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Effect of alpha-tocopherol supplementation on plasma homocysteine and oxidative stress in highly trained athletes before and after exhaustive exercise

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Abstract

The interrelationship between physical exercise, antioxidant supplementation, oxidative stress and plasma levels of homocysteine (Hcy) has not been adequately examined. The purpose of this study was to examine the effect of 2 months of vitamin E supplementation (800 IU/day alpha-tocopherol) (E) or placebo (P) in 38 triathletes on plasma Hcy concentrations, antioxidant potential and oxidative stress. It was hypothesized that vitamin E supplementation would reduce plasma Hcy and oxidative stress markers compared to placebo. Blood samples were collected 1 day prior to the race, immediately postrace and 1.5 h postrace. Plasma alpha-tocopherol was 75% higher ($P<.001$) in E versus P prerace (24.1±1.1 and 13.8±1.1 μmol/L, respectively), and this group difference was maintained throughout the race. Cortisol was significantly increased in both E and P ($P<.001$), but there was no difference in the pattern of change. There were no significant time, group or interaction effects on plasma Hcy concentrations between E and P. Plasma F_2-isoprostanes increased 181% versus 97% during the race in E versus P, and lipid hydroperoxides were significantly elevated ($P=.009$) 1.5 h postrace in E versus P. Plasma antioxidant potential was significantly higher 1.5 h postrace in E versus P ($P=.039$). This study indicates that prolonged large doses of alpha-tocopherol supplementation did not affect plasma Hcy concentrations and exhibited pro-oxidant characteristics in highly trained athletes during exhaustive exercise.

Keywords: Homocysteine; Oxidative stress; F_2-isoprostanes; Endurance exercise; Vitamin E; Lipid hydroperoxides

1. Introduction

Homocysteine (Hcy) is an intermediate formed during metabolism of methionine, an essential amino acid obtained from the diet. During metabolism, Hcy ultimately enters either a transsulfuration pathway or remethylation cycle. Folate, vitamin B_{12} and vitamin B_6 are important cofactors in the metabolic pathways of Hcy, and supplementation with folate has been found to be an effective measure for lowering plasma Hcy [1]. The influence, if any, of vitamin E supplementation on the transsulfuration pathway or remethylation cycle is unknown. However, vitamin E might indirectly affect the pathways by reducing oxidative stress and preventing the oxidative destruction of folate, which is involved in the remethylation of Hcy to methionine [2]. In support of this concept, Can et al. [3] found that vitamin E administration of 100 mg/kg day to arthritic rats significantly decreased serum Hcy levels with respect to nontreated arthritic rats. The exact mechanism for this observation was not elucidated in the study. In contrast, Brude et al. [4] and Baydas et al. [5] found no effect of vitamin E supplementation on Hcy in smokers and rats, respectively.

Homocysteine has been identified as a positive independent risk factor for cardiovascular disease (CVD) [6,7]. Exercise is thought to reduce the risk of developing CVD by reducing resting heart rate, blood pressure and low-density

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lipoprotein (LDL) cholesterol levels, and increasing high-density lipoprotein (HDL) cholesterol levels [8]. However, the effects of exercise on Hcy concentration are not clear and have been minimally studied [9–12]. De Cree et al. [11] found that an acute 1-h bout of cycle ergometer exercise at 60% maximal oxygen consumption (VO_2max) in young male subjects did not significantly change Hcy concentration. Conversely, in another study involving young women, De Cree et al. [10] found that bicycle ergometry exercise to exhaustion significantly increased Hcy concentrations. Konig et al. [13] found that a short sprint triathlon significantly increased Hcy concentration but that high levels of chronic training significantly reduced Hcy compared to lower levels of training. The effect of an episode of very intense, long duration exercise, such as an ultramarathon or triathlon on plasma Hcy, is not known.

Oxidative stress is typically increased as a result of exercise [14–16]. This increase is thought to result from an imbalance between the production of oxidants and the available antioxidant defenses [17]. These oxidants primarily include reactive oxygen species (ROS) and reactive nitrogen species (RNS) [18,19]. Homocysteine is known to induce oxidative stress in the endothelium [20]. It is unclear what effects supplementation with antioxidants, such as vitamin E, might have on plasma Hcy concentration during exhaustive long-duration exercise in humans. Therefore, the purpose of this study was to examine the effects of 800 IU of vitamin E (alpha-tocopherol) supplementation daily for 2 months prior to and after the Hawaii Ironman triathlon in highly trained athletes. We hypothesized that alpha-tocopherol would reduce plasma Hcy and oxidative stress and increase plasma antioxidant potential.

2. Materials and methods

2.1. Participants

Thirty-eight subjects were recruited from applicants for the 2002 Kona Triathlon World Championship held October 19, 2002, in Kailua-Kona, HI. Informed written 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 (ASU). The race began at 7:00 a.m. and consisted of a 2.4-mile ocean swim, a 112-mile bike race and a 26.2-mile run.

2.2. Supplementation

Subjects were randomized into either a vitamin E (E) or placebo (P) group. From August 19 to October 19, E ingested 800 IU of alpha-tocopherol daily as a gel cap. P ingested a gel cap daily containing only soybean vegetable oil that provided 0.075 IU of alpha-tocopherol. During the 2 months of vitamin E/placebo supplementation, subjects followed a diet (using a food list) that prohibited consumption of foods high in vitamin E. During the last week of supplementation, all subjects recorded a 7-day food record to determine if both groups had similar diets. The food records were analyzed using the ESHA version 7.9 food processor software program (ESHA Research, Salem, OR). During the race, subjects were allowed to eat and drink ad libitum, and a postrace questionnaire was used to estimate food intake during the race. Subjects were instructed to avoid foods and supplements high in vitamin E or C during the race.

2.3. Sample collection

Prerace blood samples were collected between 4:00 p.m. and 5:00 p.m. the day prior to the race; height and weight measures were taken at this time. The remaining blood samples were collected about 5 min postrace and 1.5 h postrace. Blood samples were taken at these times for the following reasons: 5 min was the approximate amount of time it took to get the subjects from the finish line to our research site, and we had previously observed that oxidative measures generally peak around this time (5 min postexercise) and are in decline by 1.5 h postexercise. Additionally, we collected immune data (reported elsewhere) that peak about 1.5 h postexercise. Lastly, we had concerns of trying to keep subjects around longer than 1.5 h postrace. Blood samples were collected into heparinized and EDTA vacutainer tubes. The tubes were immediately placed on ice and then spun at $1500\times g$ for 10 min at 4°C. The plasma from the heparin tubes was aliquoted into cryotubes, snap frozen in liquid nitrogen and stored at −80°C until analysis for vitamin E, cortisol, F_2-isoprostanes, lipid hydroperoxides (ROOH) and ferric-reducing ability of plasma (FRAP). The plasma from the EDTA tube was aliquoted into cryotubes, snap frozen in liquid nitrogen and stored at −80°C until analysis for Hcy. All assay determinations were accomplished within 1 month of the end of the study and were corrected for plasma volume shift according to the methodology of Dill and Costill [21].

2.4. Analytical measurements

2.4.1. Alpha-tocopherol and cortisol

Plasma alpha-tocopherol was determined by HPLC (Kronos Laboratories, Phoenix, AZ) and plasma cortisol was assayed in duplicate using the competitive solid-phase ^{125}I radioimmunoassay (RIA) technique (Diagnostic Products, Los Angeles, CA).

2.4.2. Homocysteine

Plasma Hcy was determined by an ELISA kit from Axis-Shield Laboratories (Bickbeergrund, Germany).

2.4.3. F_2-isoprostanes

Plasma F_2-isoprostanes were determined using gas chromatography–mass spectrometry according to the methodology of Morrow and Roberts [22]. Briefly, free F_2-isoprostanes were extracted from 1 ml of plasma. One to five picomoles of deuterated [$^2H_4$]PGF_2 internal standard

was added and the mixture vortexed. This mixture was then added to a C_{18} Sep Pak column, followed by silica solid phase extractions. F_2-isoprostanes were converted into pentafluorobenzyl esters, subjected to thin layer chromatography and then converted to trimethylsilyl ether derivatives. Samples were then analyzed by a negative ion chemical ionization GC-MS using a Nermag R10-10C mass spectrometer interfaced with a DEC-PDP 11/23 Plus computer system.

2.4.4. Lipid hydroperoxides

ROOH of duplicate samples was determined after chloroform extraction using spectrophotometric analysis and a kit (#705002) obtained from Cayman Chemical (Ann Arbor, MI). Briefly, 100 μl of plasma sample was pipetted into duplicate test tubes, and 100 μl of extract R saturated methanol (Fisher Scientific, Pittsburgh, PA) was added to each tube. All tubes were then vortexed and had 750 μl of cold chloroform (Fisher Scientific) added, and the tubes were again vortexed. All tubes were then centrifuged at 1500×*g* for 5 min at 4°C according to protocol instructions. Then, 500 μl of the bottom chloroform layer was extracted from each tube and immediately placed in ice. At this point, 450 μl of a 2:1 ratio deoxygenated chloroform–methanol mixture was added to each tube, and the tubes were vortexed. A standard curve was prepared using a hydroperoxide standard and varying amounts of the 2:1 ratio deoxygenated chloroform–methanol mixture giving a range of 0–5 nmol and a total volume of 950 μl. Lastly, 50 μl of chromogen was added to each sample and standard in duplicate, and the tubes were vortexed. All tubes were incubated at room temperature for 5 min, and then 300 μl of each standard and sample were removed and placed in a 96-well glass plate and read at 500 nm in a microplate reader (Biotek uQuant, Winooski, VT). ROOH concentration was determined from a linear regression line generated from the standard curve of cumene hydroperoxide.

2.4.5. Total plasma antioxidant potential

Total plasma antioxidant potential was determined by the FRAP assay according to the methodology of Benzie and Strain [23]. The basis of this assay is that water soluble reducing agents (antioxidants) in the plasma will reduce ferric ions to ferrous ions, which then react with an added chromogen. Working FRAP solution was prepared daily and consisted of 300 mmol/L acetate buffer with the pH adjusted to 3.6 [3.1 g sodium acetate (Sigma, St. Louis, MO) and 16 ml of 1N acetic acid (Sigma) per liter of buffer solution]; 10 mmol per liter 2,4,6-tripyridyl-*s*-triazine [TPTZ (Sigma) in 40 mmol HCl (Fisher Scientific)]; and 20 mmol iron trichloride hexhydrate (Sigma) in doubly distilled deionized water. Working FRAP reagent was prepared as required by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of iron trichloride hexhydrate solution. The working FRAP solution was placed in a water bath and warmed to 37°C.

Then, 100 μl of either standard, sample or blank (doubly distilled deionized water), respectively, were added to glass test tubes containing 3.0 ml of warmed FRAP reagent and vortexed. All tubes were then incubated at 37°C for 4 min and read at 593 nm in a spectrophotometer (Genesys-5, Thermo Spectronic, Rochester, NY). Samples and standards were analyzed in duplicate, and FRAP values were expressed as vitamin C equivalents as determined by linear regression from a vitamin C standard curve (0–1000 μmol).

2.5. Statistical analysis

Statistical significance was set a priori at the $P<.05$ level, and values were expressed as means±S.E.M. All statistical analyses were done using Instat version 1.01(San Diego, CA) and SPSS version 11.5 (Chicago, IL). Vitamin E and placebo groups were compared for subject characteristics and race performance measures using independent *t* tests (Table 1). Oxidative measures and hormone values were analyzed using 2 (vitamin E and placebo groups)×3 (times of measurement) repeated measures analysis of variance (ANOVA). Homocysteine values were analyzed using 2 (vitamin E and placebo groups)×2 (times of measurement) repeated measures ANOVA. If the Group×Time interaction *P* value was <.05, the change from prerace for

Table 1
Subject characteristics (means±S.E.M.)

	Vitamin E ($N=19$)	Placebo ($N=17$)	*P* value
Age (years)	35.2±1.6	39.2±1.4	.064
Height (m)	1.75±0.02	1.77±0.02	.405
Body mass (kg)	71.4±2.4	73.1±2.6	.636
Body fat (%)	12.7±1.5	11.3±0.8	.437
Training (years)	8.4±1.2	8.9±1.4	.823
Ironmans	5.5±1.4	4.6±1.0	.599
Ironman PR (min)	683±24	652±20	.326
Swim (km/week)	7.2±0.9	8.1±0.5	.38
Run (km/week)	50.1±4.0	53.8±5.6	.585
Bike (km/week)	283±27	263±28	.614
Race times (min)	721±24	719±27	.959
Intensity bike, BPM (~80% maximum heart rate)	145±3	146±2	.780
Intensity run, BPM (~80% maximum heart rate)	143±4	148±3	.233
Mean plasma shift (AS)	1.5±0.6%		>.05
Mean macronutrient intake (kcal) (AS)	3069±174		>.05
Mean macronutrient intake carbohydrate (g) (AS)	423.5±2.7		>.05
Mean macronutrient intake fat (g) (AS)	95.1±2.2		>.05
Mean macronutrient intake protein (g) (AS)	521.7±0.39		>.05

PR, personal record; BPM, beats per minute; AS, all subjects.

the postrace and 1.5-h postrace values was calculated and compared using Student's *t* tests. For these comparisons, a Bonferroni adjustment was made with statistical significance set at $P<.025$. Pearson product-moment correlations were used to test the relationship between plasma levels of alpha-tocopherol, Hcy, cortisol, plasma antioxidant potential, F_2-isoprostanes and ROOH measures. Statistical power was calculated to be at 85% or better for all variables.

3. Results

3.1. Subject demographics

All athletes, except two, complied with research requirements and completed the triathlon ($N=36$). Subject characteristics, racing and training data are listed in Table 1 for E ($N=19$) and P ($N=17$). No significant group differences were found for basic demographics and weekly training characteristics. All subjects had previously competed in several Ironman competitions, but the number of races and personal records were not significantly different (Table 1). Since none of the weekly training parameters or personal records were significantly different, we conclude that subjects in the E and P were a homogenous sample pool and that none of our findings are likely connected to differences in training or fitness level.

Race times, mean heart rate intensities and mean plasma volume shifts are listed in Table 1. Race times were not different between E (721±24 min) and P (719±27 min, $P=.959$). Subjects in E and P maintained an intensity of about 80% of predicted maximum heart rate during the bike (145±3 and 146±2 beats/min, $P=.780$) and run (143±4 and 148±3 beats/min, $P=.233$) portions, respectively. Mean plasma volume shift for all subjects was 1.5+0.6% and was not significantly different at any time.

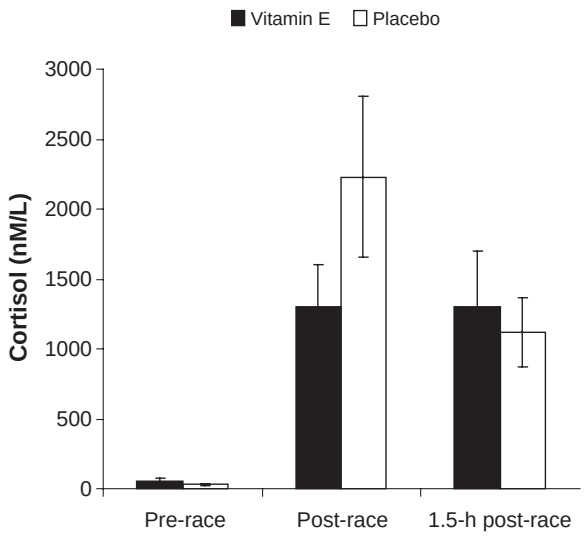

Fig. 2. Cortisol was significantly elevated over time in both vitamin E ($N=19$) and placebo groups ($N=17$) ($P<.001$). There was no difference in the pattern of change in group effects ($P=.491$) and interaction effects ($P=.100$). Values are means±S.E.M.

3.2. Diet

Energy and macronutrient intake did not differ between groups and averaged 3069±174 kcal/day 2 weeks prior to the race event. This caloric average breaks down to 423.5±2.7 g carbohydrate, 85.6±2.2 g fat and 214.8±1.5 g protein daily. The daily percentage breakdown was 55.2±1.6% carbohydrate, 27.9±1.3% fat and 17.0±0.9% protein (Table 1).

3.3. Biochemical measures

Plasma alpha-tocopherol was 75% higher in E versus P prerace (24.1±1.1 and 13.8±1.1 mol/L, $P<.001$, respectively), and this group difference was maintained throughout

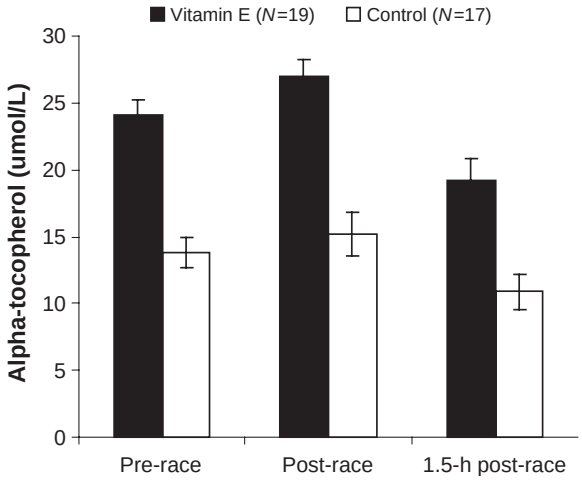

Fig. 1. Plasma alpha-tocopherol was significantly higher in the vitamin E ($N=19$) compared to placebo group ($N=17$) before and after the race event (group effects $P<.001$, interaction effects $P<.047$, time effects $P<.001$). Values are means±S.E.M.

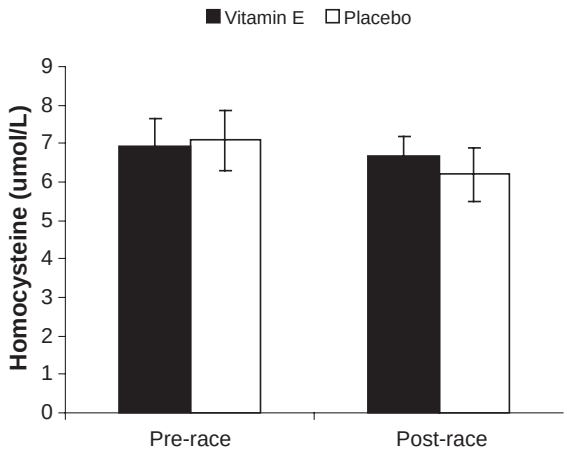

Fig. 3. There were no significant differences in plasma Hcy in vitamin E ($N=10$) versus placebo ($N=10$) groups prerace or immediately postrace. Overall group effects ($P=.831$), interaction effects ($P=.582$) and time effects ($P=.365$).

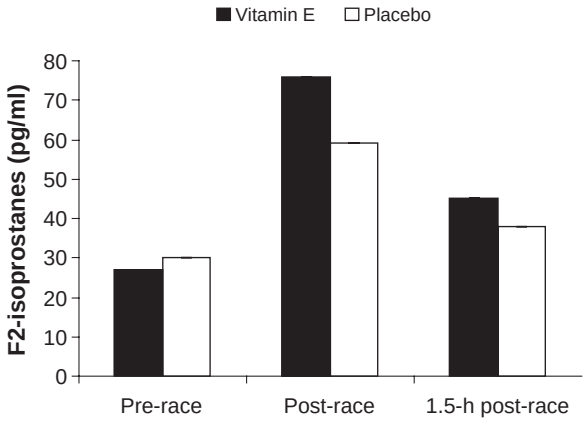

Fig. 4. Plasma F_2-isoprostanes increased 181% versus 97% during the race in the vitamin E ($N=19$) compared to the placebo group ($N=17$) immediately postrace. Overall time effects ($P<.001$) and interaction effects ($P=.044$) were significant. Values are means±S.E.M.

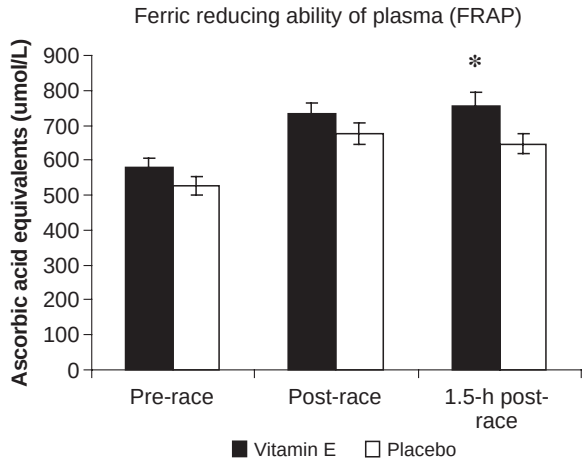

Fig. 6. The FRAP was significantly higher ($P=.039$) 1.5 h postrace in the vitamin E group ($N=19$) versus the placebo group ($N=17$). Overall interaction ($P=.013$) and time effects ($P<.001$) were significant. Values are means±S.E.M.

the race (Fig. 1). Plasma cortisol increased significantly after the race ($P<.001$), but there were no differences in the pattern of change between E and P (group effect, $P=.491$; interaction effect, $P=.100$) (Fig. 2). Homocysteine was unchanged in both E and P 5 to 10 min postrace, and there were no significant time ($P=.365$), group ($P=.831$) or interaction effects ($P=.582$) (Fig. 3). F_2-isoprostanes (data reported elsewhere) increased 181% versus 97% during the race in the E versus P groups, respectively ($P<.05$), and interaction effects were significant ($P=.044$) (Fig. 4). ROOH were elevated in E versus P at 1.5 h postrace ($P=.009$) (Fig. 5). The pattern of change in the FRAP as a measure of plasma antioxidant potential was significantly higher 1.5 h postrace in E versus P ($P=.039$), as well as exhibiting interaction ($P=.013$) and time ($P<.001$) effects (Fig. 6).

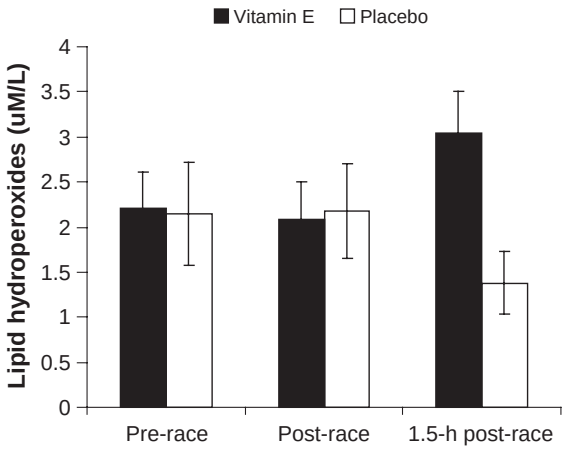

Fig. 5. Plasma lipid hydroperoxides were significantly higher ($P=.009$) 1.5 h postrace in the vitamin E group ($N=19$) versus the placebo group ($N=17$). Overall, group ($P=.229$), interaction ($P=.065$) and time ($P=.978$) effects were not significant. Values are means±S.E.M.

3.4. Correlations

There were no significant correlations among variables, except for a positive correlation between cortisol and F_2-isoprostanes in the P group ($r=.646$, $P=.000$).

4. Discussion

Two months of supplementation with 800 IU of vitamin E as alpha-tocopherol significantly increased plasma vitamin E levels compared to placebo group; this increase was maintained throughout the race and afterward. The increased plasma vitamin E neither exerted any group differences in performance times nor was overall systemic stress affected because there were no group differences in plasma cortisol. The observation in our study of no differences in performance times between E and P is in agreement with those of Rokitzki et al. [24] who found no effect of alpha-tocopherol supplementation on performance in racing cyclists. Most available research indicates that alpha-tocopherol supplementation over a wide range of values (200–1200 IU/day) has little impact on performance and a variable impact or effect on exercise-induced oxidative stress [19,25].

We observed no effect or correlation of vitamin E supplementation on Hcy levels. This is in agreement with Baydas et al. [5]. However, Brude et al. [4] found a negative correlation with plasma Hcy and dietary intake of vitamin E. We also observed no change in Hcy levels due to the intense exercise. Although using exercise protocols of lower intensity and much lower durations, Wright et al. [12] and De Cree et al. [10] found that exercise did not affect Hcy levels. In contrast, Bailey et al. [9] found that normoxic cycling three times per week for 20–30 min at 70–85% of maximum heart rate significantly elevated Hcy levels.

Apparently, more research is needed to clarify the effects of different exercise protocols on plasma Hcy.

Our study found that both E and P exhibited significant increases in plasma antioxidant potential, and this potential was significantly higher in E versus P 1.5 h postrace. Unfortunately, a Hcy measurement could not be made at this time, so no assessment of the effect (if any) of the significantly increased antioxidant potential in E versus P on Hcy can be made. The increase in antioxidant potential is primarily a result of uric acid and vitamin C release into the plasma [16]. This observation is in agreement with findings we observed in a previous study [15].

Homocysteine concentrations were not different between E and P, despite significant differences in oxidative stress markers. This indicates that Hcy was neither affected by increased oxidative stress nor contributed to the oxidative stress. Furthermore, there were no significant correlations to support any relationship. Our finding is in agreement with Dudman et al. [26] who found that hyperhomocysteinemia did not increase levels of lipid hydroperoxides or generate vascular damage by this mechanism, although the Dudman study was not an exercise study. Interestingly, Voutilainen et al. [27] found a significant association with elevated fasting Hcy levels (mean of 11.0 μmol) and F_2-isoprostanes (mean of 29.6 pg/ml) in men. Homocysteine concentrations in our study were consistently in the 6- to 7-μmol range (low–normal) pre- and postrace, despite the fact that F_2-isoprostanes rose to a range of 60 to 70 pg/ml postrace. This suggests that acute increases in F_2-isoprostanes do not exacerbate Hcy levels. However, it is still possible that elevations of Hcy could increase F_2-isoprostanes by initiation of oxidative stress induced by Hcy [20]. The research is clearly not complete with regard to effects of vitamin E supplementation and intense exercise on Hcy concentration. A possible limitation to our study is that we did not measure two important vitamins known to influence plasma Hcy values. It would have been interesting to examine changes in blood folate and vitamin B_{12} values in relation to plasma Hcy. This might have provided further insight to the lack of change in Hcy associated with exercise.

Interestingly, F_2-isoprostanes and ROOH were higher in E versus P. Although we are unaware of any vitamin E supplementation studies with long-duration aerobic exercise in which vitamin E acted as a pro-oxidant and enhanced lipid peroxidation, several nonexercise investigations have found large doses of vitamin E to exert pro-oxidative effects in humans [28,29]. The data from our study indicate that vitamin E supplementation caused a pro-oxidative state.

A pro-oxidative effect may be defined as a reaction that can promote oxidative activity and induction of oxidative stress [30]. Several possibilities exist that may explain the pro-oxidative outcome of the alpha-tocopherol supplementation in our study. As a part of its antioxidant function, alpha-tocopherol temporarily becomes a radical species known as the alpha-tocopherol radical. In the absence or decreased concentration of co-antioxidants (such as ascorbate) capable of reducing the alpha-tocopherol radical, alpha-tocopherol can exhibit pro-oxidant activity [29]. Cooney et al. [28] found that malondialdehyde (MDA) equivalents were increased in individuals with high plasma alpha-tocopherol. The reason was postulated to be from endogenous oxidants associated with enhanced immune function from the higher alpha-tocopherol. Antioxidant balance seems to be critically important, as supplementation with large amounts of one antioxidant may change this balance and create a pro-oxidative state. Relevant to our study is that supplementation with alpha-tocopherol has been shown to decrease plasma concentrations of gamma-tocopherol, a minor unmethylated tocopherol [31]. Gamma-tocopherol has been found to be more effective than alpha-tocopherol in detoxifying RNS [32].

Only a few others have examined vitamin E, exercise and F_2-isoprostanes. Mastaloudis et al. [33] showed that plasma F_2-isoprostanes increased in 11 athletes during a 50-km race with a concomitant increase in vitamin E disappearance. Conversely, Sacheck and Blumberg [25] found that vitamin E supplementation (1000 IU/day for 12 weeks) did not attenuate increases in plasma F_2-isoprostanes in 16 young males following 45 min of downhill running at 75% VO_2max. F_2-isoprostanes are a recently described class of prostaglandin-like compounds produced by noncyclooxygenase free radical-mediated lipid peroxidation of arachidonic acid. F_2-isoprostanes are recognized as a sensitive and stable marker of oxidative stress and are biologically active as well [22]. We have demonstrated that exercise causes significant increases in these compounds [15,16,34,35]. The acute and chronic physiological and biochemical effects of increased F_2-isoprostanes after exercise are not currently known. High concentrations of F_2-isoprostanes are found after cardiac arrest, in individuals with diabetes and in smokers [22]. Additionally, our results indicate that an increased antioxidant potential from alpha-tocopherol supplementation did not suppress F_2-isoprostanes, but actually increased F_2-isoprostanes in the E group. The exact mechanism of the increase in plasma antioxidant potential and the resulting increase in lipid peroxidation cannot be discerned from this study. It was hypothesized that an increase in antioxidant potential would decrease oxidative stress, but this was not the case. In contrast, total plasma antioxidant potential has been found to be inversely related to oxidative stress in some disease states [36].

ROOH are highly reactive compounds and interact with proteins, amino acids, amines and DNA [37]. Additionally, ROOH originate primarily from oxidation of omega-3 and omega-6 fatty acids found in lipoproteins. These compounds have been found to increase as a result of exercise [34,38]. ROOH were significantly increased in E versus P similar to the F_2-isoprostanes and were not diminished by the increased plasma antioxidant potential.

In summary, a long strenuous bout of exercise did not affect Hcy concentration, despite significant increases in oxidative stress. Furthermore, 2 months of vitamin E

supplementation did not affect Hcy concentration but resulted in a pro-oxidative state and significantly increased oxidative stress markers, despite an increased plasma antioxidant potential. We believe this is the first study to examine changes in plasma Hcy after intense-long duration exercise and to demonstrate a pro-oxidative effect in vivo from large-dose vitamin E supplementation. Our results indicate that athletes engaged in intense long-duration exercise should avoid high intakes of alpha-tocopherol. Future research should examine the effect of large-dose antioxidant combinations in comparison to the outcomes to our use of a single large-dose antioxidant.

Acknowledgments

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