Effects of dietary antioxidants on training and performance in female runners

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ORIGINAL ARTICLE

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Abstract
Exercise-induced oxidative stress is implicated in muscle damage and fatigue which has led athletes to embark on antioxidant supplementation regimes to negate these effects. This study investigated the intake of vitamin C (VC) (1 g), blackcurrant (BC) juice (15 mg VC, 300 mg anthocyanins) and placebo in isocaloric drink form on training progression, incremental running test and 5-km time-trial performance. Twenty-three trained female runners (age, 31 ± 8 y; mean $\pm$ SD) completed three blocks of high-intensity training for 3 wks and 3 days, separated by a washout (~ 3.7 wks). Changes in training and performance with each treatment were analysed with a mixed linear model, adjusting for performance at the beginning of each training block. Markers of oxidative status included protein carbonyl, malondialdehyde (in plasma and *in vitro* erythrocytes), ascorbic acid, uric acid and erythrocyte enzyme activity of superoxide dismutase, catalase and glutathione peroxidase were analysed. There was a likely harmful effect on mean running speed during training when taking VC (1.3%; 90% confidence limits ± 1.3%). Effects of the two treatments relative to placebo on mean performance in the incremental test and time trial were unclear, but runners faster by 1 SD of peak speed demonstrated a possible improvement on peak running speed with BC juice (1.9%; ± 2.5%). Following VC, certain oxidative markers were elevated: catalase at rest (23%; ± 21%), protein carbonyls at rest (27%; ± 38%) and superoxide dismutase post-exercise (8.3%; ± 9.3%). In conclusion, athletes should be cautioned about taking VC chronically, however, BC may improve performance in the elite.

Keywords: *Sports performance, ergogenic aids, sports nutrition*

Introduction

Exercise-induced oxidative stress, due to an accumulation of reactive oxygen species, has been implicated in muscle damage, immune dysfunction and fatigue (Powers & Sen, 2000). Given the conventional wisdom that oxidative stress is harmful, many athletes supplement their diet with vitamin C (VC) and other antioxidants to protect against the negative consequences of exercise (Schwenk & Costley, 2002). However, athletes may not be aware that exercise-induced reactive oxygen species not only cause damage but may also play a role in cell signalling for training adaptation (Gomez-Cabrera et al., 2008; Ristow et al., 2009) and may be beneficial. Recent research has shown that antioxidant supplements block expression of proteins'

RNA related to exercise adaptation, including antioxidant enzymes and mitochondrial biogenesis in sedentary and trained males (Ristow et al., 2009). Reducing the concentration of reactive oxygen species via antioxidant supplementation may have negative consequences by attenuating adaptations to training. However, Higashida, Kim, Higuchi, Holloszy and Han (2011) found little difference in an important mitochondrial growth factor, PGC-1α expression, and other mitochondrial proteins, following three days of training despite a large dose of VC and VE. While biochemical data support a negative consequence of VC on markers of training adaptations (Gomez-Cabrera et al., 2008; Ristow et al., 2009), studies on active human participants have not yet demonstrated the negative consequences of

supplementation (Roberts, Beattie, Close, & Morton, 2011; Teixeira, Valente, Casal, Marques, & Moireira, 2009).

While single antioxidant nutrients can be clearly defined, foods that are naturally antioxidant rich have not been studied extensively. Blackcurrants (BCs) are very high in antioxidant activity and are amongst the top dietary sources of antioxidants (Halvorsen et al., 2006; Lister, Wilson, Sutton, & Morrison, 2002). BC flavonoids protect against exercise-induced oxidative stress in humans (Skarpanska-Stejnborn, Basta, & Pilaczynska-Szczesniak, 2006). Foods naturally high in antioxidants may offer a range of phytonutrients that confer benefits to performance not seen in a single nutrient supplement.

The aim of the present study was to investigate whether a little over 3 wks of antioxidant supplementation alters training-induced improvements in exercise performance by measuring the change in repetition times completed during training, 5-km time trial (TT) and laboratory run to exhaustion. The randomised antioxidant treatments included BC, containing a daily intake of 300 mg anthocyanin and 15 mg VC, and VC alone containing 1 g and were compared to a commercial drink with little antioxidant content or activity. To date, the impact of training and antioxidant supplementation on training efficacy and performance in competitive females has not been investigated.

Methods

Subjects and study design

Twenty-three trained female runners volunteered to take part in this study. The characteristics of the runners are presented in Table I. Using a randomised three-treatment, placebo cross-over design, each runner completed three, 3.3-week-long blocks of high-intensity training separated by a washout period of normal training lasting 3.7 weeks. Each

Table I. Descriptive characteristics of the 23 runners who completed the study.

	Mean ± SD
Age (y)	31 ± 8
Weight (kg)	61 ± 7
Years of competition	7 ± 5
Maximal oxygen uptake (ml min^{-1} kg^{-1})	49 ± 4
Average training pre-study (min wk^{-1})	245 ± 130
Average training[a] (min wk^{-1})	330 ± 100
Repetition run time long course[a] (min:s)	04:37 ± 00:22
Repetition run time short course[a] (min:s)	02:52 ± 00:14
Peak running speed[b] (km hr^{-1})	16.8 ± 1.7
5-km race time[b] (min:s)	25:06 ± 2:51

[a]Mean over the 3 wk of training on placebo.
[b]After the 3 wk of training on placebo.

block was preceded by a 5-km TT. The runners were non-smokers and did not take any antioxidant supplements for 1 month prior to the study. See Figure 1 for an overview of the experimental schedule design. There was one withdrawal from the study as a result of injury. Runners completed a training log and drink compliance tickbox daily. The protocol was approved by Auckland University of Technology Ethical Committee.

Exercise performance tests

Familiarisation testing occurred for both the 5-km TT and incremental treadmill test. All laboratory tests were performed on a calibrated treadmill (Powerjog, Mid Glamorgan, UK) in a temperature-controlled laboratory (21°C). Following the familiarisation testing, the 5-km TT was completed at the beginning and end of every 3.3-week training block, the incremental laboratory test was completed at the end of each training block, in addition to the familiarisation testing.

The 5-km TT was performed on a loop road circuit close to the testing laboratory. The profile of the course was undulating including an ascent of 56 m covering 2.5 km of the total race and a descent of −57 m covering 1.5 km of the total race. All 5-km trials were held at the same time of the day (4 pm in the afternoon) in similar environmental conditions (except for one hot day which was accounted for in the analysis) following a jog and stretching. TTs were run as a group to maximise the impact of competition on performance.

For the incremental treadmill test, runners warmed up and ran five to eight stages at an initial gradient of 1% and speed set relative to each runner's best 10-km race time. The speed of the treadmill was increased by 1 km h^{-1} for each subsequent stage for the first four stages, then in incline by 1% every minute until exhaustion.

Training

Compulsory training sessions were completed 2–3 times a week and consisted of 4 near-maximal 3- to 5-min timed repetitions of a hilly course (1 km or 650 m) and 6 additional 2-min intense hill repetitions (400 m), in which runners were categorised into one of the two training groups, long or short session, based on their incremental run test during familiarisation. The long session involved 3- to 5-min repetitions of 1 km, the short 650 m, both including steep inclines. Each compulsory training session was completed under supervision accompanied by one of the researchers, with one session a week timed. Additional training was individualised for each runner according to their fitness level and

Familisation-once only	Pre-testing			Intervention-Dietary drink provided daily					
Incremental test	5-km TT	5-km TT	Rest day	Training block	Rest day	Incremental test	Rest day	5-km TT	Washout 3.7 wks
		B	B					B	B

Figure 1. Experimental schedule design. The pre-testing and intervention were repeated three times, with a different dietary drink each training block, assigned randomly. B = blood test, taken before and after the 5-km TT.

current training load. Examination of the training diaries showed all runners complied with the standardised training programme.

Heart rate monitors (Polar S625x or FSC3$_c$; Polar Electro, Kempele, Finland) and training logs were provided and runners instructed to record every session, including duration, average and maximum heart rates. Morning resting heart rate was also recorded and averaged for the week (HR_{rest}). Calculations for the estimation of the training impulse (TRIMP) were derived from Banister (Banister, Morton, & Fitz-Clarke, 1992). For females, $TRIMP = training\ duration \times [(HR_{mean} - HR_{rest})/(HR_{max} - HR_{rest})] \times 0.86\ exp[(HR_{mean} - HR_{rest})/(HR_{max} - HR_{rest}) \times 1.67]$, where training duration is the minutes spent training, HR_{mean} is the mean heart rate, HR_{rest} is the morning heart rate at rest and HR_{max} is the maximum heart rate derived from the laboratory exercise test.

Antioxidant drinks

The three dietary treatments contained equal volumes of fluid and calories. The VC treatment was prepared combining a commercially available fruit drink (Raro, Cerebos Gregg's Ltd., Auckland, New Zealand) with VC powder (Melrose ascorbic acid, Melrose Laboratories Pty Ltd., Mitcham, Australia) to provide 1 g of VC daily. The dose of 1 g of VC was chosen as previous studies investigating performance used this dosage (Gomez-Cabrera et al., 2008; Ristow et al., 2009). The BC treatment was prepared by combining a fruit drink concentrate (Barkers Fruit, Berrylife, Geraldine, New Zealand) with BC juice powder (Tasman Extracts, Nelson, New Zealand) and BC extract (containing the following anthocyanins; delphinidin-3-glucoside 4.8%, delphinidin-3-rutinoside 17.6%, cyaniding-3-rutinoside 14.7% and cyaniding-3-glucoside 2.1%; from Tasman Extracts, Nelson New Zealand). The final nutrient content provided from the BC treatment each day was 300 mg anthocyanin and 15 mg VC. Placebo treatment (PL) was prepared using a commercially available sports drink, orange flavour (Replace, Horleys, Auckland, New Zealand). The BC dose was chosen as a standard drink and nutrient intake that we would have expected an athlete to consume on a daily basis. Treatment was blinded to the researchers. Runners were instructed

to drink half the drink in the morning and half in the evening, providing a total fluid volume of 0.5 L daily. Two of the runners reported gastrointestinal upset on the BC treatment, however, not serious enough to limit consumption.

The runners completed a previously validated antioxidant food frequency questionnaire prior to commencing the study, designed to assess the overall antioxidant intake over the previous 3 wks (Braakhuis, Hopkins, Lowe, & Rush, 2011). The antioxidant questionnaire was repeated at the conclusion of each 3-wk training block to determine any changes in dietary habits. On the day of blood testing, runners were asked to refrain from foods high in antioxidants including red wine, fruit juice, coffee, tea, chocolate drinks, chocolate and no more than three pieces of fruit. A food diary was completed on the day of blood testing to ensure compliance to the low-antioxidant consumption and determine dietary habits for that day. The food diary included all food consumed until the completion of testing, so excluded the evening meal and supper. Examination of the food diaries shows all runners complied with the pre-trial standardisation protocol. The runners completed a menstrual cycle log at the completion of each 3-wk training block.

Blood testing

Venous blood samples (10 mL) were obtained from the antecubital vein of the runner into an Ethylene-diaminetetraacetic acid (EDTA) containing vacutainer. Venous samples were obtained between 1 and 3 hours before and 20 and 45 minutes after finishing the 5-km TT. Blood samples were taken at the same time, thus standardised for each runner on every trial.

A 1-ml aliquot of whole blood was refrigerated for in vitro erythrocyte antioxidant activity. Collected blood was centrifuged at 2700 g at room temperature for 2 min. A 200 μL sample of plasma was immediately transferred to 250 μL of metaphosphoric acid/EDTA for later analysis of VC, and two 200 μL plasma samples were transferred to 40 μL of GSH/EDTA for later analysis of protein carbonyls (PC) and malondialdehyde (MDA). These samples and the remaining plasma were frozen on dry ice and stored at $-80°C$. The packed erythrocyte phase was washed three times with Phosphate

buffered saline (PBS) and centrifuged as above, then frozen on dry ice and stored at $-80°C$ before enzyme analysis. Ascorbic acid and uric acid analysis were completed within a week of sample collection.

Antioxidant enzyme activities and plasma determinations

Superoxide dismutase (SOD) was measured by the method described by Kakkar, Das, and Viswanathan (1984). The erythrocytes were first treated with ice cold chloroform and ethanol (ratio 62.5:37.5) and the supernatant removed before measuring SOD activity. Glutathione peroxidise (GPx) was measured by the method described by Mannervik (1985). Catalase (CAT) was measured using the method by Abei (1984). Haemoglobin was measured using Drabkins solution as described by Dacie and Lewis (1984). The average within-run coefficient of variation obtained for all samples, done in triplicate was 5.9% and 2.11% for GPx and SOD activities, respectively, and expressed relative to haemoglobin concentration. CAT was completed as single measurements, and thus coefficients of variation were not determined.

Ascorbic acid and uric acid were measured by high-performance liquid chromatography (HPLC) with electrochemical detection as described by Lykkesfeldt (2002).

Oxidative stress markers

Plasma PC was measured using a protocol described by Morabito et al. (2005). Briefly, plasma (100 μL) was added to an equal volume of 2,4-dinitro-phenylhydrazine (DNPH, Sigma-Aldrich, Auckland, New Zealand) in 2 M HCl (control = DNPH/HCl in the absence of plasma) and incubated in the dark for 1 h. Protein was precipitated with 50% trichloroacetate (TCA, Sigma-Aldrich, Auckland, New Zealand) and washed with equal amounts of ethanol:ethylacetate and resuspended in 1 mL 6 M guanidine (Merck, NZ Ltd., Palmerston North, New Zealand), and the absorbance of the final suspension measured at 360 nm in a UV Visible 1601 spectrophotometer (Shimadza Corporation, Kyoto, Japan). Carbonyl levels were calculated using the absorbance difference between test and control using the molar absorption coefficient (ε): 22,000 M^{-1} cm^{-1}. Plasma protein levels were measured using the Bradford Method (Bradford, 1976) using commercial Bradford reagent (BioRad Laboratories Ltd., Auckland, New Zealand). Results are expressed as nmoles of PC/mg total protein.

Total MDA was determined as follows: briefly, 100 μl plasma was added to 100 μl 2 M sodium hydroxide and 20 μl methanol [0.005% butylated hydroxy toluene (BHT); ICN Eschwege, Germany]

and incubated at $60°C$ for 30 min, then 65 μl 4 M perchloric acid (PCA) was added. The sample was immediately vortexed and centrifuged at 10,000 g for 2 min. To 240 μl of the supernatant 24 μl 2 M HCL 10 mmol DNPH was added, and the mixture was incubated at $60°C$ for 30 min. The sample was then extracted with hexane:dichloromethane (80:20). The samples were blown dry in nitrogen and reconstituted in 15% methanol. The samples were then analysed by HPLC with UV detection as described by Pilz, Meineke, and Gleiter (2000).

The erythrocyte in vitro antioxidant capacity was determined as follows: whole blood was refrigerated at $4°C$ overnight. Samples were centrifuged for 5 min, 1000 g at $4°C$ and three times between saline washes. Thirty-three microlitres of the erythrocyte packed cells were added to 300 μl saline and incubated for 5 min at $37°C$, then vortexed. Twenty-five microlitres of the erythrocyte pack cell sample were added to a 1-ml cuvette, then 1.2-ml Drabkins added to the cuvette. Absorbance was measured at 540 nm (Dacie & Lewis, 1984). Following a 20-fold dilution, the remaining packed erythrocytes were challenged for 30 min at $37°C$ with a final concentration of 0.2 mmol cumene hydroperoxide. The challenge was stopped by precipitating 308 μl of the incubating solution with the addition of 300 μl of 0.6 M PCA. The solution was immediately centrifuged and 500 μL of the clear supernatant added to 100 μL of 8 mmol EDTA. Samples were frozen at $-80°C$ until analysis for MDA. The HPLC method described by Pilz et al. (2000) was used to measure the MDA

Statistical analyses

Descriptive data on the runners are raw means and standard deviations (SD). The performance and blood data were analysed using a mixed linear modelling procedure (Proc Mixed) in the Statistical Analysis System (version 9.2, SAS Institute, Cary, NC, USA) to derive estimates of changes in the mean, using 5-km performance before each treatment as a covariate to adjust for individual differences in washout and maintenance of fitness between treatments. The test date, energy and carbohydrate intake were used as covariates for the 5-km-TT post-block run, as was the stage of the menstrual cycle, where days 18–24 inclusive of the menstrual phase where coded as mid-luteal and remaining days were coded as other. Average time in weekly running repetitions (running speed in training) was included as a mechanistic covariate in separate analyses to determine the effect of training performance on 5-km race and laboratory speed.

Following a growing trend in methods of inferential statistics, we report uncertainty in the outcomes

as 90% confidence limits (CL) and make probabilistic magnitude-based inferences about true values of outcomes using methods described below and in more detail elsewhere (Hopkins, Marshall, Batterham, & Hanin, 2009; Sterne & Smith, 2001).

The thresholds for small, moderate, large and very large effects on performance were assumed to be 0.3, 0.9, 1.6 and 2.5 of the race-to-race within-athlete variability in competitive performance of top athletes (Hopkins et al., 2009). The between-athlete variability previously calculated on female elite athletes running 3–10 km is 1.3% with a smallest worthwhile effect of ~0.3 (0.2 times the between-athlete variability, based on Cohen's effects sizes) (Hopkins, 2005). The plasma and erythrocyte markers of antioxidant status were standardised following log transformation, with thresholds for small, moderate, large as 0.20, 0.60 and 1.20, respectively, based on standard Cohen effect sizes. Trivial is less than small (Hopkins et al., 2009).

Results

Examination of training logs showed all the runners complied with the standardised training programme, with only trivial differences between dietary interventions on total TRIMP scores for the 3 wks of training. Weekly training loads in trimps (intensity, minutes) averaged over the three treatments were: baseline, 640 ± 410; week 1, 1090 ± 390; week 2, 880 ± 290; week 3, 630 ± 260, and for each dietary treatment were BC 890 ± 270; VC 850 ± 360; PL 850 ± 290 (mean $\pm$ SD). Standardised differences between treatments in each week were at most small. The training completed during the intervention was a small to moderate increase in TRIMP compared with the runners' usual training. There was a moderate increase in training from baseline to week 1 and week 2 of the study.

All the runners reported 100% compliance with the daily drink consumption. Excluding the antioxidant drinks, all runners consumed a low-antioxidant diet on testing day, with trivial differences between dietary interventions. Over the 3-wk intervention period, there were trivial differences in antioxidant intake on all treatments, from non-treatment sources (see Table II).

Differences in energy intake on the day of testing were at most small (mean $\pm$ SD; BC 4.4 ± 1.2 MJ; VC 4.6 ± 1.9 MJ; PL 4.1 ± 1.5 MJ). Differences in carbohydrate intake on the day of testing were also small at most (mean $\pm$ SD; BC 147 ± 44 g; PL 134 ± 47 g; VC 169 ± 84 g); carbohydrate intake was, nevertheless, included as a covariate in the analysis of TT time and was shown to improve performance (1.0% per 100 g of carbohydrate consumed; 90% CL ± 1.1%).

Table II. Dietary antioxidant intake during the 3-wk training block and on 5-km race day.

Treatment period	During (mmol wk^{-1})		On race day (mmol)	
	From diet[a]	From treatment	From diet[b]	From treatment
Baseline	45 ± 22	–	–	–
BC	43 ± 24	36	2.5 ± 1.3	5.1
VC	45 ± 29	10	2.2 ± 1.0	1.4
PL	47 ± 32	0	2.6 ± 1.5	0.0

Data from diet are mean $\pm$ SD; data from treatment represent content in the supplements.
[a]Determined via an antioxidant food-frequency questionnaire.
[b]Determined via food diary.

Interval repetition running times were slower during the VC and BC compared to placebo for the average runner (see Table III). Faster runners (defined by +1 standard deviation of mean speed on the incremental running test) were also slower on VC and on BC, compared with placebo (see Table III). By week three, the differences in repetition times were trivial between each dietary intervention.

Each 3-wk training block had a moderate to large improvement in 5-km race times (mean $\pm$ SD; Block 1, $4.0\% \pm 3.1\%$; Block 2, $3.0\% \pm 4.6\%$; Block 3, $2.4\% \pm 3.8\%$). The average runners were possibly slower on BC, but very fast runners (+2 SD) had improved running performance in the 5-km race (3.0%, $\pm$90% CL 2.9%) and the incremental running test (2.3%, ± 3.6%). The effect of the menstrual cycle on 5-km TT performance was trivial but unclear (0.3%, ± 2.5%). The results of the laboratory incremental running test suggest faster runners benefit from BC, but unclear on VC (see

Table III. Effects (%) on running speed of BC and VC supplements compared with PL.

	BC vs. PL	VC vs. PL
Intervals in training		
All runners	−1.0; ± 1.3 (possibly slower)	−1.3; ± 1.3 (likely slower)
Faster runners	−1.2; ± 1.8 (possibly slower)	−2.1; ± 1.8 (likely slower)
Peak incremental test		
All runners	0.8; ± 1.7 (unclear)	0.7; ± 1.8 (unclear)
Faster runners	1.9; ± 2.5 (possibly faster)	0.9; ± 2.5 (unclear)
5-km TT		
All runners	−0.5; ± 1.5 (unclear)	0.3; ± 1.5 (unclear)
Faster runners	−1.0; ± 1.6 (unclear)	0.5; ± 1.9 (unclear)

Note: Data are mean per cent effect with 90% CL; qualitative clinical inference is shown in parentheses. Faster runners are those whose mean performance in the three pre-treatment incremental tests was 1 SD faster than the mean for all runners.

Table III). The effect of the menstrual cycle on the laboratory running test was trivial, but unclear ($-0.1\% \pm 1.7\%$). The 5-km times had a moderate to large improvement on all dietary treatments (mean $\pm$ SD; PL $3.8\% \pm 4.3\%$; BC $2.4\% \pm 3.9\%$; VC $3.2\% \pm 3.6\%$), with differences between treatments unclear. Training-performance time included in the analysis as a mechanistic covariate revealed a clear relationship between per cent change in training-performance time and per cent change in 5-km time between treatments (0.8; $\pm 0.4\%/\%$), accompanied by a slight reduction in the effects of the antioxidant drinks. The effect of inclusion of training performance in the analysis of laboratory run speed was trivial (-0.3; $\pm 0.6\%/\%$), and there was little accompanying reduction in the effect of the drinks.

Plasma concentrations of the antioxidants ascorbic acid and uric acid displayed clear, small to moderate rise with exercise (Figure 2A and B). Following VC runners had a small increase in ascorbic acid levels in the plasma both at rest and following exercise. There were only trivial differences between the plasma levels of uric acid on each of the dietary treatments, at rest and after exercise. There was a possibly small effect of the menstrual cycle on ascorbic acid (8%; $\pm 12\%$) and uric acid (5.8%; $\pm 10.2\%$).

The markers of oxidative stress, MDA and PC and an oxidative challenge test using erythrocytes (MDA-erythrocytes) were measured. BC had a small, possibly beneficial effect on plasma MDA at rest, compared with placebo (see Figure 2D). Trivial differences were seen with VC and BC in plasma MDA, following exercise. Compared with placebo, VC and BC had trivial differences between erythrocyte MDA, following in vitro stimulation, at rest and post-exercise (see Figure 2E). PC at rest showed a small, possibly harmful effect after VC (see Figure 2C).

The activity of erythrocyte enzymes responsible for the removal of reactive oxygen species included GPx, SOD and CAT. GPx activity had a trivial difference following any of the dietary interventions (see Figure 2H). There was a small, possible effect of the menstrual cycle on GPx activity (5.8%, $\pm 7.6\%$). CAT activity showed a clear decrease with training,
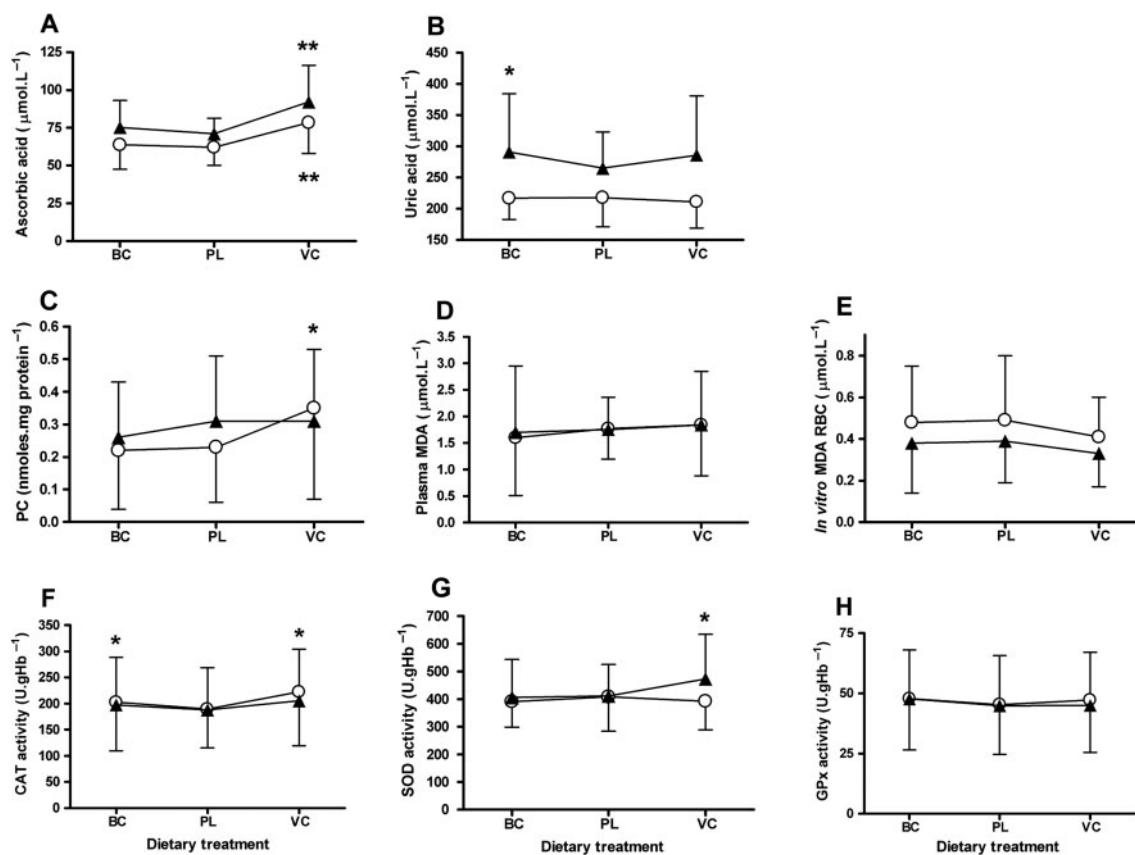

Figure 2. Biomarkers of antioxidant status and oxidative stress, pre ($\bigcirc$) and post ($\blacktriangle$) exercise, in three dietary antioxidant supplements (BC, blackcurrant, PL, placebo, VC, Vitamin C). Levels of antioxidants in plasma (plasma ascorbic acid and uric acid concentration in plasma (A and B, respectively). Oxidative stress markers [protein carbonyl and malondialdehyde concentration in plasma (C and D, respectively) and in vitro malondialdehyde concentration in erythrocytes (E)]. Erythrocyte antioxidant enzyme activity (F, catalase; G, superoxide dismutase and H, glutathione peroxidise). Data are means; error bars are SD. Abbreviations: SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase. *Small, clear difference from placebo treatment; **Moderate, clear difference from placebo treatment.

from Block 1 to 2 to 3 (mean at rest $\pm$ SD; 269 ± 74; 188 ± 77; 162 ± 57 U.gHb^{-1}). VC had a clear, harmful effect on CAT activity at rest (see Figure 2F) and SOD activity post-exercise (see Figure 2G). There was a small, possible effect of the menstrual cycle on SOD activity (-6.3%, $\pm 9.1\%$).

Discussion

The aim of this study was to investigate the effects of 3 wks of hard training in combination with dietary antioxidant intake on training speed, racing speed and antioxidant status of the blood. VC impaired training intensity clearly by $\sim 1\%$, but in spite of the large sample size and sophisticated analysis, mean effects on the performance tests were unclear (i.e., possibility of small beneficial effects but unacceptable risk of small harmful effects). BC had little effect on biomarkers of antioxidant status, but VC appeared to increase markers of oxidative stress, including PC, CAT and SOD activity, suggesting it may have acted as a pro-oxidant.

The weekly training load represented by the TRIMP scores (a mean of 938) was similar to that in another study of competitive runners (900) (Iwasaki, Zhang, Zuckerman, & Levine, 2003). This training resulted in large gains in performance in all the three treatment conditions. Similar high-intensity training for as little as 1–2 wk produced substantial changes in mitochondrial enzymes (Little, Safdar, Wilkins, Tarnapolsky, & Gibala, 2010) and muscle capillarisation (Irrcher, Adhinetty, Joseph, Ljubicic, & Hood, 2003) that could explain the improvements in aerobic performance in the present study.

The ability of reactive oxygen species to influence the expression of proteins involved in training adaptation is a result of the regulation of various intermediate kinases and phosphatases. Reactive oxygen species increase kinase activity (including ERK, JNK and p38), while reducing phosphatase activity (including PTEN and calcineurin), which activates transcription factors (p53, NK-κB and ATF2) (Cuschieri & Maier, 2005; Powers & Jackson, 2008) resulting in increases in mitochondrial growth factors, cell survival proteins (B-cell lymphoma 2), reduction in muscle atrophy and proteins involved cell death signalling pathways (Cuschieri & Maier, 2005; Powers & Jackson, 2008). Reactive species are an important trigger for training adaptations.

VC is recognised as an excellent antioxidant, but the consumption of excessive amounts may be detrimental to athletic performance via the blockage of exercise-dependent production of reactive oxygen species (Gomez-Cabrera et al., 2008). Previous research has demonstrated the ability of dietary antioxidants to negate training improvements. VC

has been shown to reduce athletic performance in racing greyhound dogs, given 1 g of VC daily for 4 wks, with supplemented dogs running an average 0.2 s slower (Massafra, 2002). Roberts et. al. (2011) compared 10 km TT performance after 4 wks of supplementing with 1 g of VC daily and found insignificant differences between the placebo and supplemented groups, although with a sample size of eight in the supplemented and seven in placebo, it is difficult to make decisive conclusions from this data. Teixeira et al. (2009) supplemented athletes with a daily cocktail of antioxidants, including 0.2 g of VC for 1 month. Although results were insignificant, authors report an average 1.5% drop in 1000 m kayak TT performance, similar to the performance drops seen by our runners. Senturk et al. (2005) supplemented athletes with 0.8 g of VC daily for 2 months and found no significant change in cycle time to fatigue during an incremental test, despite a 4.7% poorer time to fatigue. Yfanti et al. (2010) supplemented untrained subjects with 0.5 g of VC daily for 16 wks and reported an insignificant improvement in maximal power of 4% during an incremental cycle test. Certainly, our results concur with those completed on athletes, using a maximal effort TT testing protocol, which in our opinion represents the type of competition an athlete is most likely to conduct. However, it is difficult to compare studies on different exercise protocols and athletic populations.

It is possible high doses of single antioxidant nutrients may be detrimental to athletic performance. The mechanisms for the decrements in performance may be a result of sub-optimal mitochondrial biogenesis and/or the large dose antioxidants acting as a pro-oxidant and hampering muscle force generation or exercise recovery (Gomez-Cabrera et al., 2008).

In high concentrations, VC is capable of pro-oxidant action in the presence of available iron (Podmore, Griffiths, Herbert, Mistry, & Lunec, 1998). It is debated whether these findings are relevant to the plasma of athletes, where most of the transition metals are not free but attached to binding proteins, and thus prevented from participating in free radical reactions (Duarte & Lunec, 2005). Certainly, in trained athletes, serum iron has been shown to increase after exercise, without a concomitant increase in iron binding capacity (Smith & Roberts, 1994), providing a milieu capable of pro-oxidant activity. The concentrations of VC showing pro-oxidant effects (~ 1 mM) are those that could be achieved with supplementation of 1–2 g of VC daily (Rietjens et al., 2002). VC may be a pro-oxidant and increase tissue damage or reduce force production within the muscle.

The VC increased the activity of CAT at rest and SOD post-exercise. We also found CAT activity was

higher at rest on BC. A marker of oxidative stress (PC) was higher at rest on VC. The results of the blood assays suggest VC increase oxidative stress, supporting the theory that VC is acting as a pro-oxidant.

In contrast to VC, BC appeared to assist performance in faster runners completing the laboratory incremental running test. Mechanisms by which polyphenols may confer performance benefit involve changes in vascular function such as improvement of the endothelial function and proliferation in blood vessels (Ghosh & Scheepens, 2009; Stoclet et al., 2004). Studies have indicated that plant polyphenols, independently of their antioxidant effects, can enhance the synthesis of vasodilators and inhibit the synthesis of vasoconstrictors by endothelial cells (Schini-Kerth, Auger, Etienne-Selloum, & Chataigneau, 2010). Skarpanska-Stejnborn et al. (2006) reported no improvement in 2000 m ergometer test in athletes taking BC fruit for 6 wks, however, parameters of blood flow did. The performance improvements became clear only in the faster runners, a likely combination of higher training load in combination with the BC. Therefore, we speculate that polyphenols enhance performance when combined with a greater training load, which manifests in the faster runners.

The menstrual cycle was found to influence blood measures of ascorbic acid, uric acid, GPx and SOD activity. Southam and Gonzaga (1965) reported decreased ascorbic acid excretion during the luteal phase of the menstrual cycle, although not widely reported in more recent literature. Previous research has found menstrual cycle phase-dependent changes in erythrocyte GPx activity, but not in SOD or CAT (Massatra et al., 2000). The menstrual cycle phase did not affect the oxidative stress markers (PC and MDA), as reflected in another study (Chung, Goldfarb, Jamurtas, Hegde, & Lee, 1999). The running parameters had a trivial change with the menstrual cycle, similar to results reported in other studies investigating the impact of the menstrual cycle of endurance performance (De Jonge, 2003).

Conclusion

VC decreased training speed and increased markers of oxidative stress, including PC, CAT and SOD activity, suggesting pro-oxidant action. However, there is a possibility that the faster runners benefitted from BC in the 5-km TT and the laboratory test. Further research is needed on acute ingestion of BC, in case any harmful effect on training offsets possible benefit of ingestion on race day.

References

Abei, H. E. (1984). Catalase. In H. U. Bergmeyer (Ed.), *Methods in enzymatic analysis* (pp. 273–286). Basel: Verlag Chemie.

Banister, E. W., Morton, R. H., & Fitz-Clarke, J. (1992). Dose/response effects of exercise modelled from training: Physical and biochemical measures. *Annals of the Physiological Society of Anthropology*, 11(3), 345–356. doi:10.2114/ahs1983.11.345

Bradford, M. M. (1976). A rapid and sensitive test for the detection of ug quantities of proetin utilizing the principle of protein-dye binding. *Analytic Biochemistry*, 72(1–2), 248–254. doi:10.1016/0003-2697(76)90527-3

Braakhuis, A. J., Hopkins, W. G., Lowe, T. E., & Rush, E. C. (2011). Development and validation of a food-frequency questionniare to assess short-term antioxidant intake in athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, 21(2), 102–112. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21558572

Chung, S. C., Goldfarb, A. H., Jamurtas, A. Z., Hedge, S. S., & Lee, J. (1999). Effect of exercise during the follicular and luteal phases on indices of oxidative stress in healthy women. *Medicine and Science in Sports and Exercise*, 31(3), 409–413. doi:10.1097/00005768-199903000-00009

Cuschieri, J., & Maier, R. V. (2005). Mitogen-activated protein kinase (MAPK). *Critical Care Medicine*, 33(12), S417–S419. doi:10.1097/01.CCM.0000191714.39495.A6

Dacie, J. V., & Lewis, S. M. (1984). *Practical haemotology*. Edinburgh: Churchill Livingstone.

De Jonge, J. (2003). Effects of the menstrual cycle on exercise performance. *Sports Medicine*, 33(11), 833–851. doi:10.2165/00007256-200333110-00004

Duarte, T. L., & Lunec, J. (2005). When is an antioxidant not an antioxidant? A review of actions and reactions of vitamin C. *Free Radical Research*, 39(7), 671–686. doi:10.1080/10715760500104025

Ghosh, D., & Scheepens, A. (2009). Vascular action of polyphenols: Review. *Molecular Nutrition and Food Research*, 53(3), 322–331. doi:10.1002/mnfr.200800182

Gomez-Cabrera, M. C., Domenech, E., Romagnoli, M., Arduini, A., Borras, C., Pallardo, F. V., ... Vina, J. (2008). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *American Journal of Clinical Nutrition*, 87, 147–149.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bohn, S. K., Holte, K., Jacobs, D. R., Jr. ... Blomhoff, R. (2006). Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *American Journal of Clinical Nutrition*, 84(1), 95–135. Retrieved from http://www.ajcn.org/cgi/content/abstract/84/1/95

Higashida, K., Kim, S. H., Higuchi, M., Holloszy, J. O., & Han, D.H. (2011). Normal adaptations to exercise despite protection against oxidative stress. *American Journal of Physiology*, 301(5), E779–E784. Retrieved from http://ajpendo.physiology.org/content/301/5/E779.short

Hopkins, W. G. (2005). Competitive performance of elite track-and-field athletes: Variability and smallest worthwhile

enhancements. *Sports Science*, 9(17), 17–20. Retrieved from http://www.usetu.htmww.sportsci.org/jour/05/wghtrack.htm

Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*, 41(1), 3–13. doi:10.1249/MSS.0b013e31818cb278

Irrcher, I., Adhihetty, P. J., Joseph, A.-M., Ljubicic, V., & Hood, D. A. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. *Sports Medicine*, 33(11), 783–793. doi:10.2165/00007256-200333110-00001

Iwasaki, K., Zhang, R., Zuckerman, J. H., & Levine, B. D. (2003). Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: How much training for what benefit? *Journal of Applied Physiology*, 95(4), 1575–1583. Retrieved from http://jap.physiology.org/cgi/content/abstract/95/4/1575

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indiian Journal of Biochemirstry and Biophysics*, 21(2), 130–132. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6490072

Lister, C. E., Wilson, P. E., Sutton, K. H., & Morrison, S. C. (2002). Understanding the health benefits of blackcurrants. *Acta Hortic*, 585, 443–449.

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A., & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: Potential mechanisms. *Journal of Physiology*, 588(6), 1011–1022. doi:10.1113/jphysiol.2009.181743

Lykkesfeldt, J. (2002). Measurement of ascoarbic acid and dehydroascorbic acid in biological samples. *Current Protocols Toxicology*, 12, 7.6.1–7.6.15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20954159

Mannervik, B. (1985). Glutathione peroxidase. *Methods Enzymol*, 113, 490–495. doi:10.1016/S0076-6879(85)13063-6

Massatra, C., Gioia, D., Felice, C. D., Picciolini, E., De Leo, V., Bonifazi, M., . . . Bernabei, A. (2000). Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalase and glutathione peroxidase activities during the menstrual cycle. *Journal of Endocrinology*, 167(3), 447–452. doi:10.1677/joe.0.1670447

Morabito, F., Tomaino, A., Cristani, M., Cimino, F. F., Martino, A., Minciullo, P. L., . . . Gangemi, S. (2005). Modification of the content of plasma protein carbonyl groups in donors after granulocyte colony stimulating factor-induced stem cell mobilisation. *Transfus Apheresis Science*, 33(2), 141–146. doi:10.1016/j.transci.2005.05.003

Pilz, J., Meineke, I., & Gleiter, C. H. (2000). Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. *Journal of Chromatography B: Biomed Sci Application*, 742(2), 315–325. doi:10.1016/S0378-4347(00)00174-2

Podmore, I. D., Griffiths, H. R., Herbert, K. E., Mistry, N., & Lunec, J. (1998). Vitamin C exhibits pro-oxidant properties. *Nature*, 392(6676), 559. doi:10.1038/33308

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiology Reviews*, 88(4), 1243–1276. doi:10.1152/physrev.00031.2007

Powers, S. K., & Sen, C. K. (2000). Physiological antioxidants and training. In C. K. Sen, L. Packer, & O. O. P. Hanninen (Eds.), *Handbook of oxidants and antioxidants in exercise* (1st ed., pp. 221–242). Amsterdam: Elsevier.

Rietjens, I. M. C. M., Boersma, M. G., De Haan, L., Spenkelink, B., Awad, H. M., Cnubben, N. H. P., . . . Koeman, J. H. (2002). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology*, 11(3,4), 321–333. doi:10.1016/S1382-6689(02)00003-0

Ristow, M., Zarse, K., Oberbach, A., Klothing, N., Birringer, M., Kiehntopf, M., . . . Bluher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *PNAS*, 106(21), 8665–8670. doi:10.1073/pnas.0903485106

Roberts, L. A., Beattie, K., Close, G. L., & Morton, J. P. (2011). Vitamin C consumption does not impair training-induced improvements in exercise performance. *International Journal of Sport Performance and Physiology*, 6, 58–69.

Schini-Kerth, V. B. C., Auger, J. H., Etienne-Selloum, N., & Chataigneau, T. (2010). Nutritional improvement of the endothelial control of vascular tone by polyphenols: Role of NO and EDHF. *Pflugers Archiv*, 459(6), 853–862. doi:10.1007/s00424-010-0806-4

Schwenk, T. L., & Costley, C. D. (2002). When food becomes a drug: Nonanabolic nutritional supplement use in athletes. *American Journal of Sports Medicine*, 30(6), 907–916. Retrieved from http://ajs.sagepub.com/content/30/6/907.short

Senturk, U. K., Gunduz, F., Kuru, O., Kocer, G., Ozkaya, Y. G., Yesilkaya, A., . . . Baskurt, O. K. (2005). Exercise-induced oxidative stress leads hemolysis in sedentary but not trained humans. *Journal of Applied Physiology*, 99(4), 1434–1441. doi:10.1152/japplphysiol.01392.2004

Skarpanska-Stejnborn, A., Basta, P., & Pilaczynska-Szczesniak, P. L. (2006). The influence of supplementation with the black currant (*Ribes nigrum*) extract on selected prooxidative balance parameters in rowers. *Studies in Physical Culture and Tour*, 13(2), 51–58. Retrieved from http://www.wbc.poznan.pl/Content/53917/Skarpanska_Stejnborn.pdf

Smith, D. J., & Roberts, D. (1994). Effects of high volume and/or intense exercise on selected blood chemistry parameters. *Clinical Biochemistry*, 27(6), 435–440. doi:10.1016/0009-9120(94)00055-Z

Southam, A. L, & Gonzaga, F. P. (1965). Systematic changes during the menstrual cycle. *American Journal of Obstetrics and Gynecology*, 91(142), 61–67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14245873

Sterne, J. A. C., & Smith, G. D. (2001). Sifting the evidence-what's wrong with significance testing? *British Medical Journal*, 322(7280), 226–261. doi:10.1136/bmj.322.7280.226

Stoclet, J. -C., Chataigneau, T., Ndiaye, M., Oak, M. H., El Bedoui, J., Chataigneau, M., & Schini-Kerth, V. B. (2004). Vascular protection by dietary polyphenols. *European Journal of Pharmacology*, 500(1–3), 299–313. doi:10.1016/j.ejphar.2004.07.034

Teixeira, V. H., Valente, H. F., Casal, S. I., Marques, A. F., & Moireira, P. A. (2009). Antioxidants do not prevent post exercise peroxidation and may delay muscle recovery. *Medicine and Science in Sports & Exercise*, 41(9), 1752–1760. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19657294

Yfanti, C., Akerstrom, T., Nielsen, S., Nielsen, A. R., Mounier, R., Mortensen, O. H., . . . Pedersen, B. K. (2010). Antioxidant supplementation does not alter endurance training adaptation. *Medicine and Science in Sports and Exercise*, 2(7), 1388–1395. Retrieved from http://www.jappl.org/content/112/6/990.short