Oxidative Stress Response to Aerobic Exercise: Comparison of Antioxidant Supplements

RICHARD J. BLOOMER[1], ALLAN H. GOLDFARB[2], and MICHAEL J. MCKENZIE[2]

[1]*Department of Health and Sport Sciences, University of Memphis, Memphis, TN; and* [2]*Exercise Physiology Laboratory, Department of Exercise and Sport Science, University of North Carolina Greensboro, Greensboro, NC*

ABSTRACT

BLOOMER, R. J., A. H. GOLDFARB, and M. J. MCKENZIE. Oxidative Stress Response to Aerobic Exercise: Comparison of Antioxidant Supplements. *Med. Sci. Sports Exerc*., Vol. 38, No. 6, pp. 1098–1105, 2006. **Purpose:** To compare the effects of two antioxidant formulas on biomarkers of oxidative stress before and after aerobic exercise. **Methods:** Aerobically trained men (N = 25) and women (N = 23) were assigned to one of three treatments: 400 IU of vitamin E + 1 g of vitamin C (V; N = 15), a fruit and vegetable juice powder concentrate (FV; N = 16), or a placebo (P; N = 17). Subjects ran for 30 min at 80% $\dot{V}O_{2max}$ before, after 2 wk of supplementation, and after a 1-wk washout period. Blood samples were taken before and immediately after exercise and analyzed for protein carbonyls (PC), malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and vitamins C and E. **Results:** The V treatment increased plasma vitamin C and E after 2 wk ($P \leq 0.05$), with no change in the FV or P. Postexercise PC values were elevated for all treatments after all exercise bouts ($P < 0.0001$). Both V and FV attenuated the exercise-induced increase in PC after 2 wk of supplementation (V = 21%, FV = 17%), and after the 1-wk washout (V = 13%, FV = 6%) compared with P ($P < 0.05$), with no differences between V and FV. MDA was unaffected by exercise and treatment. A treatment main effect for 8-OHdG was noted, with values for V lower than for FV and P (4.5 ± 2.5, 5.5 ± 2.7, and 6.0 ± 2.5 ng·mL^{-1}, respectively; P = 0.0002). No exercise session or time main effect was noted for 8-OHdG, suggesting that the lower mean value for the V treatment group was not a result of the supplementation. **Conclusion:** These data suggest that V and FV supplementation for 2 wk can attenuate the rise in PC after 30 min of aerobic exercise, even after a 1-wk washout, without an impact on plasma MDA or 8-OHdG. **Key Words:** DIETARY SUPPLEMENTS, FREE RADICALS, PROTEIN CARBONYLS, MALONDIALDEHYDE, 8-OHDG

Aerobic exercise of sufficient intensity and duration can result in increased generation of reactive oxygen/nitrogen species (RONS) (32). Production of RONS in quantities that overwhelm the endogenous antioxidant defense system has been referred to as oxidative stress. An increasing body of evidence implicates oxidative stress in the pathogenesis of numerous diseases, including diabetes, certain cancers, and cardiovascular disease.

Biomarkers of oxidative stress have been studied as indices of the oxidative stress status following an acute exercise bout. These biomarkers include lipid (e.g., malondialdehyde), protein (e.g., carbonyl derivatives), and nucleic acid (e.g., 8-hydroxydeoxyguanosine) as well as antioxidant vitamins and enzymes. The majority of reports suggest a transient increase in macromolecule oxidation after aerobic exercise, often at the expense of the antioxidant defense system (11,20,32).

Methods to reduce RONS formation and subsequent macromolecule oxidation in an attempt to maintain health and physical function have included the performance of regular exercise training (21) in addition to supplementation with exogenous antioxidant micronutrients (20). Aerobic training may result in suppression (not elimination) in macromolecule oxidation after acute bouts of exercise (12) due to increased antioxidant defenses (e.g., upregulation of antioxidant enzymes and thiols) and/or reduced production of oxidants during and after the acute exercise bout. The supplementation of exogenous substances may provide support for the endogenous antioxidant defense system to assist in the handling of the increased production of RONS.

Although the majority of investigations have noted lower levels of oxidative stress biomarkers as a result of aerobic training, the effectiveness of using antioxidant supplements to suppress exercise-induced oxidative stress is less clear. Some reports suggest a clear benefit with either an antioxidant-rich diet or antioxidant supplementation (26,28,34), whereas others have suggested no positive effect (33) or even negative consequences (5), depending on the vitamin supplemented (e.g., C vs E), compared with subjects receiving a placebo. The controversy as to the benefits of using antioxidant supplementation to protect

Address for correspondence: Richard J. Bloomer, Department of Health and Sport Sciences, 161F Elma Neal Roane Field House, The University of Memphis, Memphis, TN 38152; E-mail: rbloomer@memphis.edu.
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against exercise-induced oxidative stress lies in part with the type of antioxidants utilized as well as the amounts taken (11).

It has been suggested that certain antioxidants, when used alone or in high dosages, may act as prooxidants, potentially exacerbating the oxidative stress response to acute exercise (7). In addition, the timing of antioxidant administration may have an impact (i.e., postexercise vs prophylactic administration). We have recently reported that vitamin C (1 g) in combination with vitamin E (400 IU) can attenuate eccentric-exercise–induced oxidative stress (9). These antioxidants function within different body compartments (aqueous for vitamin C vs lipid for vitamin E). Despite this, these antioxidant vitamins have multiple and synergistic biological activities that likely complement each other *in vivo*, as vitamin C can assist in maintaining vitamin E in the reduced and active state (19). It has been suggested to supplement antioxidants in ratios and quantities that closely mimic the amounts consumed through a quality whole-food diet including fruits and vegetables to maximize the effectiveness and to minimize adverse outcomes (11,35).

Nutritional products are now available that contain concentrates of juiced fruits and vegetables and are laboratory verified to contain a variety of micronutrients, with excellent bioavailability (35). This blend of nutrients was shown to increase plasma antioxidant vitamin status in healthy men (25). However, the efficacy of this blend has not been tested with acute exercise. Moreover, no study to date has compared the effects of a combined antioxidant vitamin mixture with this product in relation to exercise-induced oxidative stress. Therefore, the purpose of this investigation was to compare the effects of vitamin C + vitamin E (the most examined antioxidants studied in relation to exercise) and an encapsulated fruit and vegetable juice powder concentrate on the oxidative stress response to acute aerobic exercise. We hypothesized that the vitamin C + vitamin E mixture would provide greater protection against exercise-induced oxidation compared with the fruit and vegetable juice concentrate because the amount of these antioxidants are much greater than what is found in the fruit and vegetable juice concentrate alone.

METHODS

Subjects. Fifty-five men or women volunteered to participate as subjects after explanation of all experimental procedures. All volunteers completed a medical history, diet and supplementation history, and physical activity questionnaire to determine eligibility. No subject was a smoker, used tobacco products or antiinflammatory drugs during the course of the study, or took antioxidant supplements for at least 6 months prior to the study or during the study period. All subjects trained aerobically at least 3 $d \cdot wk^{-1}$ for a minimum of 6 months prior to participation and had a minimum level of aerobic fitness as assessed with maximal testing (men $\geq$ 45 $mL \cdot kg^{-1} \cdot min^{-1}$, women $\geq$ 40 $mL \cdot kg^{-1} \cdot min^{-1}$). Two potential volunteers did not meet the enrollment criteria, and five did not complete the study. None of their data were included in the analysis. Therefore, 48 subjects were included in the analysis. Table 1 provides the descriptive characteristics of the subjects. All experimental procedures were performed in accordance with the policy statement of the American College of Sports Medicine on research with human subjects as published by *Medicine & Science in Sports & Exercise®*. The human subjects committee at the University of North Carolina at Greensboro approved all experimental procedures. All subjects provided both verbal and written consent prior to participating in this investigation.

Baseline measurements and screening for aerobic capacity. Subjects reported to the laboratory and their height and weight were measured using a Seca stadiometer and electronic scale (Hanover, MD), respectively. Body fat percentage was estimated using a seven-site (chest, triceps, midaxillary, suprailium, abdominal, subscapular, thigh) skinfold tests using Harpenden calipers (Creative Health Products, Ann Arbor, MI).

Subjects then completed a maximal graded exercise test using a motorized treadmill for determination of $\dot{V}O_{2max}$. Following a 5-min warm-up period, all tests began at a grade of 0% at subjects' self-selected running speed. We increased the treadmill grade every 2 min while keeping the speed constant. This allowed subjects to reach exhaustion within 8–12 min. The highest mean 30-s $\dot{V}O_2$ value obtained during testing was used to calculate the workload to be used during the submaximal exercise bouts. Prior to each test (both maximal and submaximal), both the flow meter and the gas analyzers on the SensorMedics Vmax 229 metabolic system (SensorMedics, Yorba Linda, CA) were calibrated, and the environmental conditions in the laboratory were measured. The $\dot{V}O_{2max}$ data were used not only to calculate the workload for the submaximal exercise bouts but to ensure a minimum level of aerobic capacity as described above.

Antioxidant therapy. Subjects were randomly assigned in a double-blind manner to one of three treatments: 400 IU of vitamin E (100% d-α-tocopherol) + 1000 mg of vitamin C (V; N = 15), a mixed fruit and vegetable juice powder

TABLE 1. Characteristics of 48 men or women.

Variable	Vitamin (N = 15) (8 Men, 7 Women)	Fruit and Vegetable Juice Concentrate (N = 16) (7 Men, 9 Women)	Placebo (N = 17) (11 Men, 6 Women)
Age (yr)	23.5 ± 6.6	23.3 ± 5.0	23.3 ± 5.2
Height (cm)	172.0 ± 7.3	172.2 ± 9.9	176.0 ± 9.3
Weight (kg)	63.7 ± 10.6	66.7 ± 7.9	71.9 ± 15.4
Percent body fat	11.1 ± 3.8	12.5 ± 5.8	14.7 ± 9.8
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	49.9 ± 12.9	46.3 ± 8.9	50.7 ± 7.4
Weekly aerobic exercise (h)	5.6 ± 2.8	4.0 ± 2.3	4.1 ± 1.9

Values are mean ± SD. No statistically significant differences were noted between treatments groups for the above variables ($P > 0.05$).

concentrate (FV; $N = 16$), or a cellulose placebo (P; $N = 17$). The FV treatment consisted of a proprietary blend of fruit, vegetable, and berry juice powder concentrates, encapsulated and taken orally (Juice Plus+®, NSA, Inc., Memphis, TN). This combination of capsules was stated to provide 12,500 IU of vitamin A activity (100% from beta-carotene), 276 mg of vitamin C, and 108 IU of vitamin E per day. The nutrient content of the treatments was verified by the manufacturer following production; however, this was not independently verified. Capsules were consumed $2\times\ d^{-1}$ (for a total of six capsules) to provide for the above dosages, and were identical in appearance for all treatments. Supplementation was started after the completion of the initial submaximal exercise bout, was continued for 14 d prior to undergoing the second submaximal exercise bout as described below, and was not consumed on the morning of the second submaximal exercise bout. The final (third) submaximal exercise bout was performed after a 1-wk washout phase. The period of supplementation was chosen based on our previous work using vitamin E and C supplementation (9). In addition, vitamin E levels have been reported to be elevated in both plasma and skeletal muscle after a similar supplementation time and vitamin C has been reported to be elevated in plasma by this time. All subjects were instructed to maintain their normal diet during the study period and completed daily food records for 3 d prior to each submaximal exercise bout to allow for nutrient intake comparisons between the groups (Diet Analysis Plus, ESHA Research, Salem, OR). Adherence was > 95% for all groups based on the number of capsules provided and the number of capsules returned, with no difference in compliance between groups.

Experimental procedures. The procedures for this investigation included the performance of three submaximal exercise bouts at 80% $\dot{V}O_{2max}$: before, after 2 wk of supplementation, and after a 1-wk washout period. Blood samples were only obtained before and immediately after each submaximal exercise bout and were immediately processed for vitamin C and plasma. The stored plasma (stored at −80°C in separate microtubes) was analyzed for plasma protein, plasma protein carbonyls (PC), malondialdehyde (MDA), and vitamin E. Serum was obtained from one vacutainer tube and then immediately stored in separate microtubes at −80°C until being analyzed for 8-hydroxydeoxyguanosine (8-OHdG). Whereas additional blood samples beyond the immediate postexercise period would have provided further insight into the oxidative status of the system, we chose simply to sample the immediate postexercise time point. This decision was based on our previous work reporting the greatest elevation in oxidative stress biomarkers to occur immediately postexercise, when performing relatively short-duration aerobic exercise (4,10). Indeed, a longer time course of elevation for certain oxidative stress biomarkers has been observed following high-intensity resistance exercise and long-duration aerobic exercise; however, for aerobic exercise of the intensity and duration used in the present study, we have noted the peak to occur immediately postexercise (4,10). Others have reported similar findings following graded exercise testing (8). Hence, we chose only to analyze samples at the times presented.

Within 2 wk of the baseline assessments, subjects returned to the laboratory (between 7 and 10 a.m.) and performed their first submaximal exercise bout. Subjects were instructed not to perform any exercise for 48 h preceding each submaximal exercise bout and were overnight fasted (8–12 h). On the day of testing, the weight of each subject was obtained, and it was confirmed that no changes had occurred since the baseline assessments. After a minimum of 10 min of rest, an initial resting blood sample was taken from an antecubital vein by a trained phlebotomist. Immediately after each submaximal exercise bout, an additional blood sample was taken from the opposite arm. At all blood collection times, approximately 10 mL of blood was obtained by vacutainer. These procedures remained identical for all subjects and all three submaximal exercise bouts.

Prior to each submaximal exercise bout subjects were fitted with a HR monitor (Polar) to record HR during the exercise. Subjects warmed up for 5 min at a moderate intensity (50% $\dot{V}O_{2max}$). The workload (i.e., treadmill speed) was then increased to elicit 80% $\dot{V}O_{2max}$ and was adjusted every 5 min as needed to maintain this intensity as $\dot{V}O_2$ values were continuously monitored throughout the exercise. Subjects were allowed to consume water *ad libitum* during the exercise. Heart rate and rating of perceived exertion (RPE) were recorded every 5 min during testing.

Dietary records. All subjects were instructed to maintain their normal diet during the study period and to complete daily food records (for 3 d prior to the exercise tests) to allow for nutrient intake assessment between the submaximal exercise bouts. The same researcher provided instruction regarding portion sizes and recording of foods and beverages consumed. Subjects received copies of their 3-d diet records and were instructed to replicate the diet prior to the next visit. The diet records were analyzed for total calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake (Diet Analysis Plus, ESHA Research, Salem, OR).

Blood collection and analysis. Plasma proteins, protein carbonyls, malondialdehyde, and vitamin E were determined from blood collected via vacutainer and immediately centrifuged at 3000 rpm for 15 min at 4°C in a Beckman (J2–21) centrifuge (Fullerton, CA). The plasma was then stored in microtubes at −80°C until analyzed. Additionally, 5 mL of blood was collected into serum collection vacutainers for analysis of 8-OHdG. Blood was allowed to clot at room temperature for 30 min, then serum was separated by centrifugation as above and stored at −80°C until analyzed for 8-OHdG. All assay procedures described below were performed in duplicate. Intra- and interassay variability was less than 5 and 8% for all assays, respectively.

Plasma protein was determined by comparing samples against known standards. Plasma samples were then adjusted to 4 $mg{\cdot}mL^{-1}$ protein using a phosphate buffer (100 mM potassium phosphate + 100 uM EDTA) for the assay of

TABLE 2. Mean dietary intake assessed during the 3 d preceding submaximal exercise.

Treatment	Session No.	Kilocalories	PRO	CHO	Fat	Vitamin C	Vitamin E	Vitamin A
Vitamin	1	2274 (797)	17 (5)	49 (11)	34 (7)	144 (153)	5.8 (4)	839 (518)
	2	2248 (717)	15 (5)	50 (6)	35 (6)	52 (38)	4.4 (3)	875 (473)
	3	2074 (800)	15 (4)	53 (10)	32 (8)	91 (102)	7.2 (6)	911 (577)
Fruit and vegetable juice concentrate	1	2054 (884)	16 (4)	50 (9)	34 (8)	111 (96)	6.4 (8)	1006 (109)
	2	1938 (1152)	17 (5)	47 (11)	36 (12)	148 (144)	6.9 (9)	876 (515)
	3	1929 (1077)	15 (4)	47 (11)	38 (12)	109 (105)	8.7 (14)	1088 (1185)
Placebo	1	2486 (792)	15 (4)	51 (11)	34 (7)	154 (208)	11.8 (10)	1299 (801)
	2	2180 (628)	17 (5)	51 (9)	32 (11)	116 (143)	6.5 (5)	1037 (555)
	3	2256 (749)	16 (4)	53 (9)	31 (10)	169 (175)	5.7 (5)	1117 (595)

Values are mean ± SD. Percentages for each macronutrient are provided. Vitamin C and vitamin E values are provided in milligrams; vitamin A values are provided in retinol equivalents. No statistically significant differences were noted between treatments or across exercise sessions for any measured variable ($P > 0.05$).

plasma protein carbonyls. Plasma samples were also used for the measurement of malondialdehyde. Both of these assay procedures have been previously described in detail by Goldfarb et al. (9).

Measurement of 8-OHdG was performed using an ELISA from Genox Corporation (Baltimore, MD) and followed the procedures provided by the manufacturer (Japan Institute for the Control of Aging).

Vitamin C analysis was performed immediately on plasma obtained from heperinized vacutainer tubes. The plasma was added to 5% TCA, 85% orthophosphoric acid o-H_3PO_4, and α,α' dipyridyl (1%), and aqueous ferric chloride (3%). All samples were read at 525 nm and compared against a standard curve as described by Zannoni et al. (36).

Plasma vitamin E was determined by HPLC using a modified procedure by Talwar et al. (27). Plasma proteins were first removed from the plasma using an ethanol precipitation and then extracting the lipid portion in hexane. The hexane was taken to dryness in a speed vacutainer and reconstituted with mobile phase (methanol) and run through a reverse phased Shimadzu system at a flow rate of 1.0 $mL \cdot min^{-1}$, scanned at 290-nm wavelength, and compared against a standard curve.

Statistical analyses. The data obtained for PC, MDA, 8-OHdG, vitamin C, and vitamin E were analyzed using a 3 (treatment) × 3 (exercise session) × 2 (time) repeated-measures ANOVA. Significant interactions and main effects were analyzed using Tukey's *post-hoc* tests. Performance data were analyzed using a 3 (treatments) × 3 (exercise session) ANOVA. Dietary variables were compared using a one-way ANOVA. All analyses were performed using JMP statistical software version 4.0 (SAS Institute, Cary, NC). Statistical significance was set at $P \leq 0.05$. All data are presented as mean ± SEM, except for subject characteristics and dietary variables, which are presented as mean ± SD.

RESULTS

Submaximal Exercise Data

All subjects in the data analysis completed all aspects of the submaximal exercise sessions with ≥ 95% supplement compliance. No differences were observed for submaximal $\dot{V}O_2$ between treatments (V = 36.9 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$; FV = 37.1 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$; P = 38.4 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$) or across times (first, 37.4 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$; second, 37.7 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$; third, 37.2 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$). Likewise, no differences were noted for RPE or heart rates between treatments or across time ($P > 0.05$), with consistent values of 13.5–14 for mean RPE and 168–170 bpm for mean heart rates during exercise.

Dietary Data

The mean daily calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake during the 3 d preceding the submaximal exercise bouts are presented in Table 2. Subjects' diets were comprised of adequate calories, macronutrients, and vitamins (C, E, and A). A high degree of variability existed between subjects. No statistically significant differences were noted between treatments or across exercise sessions for any measured dietary variable ($P > 0.05$).

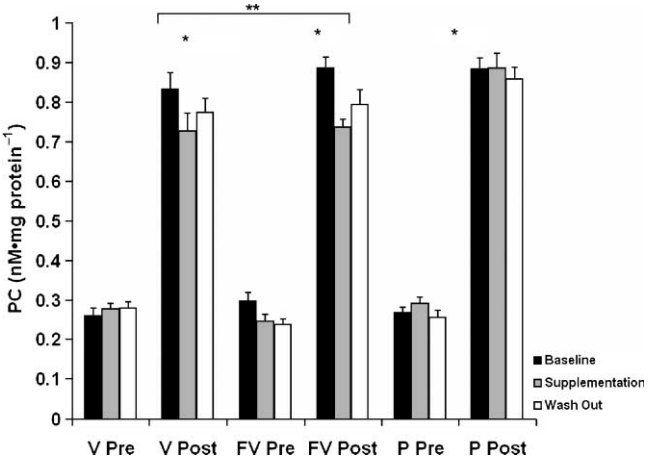

FIGURE 1—Protein carbonyl values pre- and postexercise for three treatments across three exercise sessions. *Pre* and *post* refer to preexercise and postexercise, respectively. Baseline data are for the initial submaximal exercise bout (prior to supplementation); supplementation data are for the second submaximal exercise bout (following 2 wk of supplementation); washout data are for the third submaximal exercise bout (following a 1-wk washout period from supplementation). V, treated with 400 IU vitamin E + 1 g vitamin C; FV, treated with fruit and vegetable juice powder concentrate; P, placebo; PC, protein carbonyls. * Postexercise values greater than preexercise values for all treatments at all submaximal exercise bouts ($P < 0.0001$); ** postexercise V and FV values lower than P at supplementation and washout ($P < 0.05$).

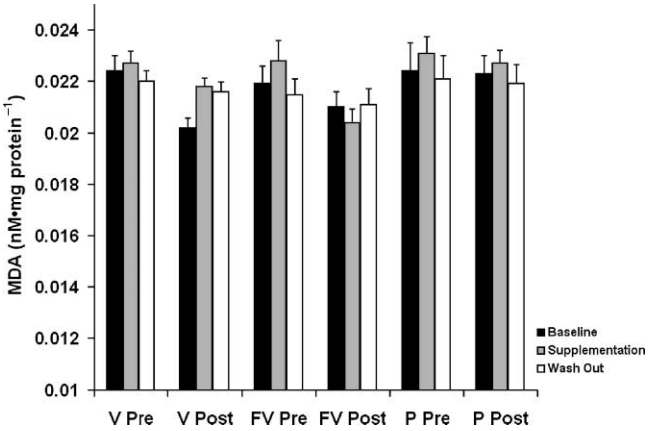

FIGURE 2—Malondialdehyde values pre- and postexercise for three treatments across three exercise sessions. See note for Figure 1.

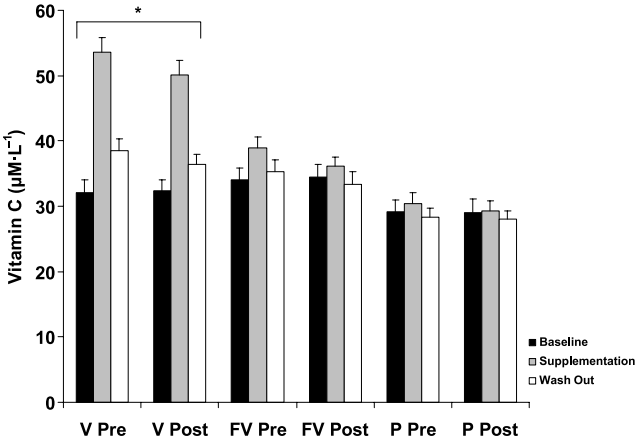

FIGURE 4—Vitamin C values pre- and postexercise for three treatments across three exercise sessions. See note for Figure 1. * Values greater for V at supplementation compared with baseline; values greater for V compared with FV and P at supplementation ($P < 0.05$).

Oxidative Stress Biomarkers

Protein carbonyls. There were no differences in resting PC across visits and between groups and ranged from 0.25 to 0.30 nM·mg protein^{-1}. Postexercise PC values were elevated for all treatments at all times ($P < 0.0001$). Both the V and the FV treatments attenuated the increase in PC after 2 wk of supplementation (V = 21%, FV = 17%), and after the 1-wk washout period (V = 13%, FV = 6%) compared with placebo ($P < 0.05$), with no significant differences between the V and FV at any times. The data are shown in Figure 1.

Malondialdehyde. Malondialdehyde was unaffected by exercise and treatment ($P > 0.05$). Values for MDA remained relatively stable at all times at approximately 0.02 nM·mg protein^{-1} (Fig. 2).

8-hydroxydeoxyguanosine. A treatment main effect was noted for 8-OHdG, with lower values for the V group compared with the FV and P groups ($P = 0.0002$). No exercise session or time main effect was noted for 8-OHdG, suggesting that the treatment main effect was not a result of the supplementation protocol, as values for the V treatment were lower than FV and P prior to supplementation. Data are shown in Figure 3.

Vitamin C. A treatment by exercise session effect was noted for vitamin C ($P < 0.0001$), with values greater after 2 wk of supplementation compared with baseline for the V group only (Fig. 4). Vitamin C returned to baseline in the V group after the 1-wk washout ($P > 0.05$). No significant differences were observed across exercise sessions for either the FV or P groups.

Vitamin E. A treatment by exercise session effect was obtained for vitamin E ($P = 0.0006$), with higher vitamin E in the V group after supplementation and washout compared with baseline. No significant changes were evident from baseline for the FV or P groups ($P > 0.05$). No significant differences existed between the treatments at any of the exercise sessions. A time main effect was also noted, with

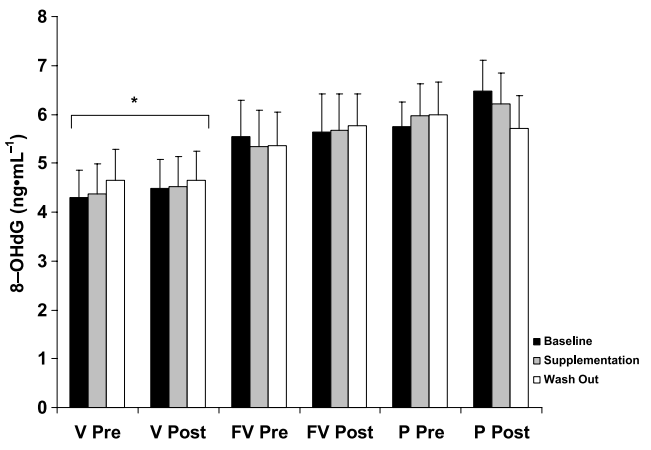

FIGURE 3—Eight-hydroxydeoxyguanosine values pre- and postexercise for three treatments across three exercise sessions. See note for Figure 1. * Treatment main effect with lower values for V than for FV and P ($P = 0.0002$).

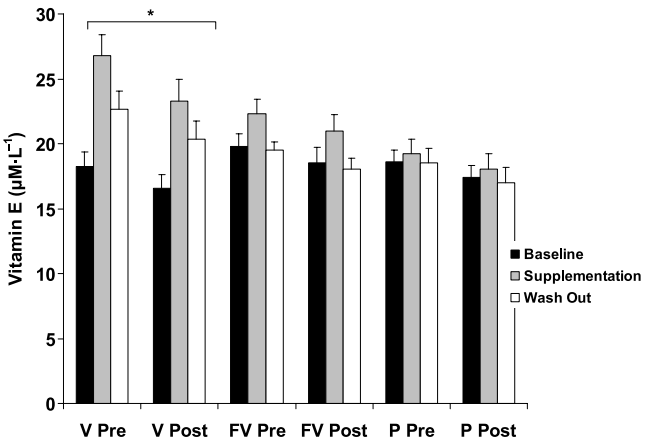

FIGURE 5—Vitamin E values pre- and postexercise for three treatments across three exercise sessions. See note for Figure 1. * Values greater for V at supplementation and washout compared with baseline ($P < 0.05$); a time main effect was also noted with postexercise values lower than preexercise ($P = 0.0025$).

values lower postexercise compared with preexercise (P = 0.025). Vitamin E data are presented in Figure 5.

DISCUSSION

Data from this investigation indicate that 1) both the V and FV treatments attenuated the rise in PC after 30 min of aerobic exercise; 2) the suppression of PC following exercise was partially maintained for both of these treatments during the third exercise bout, which occurred after a 1-wk washout period; and 3) neither treatment impacted MDA or 8-OHdG in this population of aerobically trained subjects. These data suggest that a mixed phytonutrient based fruit and vegetable juice concentrate can be as effective as high-dose vitamin C + E in attenuating the rise in protein oxidation to 30 min of intense aerobic exercise. This is the first investigation that we are aware of to compare the effects of these two forms of antioxidant treatments on exercise-induced oxidative stress.

Oxidation reactions related to proteins may involve global modifications, as in the conversion to carbonyl derivatives as measured in the present study. Such modifications can lead to loss of catalytic or structural function in the affected proteins, making them highly susceptible to proteolytic degradation (14). In certain pathological states, oxidized proteins appear to accumulate in cells and can account for over 50% of the total cellular protein content, contributing to the progression of disease. Therefore, minimizing protein oxidation appears important (2).

It was initially hypothesized that the vitamin C + E supplement at this dosage would provide greater protection against exercise-induced oxidation than the FV capsules. The rational for this was that the amount of antioxidants in the vitamin supplement was approximately three- to fourfold greater than the amount reported to be in the FV supplement (1000 mg of vitamin C and 400 IU of vitamin E vs approximately 276 mg and approximately 108 IU, respectively). However, this was not supported by our findings, as both treatments yielded similar results in relation to suppressing protein oxidation for the 2-wk supplementation period. Protection remained after a 1-wk washout for both treatment groups, with no statistical difference but a greater absolute percentage protection with the V treatment. This was apparent despite the fact that plasma levels of vitamins C and E were elevated to a greater extent in the vitamin compared with the concentrate treatment (Figs. 4 and 5). It is possible that antioxidants other than vitamins C and E may have assisted in the protection against RONS produced during the acute aerobic exercise. Perhaps the phytonutrients within the FV treatment could have served to protect against protein oxidation, although additional research is needed to confirm this hypothesis. Specifically, the FV treatment consisted of a blend of fruit, vegetable, and berry juice powder concentrates containing 276 mg of vitamin C, 108 IU of vitamin E, and 12,500 IU of vitamin A activity, in addition to other micronutrients. In contrast, the vitamin C + E treatment contained only these vitamins, albeit at a higher dosage.

Despite our subjects having an adequate dietary intake (Table 2), the additional antioxidants appeared necessary to suppress protein oxidation. It should be noted, however, that because dietary intake was not totally controlled by the investigators, we assumed accuracy in subject reporting. This, of course, is a limitation of our study, as variation in dietary intake can influence both the antioxidant capacity of blood (6) as well oxidative stress biomarkers (29,34). Multiple factors, including suppressed cytokines and chemokines, in addition to enhanced membrane stability, could have contributed to the reduced formation of protein carbonyls following supplementation. Our data suggest that although using higher dosages of isolated vitamin C + E may be beneficial, similar effects can be seen with lower concentrations of these vitamins when consumed as part of a fruit and vegetable juice powder concentrate. Phytonutrients, including polyphenols and carotenoids such as lutein and lycopene, in conjunction with the vitamin C and E, may work synergistically to protect macromolecules such as proteins from oxidation. This may be the case in this study but requires confirmation. Admittedly, without an additional treatment group assigned to simply a lower dosage of vitamins C and E without the additional nutrients found in the concentrate, we cannot conclude that these additional nutrients were of significance in allowing for the reduction in protein carbonyls. That is, a lower intake of vitamin C and E alone may account for such a reduction in protein oxidation. Further study would be needed to confirm this hypothesis.

No increase was observed for either MDA or 8-OHdG after any of the exercise bouts in the present investigation. Prior work has suggested that these markers may be affected by both exercise duration (1,23) as well as intensity (13,22). However, not all studies have demonstrated increases with exercise with these variables. Because samples were only analyzed pre- and immediately postexercise, it is unknown whether differences between treatments occurred at times beyond the immediate postexercise period. This infrequent sampling is a limitation of the current study. However, we have previously noted the greatest rise in both PC and MDA to occur immediately postexercise in subjects following a 30-min bout of aerobic exercise performed at 70% $\dot{V}O_{2max}$, with a return towards baseline at 60 min postexercise (4).

Supplementation with vitamin E at dosages $\geq$ 400 IU·d^{-1} for periods of 3 wk or longer has reported attenuation in the MDA increase observed with exercise (18,24,26). Studies using vitamin C have yielded mixed findings in relation to suppressing exercise-induced oxidative stress. Thompson et al. (30) reported that 400 mg of vitamin C given for 2 wk did not attenuate the MDA changes brought about by a shuttle run. Goldfarb et al. (10) recently reported that both a 500- and 1000-mg dose of vitamin C given for 2 wk did not alter lipid peroxidation following 30 min of aerobic exercise similar to the intensity of exercise used in the present study, but did attenuate the increase in protein carbonyls. Bryant et al. (5) reported that vitamin E could attenuate the rise in plasma MDA in response to acute

exercise, whereas vitamin C actually increased MDA, both at rest and postexercise compared with placebo. Moreover, the combined treatment of vitamins C + E had little impact on MDA, similar to that reported in the present study.

Rokitzki et al. (24) reported that a combined vitamin E (400 IU) and C (200 mg) mixture for 4.5 wk prior to competition did not attenuate the MDA rise in response to a marathon compared with placebo. In contrast, we recently reported that the combination of vitamin E (400 IU) and vitamin C (1000 mg) could attenuate the increase in protein carbonyls following eccentric exercise (9). Collectively, the data are mixed in relation to antioxidant supplementation and exercise-induced oxidative stress, and indicate that vitamin E may act to suppress lipid peroxidation, whereas vitamin C alone does not appear to have this ability. Furthermore, vitamin C alone and in conjunction with vitamin E appears to suppress protein oxidation, in agreement with the findings presented here. It should be noted that in the present investigation, the changes in lipid and DNA oxidation were minimal. Additionally, neither the antioxidant treatments nor the placebo had any influence on these measures. It is possible that this group of moderately aerobically trained subjects had improved oxidative stress protection, as aerobically trained subjects can increase their antioxidant defenses (21). Other investigators have reported increases in both lipid (17) and DNA (23,31) oxidation following longer-duration exercise sessions performed at a similar intensity as in the present study. Perhaps our subjects might have demonstrated increased lipid and DNA oxidation if the duration of exercise were longer.

Aside from the macromolecules discussed above, the exercise bouts resulted in no change in plasma levels of vitamin C and only a modest decrease in plasma vitamin E from pre- to postexercise. Previous studies have found a decrease in plasma concentrations of antioxidants after chronic (3) and acute (17) aerobic exercise, as well as no change or an increase in blood concentrations of antioxidants to acute aerobic exercise (15). Although discrepancies in findings do indeed exist, it is important to keep in mind that changes in blood levels of these antioxidants is only suggestive of cellular redox balance. Therefore, caution needs to be taken when interpreting plasma antioxidant changes after acute exercise bouts.

In conclusion, it was shown that antioxidant supplementation with vitamin C + E or a mixed fruit and vegetable juice concentrate can attenuate the rise in protein oxidation observed after an acute 30-min run at 80% $\dot{V}O_{2max}$. This exercise resulted in no change in plasma MDA or 8-OHdG. It should be noted that despite the effect of the two antioxidant treatments on postexercise protein oxidation, supplementation did not eliminate the elevation in protein carbonyl values, but rather reduced this slightly. Furthermore, the antioxidant supplements did not have any impact on exercise-associated variables (e.g., heart rate and perceived exertion), although performance variables were not measured in the present study. Taken together with the MDA and 8-OHdG data, it appears as though antioxidant supplementation within a population of aerobically trained men and women demonstrates only modest protection for protein oxidation, with little impact on the other markers. This is true for both the FV as well as the V treatment. It is believed that antioxidants other than vitamins C and E could have provided protection against protein oxidation, but further research is warranted to determine which phytonutrients were involved. The finding that supplementation with a fruit and vegetable juice concentrate can provide protection against exercise-induced oxidative stress in a similar manner as higher dosages of vitamins C and E deserves attention. This is true especially in light of the recent report suggesting adverse health outcomes with higher-dose, long-term vitamin E supplementation (16), a routine practice.

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