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John C. Quindry
Appalachian State University

Steven R. McAnulty
Appalachian State University

Matthew B. Hudson
Appalachian State University

Peter Hosick
Appalachian State University

Charles Dumke
University of Montana - Missoula, charles.dumke@umontana.edu

See next page for additional authors

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Oral Quercetin Supplementation and Blood Oxidative Capacity in Response to Ultramarathon Competition

John C. Quindry, Steven R. McAnulty, Matthew B. Hudson, Peter Hosick, Charles Dumke, Lisa S. McAnulty, Dru Henson, Jason D. Morrow, and David Nieman

Previous research indicates that ultramarathon exercise can result in blood oxidative stress. The purpose of this investigation was to examine the efficacy of oral supplementation with quercetin, a naturally occurring compound with known antioxidant properties, as a potential countermeasure against blood oxidative stress during an ultramarathon competition. In double-blind fashion, 63 participants received either oral quercetin (250 mg, 4×/day; 1,000 mg/day total) or quercetin-free supplements 3 weeks before and during the 160-km Western States Endurance Run. Blood drawn before and immediately after (quercetin finishers n = 18, quercetin-free finishers n = 21) the event was analyzed for changes in blood redox status and oxidative damage. Results show that quercetin supplementation did not affect race performance. In response to the ultramarathon challenge, aqueous-phase antioxidant capacity (ferric-reducing ability of plasma) was similarly elevated in athletes in both quercetin and quercetin-free treatments and likely reflects significant increases in plasma urate levels. Alternatively, trolox-equivalent antioxidant capacity was not altered by exercise or quercetin. Accordingly, neither F_2-isoprostances nor protein carbonyls were influenced by either exercise or quercetin supplementation. In the absence of postrace blood oxidative damage, these findings suggest that oral quercetin supplementation does not alter blood plasma lipid or aqueous-phase antioxidant capacity or oxidative damage during an ultramarathon challenge.

Keywords: antioxidant, endurance exercise, flavonol

Acute physical activity is generally marked by an identifiable oxidative stress in the blood plasma (Powers, DeRuisseau, Quindry, & Hamilton, 2004). This oxidative stress is often accompanied by acute changes in blood redox status, which, to some extent, are thought to reflect alterations in muscle redox status. Subtle redox perturbations in response to acute physical activity, moreover, are held to be

Quindry, S. McAnulty, Hudson, Hosick, Dumke, and Nieman are with the Dept. of Health, Leisure, and Exercise Science; L. McAnulty, the Dept. of Family and Consumer Sciences; and Henson, the Dept. of Biology, Appalachian State University, Boone, NC 28608. Morrow is with the Dept. of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

beneficial. Indeed, the acute oxidative stress incurred during exercise of moderate intensity and duration promotes some of the beneficial adaptations to exercise training (Gomez-Cabrera et al., 2005; Ji, Gomez-Cabrera, & Vina, 2006; Powers, Ji, & Leeuwenburgh, 1999). In contrast, severe or prolonged redox fluctuations as experienced during ultramarathon events are often considered deleterious to athletes' health and performance (Powers et al., 2004), although a direct cause-and-effect relationship is difficult to establish. In support, recent investigations of the blood oxidative-stress response to ultraendurance exercise have demonstrated elevations in various markers for oxidative damage (Liu et al., 1999; Nieman et al., 2003, 2004). These findings suggest an associative link between oxidative stress and fatigue experienced during ultramarathon participation. Accordingly, research efforts should be made to seek efficacious antioxidant countertherapies against the oxidative stress induced by prolonged exercise. Recent efforts have focused on the antioxidant properties of flavonols, molecules found abundantly in well-rounded diets including whole foods (Erdman et al., 2007; Manach, Williamson, Morand, Scalbert, & Remesy, 2005). It is largely unknown whether supplementation with flavonol compounds in the existing diets of ultraendurance athletes would prove beneficial to health and performance.

Recent evidence demonstrates the role flavonols play in preventing degenerative processes through anti-inflammatory, cardioprotective, anticarcinogenic, and antioxidant activities (Erdman et al., 2007; Manach et al., 2005). Quercetin ranks high among flavonols thought to promote good health through antioxidant capabilities (Silva et al., 2002). The current interest in quercetin is founded by its high degree of bioactivity and because it is highly concentrated in whole foods associated with good health: onions, apples, berries, red wine, broccoli, and tea (Erdman et al.; Manach et al.). Early studies indicate that quercetin has measurable effects on plasma antioxidant and oxidation biomarkers (Filipe et al., 2001; Hou, Zhou, Yang, & Liu, 2004). Furthermore, MacRae and Mefferd (2006) attributed improvements in cycle performance to the antioxidant properties of a quercetin-containing supplement. In support, quercetin has been shown to possess potent antioxidant properties through a variety of reactive sites primarily associated with two hydroxyl groups found on the catachol-type B-ring (Rice-Evans & Miller, 1996). Previous work by Quercegen Pharma indicates that quercetin potency is enhanced by cosupplementation with both vitamin C and niacin (Lines, 2006). Accordingly, there is reason to suspect that quercetin supplementation might fortify antioxidants in the aqueous phase of blood plasma. Further benefits might be conferred by preventing elevations in the inflammatory mediators COX-2 and NF-κB in the presence of quercetin (Garcia-Mediavilla et al., 2007; Martinez-Florez, Gutierrez-Fernandez, Sanchez-Campos, Gonzalez-Gallego, & Tunon, 2005). We have previously shown that quercetin supplementation reduced the incidence of upper respiratory tract infection in the weeks after 3 days of intensive cardiovascular exercise (Nieman et al., 2007). Whether the antioxidant and anti-inflammatory properties of quercetin are mechanistically linked, perhaps through redox perturbation, is currently unknown.

Based on these previous works demonstrating the antioxidant potential of quercetin, we conducted the current investigation to test whether oral quercetin supplementation would prove beneficial against the blood oxidative stress incurred during an ultramarathon foot race. Specifically, we chose to test the effects of a 1,000-mg daily quercetin dose on exercise-induced oxidative stress in the 160-km

Western States Endurance Race (WSER). This dosage was chosen to achieve plasma quercetin levels similar to those in previous, unpublished in vitro studies in which laboratory mice ingested plasma quercetin dosed to 12 mg/kg body weight (personal communication, J. Mark Davis, University of South Carolina, November 2005). The WSER is an annual ultramarathon held in the Sierra Nevada mountain range and is widely regarded as one of the most challenging events of its type. Furthermore, we have previously observed elevations in blood oxidative stress in participants after running in the WSER (McAnulty et al., 2007; Nieman et al., 2003). Accordingly, we hypothesized that participation in the WSER ultramarathon would elevate blood markers of oxidative stress. We further hypothesized that quercetin supplementation would attenuate the oxidative stress associated with ultramarathon participation.

Methods

Participant Recruitment and Race Description

To investigate the oxidative-stress response to ultraendurance physical activity we sampled participants in the 160-km WSER, a point-to-point trail run. Held in the Sierra Nevada Mountains of northern California, the WSER is regarded as one of the most arduous organized running events in the United States. Beginning in Squaw Valley, CA (1,890 m altitude), and concluding in Auburn, CA (366 m), race finishers experience a total altitude gain and loss of 5,500 m and 6,700 m, respectively. After a 5 a.m. race start, runners must reach the finish line within 30 hr to be eligible for an official finish time. Sixty-three participants (15 women, 48 men) were recruited for this investigation from the registered entrants of the 2006 WSER field, and written informed consent was obtained. The study informed-consent document was approved by the Appalachian State University Institutional Review Board in addition to the Western States Endurance Run Medical Research Board.

Research Design

Supplementation. After recruitment, participants were placed in either quercetin or quercetin-free treatments using a randomized double-blind assignment. Participants ingested soft chews with or without 1,000 mg/day quercetin under double-blinded procedures for 3 weeks before and the morning of the WSER (Figure 1). Specifically, participants ingested four individually wrapped chews each day (two before breakfast and two before dinner). Chews also contained 250 mg pure quercetin, 250 mg vitamin C, 20 mg niacin, and 20 kcal of sugars in a carnauba wax and soy lecithin base colored with FD&C yellow #6 (Nutravail Technologies Inc., Chantilly, VA, and Quercegen Pharma, Newton, MA). Quercetin-free supplements were prepared identically minus the quercetin, vitamin C, and niacin to control for researcher or participant bias. Previous data from Quercegen Pharma indicate that vitamin C and niacin are necessary to enhance quercetin bioavailability. Thus this study tested whether quercetin-containing chews that included vitamin C and niacin had an influence on plasma oxidative stress and time to race completion.

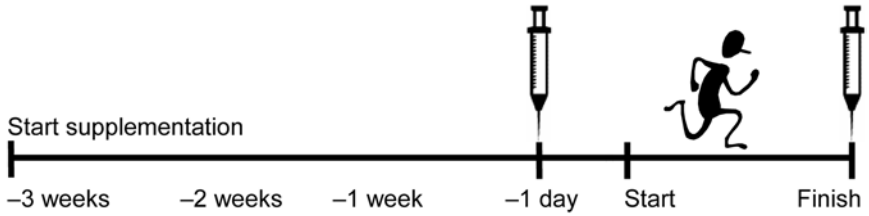

Figure 1 — Study supplementation and sample-collection timeline.

On race day, participants ingested all four chews before the 5 a.m. start time. During the supplementation period, they were instructed to ingest their normal diet of food, beverages, and supplements. By maintaining their dietary-intake routines during supplementation, participants avoided additional nonsupplementation quercetin intake. A 3-day food record was obtained during the 3-week prerace supplementation period. That is, participants were instructed to record their food intakes during days randomly assigned for the supplementation period. Food records were analyzed using the computerized nutrition software program Food Processor (ESHA Research, Salem, OR). Food records did not include the supplements provided to quercetin and quercetin-free groups. In regard to vitamin C concentrations, however, both quercetin and quercetin-free participants received equal amounts of vitamin C from their chews.

Blood Collection, Body Mass, and Questionnaires. Blood samples were drawn during registration in Squaw Valley, CA, the morning before the race and 15–30 min postrace in Auburn, CA. They were drawn from the antecubital vein with participants in the supine position. Plasma aliquots were immediately obtained, frozen, and maintained at –80 °C until biochemical analysis. In addition, a frozen aliquot of plasma was sent to a clinical laboratory for a comprehensive diagnostic chemistry panel, including uric acid, using an LX-20 clinical analyzer (Beckman, Brea, CA). During race registration, body mass was measured, and participants completed a questionnaire designed to survey basic demographics and training history. On race day, body mass was measured at the 90-km aid station (Michigan Bluff, 1,220 m) and within 5–15 min postrace at Auburn, CA. Participants completed a postrace questionnaire indicating adherence to the research design. They consumed food and beverages ad libitum during the race.

Plasma Quercetin

Total plasma quercetin (quercetin and its primary conjugates) was measured after solid-phase extraction via reverse-phase high-performance liquid chromatography (HPLC) with UV detection as described by Quercegen Pharma (Newton, MA, personal communication). This procedure is similar to that previously published by Ishii, Furuta, and Kasuya (2003). Quercetin conjugates were hydrolyzed by incubating 250- to 500-µl plasma aliquots with 10 µl of 10% DL-dithiothreitol (DTT) solution, 50 µl of 0.58-M acetic acid, a 50-µl mixture of β-glucuronidase and arylsulfatase, and crude extract from *Helix pomatia* (Roche Diagnostics GmbH, Mannheim, Germany). After incubation, 500 µl of 0.01-M oxalic acid was

added and mixed, samples were centrifuged, and supernatants were applied to solid-phase extraction cartridges (Oasis HLB 1-cc [30-mg] SEP cartridge; Waters Corp., Milford, MA). Eluant was collected and placed in a vacuum concentrator (Savant Speed Vac SC 110, Savant Instruments Inc., Farmingdale, NY). Residue was reconstituted with methanol for HPLC analysis using a Waters Breeze system (Waters Corp.) consisting of a Waters 1525 binary HPLC pump, 2487 UV detector, and Symmetry C18 5-μm 4.6 × 150-mm column. Prepared samples of 100-μl final volumes were loaded into the HPLC device with a mobile phase of acetonitrile (0.1% HCOOH) H_2O (0.1% HCOOH) at a flow rate of 1 ml/min. Data were acquired and processed using Breeze software (ver. 3.02). Quantification of the quercetin peak was based on the standard controls. Standards and samples were treated identically.

Plasma Oxidative Stress and Antioxidant Biomarkers

Ferric-Reducing Antioxidant Potential. Total plasma antioxidant potential was determined by the ferric-reducing ability of plasma (FRAP) assay according to the methodology of Benzie and Strain (1996). This assay utilizes water-soluble antioxidants native to the plasma to reduce ferric iron to the ferrous form, subsequently producing a spectrophotometrically identifiable chromogen. Samples and standards were expressed as ascorbate equivalents based on a physiologic ascorbate standard curve. Intra-assay and interassay coefficients of variation were less than 5% and 7%, respectively.

Trolox-Equivalent Antioxidant Capacity. Antioxidant activity was determined in EDTA-treated plasma samples using the trolox-equivalent antioxidant-capacity (TEAC) method as described by Cao and Prior (1998). Briefly, a free-radical-producing enzymatic system was created using horseradish peroxidase enzyme and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), hydrogen peroxide, and peroxidase. The free-radical-quenching capacity of plasma samples added to this free-radical-generating reaction was assessed spectrophotometrically, quantified, and expressed as trolox equivalents. Intra-assay and interassay coefficients of variation were less than 3% and 4%, respectively.

F_2-Isoprostanes. Plasma F_2-isoprostanes were determined using gas-chromatography mass spectrometry (GC-MS) according to the methodology of Morrow and Roberts (1999). Briefly, free F_2-isoprostanes were extracted from 500 μl plasma added to a deuterated $[^2H_4]$ $PGF_{2\alpha}$ internal standard. The mixture was then added to a C_{18} SEP Pak column, followed by silica solid-phase extractions. F_2-isoprostanes were converted to pentafluorobenzyl esters, subjected to thin-layer chromatography, and converted to trimethylsilyl ether derivatives. Samples were analyzed by negative-ion chemical-ionization GC-MS using a Nermag R10-10C mass spectrometer interfaced with an Agilent computer system.

Protein Carbonyls. Protein carbonyls, a measure of protein oxidation, were analyzed using a commercially available ELISA kit (Zentech Technology, distributed by Northwest Life Science Specialties, Vancouver, WA). Before analysis all plasma samples were assayed for protein concentration based on the methods of Bradford (1976) and adjusted to 4 mg/ml protein using phosphate-buffered saline before assay using a phosphate buffer (Bradford).

Statistical Analysis

Participant descriptive data were compared between groups using Student's *t* tests. Blood-variable data were analyzed using a 2 (groups) × 2 (time points) repeated-measures ANOVA and expressed as $M \pm SE$. When the interaction effect was significant ($p \leq .05$), pre- to postrace changes were calculated and compared between quercetin and quercetin-free groups using a Tukey's post hoc test, with significance set at $p \leq .05$. All statistical analyses were accomplished using Instat version 1.01 (San Diego, CA) and SPSS version 13.0 (Chicago).

Results

Participant Characteristics and Race Performance

Air temperature was 22 °C at the 5 a.m. race start (Squaw Valley, CA) and increased to 38 °C by 2 p.m. (Michigan Bluff), 27 °C by 12 a.m. (approximate time of the overall race winner, Auburn, CA), and 31 °C by 11 a.m. (30-hr official finish time limit). The humidity ranged from 25% to 58% during the last half of the race.

Participant characteristics for quercetin and quercetin-free groups are presented in Table 1. Thirty-nine of 63 participants ($n = 18$ for quercetin, $n = 21$ for quercetin-free) completed the 160-km race event and adhered fully to the study design. Notably, the attrition rate, approximately 35% for both treatments, was typical for this event.

Data indicate no significant differences in age, training volume, racing history, race time, and body weight pre-, during, and postevent for the two groups. Moreover, male ($n = 32$) and female ($n = 7$) runners did not differ significantly in race finish time (27.0 ± 0.5 vs. 27.0 ± 1.4 hr, respectively, $p = .992$) or any of the other measured variables save body mass and composition. Thus male and female competitors' data were combined for these analyses. Plasma volume did not change appreciably and did not differ significantly between groups (–0.3% ± 0.5% and –0.3% ± 0.4%, respectively, $p = .928$). Taken in combination with body-mass data (Table 1), which was maintained near prerace levels for both groups, participants maintained an adequate hydration level during the race.

Table 1 Participant Characteristics

	Quercetin ($n = 18$)	Quercetin-free ($n = 22$)	*p*
Age (years)	44.2 ± 2.0	46.0 ± 2.3	.575
Years of running	13.0 ± 2.3	13.3 ± 1.7	.780
Ultras raced	39.6 ± 10.6	37.9 ± 6.0	.988
Training (miles/week)	51.8 ± 3.1	47.1 ± 4.4	.398
Race time (hr)	26.4 ± 0.7	27.5 ± 0.6	.237
Weight prerace (lb)	152 ± 6	163 ± 5	.168
Weight at 55 miles (lb)	152 ± 5	162 ± 5	.169
Weight postrace (lb)	150 ± 5	161 ± 5	.140

Plasma Quercetin and Dietary Recall

After 3 weeks supplementation, plasma quercetin levels were 6.6-fold higher in the quercetin group than in the quercetin-free group and 3.1-fold higher postrace (interaction effect, $p < .001$; Figure 2). Note that both treatment groups experienced a significant pre- to postrace drop in plasma quercetin, although the magnitude of quercetin loss was greatest in the quercetin-supplemented participants. A poststudy questionnaire revealed that the double-blind methods used in the study were successful in that 22% of the quercetin-supplemented participants indicated they believed they had consumed quercetin, 17% thought they were quercetin free, and 61% were unsure of their supplement status. Of the participants receiving no quercetin, 38% suspected quercetin, 29% thought they were quercetin free, and 33% were uncertain of their treatment.

Three-day food records before the race indicated no significant differences in macronutrient intakes between groups, with a mean energy intake for all participants combined of 11.0 ± 0.8 MJ/day (2,620 ± 184 kcal/day) and percent of energy intakes of 51.1% ± 2.3% carbohydrate, 31.5% ± 1.8% fat, and 18.0% ± 1.2% protein. In addition, food-record analysis revealed no significant differences in the intakes of the antioxidants vitamin E (quercetin-free = 28.0 ± 10.2 mg, quercetin = 34.2 ± 14.7 mg; $p = .728$), vitamin A (quercetin-free = 772.2 ± 109.0 IU, quercetin = 780.1 ± 160.4 IU; $p = .968$), vitamin C (quercetin-free = 353.5 ± 143.5 mg, quercetin = 224.4 ± 51.8 mg; $p = .413$), or selenium (quercetin-free = 113.8 ± 21.7 μg, quercetin = 111.1 ± 19.0 μg; $p = .926$).

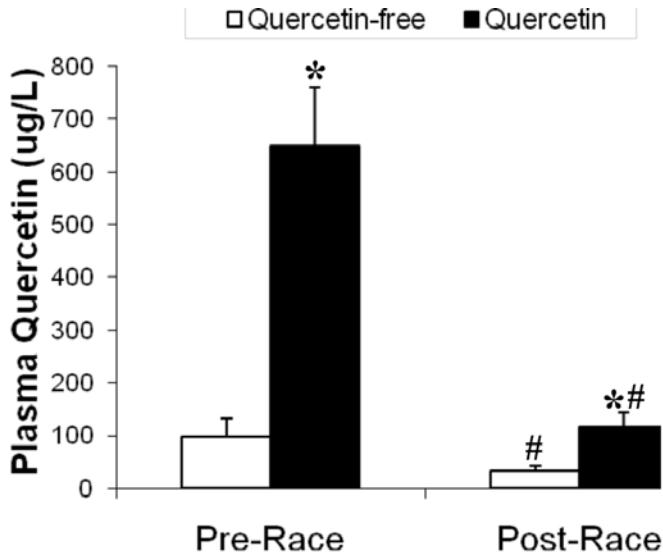

Figure 2 — Plasma quercetin values pre- and postrace, *M* ± *SEM*. *Significantly different from quercetin-free, $p \leq .05$. #Time effect, $p \leq .01$.

Biomarkers of Plasma Antioxidant Status

As compared with baseline values, plasma FRAP increased significantly after the ultramarathon (Figure 3[a]). This finding was independent of quercetin supplementation. Similarly, a significant pre- to postrace rise in plasma uric acid was observed (Figure 3[b]). Notably, the pre- to postrace changes (%) in FRAP and uric acid were calculated and found to correlate for both the quercetin-free (r = .693, p = .001) and quercetin-supplemented (r = .621, p = .005) groups (Figure 4). In contrast, TEAC was not altered by either the ultramarathon participation or quercetin supplementation (Figure 5).

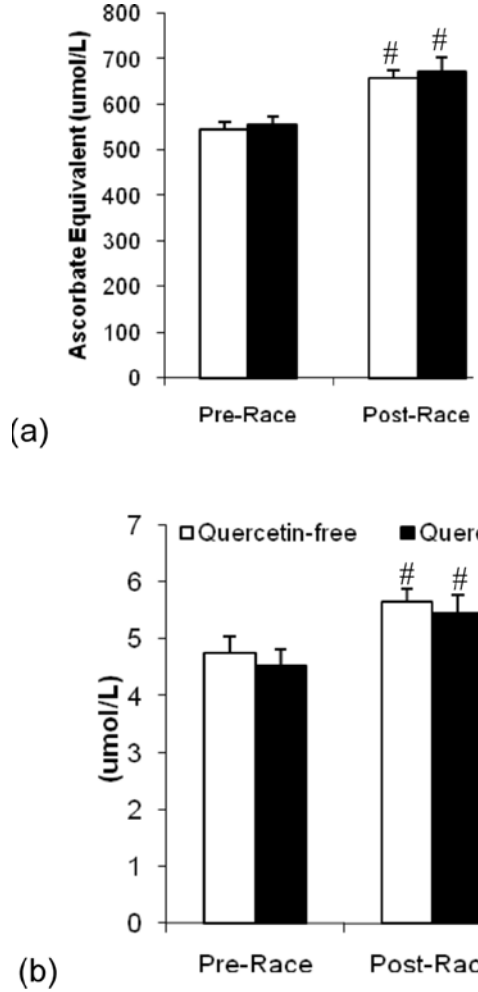

Figure 3 — (a) Ferric-reducing ability of plasma pre- and postrace, $M \pm SEM$. #Significant time effect, $p \leq .05$. (b) Plasma uric acid values pre- and postrace, $M \pm SEM$. #Significant time effect, $p \leq .05$.

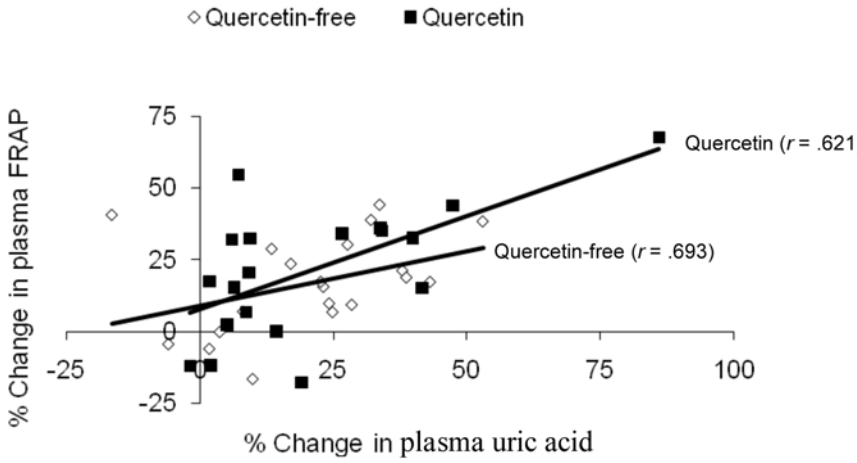

Figure 4 — Pre- to postexercise % change in ferric-reducing ability of plasma (FRAP) and % change in plasma urate.

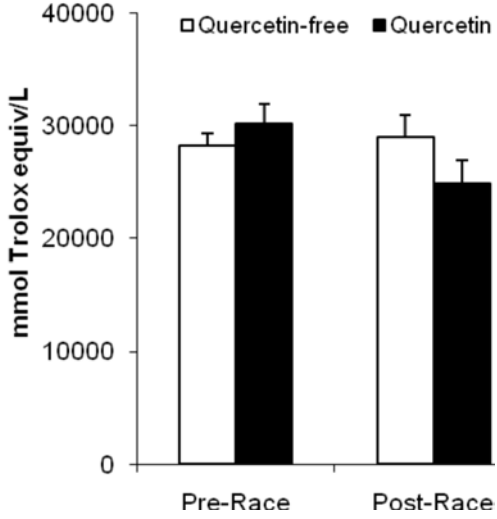

Figure 5 — Plasma trolox-equivalent antioxidant capacity pre- and postrace, $M \pm SEM$.

Biomarkers of Plasma Oxidative Damage

Despite the apparent trends, neither quercetin nor race participation elicited a change in plasma F_2-isoprostane values (Figure 6[a]). Similarly, plasma protein carbonyls were unaffected by either the quercetin treatment or the race event (Figure 6[b]).

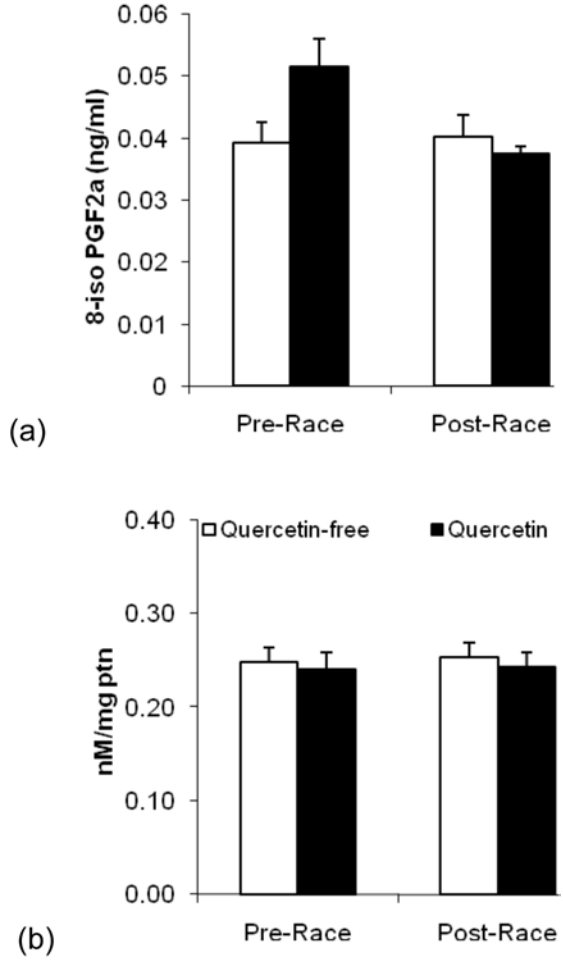

Figure 6 — (a) Plasma F_2-isoprostane values pre- and postrace, $M \pm SEM$. (b) Plasma protein carbonyl values pre- and postrace, $M \pm SEM$.

Discussion

Oxidative stress is a well-defined outcome of acute physical activity (Powers et al., 1999). Many recent investigations of exercising humans have used blood markers of oxidative stress because of the minimally invasive nature of phlebotomy relative to muscle biopsy. Using currently available biomarkers, however, identification of blood oxidative stress after exercise often requires participants to perform either high-intensity or long-duration exercise (Nieman et al., 2003; Quindry, Stone, King, & Broeder, 2003). Although these redox perturbations are thought to be a necessary part of the stimulus-adaptation response to exercise training, the

oxidative-stress response to prolonged ultraendurance-type exercise is held by some to be detrimental to athlete health and training status (Powers et al., 2004), although these effects have been difficult to quantify. In support, the thriving antioxidant-supplement business is a testament to the belief of some athletes and scientists that the oxidative stress associated with ultramarathon exercise is counterproductive to the aims of exercise training. Accordingly, ongoing research is aimed at uncovering interventions to counter the exercise-induced oxidative stress of ultraendurance exercise with the intent of improved athlete performance or health during the postcompetition recovery. This rationale led to the current investigation of supplementation with quercetin, a naturally occurring flavonol compound known to have powerful antioxidant properties (Silva et al., 2002). Specifically, a combined daily dose of 1,000 mg quercetin (which also included niacin and the powerful antioxidant vitamin C) was investigated as an antioxidant countertherapy in athletes competing in the WSER 160-km trail race. The addition of vitamin C and niacin to the quercetin supplement was intended to potentiate the bioavailability of quercetin (Lines, 2006). The WSER is widely held to be among the most demanding ultramarathon foot races in the United States and, as demonstrated previously, is a prime venue for an oxidative-stress field study (McAnulty et al., 2007; Nieman et al., 2003). Contrary to our hypotheses, findings from this investigation do not support the contention that a combined quercetin, niacin, and vitamin C supplement fortifies plasma antioxidant levels against ultramarathon-induced oxidative stress in blood plasma. In respect to ergogenics and performance, this finding is supported by the fact that quercetin-supplemented participants did not perform better than participants receiving quercetin-free supplements.

Quercetin and Blood Plasma Antioxidant Fortification

In a mixed biological solution such as human plasma, a classic approach to the study of oxidative stress incorporates measures of both taxation of antioxidant defenses and the generation of oxidative-damage products (Buettner, 1993). Accordingly, multiple redox-sensitive bioassays were used to test the antioxidant potential of oral quercetin supplementation on plasma redox status both before and after an ultramarathon. Supporting the rationale that quercetin supplementation would fortify the aqueous antioxidant defenses in plasma, several investigations have demonstrated that the presence of quercetin and quercetin metabolites in human plasma effectively prevented copper-catalyzed LDL peroxidation (Filipe et al., 2001; Hou et al., 2004). MacRae and Mefferd (2006) examined a quercetin-containing compound in athletes performing cycling time trials. In contrast to the current study, their results demonstrated modest but significant improvements in power output in quercetin-supplemented participants. Although not formally tested, this outcome was attributed to the antioxidant properties of quercetin. As an additional consideration, MacRae and Mefferd's investigation examined a mixed supplement that included 150 mg vitamin C and 300 mg of green tea, B vitamins, and quercetin. As with the current investigation, discerning the individual effects of quercetin from the potential synergistic effects with the other compounds examined by MacRae and Mefferd requires further study. In addition, the current study design of ultramarathon competition in extreme conditions varied considerably from that of MacRae and Mefferd's investigation. Thus, it is

plausible that the quercetin efficacy observed previously might have been masked by the physical demands of the WSER trial in the current investigation.

For the current investigation, daily quercetin doses of 1,000 mg elicited a mean rise in plasma quercetin of more than 600% as compared with quercetin-free participants. Based on preliminary work in our laboratory, this concentration was chosen to produce a plasma quercetin similar to concentrations from previous in vitro investigations. In this investigation, however, elevated prerace quercetin levels did not produce elevations in plasma antioxidant capacity as measured by FRAP and TEAC biomarkers. Indeed, this finding casts significant doubt on the ability of quercetin supplementation to significantly alter aqueous-phase antioxidant capacity, at least to the available limits of biomarker sensitivity. During the course of the ultramarathon competition, moreover, plasma quercetin levels decreased significantly in both quercetin-free and quercetin groups, although quercetin levels remained significantly higher in the supplemented participants. Similar to prerace findings in supplemented participants, elevated quercetin levels in the postrace plasma did not influence plasma FRAP or TEAC values. In fact, mean FRAP values increased significantly from pre- to postrace in both quercetin-supplemented and quercetin-free participants. A postultramarathon rise in plasma FRAP values has been identified previously (Nieman et al., 2003). Much of this rise is thought to result from elevations in concentrations of plasma uric acid, the most significant contributor to aqueous-phase plasma antioxidant capacity (Wayner, Burton, Ingold, Barclay, & Locke, 1987). Indeed, there were significant positive correlations between the pre- and postrace percent increase in plasma FRAP and uric acid in both treatment groups. Biochemically, elevated plasma uric acid as a result of acute exercise is the direct result of accelerated purine metabolism in skeletal muscle, in which two molecules of superoxide are created for each uric acid molecule produced (Liu et al., 1999). The import of this phenomenon hinges on the fact that production of each additional uric acid molecule also yields two molecules of the free radical superoxide (Halliwell & Gutteridge, 1999). In this respect, the fact that both quercetin-free and quercetin-treated participants experienced a similar mean rise in plasma uric acid suggests that both groups experienced similar free-radical load during the ultramarathon. Finally, the rise in plasma FRAP resulting from both quercetin-containing and quercetin-free supplements could be attributed to alterations in plasma vitamin C caused by supplementation or physical exertion. This point is particularly important with respect to the current investigation, in which both quercetin-containing and non-quercetin-containing chews included vitamin C.

Oxidative Damage After the 2006 WSER Ultramarathon

The second aspect of the "free-radical pecking order" described previously (Buettner, 1993) involves oxidative-damage products. Previous work by our group at the WSER (2003) has demonstrated that this ultramarathon yielded significant elevations in the lipid oxidative-damage markers F_2-isoprostanes (Nieman et al., 2003). Specifically, when compared with prerace values, the rise in F_2-isoprostanes was most marked at midrace but remained elevated postrace (Nieman et al., 2003). Similarly, Nieman et al. (2004) have found comparable elevations in plasma oxidative-damage markers after the Ironman Triathlon. In

contrast to those previous investigations, we did not observe a rise in either plasma F_2-isoprostanes or protein carbonyl concentrations. A previous oxidative-stress study conducted during the 2005 WSER also found that athletes (no treatment) did not exhibit a mean rise in plasma F_2-isoprostanes (McAnulty et al., 2007). Similar to F_2-isoprostanes, plasma protein carbonyls in the current study were not elevated after the ultramarathon in either treatment group. Accordingly, because plasma oxidative-damage markers were not elevated by ultramarathon competition, our evaluation of quercetin efficacy as a supplemental antioxidant rests on the measures of plasma redox status (FRAP and TEAC). As discussed previously quercetin did not affect plasma FRAP or TEAC levels, indicating that quercetin does not alter the aqueous antioxidant capacity to measurable levels.

The finding that plasma oxidative-damage biomarkers were not elevated in athletes competing in the 2006 WSER ultramarathon is interesting and worthy of further discussion. First, in an effort to maximize our ability to identify plasma oxidative damage and identify the antioxidant potential of quercetin supplementation, we chose to employ two of the most sensitive biomarkers for oxidative stress, F_2-isoprostanes and protein carbonyls. The F_2-isoprostane biomarker, as assayed by GC-MS, is the gold standard for lipid peroxidation damage (Morrow & Roberts, 1999). In addition to the sensitivity of the GC-MS technique, the primary advantage to the measure of F_2-isoprostanes is that they are more stable than other lipid oxidative-damage products (Dotan, Lichtenberg, & Pinchuk, 2004). In this investigation, postrace blood was drawn immediately after medical confirmation of athlete vital status. As with our previous investigations, we employed a rapid and meticulous sample-handling protocol to minimize in vitro loss of identifiable oxidative-damage products. Finally, to account for the potential conversion of F_2-isoprostanes to other oxidative-damage products through subsequent free-radical chain reactions (Dotan et al.), protein carbonyls were measured using antibody technology. Agreement between F_2-isoprostane and protein carbonyl data might suggest that, within the current constraints of oxidative-damage-marker sensitivity, a Type II error was avoided with respect to oxidative damage after the ultramarathon. We concede, however, that blood oxidative-stress assessment in field-type studies can be difficult. Accordingly, we chose a variety of the most sensitive oxidative-stress biomarkers with the intent that positive findings could be confirmed by multiple markers. In future studies, experimental-design construction would benefit from including midrace blood sampling to more clearly identify blood oxidative-damage responses of a transient nature.

As an alternative explanation for the observation that blood oxidative-damage markers did not rise, the 2006 WSER was exceedingly hot compared with preceding years, resulting in finish times several hours slower than average. Accordingly, finish times likely reflect athlete caution as much as an outcome of hyperthermic exercise. If correct, this hypothesis could explain year-to-year variation in oxidative damage (McAnulty et al., 2007; Nieman et al., 2003). Alternatively, both quercetin-containing and quercetin-free supplements contained the potent water-soluble antioxidant vitamin C. Based on previous findings, there is good cause to suspect that the presence of vitamin C in both experimental treatments of the current investigation could have masked the oxidative-stress response observed in previous years (Goldfarb, Patrick, Bryer, & You, 2005; McAnulty et

al.; Nieman et al., 2003). Other explanations for year-to-year discrepancies in the oxidative-damage response to the ultramarathon race might include participant nutrient intakes during the event. That is, ultramarathon athletes can be quite varied in their preferred caloric and nutrient intakes during competition. Previous works have demonstrated that caloric intake during exercise attenuates blood oxidative damage (Burneiko et al., 2006). In this investigation, participants were allowed to replenish energy stores ad libitum. Individual nutrition can vary significantly in both quantity and content. The potential impact of individual variability in food volume and antioxidant content on oxidative-damage markers cannot be discounted and is a study limitation. In addition, the transient nature of oxidative stress and its associated biomarkers can make identifying oxidative stress difficult, given the competition logistics of the WSER, in which blood-sampling opportunities are limited. Nonetheless, more frequent blood sampling throughout the event might have enabled us to identify a midrace spike in F_2-isoprostanes and protein carbonyls not apparent in the posttesting of the current investigation.

Conclusions

This investigation examined daily quercetin supplementation as a preventive aid against plasma oxidative stress incurred during an ultramarathon competition. Based on the free-radical pecking-order theory of biological solutions (Buettner, 1993), the efficacy of elevated quercetin against exercise-induced oxidative stress in blood plasma would manifest first as an elevation in plasma antioxidant capacity. The lack of oxidative-damage products after the ultramarathon indicates that an effect of this sort was not observed in our participants. Thus, at this time it does not appear that oral quercetin supplementation protects against the plasma oxidative stress induced by ultramarathon exercise, although this conclusion cannot be confirmed without further examination in which oxidative damage is clearly produced by the exercise challenge. Another plausible consideration of the current findings is that the conjugated quercetin metabolites do not exhibit antioxidant activities detectable within the confines of the antioxidant assays we employed (FRAP and TEAC). Therefore it does not appear that oral quercetin supplements protect against oxidative stress induced by ultramarathon exercise, but this conclusion cannot be confirmed without further examination whereby oxidative damage is clearly produced by the exercise challenge. Future investigations might uncover heretofore-unknown mechanisms of protection by quercetin or quercetin metabolites in exercise-stress scenarios.

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