Ref. #12

Vitamin C Supplementation Attenuates the Increases in Circulating Cortisol, Adrenaline and Anti-Inflammatory Polypeptides Following Ultramarathon Running

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The effects of vitamin C supplementation on the alterations in the circulating concentrations of cortisol, adrenaline, interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1Ra) which accompany ultramarathon running were measured using immuno-chemiluminescence, radioimmunoassay and ELISA procedures. Forty-five participants in the 1999 Comrades 90 km marathon were divided into equal groups (n = 15) receiving 500 mg/day Vit C (VC-500), 1500 mg/day Vit C (VC-1500) or placebo (P) for 7 days before the race, on the day of the race, and for 2 days following completion. Runners recorded dietary intake before, during and after the race and provided 35 ml blood samples 15–18 hrs before the race, immediately post-race, 24 hrs post race and 48 hrs post-race. Twenty-nine runners (VC-1500, n = 12; VC-500, n = 10; P, n = 7) complied with all study requirements. All post-race concentrations were adjusted for plasma volume changes. Analyses of dietary intakes and blood glucose and anti-oxidant status on the day preceding the race and the day of the race did not reveal that carbohydrate intake or plasma vitamins E and A were significant confounders in the study. Mean pre-race concentrations of serum vitamin C in VC-500 and VC-1500 groups (128 ± 31 and 153 ± 34 μmol/l) were significantly higher than in the P group (83 ± 39 μmol/l). Immediate post-race serum cortisol was significantly lower in the VC-1500 group ($p < 0.05$) than in P and VC-500 groups. When the data from VC-500 and P groups was combined (n = 17), immediate post-race plasma adrenaline, IL-10 and IL-1Ra concentrations were also significantly lower ($p < 0.05$) in the VC-1500 group. The study demonstrates an attenuation, albeit transient, of both the adrenal stress hormone and anti-inflammatory polypeptide response to prolonged exercise in runners who supplemented with 1500 mg vitamin C per day when compared to ≤ 500 mg per day.

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Introduction

We have previously reported that vitamin C supplementation reduces the incidence of post-race upper respiratory tract infections amongst ultramarathon runners [21,22]. In a more recent study we observed that supplementation with 1000 mg of the vitamin over an 8 day period resulted in an average 30% reduction in post-race serum cortisol levels in these athletes [20]. We proposed that the vitamin C-associated decrease in serum cortisol might result from inhibition of enzymes involved in steroidogenesis [9,18,24]. Alternatively, because cortisol release from the adrenals may be coupled to concomitant release of vitamin C during oxidative stress [18], it is possible that supplementation with the vitamin may negate the requirement for its mobilization from body stores, with a consequent, albeit secondary, attenuation of the cortisol response [17]. Irrespective of the biochemical mechanisms involved, the apparent vitamin C-associated attenuation of the cortisol response to strenuous exercise has potentially important implications for the prevention of transient immune dysfunction in athletes.

In the current study we have again assessed the effects of oral administration of vitamin C, at different doses to those used in our previous study [20], on the increase in circulating cortisol which accompanies ultramarathon running. Moreover, we have extended our previous study to include measurements of circulating adrenaline, interleukin-10 (IL-10) and the interleukin-1 receptor antagonist (IL-1Ra).

Methods

Study design

Approval to conduct the study was obtained from the Human Ethics Committee of the University of Natal Medical School. Forty-five registered entrants for the 1999 Comrades Marathon signed informed consent forms. They were divided into three groups which were matched for age, gender, training status and expected race finishing time:

Table 1 Mean (± SD) subject characteristics (n = 29)

	Age (years)	Stature (m)	Mass (kg)	BMI (kg/m²)	Weekly training distance (km/wk)	Race time (hours)
Placebo group (n = 7)	39.6 (±7.0)	1.77 (±0.10)	70.8 (±11.6)	22.5 (±2.9)	77.9 (±26.4)	9.85 (±1.17)
VC-500 group (n = 10)	40.9 (±9.2)	1.72 (±0.07)	69.3 (±10.7)	23.4 (±2.8)	92.0 (±30.9)	9.65 (±1.15)
VC-group (n = 12)	38.7 (±5.1)	1.74 (±0.08)	71.1 (±12.4)	23.4 (±2.5)	85.0 (±22.3)	9.60 (±0.70)

Table 2 Mean (± SD) dietary carbohydrate (CHO) intakes and plasma concentrations of glucose and vitamins A and E on the day preceding the race and day of the race

	CHO (g)	Plasma glucose (mmol/l)	Plasmma vitamin E (μmol/l)	Plasma vitamin A (μmol/l)
Day preceding the race				
Placebo group (n = 7)	399 (±77)	4.69 (±0.79)	17.0 (±3.8)	2.40 (±0.42)
VC-500 group (n = 10)	499 (±162)	4.95 (±1.05)	21.1 (±4.9)	2.57 (±0.47)
VC-1500 group (n = 12)	482 (±146)	4.74 (±0.68)	20.7 (±5.7)	2.29 (±0.49)
Day of the race				
Placebo group (n = 7)	315 (±145)	6.14 (±1.50)	16.9 (±3.9)	2.21 (±0.51)
VC-500 group (n = 10)	353 (±93)	6.47 (±1.61)	20.7 (±4.8)	2.51 (±0.54)
VC-1500 group (n = 12)	488 (±227)	5.95 (±1.18)	21.2 (±6.4)	2.27 (±0.60)

Results

Subjects

Of the 45 runners recruited to the study only 29 fully complied with the protocol requirements. The characteristics of the individuals in the P, VC-500 and VC-1500 groups are shown in Table **1**. There were no significant differences between the three groups with respect to age, height, mass, body mass index, training status, and time taken to complete the ultramarathon. Carbohydrate intake just prior to and during the race averaged 401 (± 188) g and did not differ significantly between the groups ($p > 0.05$; Table **2**). Likewise, pre- and post-race plasma glucose, vitamin A and vitamin E concentrations were not different between the 3 groups ($p > 0.05$; Table **2**). Total mean Vitamin C intake on the day preceding the race (contained in supplements, beverages and foodstuffs ingested) amounted to 94.4 (± 60.4), 650 (± 102) and 1603 (± 90) mg in P, VC-500 and VC-1500 groups, respectively (data not shown).

Serum vitamin C

Pre-race serum vitamin C was significantly higher in the supplemented groups by comparison with the P group (Fig. **1**). There was also a significant increase ($\bar{X}$= 42.6 μmol/l) in serum vitamin C in the P group immediately post-race ($p < 0.05$). This increase in the mean serum vitamin C was attenuated in both of the vitamin supplemented groups (19.3 and -2.84 μmol/l in VC-500 and VC-1500 groups, respectively). At 24 and 48 hrs after completion of the race the serum vitamin C concentrations returned to values which were not significantly different ($p > 0.05$) from pre-race values.

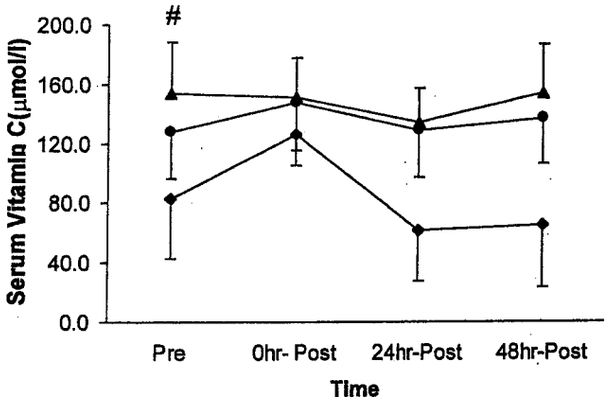

Fig. 1 Pattern of change in mean serum vitamin C concentrations before and after the 1999 Comrades 90 km ultramarathon in ♦placebo, ● VC-500 and ▲ VC-1500 groups. Data presented as means ± SD. Time effect: $p < 0.001$; group vs. time interaction effect: $p = 0.27$, group effect: $p = 0.006$, # $p < 0.05$ Bonferroni multiple comparison test between groups at time point.

Blood counts

Results of the full blood counts are shown in Table **3**. Packed cell volume and hemoglobin values indicated a varied hydration status with 27.5% presenting with an increase in plasma volume immediately following participation in the ultramarathon. The difference in plasma volume did not differ significantly between the groups. Significant immediate post-race lymphopenia and neutrophilia was present in all 3 groups with recovery to normal values at 24 and 48 hrs after completion of the race. The smaller relative magnitude of the lymphopenia and neutrophilia, as expressed in the neutrophil:lymphocyte

Table 4 Mean (± SD) stress hormone and anti-inflammatory polypeptide concentrations

	Pre-race	Post-race* (0.5–1hr)	Post-race* (24 hours)	Post race* (48 hours)	Time effect; interaction effect; group effect***
Serum Cortisol (nmol/l)					
P Group	347 (±110)	1179 (±247)	323 (±158)	329 (±188)	P < 0.001
VC-500	260 (±106)	1205 (±308)	300 (±83.2)	284 (±85.5)	P = 0.003
VC-1500	248 (±114)	770 (±224)#	262 (±75.5)	329 (±161)	P = 0.02
Plasma Adrenaline (pg/ml)					
P Group	93.4 (±44.5)	204 (±129)			
VC-500	140 (±155)	257 (±188)	ND	ND	
VC-1500	56 (±31)**	120 (±74)**			
Plasma Interleukin-10 (pg/ml)					
P Group	0.46 (±0.68)	83.1 (±60.7)	0.61 (±0.60)	0.62 (±0.79)	P < 0.001
VC-500	0.56 (±0.76)	69.6 (±59.8)	0.91 (±1.07)	0.80 (±0.84)	P = 0.001
VC-1500	0.35 (±0.52)	31.5 (±30.5)#	0.39 (±0.50)	0.30 (±0.23)	P = 0.01
Plasma Interleukin-1Ra (pg/ml)					
P Group	176 (±57.8)	2850 (±2872)	320 (±78.0)	249 (±63)	P < 0.001
VC-500	184 (±78.5)	4241 (±3322)	439 (±201)	327 (±178)	P = 0.07
VC-1500	193 (±53.5)	1519 (±1373)#	282 (±85.5)	288 (±87)	P = 0.04

# $p < 0.05$ Bonferroni multiple comparison test between groups at time point
* adjusted for plasma volume changes from pre-race
** $p < 0.05$ vs. P/500 group; Students t-test, adjusted for base-line values
*** Repeated measures ANOVA
ND = not done

[20]. In the current study we have investigated the effects of vitamin C supplementation, at different doses (500 mg and 1500 mg/daily) to those used in our previous study [20], on the cortisol response which accompanies participation in the same 90 km ultramarathon and included measurements of circulating adrenaline and those of the anti-inflammatory polypeptides, IL-10 and the IL-1Ra in an extension of this study.

As previously reported by us and others [6,10,20], vitamin C levels were increased in the placebo group on completion of the ultramarathon and subsided at 24 and 48 hrs thereafter. This apparent mobilization of vitamin C appears to represent an adaptive response to exercise-induced oxidative stress [6]. Pre-race serum vitamin C values and those measured 24 and 48hrs after completion of the race were significantly higher in the vitamin-supplemented groups. Interestingly, the difference in serum vitamin C between the placebo (51.4% higher than the pre-race value) and vitamin-supplemented groups was considerably less and statistically insignificant immediately post-race. The corresponding average changes in the VC-500 and VC-1500 groups were 13.0% and –0.02% respectively. These observations confirm our previous findings [20] that supplementation with vitamin C appears to negate the requirement for mobilization of the vitamin from the adrenal gland and other body storage sites during intensive physical stress [5].

In agreement with our previous study [20], administration of vitamin C at 1500 mg/daily, but not at 500 mg/daily, significantly attenuated (average decrease of 34.7% relative to P group) the immediate post-race increase in serum cortisol. Pre-race concentrations of serum cortisol, as well as those measured at 24 and 48 hrs after completion of the ultramarathon event, were somewhat lower, although not significantly so, in both vitamin-supplemented groups relative to the P group. These observations are also in agreement with a recent report in which administration of vitamin C (1000 mg/daily) in combination with vitamin E to healthy, elderly humans was accompanied by a significant decrease in serum cortisol and improved immune function [7] and confirm previous findings on animals [11,14,18,24].

Although blood sampling for adrenaline concentrations should ideally have been performed immediately on completion of the race, this was not logistically possible in a competitive event of this nature. It is, however, noteworthy, that circulatory adrenaline concentrations were reduced significantly following a week of supplementation with Vitamin C both prior to and following the stressful competitive event when compared to those in the unsupplemented runners. The average decrease relative to the P group was of 40% and 41% respectively in the group of athletes supplemented with 1500 mg vitamin C daily, but not significantly lower ($p < 0.05$) in those supplemented with ≤ 500 mg/daily vitamin C.

It is possible that the observed vitamin C-related attenuation of the exercise-induced increase in circulating cortisol and adrenaline may, in part, explain the reported decrease in the incidence of upper respiratory infections in vitamin C-supplemented ultramarathon athletes. Both of these adrenal hormones possess potent anti-inflammatory, immunosuppressive properties and may impact on the magnitude of the post-exercise "open-window" period [19] with a delayed manifestation of actual symptoms of infection following varying incubation

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