Effects of a Fruit/Berry/Vegetable Supplement on Muscle Function and Oxidative Stress

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ABSTRACT

GOLDFARB, A. H., R. S. GARTEN, C. CHO, P. D. M. CHEE, L. A. CHAMBERS. Effects of a Fruit/Berry/Vegetable Supplement on Muscle Function and Oxidative Stress. *Med. Sci. Sports Exerc.*, Vol. 43, No. 3, pp. 501–508, 2011. **Purpose**: This study tested the effectiveness of a fruit, berry, and vegetable concentrate (FVC), Juice Plus+® (NSA LLC, Collierville, TN), supplement on muscle function and oxidative stress in response to an acute bout of eccentric exercise (EE). **Methods**: Forty-one healthy volunteers (age = 18–35 yr) were randomly assigned to either a placebo (P) or an FVC treatment taking capsules for 28 d (6 d^{-1}) before EE and for the next 4 d. All subjects completed four sets of 12 repetitions of eccentric elbow flexion with their nondominant arm. Blood, muscle soreness (MS), range of motion (ROM), and maximal isometric force (MIF) of the elbow flexors were obtained before and immediately after exercise and at 2, 6, 24, 48, and 72 h postexercise. Plasma was analyzed for creatine kinase (CK), lipid hydroperoxides, malondialdehyde (MDA), and protein carbonyls (PC). Glutathione ratio was determined from whole-blood extracts. **Results**: MS, ROM, MIF, and plasma CK demonstrated significant time effects independent of treatment. MS and plasma CK increased over time, whereas ROM and MIF decreased over time. There was a significant time and time × treatment effect for plasma PC and MDA. PC and MDA increased over time in the P group ($P < 0.01$) but were not significantly altered in the FVC-treated group at any time. No significant changes were noted in lipid hydroperoxides. The glutathione ratio was elevated immediately postexercise in both groups ($P < 0.01$) and elevated 6 h postexercise with P compared with the FVC-treated group ($P < 0.05$). **Conclusion**: This study reports that 4 wk of pretreatment with an FVC can attenuate blood oxidative stress markers induced by EE but had no significant impact on the functional changes related to pain and muscle damage. **Key Words**: SORENESS, PROTEIN CARBONYLS, LIPID PEROXIDATION, GLUTATHIONE, ISOMETRIC FORCE

Exercise of sufficient intensity and duration can result in skeletal muscle damage. Unaccustomed lengthening-type actions (eccentric actions) typically will result in greater muscle damage than concentric exercise, which has been, in part, attributed to greater forces exerted within the muscle (34,40). This greater force within the muscle is believed to result in greater strain on the involved structures. Greater peak force during eccentric contraction resulted in greater functional impairment (40), which is thought to affect both connective structures and proteins resulting in microdamage. Chemical changes associated with both inflammatory (6,30) and oxidative stress processes have been reported with this type of damage (28,30). These processes not only degrade the damaged substances but are also thought to enhance repair processes (8,14,24,30).

Reactive oxygen/nitrogen species (RONS) have been implicated in both the initiation and the progression of muscle fiber injury after the initial mechanical insult (11,18,28). A single bout of exercise can activate several distinct radical generating systems and can be categorized into both primary as well as secondary RONS sources (18). The generation of RONS during and after eccentric exercise (EE) has been attributed to xanthine, NADPH oxidase production, ischemia reperfusion, prostanoid metabolism, phagocytic respiratory bursts, inflammatory processes, disruption of iron-containing proteins, and excessive calcium accumulation, often resulting from high force EE (6,11,21,27,29,33,38).

Increases in markers of oxidative stress within the blood (16,25,36) and muscle (27,42,43) have been reported after eccentric activities. However, not all studies report increases in oxidative stress markers, depending on the site examined (42) or the marker and exercise used (15). After eccentric resistance exercise, blood protein carbonyls (PC) and oxidized glutathione (GSSG) were reported to increase in both young men (25) and young women (16). The GSSG concentration compared with the total glutathione (TGSH) concentration (glutathione status) was elevated immediately and 2 h after EE (16). In addition, several blood lipid peroxidation markers have been reported to be elevated after EE (31). You et al. (42) reported that downhill running elevated PC in both muscle and blood and that dietary

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antioxidant treatment attenuated the PC increase in both muscle and blood.

Many substances have been purported to be potentially beneficial as a pretreatment intervention in the hopes of reducing damage or enhancing the repair of skeletal muscle from this type of insult. Unfortunately, the research substantiating protection of skeletal muscle from damage is either inconsistent or lacking. A combination of antioxidants (vitamin E ,vitamin C, and selenium) taken for 14 d before EE and for 2 d after exercise was reported to prevent the increase in both blood PC and malondialdehyde (MDA) at 48 h after exercise (4,16). This combined antioxidant treatment did not alter the immediate rise in GSSG after exercise. However, subjects reported less soreness after this treatment, and the increase of blood creatine kinase (CK) was attenuated. It was suggested that membrane stabilization and/or improved membrane integrity occurred with this treatment (4). In contrast, maximal isometric force (MIF) and range of motion (ROM) decrement was similar for both treatments (4). In contrast, a high-dose antioxidant (vitamins E and C) treatment given for 37 d before EE attenuated peak torque loss (32). This later study pretreated for a longer time, which may have contributed to the partial protection observed in the force decrement. Unfortunately no oxidative stress or inflammatory markers were assessed.

Nutritional and nutraceutical substances have been purported to have antioxidant effects as well as some anti-inflammatory processes (6,7). Several nutritional supplements have been suggested to help in the prevention and repair rate of muscle damage and soreness but have not been adequately investigated. Some substances within these supplements include isoflavones, catechins, quercetin, α-lipoic acid, and beta-carotene. A fruit, berry, and vegetable concentrate (FVC) supplemented for 2 wk before aerobic exercise attenuated oxidative stress in both men and women (3) and showed similar effects as a combined antioxidant (vitamins E and C) treatment.

Supplementation with this FVC enhanced antioxidant defense in healthy adults (3,22,23) by reducing PC as well as by enhancing plasma immunity. Flavonoids, anthocyanins, and antioxidants have been identified in several fruits, including blueberries, strawberries, and cherries. Recently, consumption of Bing sweet cherries (approximately 45 pieces per day) was reported to lower circulating inflammatory factors within the blood (20). Tart cherry juice (24 fl oz) taken for 8 d before EE reduced strength loss, with no difference in pain or ROM (12). Regrettably, no measures of oxidative stress were determined in this study. This FVC taken for 2 wk reduced oxidative stress markers in both men and women in response to 30 min of aerobic exercise (3). To provide adequate treatment of the FVC, we extended the pretreatment period to 4 wk to closer approximate the length of time of these other studies (20,32) to assess oxidative stress effects and functional outcome changes to EE. In addition, it appears that antioxidant supplementation needs to be continued after EE to help prevent secondary damage

(16). We have substantiated this with high-dose vitamin C (5) but do not know if this FVC can reduce oxidative stress as well as attenuate the muscle soreness (MS) or the muscle function loss to EE.

Therefore, we compared the effectiveness of this FVC with a placebo (P) treatment to ascertain if this juice powder concentrate, which contains modest amounts of flavonoids, anthocyanins, and antioxidants, can reduce the loss of muscle function, MS, and oxidative stress in response to acute EE. We hypothesized that the FVC would attenuate oxidative stress and would also attenuate the MS and loss of muscle function to the EE.

METHODS

Subjects. Forty-four apparently healthy college-aged subjects (age = 18–35 yr) volunteered after explanation of all experimental procedures and provided written informed consent. All subjects completed a medical history, diet, supplement, and fitness questionnaire to determine eligibility. Subjects were nontobacco users, were not on anti-inflammatory drugs or on dietary supplements (i.e., vitamins or antioxidants for at least 3 months), and refrained from these substances throughout the study. Subjects were non-resistance trained for at least 6 months. The university institutional review board for human subjects committee approved all experimental procedures, and subjects signed consent forms describing all procedures before participation.

Supplementation. Subjects were randomly assigned in a double-blind manner to either a P group (microcrystalline cellulose) ($n = 22$) or an FVC group ($n = 22$). The FVC capsules primarily contained a proprietary blend of fruit, vegetable, and berry juice powder concentrates, encapsulated (Juice Plus+®, NSA LLC, Collierville, TN). This combination powder provided approximately 7.5 mg of beta-carotene, 276 mg of vitamin C, and 108 IU of vitamin E per day as well as natural flavonoids and anthocyanins, as previously described (3). Subjects had their MIF determined on both arms using a Biodex isokinetic dynamometer (Biodex Medical Systems Inc., Ronkonkoma, NY) after meeting the initial screening criteria. They were then given a sealed bottle with instructions to take six capsules per day (three in the morning and in the evening, with meals) for 4 wk. They were not allowed to make up capsules missed on previous days and were instructed to keep a record of missed capsules. They continued to take the capsules for the 4 d after the eccentric protocol. Subjects were contacted once per week to remind them to take their capsules. All subjects were shown how to fill in their dietary records (3-d record) and bring them before the eccentric protocol. Two subjects from the P group and one subject from the FVC treatment group did not complete the study and were not included in the analysis, leaving $n = 20$ for the P group and $n = 21$ for the FVC group. No adverse effects were reported by any of the subjects in either group.

Baseline measurements. Subjects' height, weight, blood pressure, heart rate, and percent body fat (on the basis of gender) using three skinfold sites with Harpenden calipers (18) were obtained by the same trained technician. Subjects were familiarized and fitted to the Biodex isokinetic dynamometer, with all settings recorded for future reference during the eccentric protocol. Each subject performed three MIF with their nondominant and dominant arm elbow flexors, each lasting 3 s, with 60 s of rest between each effort. These efforts served as a familiarization to the isokinetic dynamometer as well as to obtain initial MIF for all subjects. ROM was assessed using a goniometer, assessing the elbow flexors on both arms by asking subjects to flex and extend their arms at the elbows.

Experimental procedures. Subjects reported back to the laboratory 4 wk after the initial assessment in the morning after an overnight fast (at least 10 h) and not having strenuously exercised 2 d before. They brought with them their 3-d diet records and sat quietly at least 15 min before having any measurements obtained. ROM and MS ratings were then obtained. MS was assessed using a visual linear scale ranging from 1 to 10 (10) in a rested position, through ROM, and in response to palpation. A resting blood sample (15 mL) was then taken from an antecubital vein and immediately processed. Subjects were then seated into the Biodex system, and their MIF obtained for both arms. They then performed four sets of 12 repetitions of eccentric actions using their nondominant arm elbow flexors at an angular velocity of $20°·s^{-1}$, with 60 s of rest between sets. The ROM for the action was set at 100° of flexion. The lever arm was programmed to move against the subjects' resistance as long as 10 N·m of torque was produced by the subject. The bar was returned to the initial flexed position by the investigator during the rest interval of 3 s. Total amount of work was recorded for each set and totaled. Peak forces and decline of forces were also obtained. Subjects were encouraged by the investigator to give their maximal force on all eccentric actions.

Blood samples were obtained before, immediately after (0), and at 2, 6, 24, 48, and 72 h after the EE protocol via vacutainer containing EDTA. Blood samples at 0 and 2 h after the EE were obtained from the exercised arm if possible and from either arm thereafter. Blood samples were analyzed for plasma CK, PC, MDA, lipid hydroperoxides, and whole-blood TGSH and GSSG.

Dietary records and analysis. All subjects were instructed to maintain their normal diet during the study period and completed food records for 3 d before EE. The researchers provided instruction regarding portion sizes and recording of foods and beverages consumed. Nutrient intake was analyzed for total calories, protein, carbohydrate, fat (total, mono- and polyunsaturated, and omega-3), vitamin C, vitamin E, and vitamin A intake (Diet Analysis Plus 8.0; ESHA Research, Salem, OR).

Blood handling and analysis. Whole blood (1 mL for both GSH and GSSG) was immediately pipetted into tubes containing 10% of 5-sulfosalicylic acid with bathophenanthrolinedisulfonic acid at a final concentration of 1 mM (1). These tubes were mixed to ensure red blood cells were lysed and destroyed enzymatic activity, then centrifuged at 3000 rpm at 4°C for 15 min (Beckman 6KR4, Fullerton, CA). The supernatant was then pipetted into microcentrifuge tubes and centrifuged at 11,000 rpm for 10 min to remove any remaining cellular debris and then stored at −80°C until analyzed for glutathione status. The remaining blood was immediately centrifuged at 3000 rpm at 4°C for 15 min to obtain plasma, and aliquots were stored at −80°C until analyzed.

Protein carbonyls. Plasma PC were determined using an ELISA kit (Biocell Ltd., Auckland, New Zealand) following the procedures of Winterbourn and Buss (41). The plasma samples were first adjusted to the appropriate protein concentration using the standard procedure outlined in the assay to ensure similar protein concentration. Each plate was loaded with standards, controls, and unknown samples and read at 450-nm wavelength on a microplate reader (BioTek Instruments, Winesski, VT). The data were processed by a KC Junior software package. All samples were measured in duplicate and compared with standards.

Malondialdehyde. MDA was determined using the Oxis (Bioxytech LPO-586, Foster City, CA) colorimetric assay kit. Briefly, this procedure is based on the reaction of the chromogen, N-methyl-2-phenylindole, with MDA at 45°C. One molecule of MDA reacts with two molecules of N-methyl-2-phenylindole to yield a stable chromophore with maximal absorbance at 586 nm. Probucol (10 μL) was added to each microcentrifuge tube, and then either standard or plasma (200 μL) was added and mixed. Then, 640 μL of N-methyl-2-phenylindole in acetonitrile was added and mixed. Concentrated HCl (150 μL) was then added to the tubes and then capped, mixed, and incubated at 45°C for 60 min. The tubes were centrifuged at 10,000 rpm in an Eppendorf microcentrifuge for 10 min, and the supernatant was loaded onto the microplate. Each microplate was read at 586-nm wavelength on a microplate reader (Biotek Instruments). The data were processed by a KC Junior software package. All samples were measured in duplicate and compared with standards.

Lipid hydroperoxides. Lipid hydroperoxides were determined from EDTA plasma using the Cayman Chemical Assay Kit (No. 750002, Ann Arbor, MI). This procedure used chloroform and methanol to extract the lipid hydroperoxides from plasma. The solutions were first prepared by bubbling nitrogen gas through the solvents to deoxygenate the extraction solutions. The extracted hydroperoxides react with a ferrous ion, which then react with thiocyanate ion as the chromogen (26). The absorbance of the samples and standards were determined using a 96-well glass plate and read on a microplate reader (Biotek Instruments) at 500-nm wavelength and compared with standards. All samples and standards were performed in triplicate.

Glutathione status. Glutathione was determined spectrophotometrically (Shimadzu UV-1601, Baltimore, MD) as

previously described by Andersen (1). This method enables both GSH and TGSH to be determined. 2-Vinyl pyridine was used to recycle all of the GSSG back to GSH to quantify TGSH concentration. The GSSG form was calculated from the following formula: $(TGSH - GSH)/2$, as previously reported (16). All samples were measured in duplicate and compared with standards.

Analysis. Data for all blood parameters, muscle forces measurements, and MS were analyzed using a 2×7 repeated-measure ANOVA. Significant interactions and main effects were further analyzed using Tukey's *post hoc* tests, if needed, to isolate where time differences occurred. Subject characteristics, dietary variables, total work, and percent decrease in work for the eccentric protocol were compared between groups using a one-way ANOVA. All analyses were performed using the Statistical Package for the Social Sciences for Windows (Version 16.0; SPSS, Inc., Chicago, IL). Statistical significance was set at $P \le 0.05$. The data are presented as mean ± SEM.

RESULTS

All subjects that were included in the data analysis ($N = 41$) successfully completed all aspects of the testing. Capsule consumption, assessed via returned capsule count and on the basis of the information of missed intake recorded by the subjects, was 98.5% ± 0.5% and 97.7% ± 0.6% for the P and FVC groups, respectively. There were no statistical differences at baseline between the groups for their characteristics, as listed in Table 1. There were 15 men and 5 women in the P group and 11 men and 10 women in the FVC group. The assessment of the 3-d dietary records period before their EE showed no significant differences between the two groups for the macromolecules and vitamins listed (Table 2).

Force and work results. There were no significant differences in the total amount of work performed during the eccentric protocol between the groups (P = 1137 ± 126 N·m, FVC = 1117 ± 130 N·m) nor the percent decrease in work performed during the eccentric protocol from the first set to the fourth set (P = 37% ± 5.3%, FVC = 38% ± 8.2%). Figure 1A shows the percent decline in MIF over time for the nondominant arm elbow flexors that performed the EE

TABLE 1. Characteristics for subjects.

Variable	P (*n* = 20)	FVC (*n* = 21)
Age (yr)	22.8 ± 0.7	23.8 ± 3.6
Height (cm)	165.6 ± 2.5	169.0 ± 1.5
Weight (kg)	66.3 ± 2.6	69.7 ± 3.8
BMI (kg·m^{-2})	24.4 ± 0.8	24.7 ± 1.5
% body fat	19.1 ± 2.0	18.2 ± 2.0
Arterial blood pressure (mm Hg)		
Systolic	118.9 ± 2.4	114.8 ± 2.6
Diastolic	71.9 ± 1.6	69.8 ± 1.6
Heart rate (bpm)	73.3 ± 2.3	71.4 ± 2.0

Values are presented as mean ± SEM. Values for blood pressure and heart rate are at rest. There was no statistical difference between the groups ($P > 0.05$).

TABLE 2. Dietary intake averages from 3-d food records.

Variable	P (*n* = 20)	FVC (*n* = 21)
Total calories (kcal)	2063 ± 172	2364 ± 114
Carbohydrate (kcal)	1005 ± 86	1185 ± 69
Carbohydrate (%)	49.4 ± 2.2	50.0 ± 1.4
Protein (kcal)	139 ± 31.0	106 ± 23.6
Protein (%)	15.5 ± 0.9	16.0 ± 0.9
Fats (kcal)	741 ± 79	812 ± 45
Fats (%)	35.2 ± 1.8	34.2 ± 0.7
Saturated fats (%)	12.3 ± 1.1	12.1 ± 0.5
Unsaturated fats (%)	13.6 ± 1.1	11.5 ± 1.0
Monounsaturated (g)	23.68 ± 3.6	23.90 ± 3.2
Polyunsaturated (g)	11.75 ± 2.4	8.67 ± 1.3
Omega-3 (g)	0.64 ± 0.11	0.43 ± 0.08
Vitamin C (mg)	83.6 ± 15.3	147.8 ± 29.8
Vitamin E (IU)	4.1 ± 0.59	4.5 ± 0.5
Beta-carotene (RE)	245.0 ± 39.3	343.3 ± 84.9

n = 20 for P and *n* = 21 for FVC. Values are presented as mean ± SEM. There was no statistical difference ($P > 0.05$) for any of the variables.
RE, retinol equivalents.

for both groups ($P \le 0.001$). There was an initial loss of MIF immediately after EE, independent of treatment, which remained depressed throughout the 72 h after EE.

ROM was significantly reduced in response to the eccentric protocol in the nondominant arm (Fig. 1B). A significant time effect ($P \le 0.001$) occurred for the loss of ROM, independent of treatment from immediately after EE through 72 h after EE.

MS was significantly increased ($P = 0.0001$) in both groups and demonstrated a time main effect (Fig. 2A). MS peaked at 48 h after EE, independent of treatment. Plasma CK response to the EE (Fig. 2B) showed a significant time effect, independent of treatment ($P = 0.031$). Both groups showed similar increases in CK over the 24–72 h after EE.
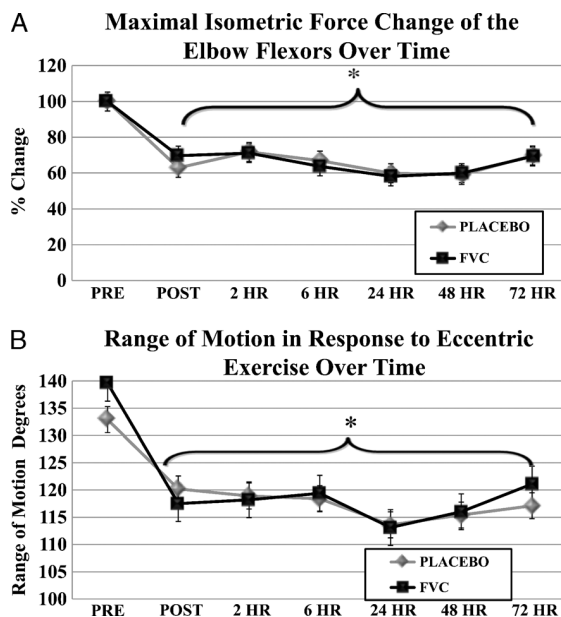

FIGURE 1—A, MIF changes in response to EE of elbow flexors over time. B, ROM changes of elbow flexors in response to EE over time. Values are presented as mean ± SEM. FVC, fruit, berry, and vegetable concentrate. *Significant time effect independent of treatment.
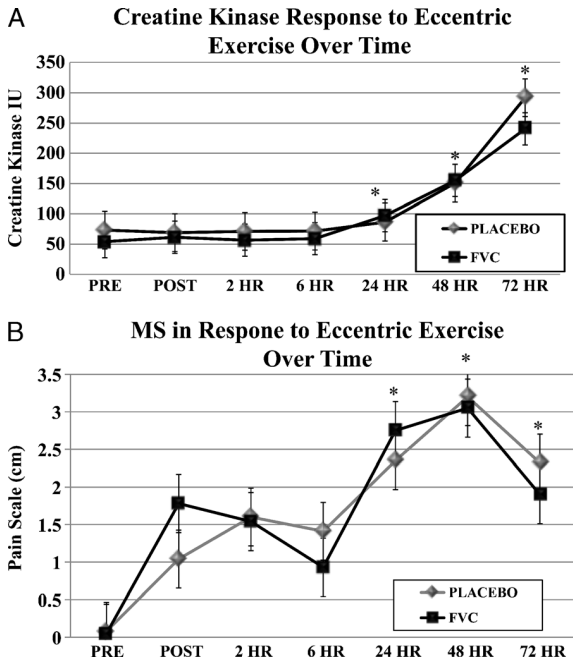
FIGURE 2—A, MS in response to EE over time. B, CK response to EE over time. Values are presented as mean ± SEM. FVC, fruit, berry, and vegetable concentrate. *Significant time effect independent of treatment.

Blood oxidative stress results. A significant time ($P = 0.0001$) and treatment ($P = 0.002$) effect occurred for plasma PC (Fig. 3A). PC increased over time, from 2 h postexercise through 72 h postexercise, in the P group. In contrast, PC were not significantly altered in the FVC-treated group over time. Therefore, there was a significant difference in PC between treatments from 2 to 72 h after EE.

A significant time and time × treatment effect ($P = 0.039$) occurred for plasma MDA. MDA increased over time in the P group, from 24 to 72 h after EE. In contrast, MDA was not significantly altered in the FVC-treated group at any time (Fig. 3B).

Lipid hydroperoxides were unaffected in either group over time, and there was no significant difference between the groups (Fig. 4A). The GSSG/TGSH ratio was significantly elevated over time (Fig. 4B). The *post hoc* test revealed that immediately after EE time, the GSSG/TGSH ratio was elevated for both groups, whereas the 6-h postexercise time was also elevated for the P group. This resulted in a significant difference between groups at the 6-h postexercise time point ($P = 0.04$).

DISCUSSION

The present study reports that this FVC compared with a P pretreatment for 4 wk before and continued for 4 d after EE can reduce blood markers of oxidative stress but did not attenuate the decrements in MIF and ROM or the
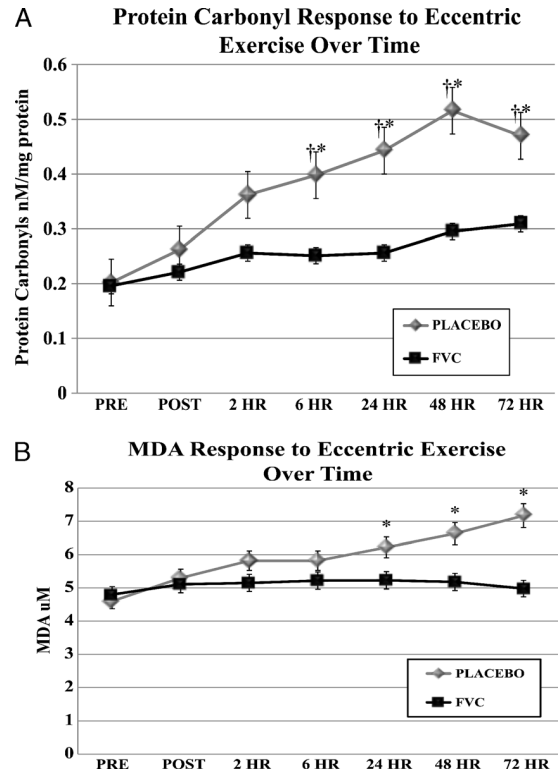

FIGURE 3—A, PC response to EE over time. B, MDA response to EE over time. Values are presented as mean ± SEM. FVC, fruit, berry, and vegetable concentrate. *Significant time effect independent of treatment. †Significant difference compared with P treatment.

increases in MS and blood CK. These data suggest that the FVC acted as an effective antioxidant. These data also suggest that the primary mechanism for muscle force loss,
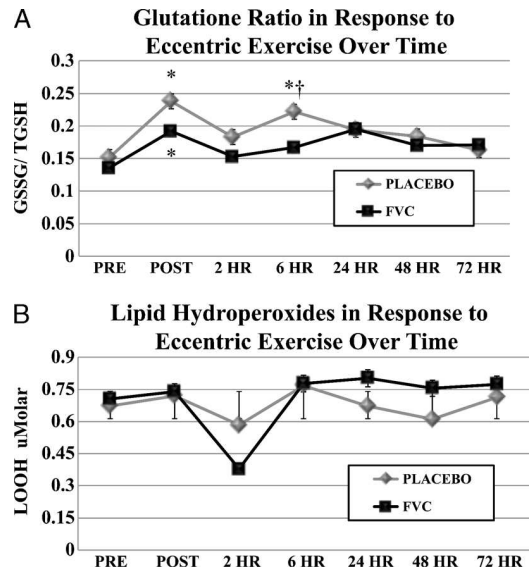

FIGURE 4—A, Lipid hydroperoxides response to EE over time. B, Glutathione ratio response to EE over time. Values are presented as mean ± SEM. GSSG, oxidized glutathione; TGSH, total glutathione; FVC, fruit, berry, and vegetable concentrate. *Significantly different from pretreatment. †Significantly different between groups.

MS, and membrane integrity damage is unrelated to blood markers of oxidative stress with EE. However, blood markers of oxidative stress may not always reflect what has happened within the muscles (38). This is the first study that we are aware of that has examined this concentrate as a potential prophylactic pretreatment agent to prevent the deleterious effects of EE.

Pretreatment of various antioxidants to prevent muscle damage and soreness has had limited success in preventing muscle force loss, MS, and mixed results in preventing oxidative stress (5,7,9,11,13,14,37). Muscle force loss was observed in both groups to a similar extent in the present study. The extent of force loss peaked 24–48 h after EE and resulted in a 40% loss of MIF. Several other studies have also reported that the peak force loss occurs from 24 to 72 h after EE and varies dependent on the number of muscle actions and the subjects used (4,14,15). It is interesting to note that Bloomer et al. (4) reported a similar force loss examining only women using this identical eccentric protocol. This suggests that both men and women exposed to EE should have similar force losses.

Although antioxidant pretreatment may attenuate oxidative stress, it is noteworthy that high-dose antioxidant treatment was ineffective in preventing skeletal muscle contractile force loss (12–14). In fact, Beaton et al. (2) reported that vitamin E supplementation of $1200~IU\cdot d^{-1}$ for 30 d was ineffective in preventing muscle damage to eccentrically biased contractions. Furthermore, it was reported that that vitamin E can stabilize membranes but did not diminish the amount of damage to eccentric contractions (36). However, an antioxidant (PEG-SOD) injected into the muscle of mice (39) was shown to prevent muscle force decline because of injury. In addition, isolated muscle treated with Tiron, a radical scavenger in the bathing medium, reduced force decline associated with Ca^{2+} handling loss (27). Therefore, it is probable that nutritional antioxidant supplementation will not be an effective route to protect against force decline within muscle.

ROM loss in our present study showed similar decrements with both treatments. The ROM loss is comparable with the loss reported by Bloomer et al. (4), using a similar eccentric protocol. Lee et al. (24) reported significant declines in ROM from 24 to 72 h after EE. This ROM loss can vary depending on the number of muscle actions.

The present study reports a similar biphasic MS response to the eccentric protocol in both groups. MS was significantly elevated from 24 to 72 h after EE and by 72 h had started to normalize, as previously reported, using an identical protocol (4). In addition, the increase in MS is similar to several other studies (4,10,15,24).

Vitamin C given for 7 d before ($3~g\cdot d^{-1}$) and after EE attenuated MS (5,17). However, despite less MS, there was still a similar increase in CK and decreased MIF with this treatment (5). In contrast, low-dose vitamin C (200 mg) taken 2 wk before downhill running did not alter increases in MS, lipid peroxidation, and CK (34). In addition, taking an acute dose of vitamin C before a shuttle run had no beneficial effects (33) nor was taking vitamin C only after the exercise beneficial (32). The present study reports similar CK and MIF responses to the study of Bryer and Goldfarb (5), but the present study reports no MS differences with this FVC. The vitamin C content in the FVC was $276~mg\cdot d^{-1}$, which probably was insufficient to alter MS, even with the combination of other ingredients.

CK increased to a similar extent, independent of treatment in the present study from 24 to 72 h after EE. The blood CK increase is typically attributed to the leakage of this enzyme out of the muscle cells because of a loss of membrane integrity. The increases observed with CK in the present investigation are similar to what has been reported in previous studies (4,9,24). It should be noted that high-dose vitamin E (2) can attenuate the CK leakage from muscle, independent of the extent of muscle damage (36). Therefore, it is probable that the amount of vitamin E (108 IU) within the FVC treatment was insufficient to attenuate the CK leakage.

In contrast to the functional measures, the present study reports that this FVC pretreatment was effective in attenuating the increase in several markers of oxidative stress within the blood. Both PC and MDA increases were not observed in the blood with the FVC treatment. The secondary increase in glutathione status was also attenuated. These data suggest that the FVC was capable of inhibiting the rise in these blood oxidative stress markers but had no effect on muscle functional outcomes because the extent of MIF and ROM loss and MS increase was similar compared with the P treatment. The results of the present study also indicate that oxidative stress was probably not the primary factor contributing to the functional changes in response to EE. It is probable that the forces within the muscle during the EE and the subsequent inflammatory-mediated factors (7,26,35) contributed to the functional changes observed in both groups. Further research is needed to more fully understand how one can minimize the damage associated with EE to help prevent the loss in function. It would be interesting to know if this FVC influenced the inflammatory-mediated response with this protocol.

In conclusion, this study reports that pretreatment with an FVC for 4 wk before and continued for 3 d after an eccentric protocol can attenuate blood oxidative stress markers but had no significant impact on the changes in functional measures and MS associated with this muscle-damaging procedure. In addition, the results suggest that blood oxidative stress may not be a good indicator of muscle damage and force declines manifested by this protocol. We suggest that supplementation of nutritional antioxidants should not be encouraged to prevent muscle damage because theses nutritional antioxidants have shown minimal beneficial functional outcomes.

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The results of the study do not constitute endorsement of the FVC product by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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