Antioxidant supplementation does not attenuate exercise-induced cardiac troponin release

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Clinically, cardiac troponins (cTn) are used as sensitive markers of cardiomyocyte damage [1,2] with any elevation in cTn being related to poor prognosis [3]. Recently, however, exercise has also been shown to stimulate the release of cTn [4–6]. The mechanism responsible for exercise-induced cTn release is not known, and is currently a matter of debate [7]. Notwithstanding this, it has been proposed, due to the relatively low post-exercise cTn concentrations and its rapid clearance, that cTn is likely released from the cytosolic pool and not from the breakdown of contractile apparatus. Previous authors [8] have suggested that oxidative stress associated with prolonged exercise may damage the cardiomyocyte membrane, resulting in cTn release from the cytosol. Antioxidant supplementation has been shown to attenuate oxidative stress, inflammation and muscle damage indices following strenuous exercise [9]; therefore, if post-exercise cTn release is related to cardiomyocyte membrane damage we hypothesised that antioxidant supplementation would reduce cTn release following marathon running.

The institutional ethics committee approved all procedures [11]. Following health-screening and gaining consent, 16 marathon runners (11 male and 5 female), a subset from a previous study [9], free from nutritional supplements and medication, volunteered to participate. Runners were assigned to either a placebo or antioxidant group in a double-blind, randomised fashion. Antioxidant or placebo supplementation was administered each day from 5 days prior to 48 h following the marathon. Indices of cardiac damage (cTnI), inflammation (IL-6 and C-RP), muscle damage (CK), and total antioxidative capacity (TAC) were taken before supplement (TAC only), before the race, immediately post, 24 and 48 h following completion of the marathon.

The antioxidant supplement (tart cherry juice, Cherrypharm, Inc, Geneva, NY) has previously been shown to reduce skeletal muscle damage [10], reduce lipid peroxidation and aid recovery following running [9]. Two servings (morning and afternoon) of approximately 225 mL (equating to ~50–60 cherries) were consumed per day; the juice contained ~600 mg of phenolic compounds such as anthocyanins and other flavonoids (quercetin, kaempferol and isoramnetin) and ~55 mmol L^{-1} Trolox equivalents [9,10]. The placebo group ingested two servings of 225 mL per day of a fruit flavoured drink matched for appearance, but lacking the phytonutrient content of the antioxidant supplement.

At each of the sampling points venous blood was drawn from an ante-cubital vein; samples were then spun and serum immediately frozen (−80 °C) for later analysis. cTnI was assessed using the

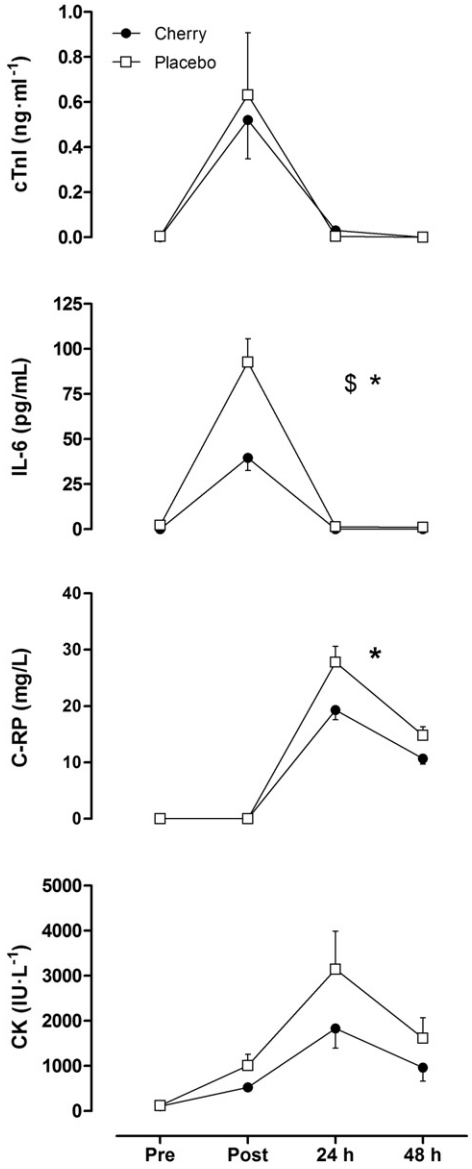

Fig. 1. Cardiac troponin (cTnI), interleukin 6 (IL-6), C-reactive protein (C-RP) and creatine kinase (CK) concentrations pre and post the marathon race. * denotes significant group effect; $ denotes a significant interaction immediately post-marathon. Values are means ± SE for 16 participants.

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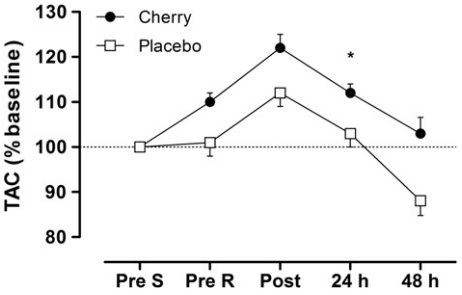

Fig. 2. Total oxidative capacity (TAC) pre-supplementation (Pre S), pre-race (Pre R) and post-marathon race. * denotes significant group effect. Values are means ± SE for 16 participants.

TnI-Ultra assay (ADVIA Centaur, Siemens Healthcare Diagnostics). The detection limit of the instrument was 0.006 μg L^{-1} and the upper limit was 50 μg L^{-1}. Serum IL-6 was determined by immunoassay ELISA technique (Quantikine, R&D Systems Europe Ltd.); normal reference ranges are reported at <3 pg mL^{-1}; intra- and inter-assay CV was <5%. Serum CRP and CK concentrations were determined using an automated analyzer (c8000, Abbott Architect, IL, USA); normal reference values for this assay are reported at <0.8 mg L^{-1} and 29–200 IU L^{-1}, respectively, with an intra-assay CV of ≤3.7%. TAC was assessed using a colorimetric assay kit (Randox Laboratories, Ltd., Antrim, UK) that was run on the aforementioned analyser; intra-assay reliability was reported as a CV of <3%.

Differences between group characteristics were examined using an independent samples Student's *t*-test. All dependent variables were analysed using a treatment by time repeated measures ANOVA and *post-hoc* analysis, where necessary. All data analyses were conducted using SPSS for windows, v16. Alpha was set at 0.05.

All participants completed the marathon and there was no significant difference in finish time between groups (3 h 36 min vs. 3 h 43 min). All dependant variables showed significant time effects ($P<0.001$). Concentrations of serum cTnI, IL-6, C-RP and CK were similar at baseline between groups (Fig. 1). TAC was significantly higher in the antioxidant group following supplementation by ~10% ($P=0.007$; Fig. 2). The post-exercise increase in cTnI did not differ between groups and returned to baseline 24 h post-exercise. Serum IL-6 showed a significant group and interaction effect ($P\leq0.003$); *post-hoc* analysis revealed smaller elevations in IL-6 (39.7 vs. 92.8 pg mL^{-1}) for the antioxidant group immediately post-race ($P<0.001$). Serum C-RP increased 24 h post-exercise in both groups, but was lower in the antioxidant group ($P=0.019$). Serum CK rose in both groups; no interaction or group effects were observed.

In agreement with previous studies [9,10] the antioxidant supplementation raised TAC and reduced markers of inflammation (IL-6 and C-RP). Despite an enhanced TAC following supplementation, and a related reduction in post-exercise inflammation, no difference in cTnI release was observed between groups. These data suggest that the mechanisms responsible for the release of cTn following prolonged exercise are unlikely related to exercise-induced oxidative stress and inflammation damaging the cardiomyocyte membrane.

Our data challenge those of Nie et al [8] who, using an animal model, suggested a temporal relationship between cTn release and lipid peroxidation in cell membranes (myocardial malondialdehyde; MDA). However, Nie et al. [8] noted that the temporal association they observed does not equal causality, and it seems plausible that the release of cTn and MDA in their study may have been coincidental. Further, Nie et al [8] also acknowledged limitations with the method for the assessment of MDA and as such their conclusions must be treated with caution. Whilst our data do not support oxidative stress as a mechanism for exercise induced cTnI release, they do not provide further insight into other potential mechanisms.

Nevertheless, both the time course and magnitude of cTnI release in the present study is similar to that previously reported [5]. The relatively low cTnI concentrations post-exercise and the normalisation within 24 h of exercise completion are consistent with cytosolic release and not breakdown of the contractile apparatus. As we have previously suggested [7], the integrity of the cardiomyocyte membrane might be compromised by mechanical stress associated with sustained elevations in heart rate, the formation of membrane blebs or stimulation of integrins. Further work is required to investigate these theories, as well as the clinical or physiological significance of post-exercise cTn release. In conclusion, increased TAC related to antioxidant supplementation does not attenuate post-exercise cTnI release. Accordingly, it seems the mechanism responsible for exercise-induced cTn release may not be related to oxidative damage to the cardiomyocyte membrane.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology (Shewan and Coats 2010; 144:1-2).

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