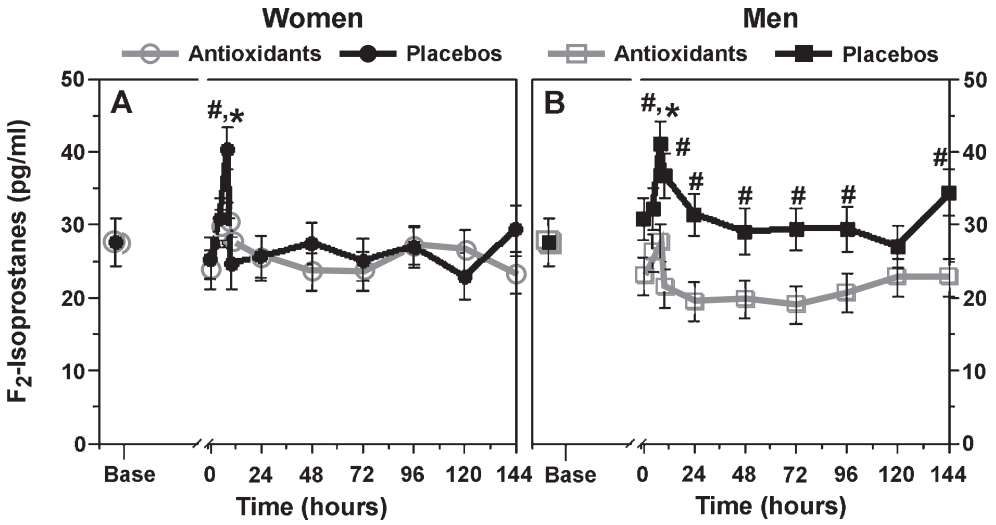

Fig. 5. Plasma F_2-IsoP concentrations in women and men 1 h prior to, during, and 6 days after competition in a 50 km ultramarathon. F_2-IsoP concentrations (means ± SE). Men and women responded differently to the treatment in the hours and days following the ultramarathon (gender × treatment interaction, $p < .03$). (A) In women, F_2-IsoP concentrations were elevated in the PL group compared with the AO group at postrace, but not at later time points (AO group vs. PL group, $p < .01$). (B) In men, F_2-IsoP concentrations were higher in the PL group compared with the AO group postrace, 2 h postrace, and 1, 2, 3, 4, and 6 days postrace (AO group vs. PL group, $p < .03$). Uncorrected baseline (Base) values used as the covariate in the model are also presented. Time 0 h = prerace. *Compared with prerace; #AO group vs. PL group.

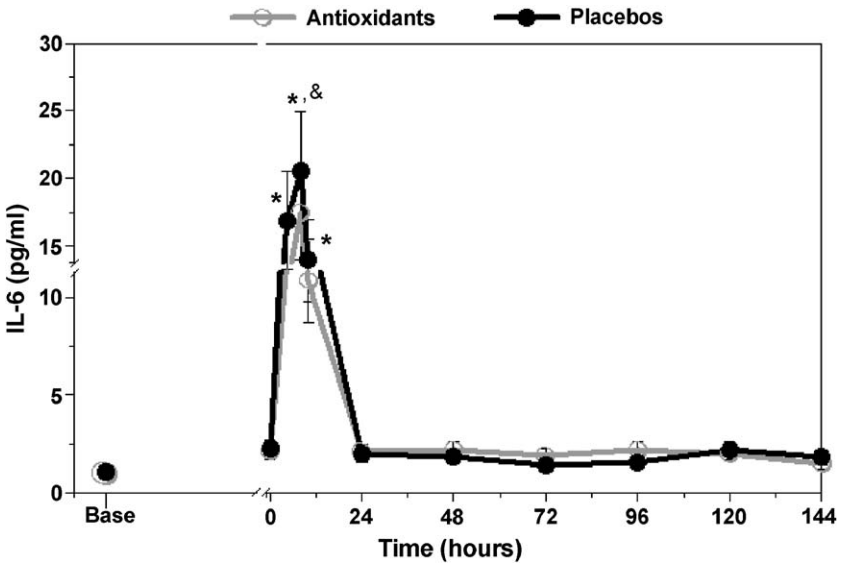

Fig. 6. Plasma IL-6 1 h prior to, during, and immediately after a 50 km ultramarathon. IL-6 concentrations (means ± SE) increased in response to the ultramarathon (time main effect, $p < .0001$) and were elevated at midrace, postrace, and 2 h postrace (compared with prerace, $p < .0001$). IL-6 concentrations were highest at postrace (compared with midrace, $p < .004$). Uncorrected baseline (Base) values used as the covariate in the model are also presented, Time 0 h = prerace. *Compared with prerace; &Compared with midrace.

F_2-IsoP concentrations were correlated with IL-6 at midrace ($R = 0.46$, $p < .03$) (Fig. 7), but not at other time points.

The time course of inflammatory markers up to 48 h postrace is depicted in Fig. 8. TNF-α behaved similarly to IL-6, increasing as early as midrace in response to the ultramarathon (time main effect, $p < .0001$). CRP increased following the run (Fig. 8, time main effect, $p < .0001$). Two days postrace, CRP plasma concentrations had declined to 8.2 ± 0.9 mg/dL ($p < .0001$), but at study end (6 days postrace), plasma CRP concentrations remained twice as high as prerace concentrations ($p < .0001$; data not shown). IL1-β did not change significantly in response to the exercise bout, nor did it appear

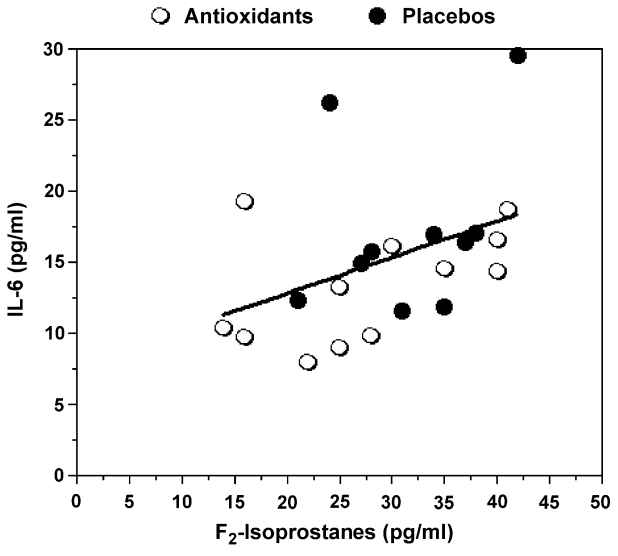

Fig. 7. At midrace, plasma IL-6 was significantly correlated to F_2-IsoP concentrations ($R = 0.46$, $p < .03$).

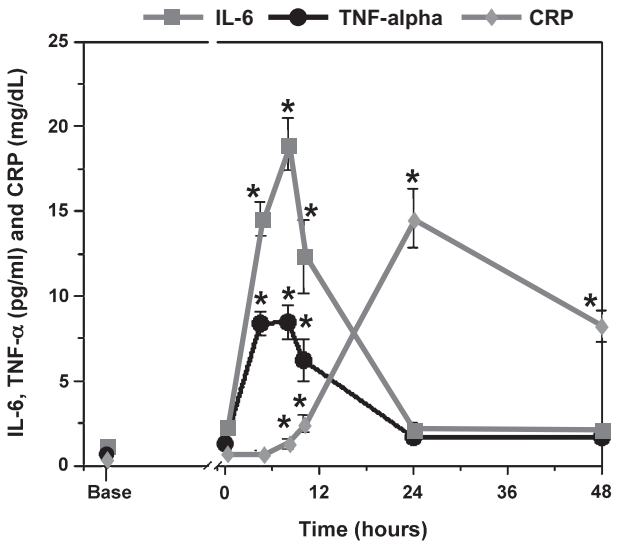

Fig. 8. Time course of inflammatory markers 1 h prior to, during, and up to 48 h postrace. Inflammatory marker concentrations (means ± SE). Plasma IL-6 and TNF-α concentrations were both elevated at midrace, and remained elevated at postrace and 2 h postrace. C-Reactive protein (CRP) was not elevated until postrace, and did not achieve maximum values until 1 day postrace, remaining elevated 48 h postrace (compared with prerace, $p < .0001$). Uncorrected baseline (Base) values used as the covariate in the model are also presented, Time 0 h = prerace. *Compared with prerace.

to be affected by antioxidant supplementation (data not shown).

DISCUSSION

Supplementation with vitamins E and C completely inhibited exercise-induced lipid peroxidation (Figs. 4 and 5). Postrace, when oxidative stress was maximal, F_2-IsoP concentrations were highly negatively correlated both with α-tocopherol/lipids ($R = -0.61$, $p < .003$) and with ascorbic acid ($R = -0.41$, $p = .05$), offering further evidence that antioxidants were responsible for preventing lipid peroxidation. These findings are in contrast to the lack of effects of antioxidants on exercise-induced DNA damage that we reported previously in these subjects [38].

Surprisingly, men and women responded differently to AO supplements in the days after the ultramarathon. PL women recovered rapidly after the race, with no differences in F_2-IsoP concentrations between the two treatment groups during the week after the ultramarathon. In contrast, elevated F_2-IsoP concentrations persisted in PL men during the recovery phase. It should be noted that subjects were instructed to refrain from physical activity for the 6 days after the run.

Plasma F_2-IsoP concentrations at baseline were negatively correlated with $VO_{2\ max}$ ($R = -0.55$, $p < .01$), suggesting that cardiovascular fitness was inversely related to lipid peroxidation as has been previously proposed [48]. Subjects were recreationally trained with an average $VO_{2\ max}$ of 58 ± 1 ml/kg/min, making results of the present study generalizable to the broader physically active population but not necessarily to sedentary persons or elite athletes. Subjects were randomly allocated to treatment groups; therefore, $VO_{2\ max}$ and F_2-IsoP concentrations were initially similar between groups and unlikely to be confounding variables.

F_2-IsoPs are specific end products of cyclooxygenase-independent, free-radical-catalyzed oxidation of arachidonic acid [49] and are a more sensitive and reliable measure of in vivo lipid peroxidation than previous assays such as those with malondialdehyde (MDA) and lipid hydroperoxides [49]. Muscle damage caused by the resumption of weight bearing activity after space flight increased urinary excretion of F_2-IsoPs, implicating oxidative stress as a cause of muscle damage [50]. In the present study, F_2-IsoPs increased only after subjects experienced an exceptional oxidative insult, the ultramarathon, and then only in the PL group (Fig. 4). We demonstrated that this oxidative stress was sufficient to cause DNA damage in both AO and PL groups [38]. Most [51–53], but not all [54], studies of the effects of high doses of vitamin E on steady-state levels of F_2-IsoPs in healthy, nonsmoking adults have concluded no effect of the antioxidant. Combinations of antioxidants have provided more positive findings. Upritchard et al. [55] reported that 111 mg/day vitamin E and 1.24 mg/day carotenoids for 11 w reduced plasma F_2-IsoPs by 15%. Supplementation with vitamins E and/or C (~500 mg/day and/or 400 IU/day, respectively) for a longer duration (8 weeks) attenuated urinary excretion of F_2-IsoPs [56].

With regard to exercise-induced lipid peroxidation, few exercise studies have used F_2-IsoPs as a biomarker of lipid peroxidation. Mori et al. [57] trained non-insulin-dependent diabetics 30 min per day at 55–65% $VO_{2\ max}$ on a cycle ergometer three times per week for 8 weeks. They reported no increase in *urinary* F_2-IsoPs in response to the moderate exercise training protocol, suggesting that the exercise bout did not elicit an oxidative stress response. Sacheck et al. [17] reported in young and elderly men effects of supplementation with 1000 IU *RRR*-α-tocopherol for 12 weeks prior to a 45-minute downhill run. Vitamin E attenuated the increase in plasma F_2-IsoPs in the elderly men 24 h postexercise, but not 72 h postexercise. In the young men, supplementation prevented any increases in plasma F_2-IsoPs, but the group started out with higher levels at baseline than the placebo group, making interpretation of the results difficult. Because F_2-IsoPs were not measured immediately postexercise, comparison with the present study, in which F_2-IsoPs peaked postrace, is not possible.

In the present study, despite similar lipid peroxidation responses during the run, men and women responded differently in the days after the race. F_2-IsoP concentrations in PL women recovered rapidly, whereas higher levels persisted in PL men for the duration of the study despite similar plasma antioxidant levels in the two PL groups. Greater oxidative stress in men than in women has been reportedly previously. Ide et al. [58] studying healthy nonsmoking adults, reported 2-fold higher excretion of urinary F_2-IsoPs in men than in women. However, after 4 weeks of supplementation with vitamins E and C, F_2-IsoP concentrations in men were reduced to those observed in the women [58]. One explanation for the observed gender difference is a higher metabolic rate in men leading to increased mitochondrial flux and increased production of ROS [58]. Women and men in the present study were of similar fitness levels, but as is typical, men exhibited a larger proportion of lean body tissue [38], likely contributing to a higher metabolic rate [41]. Another possible explanatory variable, the female hormone estrogen, is known to exhibit antioxidant properties [58] and thus may have contributed to the faster recovery observed in PL women.

Very few studies have studied the effects of antioxidants on both exercise-induced oxidative stress and inflammation [22,26]. Nieman et al. [22] reported no

effect of 1500 mg/day ascorbic acid for 1 week prior to an ultramarathon on exercise-induced increases in plasma F_2-IsoPs, lipid hydroperoxides, or IL-6. Using an eccentric arm exercise protocol known to induce muscle damage, Childs et al. [26] studied the effects of 1 week *postexercise* supplementation with ascorbic acid and *N*-acetyl-cysteine (NAC). Both F_2-IsoPs and lipid hydroperoxides increased in response to the exercise, but levels were higher in the supplemented group postexercise. Childs et al. [26] suggested a pro-oxidant effect of ascorbic acid and NAC. Nonetheless, these supplements had no effect on exercise–induced increases in IL-6. In the present study, IL-6 increases in response to the ultramarathon were not modulated by vitamin E and C supplementation. Similarly, in the present study, CRP was not attenuated by AO supplementation, in accordance with previous reports [59].

The only inflammatory marker not changed by the ultramarathon run was IL1-β; it too was unaffected by AO supplementation. While previous studies of *untrained* subjects have reported increases in IL1-β with exercise [32,60] studies have consistently reported no response in *trained* exercisers [61] except a slight but significant increase observed in ultra-endurance runners (90 km ultramarathon) [34].

As carbohydrate ingestion is known to modulate the cytokine response to exercise [62], carbohydrate intake was monitored and recorded prior to, during, and for 2 h after the race. Carbohydrate intake was higher in men than in women, but was not different between treatment groups within each gender (Table 1). Carbohydrate intake as a proportion of total calories was similar between genders and treatment groups and therefore unlikely to have influenced study outcomes with regard to cytokines.

Ascorbic acid is the most effective antioxidant in human plasma [63]; it can prevent initiation of lipid peroxidation and spare other critical antioxidants including α-tocopherol and urate [63]. In response to the 50 km ultramarathon run, plasma ascorbic acid *increased* similarly in both treatment groups, with significant increases compared to prerace, midrace, and postrace returning to prerace values by 2 h postrace. Similar increases in plasma ascorbic acid in response to vigorous exercise have been reported previously [3,6,7,36,64,65], although some studies have reported no change [16,33,66]. It has been suggested that exercise-related increases in cortisol secretion promote efflux of ascorbic acid from the adrenal gland [67] and/or the mobilization of ascorbic acid from other tissue sites such as leukocytes and erythrocytes [65]. Some [33,34,66], but not all [22] recent studies have demonstrated an attenuation of the exercise-related increase in circulating cortisol with vitamin C supplementation.

Uric acid, a primary end product of purine metabolism and a water-soluble antioxidant [11], increased in the present study, consistent with the findings of others [7,68,69]. The increase may be explained by enhanced purine oxidation with exercise [7,68,69]. Increased energy requirements characteristic of vigorous exercise upregulate various metabolic pathways including adenylate cyclase [69,70] (or myokinase in muscle [41]). This enzyme is responsible for production of 1ATP and 1AMP from 2ADP [41]. While the ATP is used for energy, the AMP is degraded to uric acid [71]. Concurrent increases in plasma ascorbic and uric acids may reflect enhanced antioxidant defenses in response to the oxidative stress of the endurance exercise. Moreover, our findings are in accordance with previous studies reporting increased antioxidant enzymes [5] and antioxidant nutrients [2,7,65] in response to extreme exercise.

We observed an increase in plasma α-tocopherol concentrations during the exercise in the AO, but not the PL group. After correcting α-tocopherol for lipids, no significant changes in α-tocopherol/lipids were observed in either group. Thus, the increase in plasma α-tocopherol concentrations during the exercise could be completely explained by fluctuations in lipids during the run. A few of the studies investigating the effects of vitamin E and/or C supplementation on endurance run-

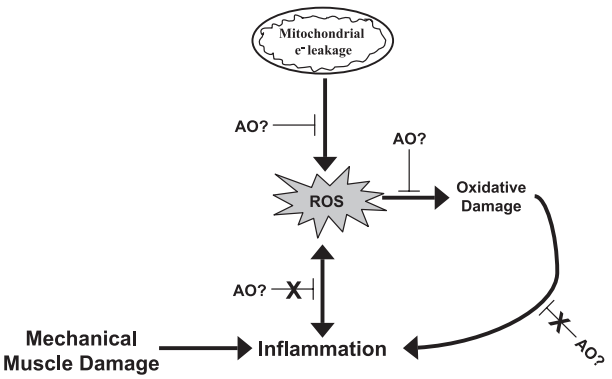

Fig. 9. Proposed model of exercise induced oxidative stress and inflammation. With exercise there is increased flux of oxygen through the mitochondria, electron (e^-) leakage, and generation of reactive oxygen species (ROS) that may exceed antioxidant defenses. These excess ROS can (1) cause oxidative damage and (2) stimulate an inflammatory response. Exercise-induced mechanical muscle damage may or may not lead to a delayed inflammatory response [31]. Hypothetically, supplementation with antioxidants (AO) prevents (⊥) exercise–induced oxidative stress and may therefore protect against both oxidative damage and subsequent inflammation. We demonstrate that supplementation with vitamins E and C *prevents (⊥) oxidative damage* (lipid peroxidation), but has no apparent effect on inflammation (X). These results suggest that oxidative damage and the inflammatory response are operating independently. It may be that ROS do not modulate inflammation in this model or that the inflammation induced by muscle damage is great enough to overwhelm the protective effects of the antioxidants.

ning have reported increases in plasma α-tocopherol concentrations in both supplemented and placebo groups following exercise [7,25,72], but most failed to report postexercise plasma α-tocopherol concentrations [16,17,32,35,60]. A single study reported no change in α-tocopherol concentrations with exercise [36]. Only one of these studies reported both absolute α-tocopherol concentrations and α-tocopherol/lipid concentrations [72] and observed differential responses in the AO supplemented groups that depended on correction for fluctuations in lipid levels.

As presented schematically in Fig. 9, ROS generated in response to exercise can cause oxidative damage [6] and stimulate an inflammatory response [32]. Therefore it has been proposed that AO supplementation could prevent exercise–induced oxidative stress and protect against both oxidative damage and inflammation [22,32]. Unlike previous studies testing this hypothesis, the present study investigated the effects of supplementation with *both* vitamins E and C, measuring oxidative stress *and* inflammatory markers in response to exercise. Supplementation *prevented* oxidative damage (lipid peroxidation), but had no apparent effect on inflammation. These results suggest that oxidative damage and the inflammatory response are operating independently [22]. Alternatively, it may be that higher doses of α-tocopherol (≥ 800 IU/day) are required to elicit an anti-inflammatory effect, as has been previously suggested [46,73].

Preventing production/enhancing clearance of F_2-IsoPs may be more biologically relevant than preventing inflammation. F_2-IsoPs have demonstrated pro-atherogenic biological activity, including vasoconstriction, and activation of platelet aggregation [22,49] and they are known to recruit proatherogenic monocytes and induce monocyte adhesion [74]. In contrast, the exercise-induced inflammatory response stimulates recovery from exercise by inducing regeneration of damaged tissue and recruitment of satellite cell proliferation [31]. Together, AO supplementation proved to prevent the damaging increase in lipid peroxidation without influencing inflammation. This is especially important because prevention of exercise-induced inflammation could actually inhibit muscular adaptation to physical activity, the so-called "training effect" of exercise.

The acute oxidative and inflammatory stress responses characteristic of vigorous aerobic exercise are analogous to the stress responses observed after acute events such as myocardial infarction, ischemic stroke, surgery, and trauma [13,30,75]. Thus, aerobic exercise provides a well-controlled, relatively noninvasive model to study the effects of AO supplementation on acute oxidative and inflammatory stress. The results from such research may be useful for the exploration of these other chronic human diseases.

Acknowledgments—Special thanks to Dr. Robert P. O'Donnell at Oregon State University for extensive help with statistics; Dr. Jeff Zachwieja at Gatorade for providing the Gatorade sports drinks for participants; Tim Corliss at Clif Bar Inc. for providing Clif Shots for subjects; Clem LaCava, the McDonald Forest Ultramarathon Race Promoter; Dr. James Clark, Cognis Health and Nutrition, for providing vitamin E capsules and matched placebos; and Dr. Klaus Krämer, BASF, for providing vitamin C tablets and matched placebos.

This publication was made possible by Grant ES-11536 from the National Institute of Environmental Health Sciences (NIEHS), NIH. Work was supported by NIH Grants GM-15431, DK-48831, CA-77839, and RR-00095 to J.D.M. and K24AT00596 to S.D. J.D.M. is the recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research. The Natural Source Vitamin E Association is gratefully acknowledged for their support of student fellowships for A.M. and D.W.H.

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