

Influence of tart cherry juice on indices of recovery following marathon running

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This investigation determined the efficacy of a tart cherry juice in aiding recovery and reducing muscle damage, inflammation and oxidative stress. Twenty recreational Marathon runners assigned to either consumed cherry juice or placebo for 5 days before, the day of and for 48 h following a Marathon run. Markers of muscle damage (creatine kinase, lactate dehydrogenase, muscle soreness and isometric strength), inflammation [interleukin-6 (IL-6), C-reactive protein (CRP) and uric acid], total antioxidant status (TAS) and oxidative stress [thiobarbituric acid reactive species (TBARS) and protein carbonyls] were examined before and following the race. Isometric strength

recovered significantly faster ($P = 0.024$) in the cherry juice group. No other damage indices were significantly different. Inflammation was reduced in the cherry juice group (IL-6, $P < 0.001$; CRP, $P < 0.01$; uric acid, $P < 0.05$). TAS was $\sim 10\%$ greater in the cherry juice than the placebo group for all post-supplementation measures ($P < 0.05$). Protein carbonyls was not different; however, TBARS was lower in the cherry juice than the placebo at 48 h ($P < 0.05$). The cherry juice appears to provide a viable means to aid recovery following strenuous exercise by increasing total antioxidative capacity, reducing inflammation, lipid peroxidation and so aiding in the recovery of muscle function.

Muscle damage, inflammation and oxidative stress typically occur in response to long-distance running races such as half-Marathons (Duthie et al., 1990), Marathons (Weight et al., 1991; Ostrowski et al., 1999; Starkie et al., 2001; Kratz et al., 2002; Suzuki et al., 2003; Smith et al., 2004) and ultraendurance running events (Kim et al., 2007) and have subsequently proven to be useful models in which to study the stress response to prolonged endurance exercise. Numerous studies have examined the effect of dietary supplements that contain antioxidants on markers of muscle damage, inflammation and oxidative stress in response to running. Because the goal of antioxidant supplementation is to boost the body's defenses against oxidative stress it is not surprising that several investigations using supplementation have shown reductions in markers of oxidative stress in response to running (Itoh et al., 2000; Mastaloudis et al., 2004; Bloomer et al., 2006; Machefer et al., 2007). Despite these positive effects, some studies have shown no effect of antioxidant supplementation on markers of running-induced oxidative stress (Rokitzki et al., 1994; Kaikkonen et al., 1998; Dawson et al., 2002).

In addition, most investigations have shown that antioxidant supplementation has no effect on the indices of muscle damage following running (Kaikkonen et al., 1998; Dawson et al., 2002; Kingsley et al., 2005; Mastaloudis et al., 2006; Machefer et al., 2007), although a few studies have shown reductions in markers of muscle damage (Rokitzki et al., 1994; Itoh et al., 2000). Furthermore, several studies have shown that antioxidant supplementation does not decrease markers of inflammation after prolonged running (Castell et al., 1997; Kaikkonen et al., 1998; Peters et al., 2001b; Mastaloudis et al., 2004; Machefer et al., 2007). However, some aspects of immune response to prolonged running may be enhanced by glutamine supplementation (Castell & Newsholme, 1997).

The efficacy of a nutritional intervention to lessen exercise-induced muscle damage, inflammation and oxidative stress might be improved by providing a food or supplement that contains phytochemicals with both antioxidant and anti-inflammatory properties. For example, cherries are known to be high in numerous different phytochemicals with both antioxidant and cyclooxygenase inhibitory properties

(Wang et al., 1999; Seeram et al., 2001). Evidence of anti-inflammatory effects from consuming cherries has been demonstrated in studies involving healthy human subjects (Jacob et al., 2003; Kelley et al., 2006). Additionally, animal investigations have also demonstrated pain inhibition (Tall et al., 2004) and anticarcinogenic (Kang et al., 2003) effects with the consumption of cherries. Only one previous piece of research has used a cherry intervention with an exercise model in hominids; Connolly et al. (2006) supplemented subjects with a tart cherry juice before and following high-intensity eccentric muscle contractions and found a reduction in some markers of muscle damage when compared with a placebo. Despite the investigators not recording measures of inflammation or oxidative stress, they speculated that the differences were due to the anti-inflammatory and antioxidant properties of the cherry juice (Connolly et al., 2006); consequently it would be of benefit for future investigations to examine measures of inflammation and oxidative stress in order to provide an insight into the potential mechanisms of cherry juice supplementation (Howatson & van Someren, 2008).

This growing body of literature (Wang et al., 1999; Seeram et al., 2001; Jacob et al., 2003; Kang et al., 2003; Tall et al., 2004; Connolly et al., 2006; Kelley et al., 2006) indicates that cherries may have potent antioxidant and anti-inflammatory effects, which make the expectation tenable that supplementation with cherries, may have beneficial effects following strenuous endurance activity. Therefore, the purpose of this study was to examine the effect of a tart cherry juice blend taken before and following running a Marathon on markers of muscle damage, inflammation and oxidative stress. It was hypothesized that consumption of cherry juice on the days before and following the Marathon would reduce the subsequent markers of muscle damage, inflammation and oxidative stress.

Materials and methods

Subjects

Before the start of the investigation all procedures were approved by the institutional ethics committee in accordance with the Declaration of Helsinki. Twenty volunteers, male ($n = 13$) and female ($n = 7$), participated in this investigation. Eighteen of the participants were accepted for, and completed the 2008 London Marathon, the environmental conditions on the day of the race were: barometric pressure, 758 mmHg; temperature, 7 °C; wind speed, 4 km/h; relative humidity, 56%; there were also intermittent showers throughout the day. Fourteen days later the remaining two volunteers completed the Marathon distance on similar terrain and similar environmental conditions: barometric pressure, 751 mmHg; temperature, 10 °C; wind speed 12 km/h; relative humidity, 50%; there were also intermittent showers throughout the day. All participants completed a health screening questionnaire and a written informed consent. In addition, the participant

characteristics, predicted Marathon time, Marathon history and training mileage leading up to the race were recorded and are presented in Table 1. We also asked participants to complete a food diary, refrain from taking nutritional supplements and strenuous exercise (other than completing training runs before the Marathon) for the duration of the study.

Experimental overview

Volunteers were equally assigned to either a placebo or cherry juice group based upon predicted finish time in a pseudo-randomized fashion. We also attempted to balance the number of male and female subjects in each group to account for possible sex differences in the response to Marathon running. Of the two participants who completed a Marathon distance over similar terrain to the London Marathon, one was randomly assigned to the cherry juice group and the other was assigned to the placebo group. Markers of muscle damage (with the exception of muscle soreness and isometric force, which were not measured pre-supplementation), antioxidative status, oxidative stress and inflammation were taken on five occasions; 6 days before the Marathon, the day before the Marathon, immediately after, and at 24 and 48 h after the Marathon. Following the initial visit to the laboratory subjects were allocated to treatment groups and were instructed to take the supplement every day before, the day of the Marathon and for the 48 h following the Marathon. This equated to 5 days supplementation before the Marathon and 8 days of supplementation in total.

Treatment groups

The placebo group was instructed to take two servings of a fruit flavoured concentrate that was mixed with approximately 8 fl oz of water and was intended to have similar visual properties but without the phytonutrient content found in the cherry juice blend. The cherry juice group took two 8 fl oz bottles of a commercially available tart cherry juice blend. The cherry juice blend was a mixture of freshly prepared tart cherry juice with commercially available apple juice in a proprietary ratio (Cherrypharm Inc., Geneva, New York, USA). Frozen tart cultivar Montmorency cherries (*Prunus cerasus*) were used to prepare the cherry juice following standard industrial processing procedures. The blended juice was pasteurized by heating it to 85 °C, hot packed into 8 oz bottles with a 3-min hold time to achieve commercial sterility, and then forced cooled in a water bath. One 8 oz bottle of the juice (containing the equivalent of 50–60 cherries) provided at least 600 mg phenolic compounds, expressed as gallic acid equivalents, 32 g carbohydrate and at least 40 mg anthocyanins, calculated as cyanidin-3-glucoside equivalents by the pH differential (Connolly et al., 2006). The remaining 560 mg of compounds is comprised of other flavonoids compounds, such as the flavonols quercetin, kaempferol and isorhamnetin and their glucosides, flavanols such as catechin, epicatechin and procyanidins and their glucosides and phenolic acids such as neochlorogenic acid, 3-coumaroylquinic acid, chlorogenic acid and ellagic acid. The oxygen radical absorbance capacity (ORAC) of a sample bottle was 55 mmol/L Trolox equivalents, which compares favorably with reported ORAC values ranging from 9.1 to 31.7 mmol/L Trolox equivalents for other commercially available juices, such as grape juice, black cherry juice, pomegranate juice, blueberry juice, acai juice and cranberry juice (Seeram et al., 2008).

Participants were instructed to take one bottle or serving in the morning and one in the afternoon for the duration of the supplementation period. In addition, participants were asked

to keep the supplement in cool dark storage, preferably refrigerated, until it was consumed to attenuate the possibility of degradation of the active compounds by light and heat.

Blood sampling and handling

Approximately 8.5 mL of blood was taken from a branch of the basilic vein at the ante-cubital fossa and collected into serum separation tubes. The blood was spun in a refrigerated (4 °C) centrifuge at 3500 g for 20 min; serum supernatant was aspirated, protected from the light and immediately frozen at –80 °C for later analysis.

Dependent variables

Indices of muscle damage were serum creatine kinase (CK) and lactate dehydrogenase (LDH), muscle soreness (DOMS) and maximum voluntary isometric contraction (MVIC). Inflammation markers were C-reactive protein (CRP), interleukin-6 (IL-6) and uric acid. Markers of antioxidative status and stress were total antioxidant status (TAS), thiobarbituric acid reactive species (TBARS) and protein carbonyls (PC) – TBARS and PC are measures of lipid and protein peroxidation, respectively. All blood measures were run in duplicate.

Muscle damage indices

Serum CK concentration was determined using an automated analyzer (c8000, Abbott Architect, Abbot Park, Illinois, USA). The normal range for CK using this assay in males and females is reported as 29–200 IU/L. Serum LDH was analyzed using a dry slide chemistry analyzer (Ektachem DT60 II and DTSC II Module, Ortho-clinical Diagnostics, Amersham, UK). The coefficient of variation (CV) of intra-sample reliability was <3% for both CK and LDH. DOMS was determined using a 200 mm visual analogue scale with “no soreness” indicated at one end and “unbearably painful” at the other. The subject stood with the hands on hips and feet approximately shoulder width apart. The participant was then asked to squat down to 90° (internal joint angle), rise to the start position and then indicate on the visual analogue scale the soreness felt in the lower limbs. MVIC of the non-dominant knee extensors was determined using a strain gauge (MIE Medical Research Ltd., Leeds, UK). Participants were seated on a platform and the non-dominant ankle was attached to the strain gauge at an internal joint angle of 80° (verified by a goniometer). Participants were given three submaximal trials at approximately 50%, 70% and 90% of their perceived maximum, followed by two maximal trials, each separated by 1 min. Each contraction lasted for approximately 3 s and all participants were given standardized verbal encouragement throughout. If there was <5% variance between the two MVICs the highest output recorded on the strain gauge was used for data analysis. On some occasions (13% of all trials) a third trial was necessary in order to attain two trials within the 5% tolerance.

Inflammation indices

Serum IL-6 concentration was determined in duplicate using a quantitative sandwich enzyme immunoassay ELISA technique (Quantikine, R&D Systems Europe Ltd., Abingdon, UK). Normal reference ranges for this assay are reported at <3 pg/mL. The serum intra- and inter-assay precision, determined by CV was <5%. Serum CRP concentration was determined using an automated analyzer (c8000, Abbott Architect). The normal reference values for this assay are reported at <0.8 mg/L with an intra-assay CV of 3.7%. Uric acid was

determined using a dry slide chemistry analyzer (Ektachem DT60 II and DTSC II Module, Ortho-clinical Diagnostics), the intra-assay CV was 4.2%.

Antioxidative status and oxidative stress

TAS was assessed using a colorimetric assay kit (Randox Laboratories Ltd., Antrim, UK) that was run on an automated analyzer (c8000, Abbott Architect); intra-assay reliability was reported as a CV of <3%. TBARS and PC were measured using commercially available kits (Cayman Chemical, Ann Arbor, Michigan, USA); the intra-assay CV was 5.5% and 4.7%, respectively.

Statistical analyses

Based on the available literature on prolonged endurance exercise, estimates were made of the expected change, and the inter-subject variation in change for each marker of muscle damage, inflammation and oxidative stress in response to running a Marathon. Assuming that the placebo group would have the expected responses, estimates were made on how much lower that response would need to be in the cherry juice group with a sample of 10 per group at an α level of 0.05 and a β level of 0.2 (80% power). Estimated effect sizes are reported as percentage with a maximum possible effect of 100% (i.e., no response in the cherry juice group). For markers of muscle damage the estimated effect sizes (percent lower response in cherry juice vs placebo) were 23% for MVIC (Suzuki et al., 2006), 45% for DOMS (Suzuki et al., 2006), 90% for CK activity (Duthie et al., 1990; Rokitzki et al., 1994; Starkie et al., 2001; Kratz et al., 2002; Suzuki et al., 2003; Smith et al., 2004) and 56% for LDH activity (Rokitzki et al., 1994; Smith et al., 2004; Kim et al., 2007). For markers of inflammation the estimated effect sizes were 85% for CRP (Weight et al., 1991; Fallon, 2001), 63% for IL-6 (Ostrowski et al., 1999; Starkie et al., 2001; Suzuki et al., 2003; Kim et al., 2007) and 27% for uric acid (Duthie et al., 1990; Rokitzki et al., 1994; Kratz et al., 2002). For markers of oxidative stress the estimated effect sizes were 69% for TBARS (Duthie et al., 1990; Machefer et al., 2007) and 35% for PC (Bloomer et al., 2006).

All data analyses were conducted using SPSS for windows, v. 15 and are reported as mean $\pm$ SD. Descriptive group characteristics were examined for differences using an independent samples Student's *t*-test. For the purposes of data analysis MVIC and TAS were expressed as a percentage change from baseline to account for inter-individual variation. All dependent variables were analyzed using a treatment (cherry juice vs placebo) by time (pre-supplement, pre-race, immediately post-race, 24 and 48 h post-race) mixed model analysis of variance (ANOVA). DOMS and MVIC were not recorded pre-supplementation so the time factor in the ANOVA had one less level. Mauchly's Test of Sphericity was used to check homogeneity of variance for all ANOVA analyses; where necessary any violations of the assumption were corrected using the Greenhouse–Geisser adjustment. Significant interaction effects were followed up using LSD *post hoc* analysis. A significance level of $P \leq 0.05$ was established before analyses.

Results

There were no significant differences in previous Marathon history, weekly mileage, longest single training run and predicted finish time (Table 1), and hence the groups were generally well matched.

Table 1. Descriptive data of the volunteer Marathon runners in the cherry juice and placebo groups

Group	Sex (M/F)	Age (years)	Height (m)	Mass (kg)	Predicted time (h:min:s)	Actual time (h:min:s)	Highest weekly mileage	Longest training run (miles)	Previous Marathons
Cherry juice	7/3	37 ± 13	1.77 ± 0.06	72.9 ± 9.8	3:41:00 ± 0:26:01	3:48:04 ± 0:48:58	33.0 ± 11.6	20.9 ± 2.6	7 ± 9
Placebo	6/4	38 ± 5	1.75 ± 0.09	73.8 ± 9.5	3:56:40 ± 0:40:37	4:15:48 ± 1:01:22	31.7 ± 8.2	19.3 ± 3.1	2 ± 7

Values are mean ± SD; $n = 10$ per group. There were no statistical differences between groups for any variable.

This was the first Marathon for three participants in the cherry juice group and for two subjects in the placebo group. The mean “actual finish time” was significantly slower than the “predicted finish time” ($t = 2.477$, $P = 0.023$); although not significant the difference between the “predicted finish time” and “actual finish time” was greater in the placebo group (19 min) than the cherry juice group (7 min). Post-race body mass was significantly lower than body mass the day before the race ($P < 0.001$) with similar declines in the cherry juice group and placebo group (1.2 ± 1.3 vs 1.7 ± 1.5 kg, respectively).

All dependent variables showed a significant time effect ($P \leq 0.009$) and demonstrated a power of ≥ 0.74 . The decrement in MVIC, expressed as a percentage of baseline (Fig. 1.), was similar between groups post-race (24.3% in cherry juice vs 26.9% in placebo) but a significant group effect indicated the recovery of strength over the following 48 h was more rapid in the cherry juice group ($F_{(1,18)} = 5.559$, $P < 0.024$). For illustrative purposes the absolute changes in MVIC are presented in Table 2. Despite the accelerated recovery in strength no other indices of muscle damage were different between groups (Table 2).

Serum IL-6 (Fig. 2.) showed a significant group effect ($F_{(1,18)} = 8.659$, $P = 0.009$) and group by time interaction ($F_{(4,72)} = 8.401$, $P < 0.001$) and was elevated immediately post-race, with a smaller elevation in the cherry juice group vs placebo (41.8 vs 82.1 pg/mL; $P < 0.001$). IL-6 values had returned to baseline by 24 h post-race. Serum CRP concentrations (group – $F_{(1,18)} = 12.920$, $P = 0.002$; group by time – $F_{(4,72)} = 10.938$, $P < 0.001$, Fig. 3.) were increased at 24 and 48 h post-Marathon with significantly smaller elevations in the cherry juice group vs placebo ($P \leq 0.025$). Serum uric acid (group – $F_{(1,18)} = 7.944$, $P < 0.011$; group by time – $F_{(4,72)} = 2.801$, $P < 0.032$, Fig. 4.) was

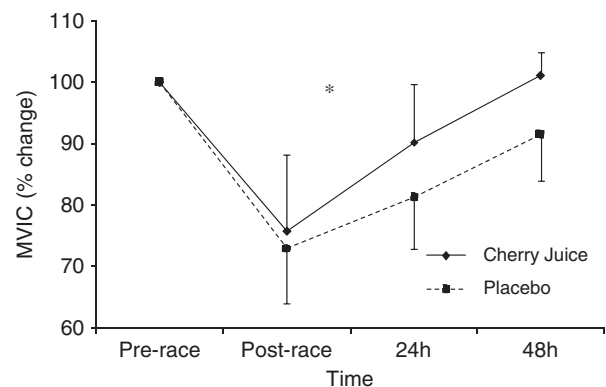


Fig. 1. Maximum voluntary isometric contraction (MVIC) for the cherry juice and placebo groups before and following the Marathon. *Significantly greater recovery of force in the cherry juice group ($P < 0.05$); values are mean ± SD ($n = 10$ per group).

Table 2. Indices of muscle function, damage and protein carbonyls for the cherry juice and placebo groups before and following Marathon running

	Pre-supplement	Pre-race	Post-race	24 h	48 h
DOMS (mm)					
Cherry juice	N/A	0	115 ± 52	91 ± 39	58 ± 39
Placebo	N/A	0	115 ± 60	82 ± 45	46 ± 28
MVIC (N)*					
Cherry juice	N/A	432 ± 114	310 ± 88	387 ± 94	435 ± 109
Placebo	N/A	384 ± 112	276 ± 69	313 ± 74	349 ± 96
CK (IU/L)					
Cherry juice	187 ± 126	109 ± 40	586 ± 315	2227 ± 1486	1118 ± 905
Placebo	201 ± 164	187 ± 220	912 ± 663	2814 ± 2235	1487 ± 1180
LDH (IU/L)					
Cherry juice	477 ± 96	487 ± 120	1084 ± 358	828 ± 265	591 ± 173
Placebo	557 ± 88	483 ± 97	1072 ± 344	761 ± 179	712 ± 234
PC (μmol/L)					
Cherry juice	24 ± 11	12 ± 11	17 ± 6	15 ± 8	17 ± 6
Placebo	21 ± 9	18 ± 6	16 ± 7	16 ± 7	18 ± 4

DOMS, muscle soreness; MVIC, maximal voluntary isometric contraction; CK, creatine kinase; LDH, lactate dehydrogenase; PC, protein carbonyls.

*Significantly more force in the cherry juice group than the placebo ($P < 0.05$); values are mean ± SD ($n = 10$ per group).

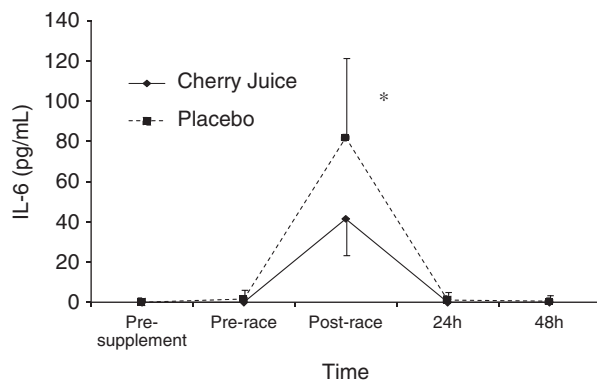


Fig. 2. Serum interleukin 6 (IL-6) concentrations for the cherry juice and placebo groups before and following Marathon running. *Significantly lower serum IL-6 in the cherry juice group than the placebo immediately post-race ($P < 0.05$); values are mean ± SD ($n = 10$ per group).

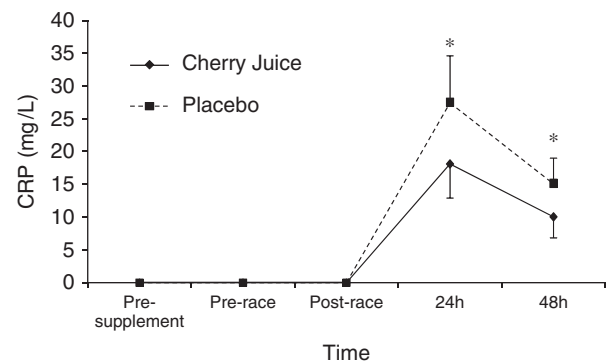


Fig. 3. Serum C-reactive protein (CRP) concentrations for the cherry juice and placebo groups before and following Marathon running. *Significantly lower serum CRP in the cherry juice group than the placebo at 24 and 48 h post-race ($P < 0.05$); values are mean ± SD ($n = 10$ per group).

elevated post-race and at 24 h in the placebo group with no increase in the cherry juice group ($P \leq 0.006$).

TAS, as a percentage of baseline, was significantly higher in the cherry juice group vs placebo (group – $F_{(1,18)} = 10.938$, $P < 0.001$; group by time – $P = 0.053$, Fig. 5.). The 5-day supplementation increased TAS in the cherry juice group (pre-race 111% of baseline, $P < 0.01$) with no change in the placebo group (101% of baseline, $P = 0.75$). TAS was increased in both groups after the marathon (cherry juice group 124% of baseline, $P < 0.01$; placebo 112% of baseline, $P < 0.01$), and remained elevated in the cherry juice group at 24 h (114% of baseline, $P < 0.01$) but not in the placebo group (103% of baseline, $P = 0.21$). By 48 h post-race TAS was below baseline in the placebo group (90%, $P < 0.01$) but not different from baseline in the cherry juice group (99% of baseline, $P = 0.82$). The TBARS response to the Marathon was different

between groups (group by time – $F_{(4,72)} = 3.199$, $P = 0.018$, Fig. 6.) with significantly higher values for the placebo group vs the cherry juice group at 48 h post-exercise (30.2 vs 21.4 μmol/L, $P < 0.01$). The PC response to the Marathon was not different between groups (Table 2) with no post-race elevations evident in either group.

Following the investigation subjects were asked to report if they knew what supplement they had been given. Half ($n = 5$) of the cherry juice group guessed correctly, the remainder of this group reported that they did not know what supplement they were taking. In the placebo group 20% ($n = 2$) thought they were on a placebo, the remainder did not know or thought they were taking the cherry juice. In addition, the food diaries provided by the participants did not allow for accurate quantification of antioxidants; however, we attempted to quantify the total number of food portions that may contain

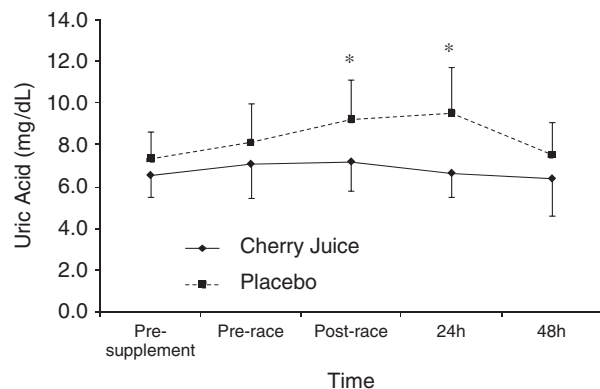


Fig. 4. Serum uric acid concentrations for the cherry juice and placebo groups before and following Marathon running. *Significantly lower serum C-reactive protein in the cherry juice group than the placebo immediately post-race and at 24 h post-race ($P < 0.05$); values are mean $\pm$ SD ($n = 10$ per group).

antioxidant compounds in the time before the race. The mean number of portions was 20 vs 21 portions for the cherry juice and placebo groups, respectively, and was not significantly different between groups.

Discussion

It was hypothesized that consumption of a tart cherry juice blend would reduce markers of muscle damage, inflammation and oxidative stress in response to running a Marathon. With respect to markers of muscle damage, the cherry juice group had a more rapid return of isometric knee extension strength than the placebo group with no differences between groups in CK, LDH or muscle soreness. Despite this, markers of inflammation showed elevations in IL-6, CRP and uric acid to be significantly smaller in the cherry juice group compared with the placebo group. Finally, with respect to oxidative stress, total antioxidant capacity was increased and lipid peroxidation was decreased in the cherry juice group compared with the placebo group; however, serum protein carbonyl concentration was not different between groups.

The fact that post-race strength loss was similar between groups, with a more rapid return of strength in the cherry juice group vs placebo, indicates that consumption of the cherry juice may have served to blunt the secondary muscle damage response. With exercise-induced muscle damage the initial injury is a mechanical disruption of myofibrils which triggers a local inflammatory response that exacerbates the initial damage (Pizza et al., 2002) and this is referred to as the secondary damage response (Howatson & van Someren, 2008). The IL-6, CRP and uric acid data indicate that the inflammatory response to

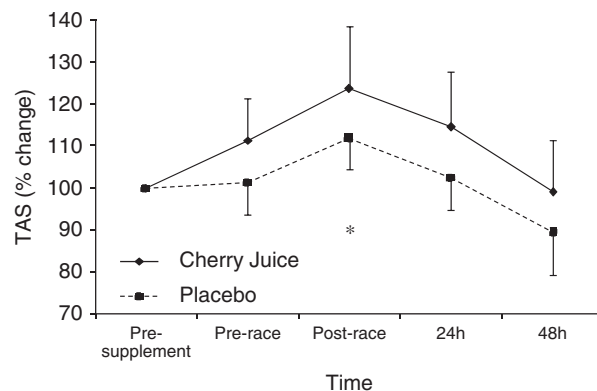


Fig. 5. Total antioxidative status (TAS) for the cherry juice and placebo groups before and following Marathon running. *Significantly higher TAS in the cherry juice group than the placebo ($P < 0.05$); values are mean $\pm$ SD ($n = 10$ per group).

running the Marathon was blunted by consuming cherry juice and that this may have limited the subsequent exacerbation of damage. While the strength data support this interpretation, the CK, LDH and DOMS data are less clear because these markers were not different between the cherry juice and placebo groups. However, there was an apparent association between the inflammatory response and the CK response. The post-race IL-6 elevation was correlated with CK elevation post-race ($r = 0.58$, $P = 0.007$), at 24 h ($r = 0.61$, $P = 0.006$) and at 48 h ($r = 0.50$, $P = 0.026$). Similarly, CRP elevation at 24 h was correlated with CK elevation at 24 h ($r = 0.56$, $P = 0.01$) and CRP elevation at 48 h was correlated CK elevation at 48 h ($r = 0.52$, $P = 0.019$).

The apparent beneficial effect of cherry juice consumption on recovery of strength is consistent with previous findings showing that consumption of cherry juice markedly reduced strength loss after a bout of high-intensity eccentric contractions of the elbow flexors (Connolly et al., 2006). In contrast, Connolly et al. (2006) also found that cherry juice consumption reduced the pain response to the eccentric exercise. However, the effect on pain was not as marked as the effect on strength. Additionally, the pain response to isolated maximum eccentric contractions typically peaks 24–48 h after the exercise bout (Connolly et al., 2006; Howatson & van Someren, 2007); while the muscle soreness reported in the present study was highest immediately post-race.

Post-race elevations in IL-6 have been consistently shown in Marathon runners (Ostrowski et al., 1999; Starkie et al., 2001; Suzuki et al., 2003). In only one of those investigations (Starkie et al., 2001) was IL-6 measured for a longer period than 24 h after the race; at 24 h, values were markedly lower than post-race. Similarly, in the present study IL-6 had returned to baseline by 24 h. Elevations in CRP (Weight et al.,

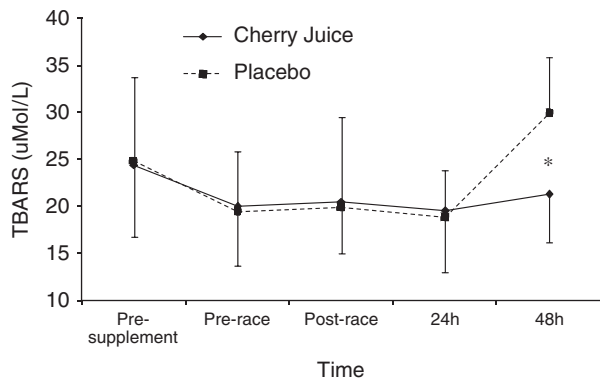


Fig. 6. Serum thiobarbituric acid reactive species (TBARS) for the cherry juice and placebo groups before and following Marathon running. *Significantly higher TBARS in the placebo group at 48 h ($P < 0.05$); values are mean $\pm$ SD ($n = 10$ per group).

1991) and uric acid (Rokitzki et al., 1994; Kratz et al., 2002) have also been demonstrated in response to running a Marathon. Similar to the present results, CRP elevations were shown to peak 24 h post-race and uric acid was shown to be elevated immediately post-race and at 24 h. In our study, post-race IL-6 elevation was 49% lower in the cherry juice group compared with the placebo group while at 24 h the CRP elevation was 34% lower in the cherry juice group. These results are clear evidence of an anti-inflammatory effect of the cherry juice.

While lower IL-6 values in the cherry juice group are attributed here to an inflammatory effect others have considered IL-6 as an anti-inflammatory cytokine (Peters et al., 2001a; Steensberg et al., 2003; Fischer et al., 2004). Infusion with recombinant human IL-6 has been shown to increase production of IL-1ra and IL-10, which are both regarded as anti-inflammatory cytokines (Steensberg et al., 2003). Furthermore, prolonged (29 days) vitamin C (500 mg/day) and vitamin E (400 IU/day) supplementation before concentric exercise (Fischer et al., 2004) blunted the exercise-induced IL-6, IL-1ra and IL-10 responses. Of note this exercise did not induce elevations in CK activity and was not considered to have caused muscle damage. High-dose vitamin C supplementation (1500 mg/day) for 7 days before and 2 days following a 90 km run lowered the IL-1ra and IL-10 responses and was interpreted as an attenuation of the anti-inflammatory response (Peters et al., 2001a). However, IL-6 values were not reported. Furthermore, in an earlier study these authors showed that vitamin C supplementation (1000 mg/day) for 7 days before and 2 days following a 90 km run resulted in an elevated CRP response compared with a placebo which is consistent with a pro-inflammatory response (Peters et al., 2001b). The possibility that IL-6 might be an anti-inflammatory cytokine

has caused some to question the benefit of a vitamin supplementation regimen that blunts the normal IL-6 response because it might compromise the immune system (Fischer et al., 2004). Neither IL-1ra or IL-10 were measured here so it is difficult to compare and contrast the current results with these previous studies. In the present study it is important to note that in addition to having a blunted IL-6 response to exercise the cherry juice group had a more rapid recovery of knee extension strength compared with placebo. The combination of improved function and lower values for various blood markers (IL-6, CRP, uric acid, TBARS) is consistent with an accelerated recovery in the cherry juice group.

Elevations in uric acid after prolonged endurance exercise have been shown consistently (Duthie et al., 1990; Rokitzki et al., 1994; Kratz et al., 2002; Rønsen et al., 2004), but the mechanism is not well understood. Increased uric acid after Marathon running may reflect (1) decreased clearance of uric acid, secondary to dehydration (Suzuki et al., 2006), (2) increased mobilization of uric acid as part of the antioxidant defences (Mastaloudis et al., 2004; Rietjens et al., 2007) or (3) increased production of uric acid as part of the inflammatory process (Kondo et al., 2005). Post-race weight loss was not different between the cherry juice and placebo groups (1.2 ± 1.3 vs 1.7 ± 1.5 kg, $P = 0.49$), therefore, it is unlikely that the differences in uric acid were attributable to dehydration effects. Previous studies have attributed exercise-induced uric acid elevations to antioxidant defence mechanisms (Mastaloudis et al., 2004; Rietjens et al., 2007). Furthermore, uric acid infusion has been shown to increase total antioxidant capacity and decrease exercise-induced oxidative stress (Waring et al., 2003). However, the cherry juice group had an increased total antioxidant capacity and decreased oxidative stress in the presence of lower uric acid levels compared with the placebo group. Therefore, the uric acid changes in response to running a Marathon were not reflective of an enhanced antioxidant defence. A third interpretation is that uric acid elevations reflected the inflammatory response to running a Marathon. CRP is correlated with uric acid in healthy subjects (Kondo et al., 2005). In the present study detectable levels of CRP were not evident until 24 and 48 h post-Marathon. CRP at 24 and 48 h was correlated with uric acid levels ($r = 0.57$, $P = 0.009$ and $r = 0.45$, $P = 0.048$, respectively) lending some support to the conclusion the uric acid elevations in response to running a Marathon reflect the inflammatory response.

In previous studies TBARS were not elevated immediately after or 24 h after a Marathon (Rokitzki et al., 1994) or at any time point up to 5 days after a half-Marathon (Duthie et al., 1990). In contrast,

following 3 weeks of multivitamin–mineral supplementation an elevation in TBARS was reported in the placebo, but not the treatment group, at 72 h during a 7-day foot race (Marathon des Sables) (Machefer et al., 2007). In the present study, TBARS was not elevated immediately post-Marathon or at 24 h. However, at 48 h a marked increase in TBARS was apparent in the placebo group but not in the cherry juice group, which shows a similar response in the elevation of TBARS following extended endurance activity (Machefer et al., 2007). It is notable in the present study that the increase in TBARS in the placebo group occurred when total antioxidant capacity had fallen below baseline (89%). This indicates that normal antioxidant defences may only be effective at preventing oxidative stress up to 24 h after Marathon running and that after that point an augmentation in antioxidant capacity was required to prevent oxidative stress. Consistent with this summation, TAS at 48 h (percentage of baseline) was negatively correlated ($r = -0.49$, $P = 0.03$) with TBARS at 48 h (percentage of baseline).

While previous studies have measured changes in TBARS as a marker of lipid peroxidation in response to a half Marathon (Duthie et al., 1990), a Marathon (Rokitzki et al., 1994) and an ultraendurance event (Machefer et al., 2007) it is recognized that TBARS lacks specificity (Urso & Clarkson, 2003). F_2 -isoprostanes is perhaps a more specific marker of lipid peroxidation that has been examined in ultramarathon events (Mastaloudis et al., 2004; McAnulty et al., 2007). Antioxidant supplementation was shown to reduce the elevation in F_2 -isoprostanes after a 50 km race, but uric acid, CRP and IL-6 were unaffected by antioxidant supplementation (Mastaloudis et al., 2004).

PC has been shown to be elevated after a 160 km run (McAnulty et al., 2007), a soccer match (Ispiridis et al., 2008) and a 30-min running (Bloomer et al., 2006). Surprisingly, in this investigation, there was no evidence of protein oxidation (based on PC data) after the Marathon despite obvious signs of inflammation and muscle damage.

The effects of the tart cherry juice on markers of muscle damage, inflammation and oxidative stress demonstrated in this study are most likely attributable to the numerous different phytochemicals in tart cherries that have been shown to have antioxidant and anti-inflammatory properties (Wang et al., 1999; Seeram et al., 2001). The high-ORAC value for the cherry juice (55 mmol/L Trolox equivalents) compared with values reported for other available juices (ranging from 31.7 to 9.1 mmol/L Trolox equivalents) (Seeram et al., 2008) indirectly indicated that the antioxidant phytochemicals in tart cherries were unlikely to have been degraded by the processing and storage procedures, although it remains to

be determined if similar effect would be evident using a concentrate.

Although it is likely that muscle damage, inflammation and oxidative stress are important factors in the adaptation process, minimizing these factors in response to strenuous or prolonged exercise may be important to the recovery process when subsequent training and performance can be inhibited. For example, the IL-6 response to the same bout of exercise is more prolonged during a period of high-intensity training compared with a period of low-intensity training (Rønsen et al., 2001). Similarly, a dramatic increase in training load to simulate overreaching was shown to increase TBARS in rats (Zoppi & Macedo, 2008). Numerous studies have examined the effects of various nutritional interventions with antioxidants on markers of muscle damage, inflammation and oxidative stress. While some studies have demonstrated reductions in markers of oxidative stress (Itoh et al., 2000; Mastaloudis et al., 2004; Kingsley et al., 2005; Bloomer et al., 2006; Machefer et al., 2007) and muscle damage (Rokitzki et al., 1994; Itoh et al., 2000) other studies have shown no effect on markers of oxidative stress (Rokitzki et al., 1994; Kaikkonen et al., 1998; Dawson et al., 2002) or muscle damage (Kaikkonen et al., 1998; Peters et al., 2001b; Dawson et al., 2002; Kingsley et al., 2005; Mastaloudis et al., 2006; Machefer et al., 2007). Additionally, antioxidant interventions have generally shown to have little or no positive effect with regards to elevations in markers of inflammation in response to prolonged running (Castell et al., 1997; Kaikkonen et al., 1998; Peters et al., 2001b; Mastaloudis et al., 2004; Machefer et al., 2007). However, reductions in neutrophilia and IL-8 were evident following glutamine supplementation (Castell & Newsholme, 1997) and vitamin C supplementation was shown to decrease IL-6 following 90 min shuttle running (Thompson et al., 2001), although there was no effect of vitamin C supplementation on CRP, uric acid or markers of muscle damage, and markers of oxidative stress were not examined. In the present study clear reductions in IL-6, CRP and uric acid were evident with the cherry juice intervention. These findings have important practical significance for distance runners considering that the inflammatory response to prolonged endurance exercise (particularly IL-6) has been linked to delayed recovery (Neubauer et al., 2008).

The 8-day supplementation period in this study is markedly shorter than most investigations examining the effects of dietary interventions on markers of exercise stress. For example, reductions in markers of oxidative stress and/or muscle damage following ultraendurance running events (Mastaloudis et al., 2004; Machefer et al., 2007), a Marathon race (Rokitzki et al., 1994) or repeated days of running

(Itoh et al., 2000) occurred with 21–32 days supplementation. The optimal dosage and supplementation period for this cherry juice remains to be determined but it is apparent that marked effects can be achieved with a relatively short duration of supplementation.

In conclusion, when compared with a placebo, cherry juice taken for 5 days before, the day of and for 2 days after running a Marathon was effective at accelerating recovery of strength, increasing total antioxidant capacity, minimizing lipid peroxidation and reducing IL-6, CRP and uric acid. This represents a broad spectrum of aiding recovery and providing protection against the inflammation and oxidative stress that is associated with prolonged running.

Perspectives

Various strategies are routinely used to accelerate recovery following strenuous physical activity. Previous work (Connolly et al., 2006) has suggested that tart cherry juice accelerates recovery following muscle damaging exercise, which was speculated to be attributable to the anti-inflammatory and antioxidant phytochemicals contained within the juice. The current investigation supports the supposition of Connolly et al. (2006) and has demonstrated that the cherry juice reduced oxidative stress and inflam-

mation and hence increases the rate of recovery. Tart cherry juice appears to provide a feasible alternative to pharmaceutical and therapeutic interventions in aiding recovery following such exercise; in addition, it may also prove useful where a number of strenuous exercise bouts are required within a relatively short period of time. Furthermore the marked reductions in inflammation afforded by tart cherry juice may also have implications for the management of other clinical pathologies that display inherently high levels of inflammation and oxidative stress, although this remains to be elucidated.

Key words: recovery, inflammation, muscle damage, antioxidants, Montmorency cherries.

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References

- Bloomer RJ, Goldfarb AH, McKenzie MJ. Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Med Sci Sports Exerc* 2006; 38: 1098–1105.
- Castell LM, Newsholme EA. The effects of oral glutamine supplementation on athletes after prolonged, exhaustive exercise. *Nutrition* 1997; 13: 738–742.
- Castell LM, Poortmans JR, Leclercq R, Brasseur M, Duchateau J, Newsholme EA. Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. *Eur J Appl Physiol Occup Physiol* 1997; 75: 47–53.
- Connolly DA, McHugh MP, Padilla-Zakour OI. Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *Br J Sports Med* 2006; 40: 679–683.
- Dawson B, Henry GJ, Goodman C, Gillam I, Beilby JR, Ching S, Fabian V, Dasig D, Morling P, Kakulus BA. Effect of Vitamin C and E supplementation on biochemical and ultrastructural indices of muscle damage after a 21 km run. *Int J Sports Med* 2002; 23: 10–15.
- Duthie GG, Robertson JD, Maughan RJ, Morrice PC. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch Biochem Biophys* 1990; 282: 78–83.
- Fallon KE. The acute phase response and exercise: the ultramarathon as prototype exercise. *Clin J Sport Med* 2001; 11: 38–43.
- Fischer CP, Hiscock NJ, Penkowa M, Bassu S, Vessby B, Kallner A, Sjöberg LB, Pedersen BK. Supplementation with vitamin C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. *J Physiol* 2004; 558: 633–645.
- Howatson G, van Someren KA. Evidence of a contralateral repeated bout effect after maximal eccentric contractions. *Eur J Appl Physiol* 2007; 101: 207–214.
- Howatson G, van Someren KA. The prevention and treatment of exercise-induced muscle damage. *Sports Med* 2008; 38: 483–503.
- Ispiridis I, Fatouros IG, Jamurtas AZ, Nikolaidis MG, Michailidis I, Douroudos I, Margonis K, Chatzinikolaou A, Kalistratos E, Ktrabakas I, Alexiou V, Taxildaris K. Time-course of changes in inflammatory and performance responses following a soccer game. *Clin J Sport Med* 2008; 18: 423–431.
- Itoh H, Ohkuwa T, Yamazaki Y, Shimoda T, Wakayama A, Tamura S, Yamamoto T, Sato Y, Miyamura M. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. *Int J Sports Med* 2000; 21: 369–374.
- Jacob RA, Spinozzi GM, Simon VA, Kelley DS, Prior RL, Hess-Pierce B, Kader AA. Consumption of cherries lowers plasma urate in healthy women. *J Nutr* 2003; 33: 1826–1829.
- Kaikkonen J, Kosonen L, Nyssönen K, Porkkala-Sarataho E, Salonen R, Korpelan H, Salonen JT. Effect of combined coenzyme Q10 and D-alpha-tocopheryl acetate supplementation on exercise-induced lipid peroxidation and muscular damage: a placebo-controlled double-blind study in marathon runners. *Free Radic Res* 1998; 29: 85–92.
- Kang SY, Seeram NP, Nair MG, Bourquin LD. Tart cherry anthocyanins inhibit tumor

- development in Apc (Min) mice and reduce proliferation of human colon cancer cells. *Cancer Lett* 2003; 194: 13–19.
- Kelley DS, Rasooly R, Jacob RA, Kader AA, Mackey BE. Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. *J Nutr* 2006; 136: 981–986.
- Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur J Appl Physiol* 2007; 99: 443–447.
- Kingsley MI, Wadsworth D, Kilduff LP, McEneny J, Benton D. Effects of phosphatidylserine on oxidative stress following intermittent running. *Med Sci Sports Exerc* 2005; 37: 1300–1306.
- Kondo N, Nomura M, Nakaya Y, Ito S, Ohguro T. Association of inflammatory marker and highly sensitive C-reactive protein with aerobic exercise capacity, maximum oxygen uptake and insulin resistance in healthy middle-aged volunteers. *Circ J* 2005; 69: 452–457.
- Kratz A, Lewandowski KB, Siegel AJ, Chun KY, Flood JG, Van Cott EM, Lee-Lewandowski E. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *Am J Clin Pathol* 2002; 118: 856–863.
- Machefer G, Groussard C, Vincent S, Zouhal H, Faure H, Cillard J, Radák Z, Gratas-Delamarche A. Multivitamin-mineral supplementation prevents lipid peroxidation during “the Marathon des Sables”. *J Am Coll Nutr* 2007; 26: 111–120.
- Mastaloudis A, Morrow JD, Hopkins DW, Devaraj S, Traber MG. Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. *Free Radic Biol Med* 2004; 36: 1329–1341.
- Mastaloudis A, Traber MG, Carstensen K, Widrick JJ. Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Med Sci Sports Exerc* 2006; 38: 72–80.
- McAnulty S, McAnulty L, Nieman D, Morrow J, Dumke C, Henson D. Effect of NSAID on muscle injury and oxidative stress. *Int J Sports Med* 2007; 28: 909–915.
- Neubauer O, König D, Wagner KH. Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress. *Eur J Appl Physiol* 2008; 104: 417–426.
- Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* 1999; 515: 287–291.
- Peters EM, Anderson R, Mieman DC, Fickl H, Jogessar V. Vitamin C supplementation attenuates the increase in circulating cortisol, adrenaline and anti-inflammatory polypeptides following ultramarathon running. *Int J Sports Med* 2001a; 22: 537–543.
- Peters EM, Anderson R, Theron AJ. Attenuation of increase in circulating cortisol and enhancement of the acute phase protein response in vitamin C-supplemented ultramarathoners. *Int J Sports Med* 2001b; 22: 120–126.
- Pizza FX, Koh TJ, McGregor SJ, Brooks SV. Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *J Appl Physiol* 2002; 92: 1873–1878.
- Rietjens SJ, Beelen M, Koopman R, Van Loon LJ, Bast A, Haenen GR. A single session of resistance exercise induces oxidative damage in untrained men. *Med Sci Sports Exerc* 2007; 39: 2145–2151.
- Rokitzi L, Logemann E, Sagredos AN, Murphy M, Wetzel-Roth W, Keul J. Lipid peroxidation and antioxidative vitamins under extreme endurance stress. *Acta Physiol Scand* 1994; 151: 149–158.
- Rønsen O, Børsheim E, Bahr R, Klarlund Pedersen B, Haug E, Kjeldsen-Kragh J, Høstmark AT. Immuno-endocrine and metabolic responses to long distance ski racing in world-class male and female cross-country skiers. *Scand J Med Sci Sports* 2004; 14: 39–48.
- Rønsen O, Holm K, Staff H, Opstad PK, Pedersen BK, Bahr R. No effect of seasonal variation in training load on immuno-endocrine responses to acute exhaustive exercise. *Scand J Med Sci Sports* 2001; 11(3): 141–148.
- Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, Dreher M, Heber D. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *J Agric Food Chem* 2008; 56: 1415–1422.
- Seeram NP, Momin RA, Nair MG, Bourquin LD. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* 2001; 8: 362–369.
- Smith JE, Garbutt G, Lopes P, Pedoe DT. Effects of prolonged strenuous exercise (marathon running) on biochemical and haematological markers used in the investigation of patients in the emergency department. *Br J Sports Med* 2004; 38: 292–294.
- Starkie RL, Rolland J, Angus DJ, Anderson MJ, Febbraio MA. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Physiol Cell Physiol* 2001; 280: C769–C774.
- Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Metab* 2003; 285: E433–E437.
- Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, Sugawara K. Impact of a competitive marathon race on systemic cytokine and neutrophil responses. *Med Sci Sports Exerc* 2003; 35: 348–355.
- Suzuki K, Peake J, Nosaka K, Okutsu M, Abbiss CR, Surriano R, Bishop D, Quod MJ, Lee H, Martin DT, Laursen PB. Changes in markers of muscle damage, inflammation and HSP70 after an Ironman Triathlon race. *Eur J Appl Physiol* 2006; 98: 525–534.
- Tall JM, Seeram NP, Zhao C, Nair MG, Meyer RA, Raja SN. Tart cherry anthocyanins suppress inflammation-induced pain behavior in rat. *Behav Brain Res* 2004; 153: 181–188.
- Thompson D, Williams C, McGregor SJ, Nicholas CW, McArdle F, Jackson MJ, Powell IR. Prolonged vitamin C supplementation and recovery from demanding exercise. *Int J Sport Nutr Exerc Metab* 2001; 11: 466–481.
- Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 2003; 189: 41–54.
- Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JJ, DeWitt DL. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *J Nat Prod* 1999; 62: 294–296.
- Waring WS, Convery A, Mishra V, Shenkin A, Webb DJ, Maxwell SR. Uric acid reduces exercise-induced oxidative stress in healthy adults. *Clin Sci (London)* 2003; 105: 425–430.
- Weight LM, Alexander D, Jacobs P. Strenuous exercise: analogous to the acute-phase response? *Clin Sci (London)* 1991; 81: 677–683.
- Zoppi CC, Macedo DV. Overreaching-induced oxidative stress, enhanced HSP72 expression, antioxidant and oxidative enzymes downregulation. *Scand J Med Sci Sports* 2008; 18: 67–76.