

Vitamin E and Immunity after the Kona Triathlon World Championship

DAVID C. NIEMAN¹, DRU A. HENSON¹, STEVEN R. MCANULTY¹, LISA S. MCANULTY¹, JASON D. MORROW², ALAA AHMED³, and CHRIS B. HEWARD³

¹Department of Health and Exercise Science, Fischer Hamilton/Nycom Biochemistry Laboratory, Appalachian State University, Boone, NC; ²Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN; and ³Kronos The Optimal Health Company, Kronos Science Laboratory, Phoenix, AZ

ABSTRACT

NIEMAN, D. C., D. A. HENSON, S. R. MCANULTY, L. S. MCANULTY, J. D. MORROW, A. AHMED, AND C. B. HEWARD. Vitamin E and Immunity after the Kona Triathlon World Championship. *Med. Sci. Sports Exerc.*, Vol. 36, No. 8, pp. 1328–1335, 2004. **Purpose:** To measure the influence of vitamin E ingestion on oxidative stress and immune changes in response to the Triathlon World Championship in Kona, Hawaii. **Methods:** Thirty-eight triathletes received vitamin E (VitE) (800 IU·d⁻¹ α -tocopherol) or placebo (Pla) capsules in randomized, double-blind fashion for 2 months before the race event. Blood, urine, and saliva samples were collected the day before the race, 5–10 min posttrace, and 1.5 h posttrace. **Results:** Race times did not differ between VitE ($N = 19$, 721 \pm 24 min) and Pla groups ($N = 17$, 719 \pm 27 min, $P = 0.959$), and both groups maintained an intensity of \sim 80% maximum heart rate during the bike and run portions. Plasma α -tocopherol was approximately 75% higher in the VitE versus Pla group prerace (24.1 \pm 1.1 and 13.8 \pm 1.1 μ mol·L⁻¹, $P < 0.001$, respectively) and posttrace. Plasma F₂-isoprostanes increased 181% versus 97% posttrace in the VitE versus Pla groups ($P = 0.044$). IL-6 was 89% higher (166 \pm 28 and 88 \pm 13 pg·mL⁻¹, respectively, $P = 0.016$), IL-1ra was 107% higher (4848 \pm 1203 and 2341 \pm 790 pg·mL⁻¹, respectively, $P = 0.057$), and IL-8 was 41% higher posttrace in the VitE versus Pla groups (26.0 \pm 3.6 and 18.4 \pm 2.4 pg·mL⁻¹, respectively, $P = 0.094$). **Conclusion:** These data indicate that vitamin E (800 IU·d⁻¹ for 2 months) compared with placebo ingestion before a competitive triathlon race event promotes lipid peroxidation and inflammation during exercise. **Key Words:** CYTOKINES, F₂-ISOPROSTANES, DNA, SALIVARY IGA

Heavy exertion increases the generation of free radicals and reactive oxygen species (ROS) through several pathways including oxidative phosphorylation, increase in catecholamines, prostanoid metabolism, xanthine oxidase, and NAD(P)H oxidase (29). Neutrophils and macrophages migrate to the site of contraction-induced muscle damage, infiltrate the muscle tissue, activate the release of cytokines, and produce additional ROS (3,24). Most ROS are neutralized by a sophisticated antioxidant defense system consisting of a variety of enzymes and nonenzymatic antioxidants including vitamin A, E, and C, glutathione, ubiquinone, and flavonoids (29). Intensive and sustained exercise, however, can create an imbalance between ROS and antioxidants, leading to oxidative stress that can be assessed by measuring lipid peroxidation, protein oxidation, change in status of antioxidant compounds and enzyme activities, and electron spin resonance (16–19). Less clear is the influence of heavy exertion on oxidative

DNA damage, with some studies reporting increases in DNA damage and strand breakage (11,28), whereas others have found no change in leukocyte or urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) or 7,8-dihydro-8-oxoguanine (8-oxoG), the most commonly used markers of oxidative DNA modifications (1,25,27).

Vitamin E functions primarily as a nonspecific, chain-breaking antioxidant that prevents the propagation of lipid peroxidation (8). The vitamin is a peroxy radical scavenger and protects polyunsaturated fatty acids within membranes and in plasma lipoproteins (8). Quantification of F₂-isoprostanes is the most reliable index of *in vivo* free-radical generation and oxidative lipid damage (17). The F₂-isoprostanes are increased in vitamin E-deficiency, and their excretion can be depressed by consuming vitamin E supplements (17). Vitamin E plays an important role in the maintenance of immune function, with a marginal deficiency impairing immunity, and supplementation with higher than recommended levels enhancing humoral and cell-mediated immunity in the elderly (4). These findings have created interest as to whether or not vitamin E supplements may attenuate oxidative stress and immunosuppression during heavy exertion.

Most research has indicated that 1–5 months of vitamin E supplementation (200–1200 IU·d⁻¹ α -tocopherol) increases plasma levels of α -tocopherol but has little or no influence on athletic performance or indices of contraction-induced muscle damage (3,6,25), and a variable effect on exercise-induced oxidative stress (12,24,25,29). The equivocal nature

Address for correspondence: David C. Nieman, Dr.P.H., FACSM, Department of Health & Exercise Science, Appalachian State University, Boone, NC 28608; E-mail: niemandc@appstate.edu.

Submitted for publication January 2004.

Accepted for publication April 2004.

0195-9131/04/3608-1328

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Copyright © 2004 by the American College of Sports Medicine

DOI: 10.1249/01.MSS.0000135778.57355.CA

of these results are related to research design issues including the timing and mode of exercise, age and fitness levels of the subjects, amount and form of the vitamin E supplement, and methodology for assessing oxidative stress (24,29).

The effect of vitamin E supplementation on the inflammatory and immune response to intensive and prolonged exercise is largely unstudied and equivocal (5,21,26). The relationship between ROS and immunity is still being described, but mounting evidence suggests a linkage (9,18,19). IL-10 has emerged as an anti-inflammatory cytokine with antioxidant properties and has been hypothesized to play a role in gene expression and the biosynthesis of oxidative stress-related cofactors, such as ROS and inflammatory cytokines (9). ROS generation and antioxidant status has been linked to immune alterations in certain disease states and the aging process, but this relationship is largely unstudied within the context of human athletic endeavor (9,15).

In a previous study by our research team, vitamin C supplementation failed to influence exercise-induced oxidative stress and perturbations in immunity during an ultramarathon (19). Vitamin C provides *in vivo* antioxidant protection primarily as an aqueous-phase peroxy and oxygen radical scavenger. We reasoned that because vitamin E prevents the propagation of lipid peroxidation, this supplement had a higher potential than vitamin C to operate as a countermeasure to exercise-induced changes in lipid peroxidation and immunity. The purpose of this study was to measure the influence of vitamin E ingestion on oxidative stress and immune changes after the Triathlon World Championship in Kona, Hawaii. Thirty-eight triathletes received vitamin E (800 IU·d⁻¹ D- α -tocopherol) or placebo capsules in randomized, double-blind fashion for 2 months before the race event. We hypothesized that within the context of severe exertion, vitamin E supplementation would attenuate exercise-induced increases in inflammatory cytokines, oxidative stress, and immune alterations.

METHODS

Subjects. The 2002 Kona Triathlon World Championship was held October 19, beginning at 7:00 a.m. The race included a 3.9-km ocean swim, 180-km bike race and 42-km run, and was held in Kailua-Kona, Hawaii. Thirty-eight male ($N = 28$) and female ($N = 10$) triathletes who had qualified for the Kona Triathlon World Championship volunteered to be subjects in this study. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements of the American College of Sports Medicine and the institutional review board of Appalachian State University. All but two athletes complied with research requirements and completed the triathlon. The afternoon before the race, subjects completed a questionnaire providing demographic and training data.

Research design and diet records. Thirty-eight triathletes received vitamin E (800 IU·d⁻¹ D- α -tocopherol) or placebo capsules in randomized, double-blind fashion for

2 months before the race event. This level and duration of supplementation was chosen based on unpublished data from a member of our research team showing that the plasma concentration of F₂-isoprostanes was reduced in high-risk subjects at this level but not below (J. D. Morrow, personal communication). The vitamin E softgel capsules contained 400 IU D- α -tocopherol, gelatin, glycerin, soybean oil, and purified water, and the placebo capsules contained the same ingredients except for D- α -tocopherol (Banner Pharmacaps, High Point, NC). Subjects ingested two capsules each day for 2 months before and the day of the race. Blood, urine, and saliva samples were collected the day before the race (4–6 p.m.), 5–10 min postrace, and 1.5 h postrace. During the 2-month period of vitamin E/placebo supplementation, subjects followed a diet (from a detailed food list) that prohibited foods or supplements that were high in vitamin E (more than 100 IU·d⁻¹), or vitamin A and C (greater than 200% of recommended levels). Two weeks before the race event, subjects listed all food and beverage intake in a 3-d food record. The athletes' food records were reviewed by the project dietitians during the first blood-collection session, and were then analyzed using the Food Processor nutrient analysis software program (ESHA Research, Salem OR). During the race, subjects ingested food and fluids *ad libitum*, with a postrace questionnaire used by project dietitians to estimate intake. Subjects were instructed to avoid supplements high in vitamins E or C on the day of the race.

Race performance data. Body mass was measured the afternoon before the race, 5–10 min postrace, and 1.5 h postrace. A three-site skinfold test was used to determine body composition the afternoon before the race. Environmental temperature and humidity data were obtained from the local weather station. During the postrace sample collection session, athletes were asked to recall swim, bike, and run race times, and bike and run mean heart rates from their personal heart rate monitors. Total race times were obtained from the race website.

Blood cell counts, hormones, glucose. Blood samples were drawn from an antecubital vein with subjects in the seated position. Routine complete blood counts (CBC) and serum glucose were performed by a clinical hematology laboratory. Other blood samples were centrifuged in sodium heparin tubes, and plasma was aliquoted, transported in dry ice, and then stored at -80°C . Plasma cortisol was assayed in duplicate using a competitive enzyme immunoassay kit provided by R&D Systems, Inc. (Minneapolis, MN) with a minimum detectable cortisol concentration of 1.6 nmol·L⁻¹. The intra-assay coefficient of variation (CV) was less than 10%. Plasma volume changes were estimated using the method of Dill and Costill (7).

Oxidative measurements. Blood samples were immediately centrifuged at 4°C for 10 min. The plasma was aliquoted into cryotubes and snap frozen in liquid nitrogen. Urine and plasma samples were transported on dry ice and then stored at -80°C until analysis. Plasma F₂-isoprostane, urinary F₂-isoprostane, 8-OHdG, and 8-oxoG, and plasma α - and γ -tocopherol were analyzed by gas chromatography

mass spectrometry (14), liquid chromatography tandem mass spectrometry (14), and high performance liquid chromatography (10), respectively. All urine measures were normalized by expressing results as a ratio to creatinine. Lipid hydroperoxides (ROOH) were analyzed using a spectrophotometric kit proved by Caymen Chemicals (Cat. 705002) (Ann Arbor, MI). The interassay CV was less than 5% and intra-assay CV less than 10%.

Lymphocyte subsets. The proportions of T cells (CD3⁺), B cells (CD19⁺), natural killer (NK) cells (CD3⁻CD16⁺CD56⁺) and activated T-cells (CD69⁺) were determined in whole blood preparations and absolute numbers calculated using CBC data to allow group comparisons of lymphocyte subset concentrations. Lymphocyte phenotyping was accomplished by two-color fluorescent labeling of cell surface antigens with mouse anti-human monoclonal antibodies conjugated to fluorescein-isothiocyanate (FITC) and phycoerythrin (PE) using Simulstest™ reagents (Becton Dickinson Immunocytometry Systems, San Jose, CA). For immunophenotyping, 60-μL aliquots of heparinized whole blood from each sample were added to five wells of a 96-well plate. Sixty microliters of CD3/CD16 + CD56 (BD, cat. 340042), CD3/CD19 (BD, cat. 349211), CD45/CD14 (BD, cat. 340040), CD69 (BD, cat. 341652) or γ1γ2a isotype control (BD, cat. 340041) were added to the wells, and the samples were incubated in the dark for 20 min on ice with orbital shaking (170 rpm). The cells were then washed with 130 μL of phosphate-buffered saline (PBS) and the plate was centrifuged for 5 min at 1500 × g. The red blood cells were lysed by adding 200 μL of 1X FACS Lysing Solution (BD, cat. 349202) for 10 min in the dark, and the cells were pelleted by centrifugation. The lysing step was repeated, the cells washed in 200 μL of PBS, and the resulting cell pellet was fixed in 200 μL of Cytotfix™ buffer (BD, cat. 554655). Samples were kept at 4°C in the dark until analyzed by flow cytometry at the Immunogenetics Laboratory with the VA–San Diego Health Care System.

Cytokine measurements. Total plasma concentrations of interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10) were determined using quantitative sandwich ELISA kits provided by R&D Systems, Inc. All samples and provided standards were analyzed in duplicate. A high sensitivity kit was used to analyze IL-6 in the prerace plasma samples. A standard curve was constructed using standards provided in the kits and the cytokine concentrations were determined from the standard curves using linear regression analysis. The assays were a two-step “sandwich” enzyme immunoassay in which samples and standards were incubated in a 96-well microtiter plate coated with polyclonal antibodies for the test cytokine as the capture antibody. After the appropriate incubation time, the wells were washed and a second detection antibody conjugated to either alkaline phosphatase (IL-6 high sensitivity) or horseradish peroxidase (IL-1ra, IL-6, IL-8, IL-10) was added. The plates were incubated and washed, and the amount of bound enzyme-labeled detection antibody was measured by adding a chromogenic substrate. The plates were then read at the appro-

priate wavelength (450 – 570 nm for IL-1ra, IL-6, IL-8, IL-10; 490 – 650 nm for IL-6 high sensitivity). The minimum detectable concentration of IL-1ra was <22 pg·mL⁻¹, IL-6 < 0.70 pg·mL⁻¹, IL-6 high sensitivity <0.039 pg·mL⁻¹, IL-8 < 10 pg·mL⁻¹, and IL-10 < 3.9 pg·mL⁻¹. The intra-assay CV was less than 5% for all cytokines.

Salivary samples. Unstimulated saliva was collected by expectoration into 15-mL plastic, sterilized vials for 4 min. Participants were urged to pass as much saliva as possible into the vials during the 4-min timed session. The saliva samples were centrifuged for 5 min at 200 × g, immediately frozen and then transported in dry ice, and stored at –80°C until analysis. Saliva volume was measured to the nearest 0.1 mL, and salivary IgA was measured by an enzyme linked immunosorbent assay according to procedures used in our laboratory in previous studies (18). The data were expressed as sIgA secretion rate (μg·min⁻¹). The intra-assay CV was less than 10% for sIgA concentration.

Statistical analysis. Statistical significance was set at the *P* < 0.05 level, and values expressed as mean ± SE. Vitamin E and placebo groups were compared for subject characteristics and race performance measures using independent *t*-tests (Tables 1 and 2). Leukocyte subset counts, oxidative and cytokine measures, and hormone values were analyzed using 2 (vitamin E and placebo groups) × 3 (times of measurement) repeated measures ANOVA. When Box’s *M* suggested that the assumptions necessary for the univariate approach were not tenable, a multivariate approach to repeated measures ANOVA was used. In the latter case, the Pillais trace statistic was used as the test statistic. If the group × time interaction *P* value was ≤ 0.05, the change from prerace for the postrace and 1.5-h postrace values was calculated and compared between groups using Student’s *t*-tests. For these two multiple comparisons across groups, a Bonferroni adjustment was made with statistical significance set at *P* < 0.025. Pearson product-moment correlations were used to test the relationship between postrace measures.

RESULTS

All but two athletes complied with research requirements and completed the Kona Triathlon World Championship race event (*N* = 36). Subject characteristics are listed in Table 1 for the vitamin E (*N* = 19, 12 males and 7 females) and placebo (*N* = 17, 14 males and 3 females) groups. No

TABLE 1. Subject characteristics.

	Vitamin E (<i>N</i> = 19)	Placebo (<i>N</i> = 17)	<i>P</i>
Age (yr)	35.2 ± 1.6	39.2 ± 1.4	0.064
Height (m)	1.75 ± 0.02	1.77 ± 0.02	0.405
Body mass (kg)	71.4 ± 2.4	73.1 ± 2.6	0.636
Body fat (%)	12.7 ± 1.5	11.3 ± 0.8	0.437
Training (yr)	8.4 ± 1.2	8.9 ± 1.4	0.823
Ironman race events	5.5 ± 1.4	4.6 ± 1.0	0.599
Ironman personal best (min)	683 ± 24	652 ± 20	0.326
Swim training (km·wk ⁻¹)	7.2 ± 0.9	8.1 ± 0.5	0.38
Run training (km·wk ⁻¹)	50.1 ± 4.0	53.8 ± 5.6	0.585
Bike training (km·wk ⁻¹)	283 ± 27	263 ± 28	0.614

TABLE 2. Serum glucose, plasma cortisol, and immune measures.

	Vitamin E (N = 19)	Placebo (N = 17)	P Values: Group; Interaction; Time
Serum glucose (mmol·L ⁻¹)			
Prerace	4.87 ± 0.16	4.70 ± 0.15	0.034; 0.08; <0.001
Postrace	5.84 ± 0.24	6.68 ± 0.19	
1.5 h postrace	6.24 ± 0.18	6.61 ± 0.25	
Plasma cortisol (nmol·L ⁻¹)			
Prerace	570 ± 200	270 ± 50	0.491; 0.100; <0.001
Postrace	1300 ± 306	2227 ± 576	
1.5 h postrace	1305 ± 392	1117 ± 250	
IL-10 (pg·mL ⁻¹)			
Prerace	4.3 ± 0.7	5.7 ± 0.8	0.432; 0.488; <0.001
Postrace	63.0 ± 11.9	49.4 ± 12.0	
1.5 h postrace	37.2 ± 7.0	28.3 ± 7.9	
IL-8 (pg·mL ⁻¹)			
Prerace	3.3 ± 1.0	1.7 ± 0.7	0.079; 0.166; <0.001
Postrace	26.0 ± 3.6	18.4 ± 2.4	
1.5 h postrace	18.3 ± 2.5	12.8 ± 2.1	
CD69 cell count (10 ⁹ ·L ⁻¹)			
Prerace	0.020 ± 0.003	0.021 ± 0.002	0.679; 0.776; 0.047
Postrace	0.023 ± 0.004	0.020 ± 0.004	
1.5 h postrace	0.015 ± 0.002	0.014 ± 0.004	
Salivary IgA secretion rate (μg·min ⁻¹)			
Prerace	186 ± 18	195 ± 17	0.406; 0.879; <0.001
Postrace	106 ± 17	116 ± 17	
1.5 h postrace	109 ± 12	131 ± 15	

significant group differences were found for basic demographic and training characteristics. Race times did not differ between vitamin E (721 ± 24 min) and placebo groups (719 ± 27 min, $P = 0.959$) (Fig. 1). Subjects in the vitamin E and placebo groups maintained an intensity of ~80% maximum heart rate during the bike (145 ± 3 and 146 ± 2 beats·min⁻¹, $P = 0.780$) and run (143 ± 4 and 148 ± 3 beats·min⁻¹, $P = 0.233$) portions, respectively. Ambient temperature and relative humidity measured 26°C and 89% at 7:00 am when the race event started, 29°C and 74% from noon to 3:00 p.m., 27°C and 79% at 6:00 p.m., and 26°C and 78% at midnight when the race was stopped. For all subjects combined, body mass was 72.2 ± 2.5 kg the afternoon before the race event, and 70.8 ± 2.4 kg at race end (mean decrease, 1.4 ± 0.3 kg), and plasma volume decreased 1.5 ± 0.6% (no significant differences were measured between groups).

Energy and macronutrient intake did not differ between groups and averaged 3069 ± 174 kcal·d⁻¹ (55.2 ± 1.6% carbohydrate, 27.9 ± 1.3% fat, and 17.0 ± 0.9% protein) 2 wk before the race event, 4393 ± 344 kcal (88.2 ± 1.5% carbohydrate, 6.4 ± 0.8% fat, and 5.2 ± 0.5% protein) during the race event, and 696 ± 93 kcal (55.0 ± 2.4% carbohydrate, 31.4 ± 2.2% fat, and 16.2 ± 1.3% protein) during the 1.5-h postrace period. Data from the 3-d dietary record obtained before the race demonstrated that vitamin E intake (including the supplement) was 894 ± 36 and 75.2 ± 27.8 IU·d⁻¹ in the vitamin E and placebo groups, respectively ($P < 0.001$). During the race, vitamin E intake was 968 ± 50 and 378 ± 122 IU in the vitamin E and placebo groups, respectively ($P < 0.001$). Vitamin C intake was 209 ± 42 and 246 ± 52 mg·d⁻¹ 2 wk before the race event, and 349 ± 96 and 784 ± 183 mg during the race event in the vitamin E and placebo groups, respectively ($P = 0.581$ and 0.046, respectively).

Plasma α-tocopherol was 75% higher in the vitamin E vs placebo group prerace, and this group difference was main-

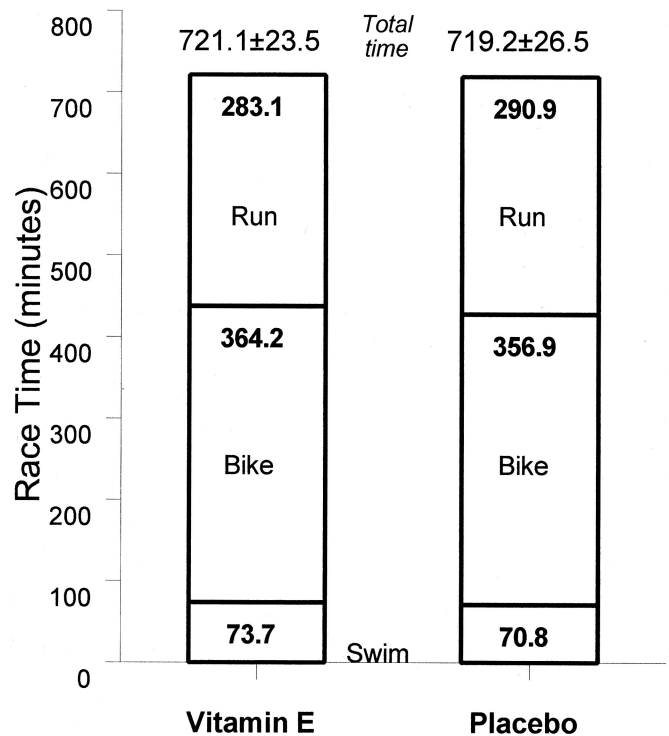


FIGURE 1— Race times for the swim, bike, and run portions of the race did not differ significantly between vitamin E and placebo groups ($P = 0.959$).

tained postrace (group main effect, $P < 0.001$) (Fig. 2A). Plasma γ-tocopherol, on the other hand, was 58% lower in the vitamin E versus placebo groups before and postrace (group main effect, $P < 0.001$) (Fig. 2B). Plasma F₂-isoprostanes increased 181% versus 97% postrace in the vitamin E versus placebo groups (interaction effect, $P = 0.044$) (Fig. 3A). Urine F₂-isoprostanes also increased postrace (132% for all subjects), but the pattern of change did not differ between groups ($P = 0.651$) (Fig. 3B). The pattern of change in plasma lipid hydroperoxides tended to differ between groups (interaction effect, $P = 0.065$) and was 2.21 ± 0.40, 2.08 ± 0.42, and 3.05 ± 0.46 compared with 2.15 ± 0.57, 2.18 ± 0.53, and 1.38 ± 0.35 μmol·L⁻¹ in the vitamin E and placebo groups prerace, postrace, and 1.5 h postrace, respectively. The 1.5-h postrace values differed significantly between groups ($P = 0.009$). No significant changes in urinary 8-OHdG and 8-oxoG were measured after the race event (time effects, $P = 0.151$ and 0.124, respectively), and no differences were noted between vitamin E and placebo groups (Fig. 4A&B).

Total blood leukocyte, neutrophil, and monocyte counts rose significantly after the race event ($P < 0.001$), but the patterns of change did not differ between the groups (Fig. 5A). Total blood lymphocyte, T lymphocytes, and natural killer lymphocytes decreased significantly after the race event ($P < 0.001$), but the patterns of change did not differ between the groups (Fig. 5B). B lymphocytes increased slightly and were significant in both groups ($P < 0.001$) (Fig. 5B). CD69 T lymphocytes dropped slightly but significantly in both groups ($P = 0.047$) (Table 2).

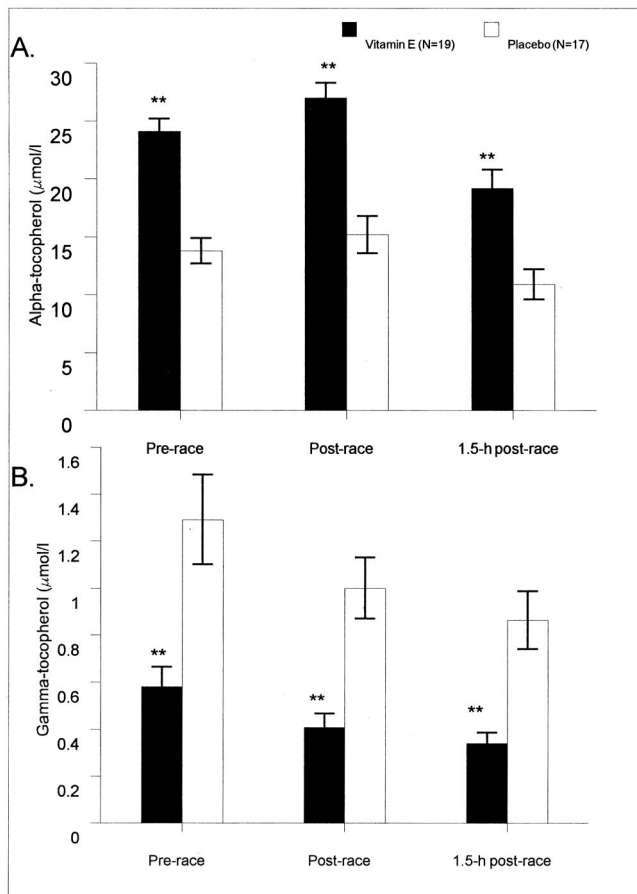


FIGURE 2—A and B. Plasma α -tocopherol was significantly higher and γ -tocopherol lower in the vitamin E compared with placebo group before and after the race (group main effects, $P < 0.001$). ** $P < 0.001$, vitamin E compared with placebo group at time point.

Plasma cortisol rose strongly in both groups following the race event, but the pattern of change did not differ between groups ($P = 0.100$), and the group main effect was not significant ($P = 0.491$) (Table 2). Serum glucose rose significantly post-race in both groups, and there was a nonsignificant tendency for higher post-race levels in the placebo group (Table 2). Increases in plasma IL-6, IL-1ra, IL-8, and IL-10 were measured in all subjects following the race ($P < 0.001$) (Figs. 6A and B, Table 2). Group main effects and interaction effects were $P = 0.017$ and $P = 0.059$ for IL-6, respectively, and $P = 0.089$ and $P = 0.057$ for IL-1ra, respectively (Fig. 6). IL-6 was 89% higher (166 ± 28 and $88 \pm 13 \text{ pg}\cdot\text{mL}^{-1}$, respectively, $P = 0.016$), IL-1ra was 107% higher (4848 ± 1203 and $2341 \pm 790 \text{ pg}\cdot\text{mL}^{-1}$, respectively, $P = 0.057$), and IL-8 was 41% higher (26.0 ± 3.6 and $18.4 \pm 2.4 \text{ pg}\cdot\text{mL}^{-1}$, respectively, $P = 0.094$), post-race in the vitamin E versus placebo group. The salivary IgA secretion rate decreased significantly by 42% following the race, but the pattern of change did not differ between groups (Table 2).

Immediately post-race, plasma α -tocopherol levels were positively correlated with plasma IL-6 ($r = 0.41$, $P = 0.013$) but not IL-1ra ($r = 0.19$, $P = 0.278$), IL-8 ($r = 0.29$, $P = 0.091$), IL-10 ($r = 0.29$, $P = 0.082$), or plasma F₂-isoprostanes ($r = 0.23$, $P = 0.185$). γ -tocopherol levels were

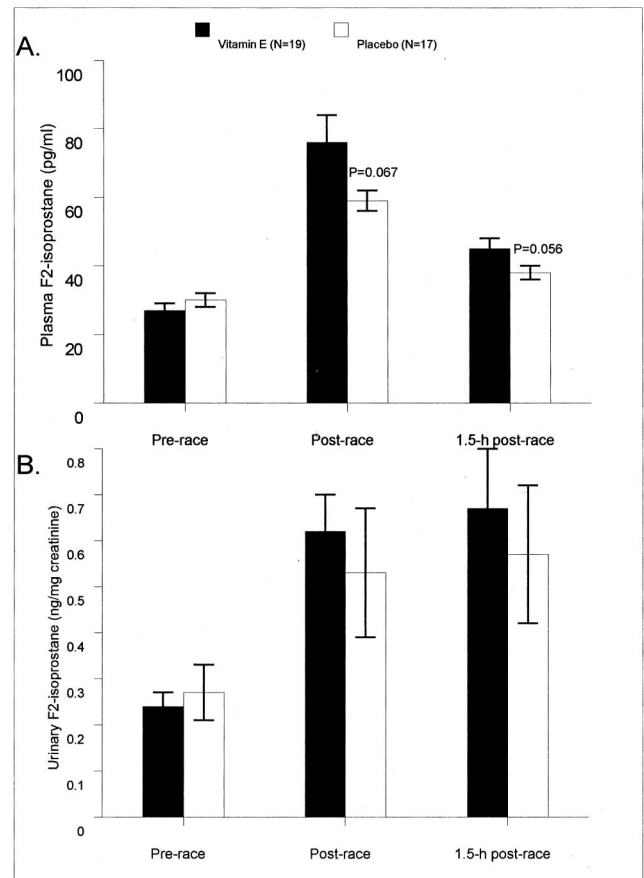


FIGURE 3—A and B. The pattern of change in plasma F₂-isoprostanes was significantly different between vitamin E and placebo groups (interaction effect, $P = 0.044$) (A). Numbers above bars represent student *t*-test P values for the change from prerace between groups. Urinary F₂-isoprostanes also increased post-race, but the pattern of change did not differ between groups ($P = 0.651$) (B).

negatively correlated with IL-6 ($r = -0.41$, $P = 0.014$) immediately post-race. Immediately post-race, plasma F₂-isoprostanes were significantly correlated with plasma IL-6 ($r = 0.56$, $P < 0.001$), IL-8 ($r = 0.56$, $P < 0.001$), and IL-1ra ($r = 0.53$, $P < 0.001$) but not IL-10 ($r = 0.30$, $P = 0.076$).

DISCUSSION

Contrary to our hypothesis, 2 months of α -tocopherol supplementation did not attenuate increases in plasma cytokines, changes in blood leukocyte subsets, decreases in salivary IgA output, or oxidative stress in athletes competing in the Triathlon World Championship in Kona, Hawaii. Unexpectedly, plasma F₂-isoprostanes increased nearly two-fold, and IL-6, IL-1ra, and IL-8 were markedly higher in the vitamin E compared with placebo group. Athletes with the highest plasma α -tocopherol levels had the highest post-race levels of plasma IL-6. Plasma cytokine levels in the vitamin E group far exceeded levels we have previously reported in subjects after competitive 42.2-km marathon race events, whereas levels in the placebo group were similar to post-marathon race values (20).

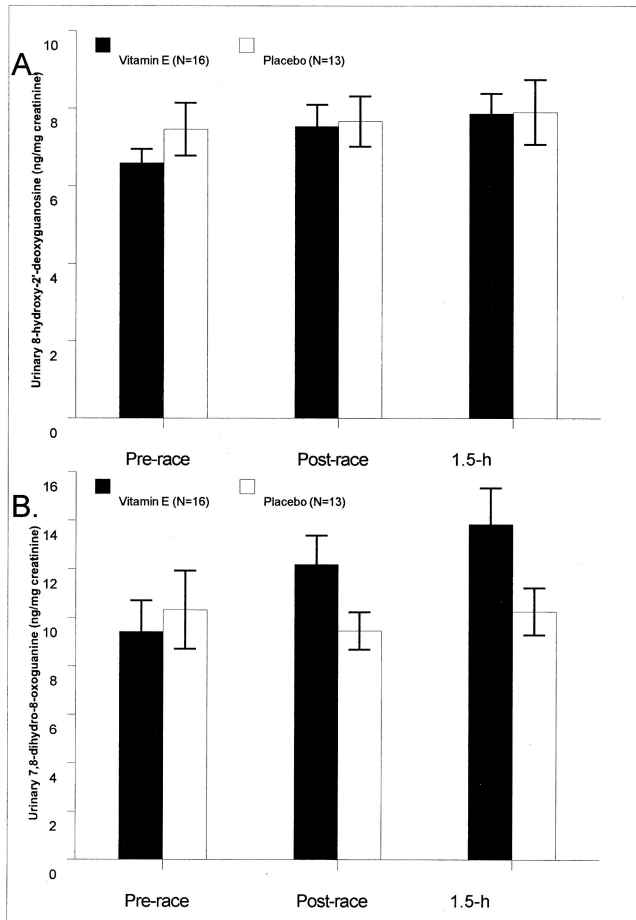


FIGURE 4—A and B. No changes in urinary 8-OHdG and 8-oxoG were measured after the race event (time effects, $P = 0.151$ and 0.124 , respectively).

There is increasing evidence that depending on the dose and experimental conditions, vitamin E can exert antioxidant, neutral, or pro-oxidant effects (8,23). High doses of α -tocopherol combined with high oxidative stress may create α -tocopherol radicals that may initiate processes of lipid peroxidation by themselves (23). When antioxidant networks are balanced, however, this pro-oxidant action of vitamin E radicals is inhibited by co-antioxidants such as vitamin C that can reduce the radical back to vitamin E (8,23). The athletes in this study avoided vitamin C supplements but still ingested high amounts of vitamin C (about 3 times recommended levels) and other nutrients, but this intake may have been insufficient to inhibit vitamin E pro-oxidant effects given the high dose of vitamin E consumed and the extreme physiological and oxidative stress experienced during the race. Vitamin C intake was substantially higher in the placebo group during the race for no apparent reason other than random outcome, but we do not know if this influenced our results. In previous studies by our team, vitamin C compared with placebo supplements did not counter exercise-induced oxidative and immune changes (19).

Of the eight naturally occurring forms of vitamin E, only the α -tocopherol form of the vitamin is maintained in human

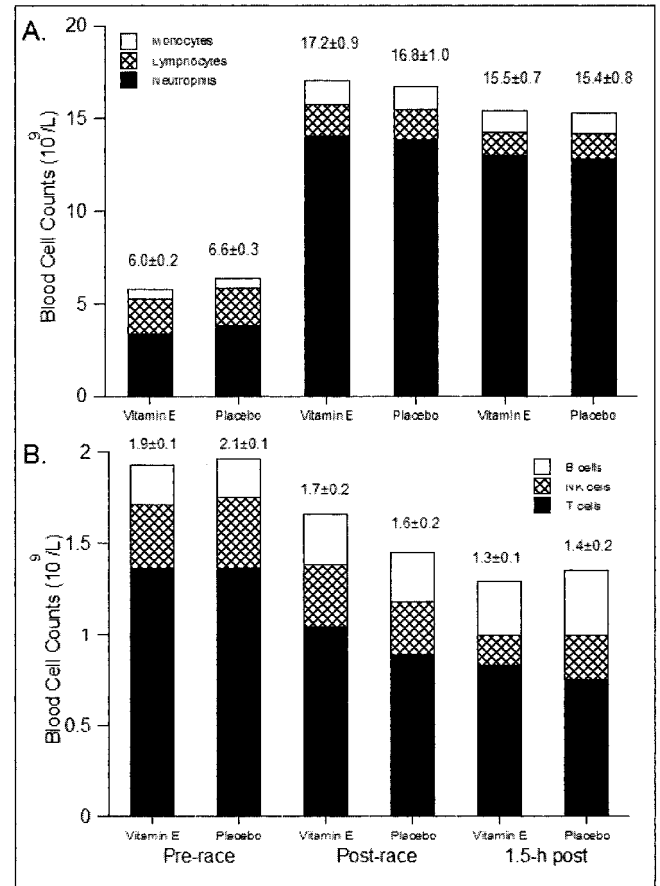


FIGURE 5—A and B. Total blood leukocyte (denoted by numbers at the top of the bars), neutrophil, and monocyte counts rose significantly after the race event (time effect, $P < 0.001$), but the patterns of change did not differ between the groups (A). Total blood lymphocyte (denoted by numbers at the top of the bars), T lymphocytes, and natural killer lymphocytes decreased significantly following the race event ($P < 0.001$), and B lymphocytes increased ($P < 0.001$), but the patterns of change did not differ between the groups (B).

plasma (8). Other naturally occurring forms of vitamin E including γ -tocopherol do not contribute toward meeting the vitamin E requirement established by the Food and Nutrition Board of the Institute of Medicine because they are not converted to α -tocopherol by humans and are poorly recognized by the α -tocopherol transfer protein in the liver (8). Nonetheless, γ -tocopherol is a major form of vitamin E in U.S. diets and has important physiological functions in the body. Recent evidence indicates that γ -tocopherol inhibits pro-inflammatory prostaglandin E₂ and leukotriene B₄, decreases TNF- α and 8-isoprostane production, and in general, attenuates inflammation-mediated damage (13). In our study, athletes in the vitamin E group experienced significantly reduced plasma γ -tocopherol levels, and this may have contributed to the pro-oxidative and pro-inflammatory changes measured post-race. It is unclear, however, by what mechanisms a reduction in plasma γ -tocopherol levels might have promoted an increase in markers of oxidative stress and inflammation after exercise.

Our findings of increased plasma and urinary F₂-isoprostane and plasma lipid hydroperoxides are indicative of increased oxidative stress. These observations are similar to

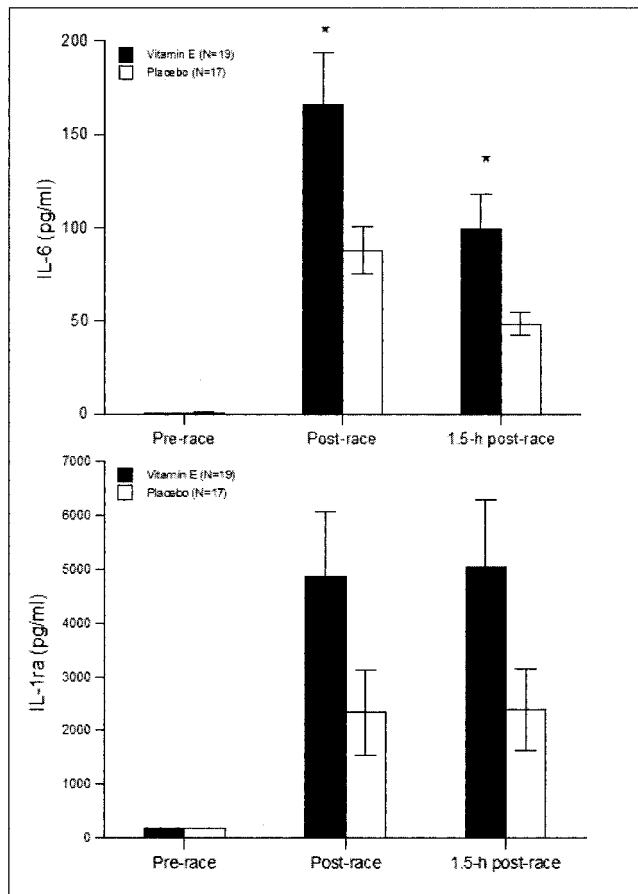


FIGURE 6—A and B. The pattern of change in IL-6 (A) and IL-1ra (B) tended to be different between vitamin E and placebo groups (group main effects and interaction effects were $P = 0.017$ and $P = 0.059$ for IL-6, respectively, and $P = 0.089$ and $P = 0.057$ for IL-1ra, respectively). * $P < 0.025$, change from prerace between groups.

those we have previously reported in two studies of athletes competing in ultramarathon races (18,19). Lipid hydroperoxides originate primarily from oxidation of omega-3 and omega-6 fatty acids found in lipoproteins, and F_2 -isoprostane from arachidonic fatty acids esterified in phospholipids (17,29). Triathletes with the highest plasma F_2 -isoprostanes levels recorded the highest plasma concentrations of IL-6, IL-1ra, and IL-8. Oxidative stress factors may interact with certain immune function factors during disease and immunosenescence and act as one mediator of inflammation by inducing apoptosis of leukocytes and biosynthesis of inflammatory cytokines (15). Little is known regarding the link between oxidative stress and immune changes induced by long-duration and high-intensity aerobic exercise. In mice, lymphocyte damage after exhaustive running has been related to oxidative damage in lymphoid tissues (2). A link between oxidative stress and suppression of cellular immu-

REFERENCES

1. ALMAR, M., J. G. VILLA, M. J. CUEVAS, J. A. RODRIGUEZ-MARROYO, C. AVILA, and J. GONZALEZ-GALLEGO. Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. *Free Radic. Res.* 36:247–253, 2002.
2. AZENABOR, A. A., and L. HOFFMAN-GOETZ. Intrathymic and intrasplenic oxidative stress mediates thymocyte and spleno-

nity has been described in men after intense, short bouts of exercise (30). In previous studies of ultramarathoners, we reported a weak but significant correlation between F_2 -isoprostane and IL-10 (18,19), a finding supported in the present study. Redox- and oxidant-mediated pathways play a role in the expression, distribution, and functional properties of IL-10, and this cytokine and other antiinflammatory cytokines may emerge as important links between oxidative stress and inflammatory processes during intensive and prolonged exercise (9).

Despite the extreme exercise stress experienced by the triathletes in this study, no significant increases were measured in DNA oxidative stress using urinary 8-OHdG and 8-oxoG, and no differences were measured between vitamin E and placebo groups. Hartmann and Niess (11) reported that vitamin E supplementation over a 2-wk period reduced the exercise-induced DNA damage measured in subjects running on treadmills to exhaustion. Other investigators have found no evidence of DNA oxidative damage after heavy exertion, but differences in methodology have made comparison between studies difficult (1,25,27). Rádak et al. (22) have shown that DNA repair enzymes are up-regulated in human skeletal muscle after a marathon race, protecting the athlete against excessive DNA damage. Our data suggest that world class triathletes do not show evidence of increased DNA oxidative stress using urinary 8-OHdG and 8-oxoG after ~12 h of intensive competition.

In summary, 2 months of vitamin E supplementation at a dose of $800 \text{ IU} \cdot \text{d}^{-1}$ α -tocopherol did not attenuate increases in plasma cytokines, perturbations in other measures of immunity, or oxidative stress in triathletes competing in the Triathlon World Championship race event. To the contrary, athletes in the vitamin E compared with placebo group experienced greater lipid peroxidation and increases in plasma levels of several cytokines after the triathlon. Despite these indications that vitamin E exerted pro-oxidant and pro-inflammatory effects, race performance did not differ between athletes in the vitamin E and placebo groups. Further research is warranted, however, to measure the potential effect of frequent large-dose vitamin E supplementation on the long-term health status of ultra-athletes.

We express appreciation to several individuals for their assistance in this study: Jeff Zachwieja, Kris Osterberg, the Gatorade Team, Laura Benezra, Cathy Nieman, Rebecca Bailey, Mary Whitlock, Linda Robinson, and Penny Mack.

This study was supported by a grant from the Gatorade Sports Science Institute.

Jason D. Morrow is supported by NIH grants GM15431, CA77839, and DK48831, and is the recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

cyte damage in acutely exercised mice. *J. Appl. Physiol.* 86: 1823–1827, 1999.

3. BEATON, L. J., D. A. ALLAN, M. A. TARNOPOLSKY, P. M. THIDUS, and S. M. PHILLIPS. Contraction-induced muscle damage is unaffected by vitamin E supplementation. *Med. Sci. Sports Exerc.* 34:798–805, 2002.

4. BEHARKA, A., S. REDICAN, L. LEKA, and S. N. MEYDANI. Vitamin E status and immune function. *Methods Enzymol.* 282:247–263, 1997.
5. CANNON, J. G., S. N. MEYDANI, R. A. FIEDLING, et al. Acute phase response to exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. *Am. J. Physiol.* 260:R1235–R1240, 1991.
6. DAWSON, B., G. J. HENRY, C. GOODMAN, et al. Effect of vitamin C and E supplementation on biochemical and ultrastructural indices of muscle damage after a 21 km run. *Int. J. Sports Med.* 23:10–15, 2002.
7. DILL, D. B., and D. L. COSTILL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.* 37:247–248, 1974.
8. FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press, 2000, pp. 186–217.
9. HADDAD, J. J., and C. S. FAHLMAN. Redox- and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? *Biochem. Biophys. Res. Commun.* 297:163–176, 2002.
10. HAIDARI, M., F. JAVADI, M. KADKHODAEI, and A. SANATI. Enhanced susceptibility to oxidation and diminished vitamin E content of LDL from patients with stable coronary artery disease. *Clin. Chem.* 47:1234–1240, 2001.
11. HARTMANN, A., and A. M. NIESS. Vitamin E prevents exercise-induced DNA damage. *Mutat. Res. Lett.* 346:195–202, 1995.
12. ITOH, H., T. OHKUWA, Y. YAMAZAKI, et al. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. *Int. J. Sports Med.* 21:369–374, 2000.
13. JIANG, Q., and B. N. AMES. Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. *FASEB J.* 17:816–822, 2003.
14. LIANG, Y., P. WEI, R. W. DUKE, et al. Quantification of 8-iso-prostaglandin-F₂ α and 2–3-dinor-8-iso-prostaglandin-F₂ α in human urine using liquid chromatography-tandem mass spectrometry. *Free Radic. Biol. Med.* 34:409–418, 2003.
15. LIN, Y., R. HUANG, N. SANTANAM, Y. G. LIU, S. PARTHASARATHY, and R. P. HUANG. Profiling of human cytokines in healthy individuals with vitamin E supplementation by antibody array. *Cancer Lett.* 187:17–24, 2002.
16. MASTALOUDIS, A., S. W. LEONARD, and M. G. TRABER. Oxidative stress in athletes during extreme endurance exercise. *Free Radic. Biol. Med.* 31:911–922, 2001.
17. MORROW, J. D., and L. J. ROBERTS. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.* 36:1–21, 1997.
18. NIEMAN, D. C., C. L. DUMKE, D. A. HENSON, et al. Immune and oxidative changes during and following the Western States Endurance Run. *Int. J. Sports Med.* 24:541–547, 2003.
19. NIEMAN, D. C., D. A. HENSON, S. R. MCANULTY, et al. Influence of vitamin C supplementation on oxidative and immune changes following an ultramarathon. *J. Appl. Physiol.* 92:1970–1977, 2002.
20. NIEMAN, D. C., D. A. HENSON, L. L. SMITH, et al. Cytokine changes after a marathon race. *J. Appl. Physiol.* 91:109–114, 2001.
21. PETERSEN, E. W., K. OSTROWSKI, T. IBFELT, et al. Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. *Am. J. Physiol. Cell Physiol.* 280:C1570–C1575, 2002.
22. RADÁK Z., P. APOR, J. PUCSUK, et al. Marathon running alters the DNA base excision repair in human skeletal muscle. *Life Sci.* 72:1627–1633, 2003.
23. RIETJENS, I. M. C. M., M. G. BOERSMA, L. DE HAAN, et al. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ. Toxicol. Pharmacol.* 11:321–333, 2002.
24. SACHECK, J. M., and J. B. BLUMBERG. Role of vitamin E and oxidative stress in exercise. *Nutrition* 17:809–814, 2001.
25. SACHECK, J. M., P. E. MILBURY, J. G. CANNON, R. ROUBENOFF, and J. B. BLUMBERG. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. *Free Radic. Biol. Med.* 34:1575–1588, 2003.
26. SINGH, A., D. A. PAPANICOLAOU, L. L. LAWRENCE, E. A. HOWELL, G. P. CHROUSOS, and P. A. DEUSTER. Neuroendocrine responses to running in women after zinc and vitamin E supplementation. *Med. Sci. Sports Exerc.* 31:536–542, 1999.
27. SUMIDA, S., K. OKAMURA, T. DOI, M. SAKURAI, Y. YOSHIKAWA, and Y. SUGAWA-KATAYAMA. No influence of a single bout of exercise on urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Biochem. Mol. Biol. Int.* 42:601–609, 1997.
28. TSAI, K., T. G. HSU, K. M. HSU, et al. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic. Biol. Med.* 31:1465–1472, 2001.
29. URSO, M. L., and P. M. CLARKSON. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 189:41–54, 2003.
30. VIDER, J., J. LEHTMAA, T. KULLISAAR, et al. Acute immune response in respect to exercise-induced oxidative stress. *Pathophysiology* 7:263–270, 2001.