Influence Of Vitamin C Supplementation On Oxidative And Salivary IgA Changes Following An Ultramarathon

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Keywords Exercise · Immune · Isoprostane · Lipid hydroperoxide · Running

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Introduction

Vitamin C (ascorbic acid) is a water-soluble vitamin found in the cytosol of cells and in the extracellular fluid. The benefits of vitamin C as a potential influence on host defense mechanisms and the immune system has generated great interest (Grimble 1997; Hughes 1999). The immune system is sensitive to the level of vitamin C intake, and supplementation of this nutrient alters many aspects of the human immune response (Anderson et al. 1980; Campbell et al. 1999; Jacob and Burri 1996). For example, Tanaka et al. (1994) reported that ascorbic acid derivatives in a culture of human peripheral blood lymphocytes acted as an immunostimulator of antibody production. Vitamin C also provides antioxidant protection as an aqueous phase peroxyl and oxygen radical scavenger and is concentrated in tissues and fluids with a high potential for radical generation (Jacob and Burri 1996).

Secretory immunoglobulins found on mucosal surfaces play a role in protection against microbial infection (Nieman and Nehlsen-Cannarella 1991). Saliva is a mucosal secretion that contains the immunoglobulin IgA. Although it has been proposed that vitamin C may increase salivary IgA (sIgA) values, this has not been confirmed in a study of healthy subjects (Anderson and

Van Wyk 1980). Heavy physical exertion suppresses sIgA (Gleeson 2000). The physiologic stress of prolonged, intense endurance exercise may be an appropriate condition to study the influence of vitamin C on sIgA.

Both sIgA concentration and secretion rate decrease following endurance exercise longer than 90 min in duration. Tomasi et al. (1982) were the first to report this in a study of elite cross-country skiers competing in 20- to 50-km races. Mackinnon et al. (1987) studied elite cyclists following 2 h of cycling at 90% of anaerobic threshold. They reported decreased sIgA concentration in both trained and untrained cyclists after exercise. Müns et al. (1989) observed a significant decrease in sIgA concentration in three males following a 31-km race. Steerenberg et al. (1997) demonstrated that salivary flow rate and total sIgA output, but not sIgA concentration, were reduced in 42 triathletes following an Olympic-distance triathlon race event in the Netherlands. The majority of the research indicates that the combination of high-intensity and prolonged-duration exercise is necessary before significant decreases in this immune parameter are detected (Mackinnon 1996). Few studies have investigated the influence of vitamin C on immune responses following intense endurance exercise. Vitamin C is highly concentrated in the adrenal cortex, and it has been hypothesized that vitamin C supplements may influence serum cortisol release and alterations in immune function following heavy exertion (Enwonwu et al. 1995; Kodama et al. 1994; Laney et al. 1990; Redmann et al.1995). In some animal models, vitamin C depletion has been associated with a significant increase in serum cortisol without an increase in adrenocorticotrophic hormone (ACTH) (Enwonwu et al. 1995; Redmann et al. 1995). In contrast, others have shown that vitamin C infusion enhances adrenal glucocorticoid production (Kodama et al. 1994) or has no effect on the serum cortisol response to ACTH (Cupps and Fauci 1982). Two studies involving ultramarathon runners have supported the hypothesis that vitamin C influences cortisol release (Nieman et al. 2000; Peters et al. 2001). However, these results may be spurious because the subjects in these two studies were not randomized to treatment groups, and carbohydrate intake was not controlled. It is not known whether vitamin C supplementation may affect post-exercise changes in sIgA through its influence on cortisol.

The production of reactive oxygen species (ROS) during exhaustive exercise is well established (Ashton et al. 1999; Niess et al. 2000; Sanchez-Quesada et al. 1998; Sen and Roy 2001; White at al. 2001). Several studies have indicated that vitamin C supplementation attenuates exercise-induced oxidative stress, but this is not a consistent finding (Ashton et al. 1999; Piercy et al. 2000; Rokitzki et al. 1994; Sanchez-Quesada et al. 1998; Thompson et al. 2001; White et al. 2001). ROS generation and antioxidant status may be linked to immune alterations following exercise, such as cell adhesion, inflammation, and lymphocyte proliferation. Conversely, certain immune changes may contribute to oxidative stress (Chen et al. 1998; Niess at al. 2000; Sen and Roy 2001; Vider et al. 2001). No data are currently available regarding how oxidative changes may influence the production of sIgA, although antioxidants such as vitamin C do play a role in preventing damage to immune cells (Hughes 1999).

The purpose of this study was to examine the influence of vitamin C compared to placebo supplementation on oxidative and sIgA changes in ultramarathoners following an 80-km race. To improve upon previous studies, subjects were randomized to treatment groups, and carbohydrate intake was carefully monitored. Other immune data from this study have been published elsewhere (Nieman et al. 2002).

Methods

Subjects

Ultramarathon runners were recruited through a letter of invitation prior to the April 7, 2001, Umstead Ultramarathon, in Raleigh, North Carolina. The Umstead Ultramarathon is conducted on a 16-km loop, which is run five times for the 80-km race. Male and female runners ranging in age from 20 to 70 years were accepted into the study if they had run at least one competitive ultramarathon, had trained and were capable of completing an 80-km race, and were willing to adhere to all aspects of the research design including randomized selection to either the vitamin C or placebo group. Informed consent was obtained from each subject, and the experimental procedures were in accordance with the policy statements of the institutional review board of Appalachian State University (ASU).

Research design

Four to six weeks prior to the ultramarathon race event, subjects reported to the ASU Human Performance Lab for orientation and measurement of body composition and cardiorespiratory fitness. Basic demographic and training data were obtained through a questionnaire. Runners agreed to avoid the use of large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), herbs, and medications, known to affect immune function, for 1 month prior to the race. Runners also agreed to avoid ingesting anti-inflammatory medications the day before or during the race. During orientation, a dietitian instructed the runners to follow a diet high in carbohydrate and moderate in vitamin C during the 7 days prior to the race event (through use of a food list) and to record dietary intake using a 7-day food record.

Body composition was assessed utilizing hydrostatic weighing, and maximal oxygen consumption ($\dot{V}O_{2max}$) was determined using a graded maximal protocol adapted for runners as described in earlier studies from our group (Nieman 2000; Nieman et al. 1997, 1998, 2001b). Oxygen uptake and ventilation were measured using the MedGraphics CPX metabolic system (MedGraphics, St. Paul, Mn., USA). Maximal heart rate (HR_{max}) was measured using a chest heart rate monitor (Polar Electro, Woodbury, N.Y., USA).

Subjects were randomized to vitamin C or placebo groups. During the 7-day period prior to the race, subjects ingested three 500-mg tablets of vitamin C or placebo each day (one at each meal). All tablets were administered in a double-blinded fashion, and compliance was verified with post-study surveys. Other than the carbohydrate beverage supplied by the research team, subjects avoided food or beverages containing calories or caffeine for 6 h prior to the race start.

On race day, 29 ultramarathon runners reported to the start area between 4:30 and 5:00 a.m. After sitting for 10–15 min, blood and saliva samples were collected. Subjects were weighed, and a chest heart rate monitor was attached to each runner. Runners

received carbohydrate beverages with or without vitamin C in a double-blinded fashion using a color code (according to the original randomization scheme). The carbohydrate-vitamin C beverage contained vitamin C at a concentration of 150 mg/l. As in earlier studies, the carbohydrate beverages, with or without vitamin C, were supplied by the Gatorade Sports Science Institute (Barrington, Ill., USA) (Nehlsen-Cannarella et al. 1997; Nieman 2000; Nieman et al. 1997, 1998; 2001b). Each runner ingested 750 ml of beverage and one 500-mg vitamin C or placebo tablet approximately 30 min prior to the start of the race (5:30 a.m). During the race, runners drank approximately 1 l of beverage each hour (60 g carbohydrate/h). Research assistants were positioned every 5 km (three aid stations on the 16-km loop) to deliver color-coded beverage bottles, which contained 500 ml fluid (with or without vitamin C). Runners ingested the fluid from two bottles per hour and also ate two to three carbohydrate gel packs per hour (each containing 25 g of carbohydrate). Runners agreed to avoid all other beverages and food before and during the race. The research assistants also recorded heart rates and ratings of perceived exertion (RPE, 6–20 scale) from each runner every 5 km.

After running 32 km, and then again after crossing the 80-km race finish line, blood and saliva samples were collected from subjects within 5 min. Secondary to extreme environmental conditions (see Results), some runners were unable to complete the race due to fatigue or the 12-h limit (imposed by the research team). Blood and saliva samples were collected from these runners if they ran at least 50 km. Subjects were weighed at 32 km and post-race. A post-race questionnaire completed by each runner verified compliance to all aspects of the research design by each runner.

Salivary samples

Unstimulated saliva was collected by expectoration into 15-ml plastic, sterilized vials for 4 min. Participants were urged to pass as much saliva as possible into the vials during the 4-min timed session. The saliva samples were frozen at −0°C until analysis. Saliva volume was measured to the nearest 0.1 ml, and saliva total protein was quantified using the Coomassie protein assay reagent, a modification of the Bradford Coomassie dye binding colorimetric method (Bradford 1976). Salivary IgA was measured by enzyme-linked immunosorbent assay according to the procedures adapted from the Hunter Immunology Unit (Royal Newcastle Hospital, Newcastle, NSW, Australia) (Gleeson et al 1999a, 1999b, 2000). The data were expressed as concentration of sIgA ($\mu g \cdot ml^{-1}$), concentration of sIgA relative to total protein concentration ($\mu g \cdot mg^{-1}$), and sIgA secretion rate ($\mu g \cdot min^{-1}$). Data on other immune measures are being published elsewhere (Nieman et al. 2002).

Blood measures and plasma volume

Blood samples were drawn from an antecubital vein with subjects in the seated position. Samples were centrifuged in sodium heparin tubes. The plasma was removed and stored at −80°C. Plasma cortisol was assayed using the competitive solid-phase ^{125}I radioimmunoassay (RIA) technique (Diagnostic Products, Los Angeles, Calif., USA). Plasma was analyzed spectrophotometrically for glucose (pre-run, 32 km and post-race). Plasma volume changes were estimated using the method of Dill and Costill (1974).

Oxidative measurements

Blood samples were immediately centrifuged at 4°C. The plasma was aliquoted into cryotubes and snap frozen in liquid nitrogen. Samples were stored at −80°C until analysis. F_2-isoprostane was analyzed by gas chromatography mass spectrometry (Morrow 2000). Lipid hydroperoxides (ROOH) were measured using a spectrophotometric kit provided by Caymen Chemicals (Cat. no. 705002).

Statistical analysis

Statistical significance was set at the $P < 0.05$ level, and values expressed as mean (SE). Vitamin C and placebo groups were compared for subject characteristics and race performance measures using Student's *t*-tests. Salivary measures were analyzed using 2 (vitamin C and placebo groups) x 3 (times of measurement) repeated-measures ANOVA. If the group x time interaction *P* value was ≤ 0.05, the change from baseline for 32 km and post-race values was compared between groups using Student's *t*-tests. For the multiple comparisons across groups, a Bonferroni adjustment was made, with statistical significance set at $P < 0.025$. Pearson product-moment correlations were used to test the relationship between all post-race measures.

Results

Twenty-eight ultramarathoners complied with all aspects of the study and completed at least 50 km of the race. Table 1 lists the subject characteristics for the vitamin C ($N = 15$) and placebo ($N = 13$) groups. The runners were experienced and committed to regular training and racing but were well below elite status. There was no significant difference between the groups for age, stature, body mass, running and racing experience, and cardiorespiratory fitness. There was no significant difference in pre-race diet with mean energy intake of 11.2 (0.2) MJ/day, carbohydrate 55.3 (2.8)% of total energy, fat 29.0 (1.8)% of total energy and vitamin C 147 (24) mg/day.

Table 1 Subject characteristics [mean (SE)]

Characteristic	Vitamin C ($N = 15$)		Placebo ($N = 13$)		*P*-value
	Mean	SE	Mean	SE	
Age (years)	49.9	(3.5)	45.2	(2.8)	0.312
Stature (m)	1.73	(0.02)	1.74	(0.02)	0.642
Body mass (kg)	76.8	(2.4)	76.4	(2.8)	0.912
Running experience (years)	14.3	(2.3)	13.7	(2.5)	0.852
Training distance ($km \cdot week^{-1}$)	58.9	(9.7)	70.3	(5.6)	0.321
Ultramarathons raced	21.2	(8.1)	20.3	(6.6)	0.934
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	47.2	(1.8)	48.4	(2.3)	0.693
VE_{max}($l \cdot min^{-1}$)	134	(6)	137	(6)	0.689
HR_{max}($beats \cdot min^{-1}$)	181	(4)	183	(4)	0.725
RER_{max}	1.15	(0.02)	1.16	(0.02)	0.841

Environmental conditions were measured four times during the race. At 6:00 a.m. readings were 18°C and 90% relative humidity (rh), at 10:00 a.m. 24°C and 60% rh, at 2:00 p.m. 31°C and 40% rh, and at 6:00 p.m. 30°C and 40% rh. The average heart rates during the race were not significantly different between the vitamin C and placebo groups [135 (4) and 136 (5) beats per minute, 75.7 (1.9)% and 75.2 (2.1)% HR_{max} respectively]. The vitamin C group ran a mean of 68.7 (3.3) km in 9.85 (0.36) h, which was not significantly different from the placebo group which completed 68.7 (3.5) km in 9.68 (3.5) h. RPE averaged 10.9 (0.3) (''fairly light'' 6–20 scale) during the first 15 km, and rose to 14.3 (1.2) for the vitamin C group and 15.0 (1.8) for the placebo group at the end of the race. There was no significant difference in fluid intake between the two groups. The vitamin C group drank 1.04 (0.08) l/h while the placebo group drank 0.85 (0.06) l/h, with a mean intake of 57 g of carbohydrate per hour for both groups. The runners also averaged 2.3 (0.16) gel packs or 58 g of carbohydrate per hour. Modest changes in body mass [vitamin C 1.88 (0.37) kg, placebo 1.79 (0.24) kg] and plasma volume [vitamin C 4.02 (0.48)%, placebo 2.74 (0.55)%] were measured but did not differ significantly between the two groups.

Plasma vitamin C was significantly higher in the vitamin C compared to placebo group pre-race, at 32 km, and post-race (Table 2). No significant group or interaction effects were measured for lipid hydroperoxides and F_2-isoprostane, but both oxidative measures rose significantly during the ultramarathon race (Table 2). Post-race lipid hydroperoxides and F_2-isoprostane were positively correlated ($r = 0.44$, $P = 0.019$). F_2-isoprostane tended to be higher in the vitamin C compared to placebo group across all time points (group effect, $P = 0.051$) (Table 2). No significant interaction effect was measured for serum glucose (data not shown). Serum cortisol rose strongly in both groups during the race, with a slightly greater increase measured in the vitamin C group (group × time interaction, $p = 0.034$) (Fig. 1). Post-race serum cortisol was positively correlated with serum vitamin C ($r = 0.50$, $P = 0.006$), but no other significant correlations were found between serum vitamin C or oxidative and sIgA parameters.

Table 3 and Figs. 2 and 3 summarize the salivary IgA data. No significant group or interaction effects were measured for saliva secretion rate, saliva protein concentration, and saliva protein IgA concentration (Table 3). The saliva secretion rate (volume of saliva in 4 min) declined in both groups at 32 km and post-race compared to the pre-race values (Table 3). Saliva protein concentration did not change significantly from pre-race values at either 32 km or post-race (Table 3). Sliva protein IgA concentration decreased 37% and 31% post-race in the vitamin C and placebo groups, respectively (Table 3). The pattern of change in sIgA concentration did not differ significantly between groups ($P = 0.713$). Saliva IgA concentration decreased in the vitamin C and placebo groups at 32 km (−38% and −47%, respectively) and post-race (−26% and −27%, respectively) (Fig. 2). The pattern of change in sIgA secretion rate did not differ significantly between groups ($P = 0.705$). Saliva IgA secretion rate decreased in the vitamin C and placebo groups at 32 km (−58% and −66%, respectively) and post-race (−45% and −40%, respectively) (Fig. 3).

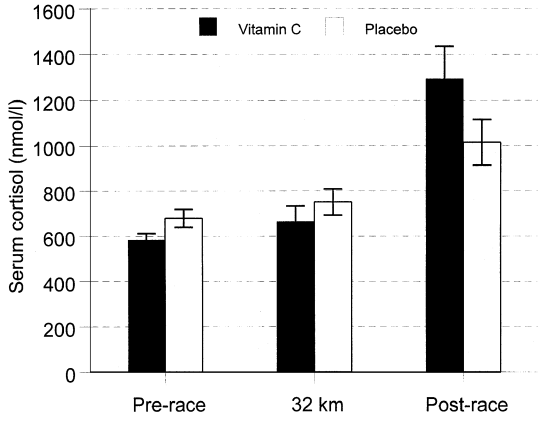

Fig. 1 Serum cortisol rose in both groups during the race, with a slightly greater increase measured in the vitamin C group (group × time interaction, $P = 0.034$)

Discussion

This study measured the influence of vitamin C supplementation (1500 mg/day for 7 days prior to and the day of the ultramarathon race) compared to placebo on

Table 2 Ascorbic acid and oxidative measures

Parameter	Pre-race		32-km		Post-race		Effect: group interaction time
	Mean	SD	Mean	SD	Mean	SD	
Plasma ascorbic acid (μg/100μl)							< 0.001
Vitamin C	1.25	(0.11)	2.56	(0.15)	3.21	(0.29)	< 0.001
Placebo	0.73	(0.10)	1.08	(0.15)	1.28	(0.12)	< 0.001
Lipid hydroperoxide (μM)							0.921
Vitamin C	31.3	(2.1)	32.1	(2.3)	39.8	(4.4)	0.079
Placebo	36.3	(0.7)	27.0	(3.1)	39.2	(3.6)	0.027
F_2-isoprostane (pg/ml)							0.051
Vitamin C	60.4	(8.2)	64.5	(7.0)	75.1	(5.6)	0.864
Placebo	44.8	(2.8)	53.7	(4.0)	62.6	(2.8)	0.003

Table 3 Salivary IgA changes inresponse to running an ultramarathon in vitamin C (N = 15) and placebo (N = 13) groups

Parameter	Pre-Race		32 km		Post-Race		Effect: group interaction time
Saliva volume (ml · 4 min^{-1})							0.515
Vitamin C	1.93	(0.09)	1.18	(0.11)	1.41	(0.08)	0.716
Placebo	1.90	(0.11)	1.01	(0.15)	1.38	(0.16)	< 0.001
Saliva protein concentration (mg · ml^{-1})							0.420
Vitamin C	1.25	(0.15)	1.15	(0.12)	1.42	(0.17)	0.918
Placebo	1.32	(0.17)	1.36	(0.18)	1.59	(0.26)	0.175
Saliva protein IgA concentration (μg · mg^{-1})							0.340
Vitamin C	520	(70.0)	297	(33.0)	330	(49.0)	0.835
Placebo	442	(39.0)	241	(47.0)	307	(68.0)	< 0.001

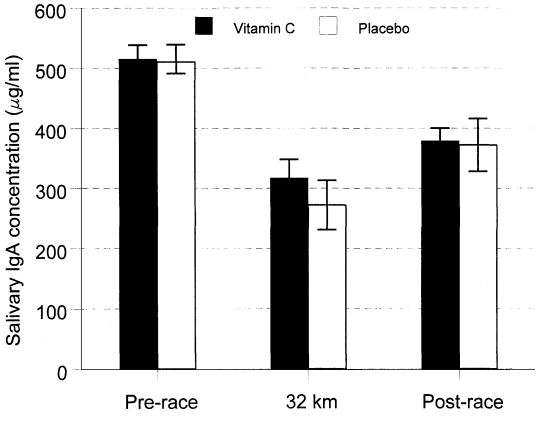

Fig. 2 The pattern of change in salivary immunoglobulin A (*sIgA*) concentration did not differ significantly between groups ($P = 0.713$). Saliva IgA concentration decreased in the vitamin C and placebo groups at 32 km (−38% and −47%, respectively) and post-race (−26% and −27%, respectively)

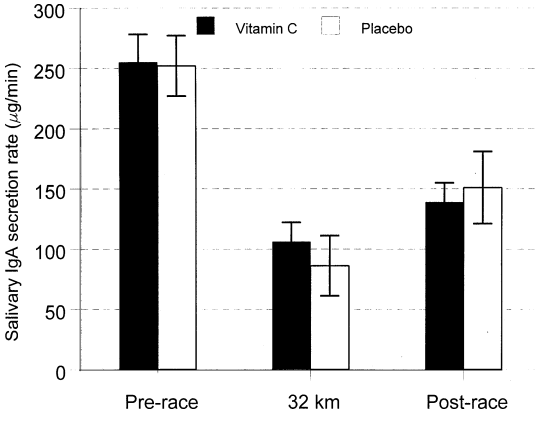

Fig. 3 The pattern of change in sIgA secretion rate did not differ significantly between groups ($P = 0.705$). Saliva IgA secretion rate decreased in the vitamin C and placebo groups at 32 km (−58% and −66%, respectively) and post-race (−45% and −40%, respectively)

oxidative and sIgA changes in carbohydrate-fed runners during and following an ultramarathon race. Vitamin C compared to placebo supplementation had no influence on any of the measured salivary and oxidative parameters in subjects running at approximately 75% HR_{max} for 10 h. No significant correlations were found between post-race plasma vitamin C, oxidative, and sIgA measures. This is the first study to test the influence of vitamin C supplementation on measures of both oxidative and sIgA following a competitive race.

Saliva IgA secretion rate, saliva IgA concentration and saliva protein IgA concentration decreased during and following the ultramarathon. This is in agreement with several previous studies that have studied the effects of endurance exercise on sIgA (Krywkowski et al. 2001; Müns et al. 1989; Nieman et al. 2001a; Tomasi et al. 1982). The decrease in the saliva values observed after exercise is dependent on both the duration and intensity of the exercise, with greater alterations observed following prolonged heavy exertion (Mackinnon and Hooper 1994; McDowell et al. 1991).

In recent years, researchers have attempted to identify nutritional countermeasures to exercise-induced changes in sIgA. Several studies have demonstrated that carbohydrate supplementation has no significant influence on sIgA changes following heavy exertion. In a study of 98 marathon runners, sIgA concentration, saliva protein IgA, and sIgA secretion rates decreased equally for both carbohydrate- and placebo-supplemented groups (Nieman et al. 2001a). The same findings were reported in a study of carbohydrate-fed female rowers engaged in 2 h of rowing drills (Nehlsen-Cannarella et al. 2000). Krzykowski et al. (2001) examined the effects of glutamine and protein supplementation on decreases in sIgA. Eleven athletes exercised at 75% of $\dot{V}O_2max$ for 2 h on a cycle ergometer on three separate days. Glutamine, protein, and placebo supplements were given during and for 2 h after exercise. Supplementation did not attenuate the exercise-induced decrease in sIgA values even though plasma glutamine values were maintained. Additional research is necessary to elucidate the mechanisms associated with the mucosal immune system and to identify other possible means for attenuating the decrease in sIgA.

Vitamin C supplementation did not attenuate exercise-induced oxidative stress in the present investigation. Similar results have been obtained from several recent studies testing the effectiveness of vitamin C at protecting against exercise-generated ROS (Peters et al. 2001; Rokitzki et al. 1994; Thompson et al. 2001). This lack of

attenuation could partially be due to the location of vitamin C within hydrophilic compartments. Radicals attacking lipid membranes are primarily scavenged by vitamin E, which is present in the phospholipid bilayer. Upon reducing the radical, vitamin E is oxidized into a weakly reactive tocopherol radical. The tocopherol radical is then reduced by vitamin C in the hydrophilic compartments. This final radical reduction acts to replenish vitamin E stores within the cell membrane. Thus, vitamin E is a much more potent defender against lipophilic radicals, while vitamin C is more effective at protecting against hydrophilic radicals (Mylonas and Kouretas 1999). This mechanism could potentially explain the apparent ineffectiveness of vitamin C at reducing lipid peroxidation by exercise-induced ROS. A recent study supports the observation that vitamin C is not effective at reducing lipid peroxidation, regardless of dose. Fifteen healthy young women who received vitamin C doses of 30–2500 mg daily had unchanged levels of plasma and urine F_2-isoprostanes (Levine et al. 2001).

Few studies have examined the relationship between oxidative stress and immunosuppression. However, a study conducted by Franci et al. (1996) found that thermally induced oxidative stress negatively affected lymphocyte function, specifically cell proliferation and immunoglobulin (Ig) function. It was also observed that the presence of vitamin E attenuated these immunosuppressive effects, while vitamin C administration elicited no response. Significantly increased pre-to-post race F_2-isoprostane concentrations demonstrated exercise-induced oxidative stress in subjects; however, the current investigation was unable to show that vitamin C supplementation demonstrated any protective effects against exercise-induced oxidative stress, nor was it able to link any oxidative measures to exercise-induced reductions in sIgA.

Most studies have failed to show an influence of vitamin C supplements on immune changes following intense endurance exercise (Krause et al. 2001; Nieman et al. 1997, 2002; Petersen et al. 2001). In the present study, we also examined immune cell counts, plasma concentrations of interleukins IL-6, IL-10, IL-1ra, and IL-8, mitogen-stimulated lymphocyte proliferation, and IL-2 and interferon gamma (IFN-γ) production (Nieman et al. 2002). As reported elsewhere, vitamin C compared to placebo supplementation had no significant effect on the pattern of change in these immune parameters following an ultramarathon (Nieman et al. 2002). A study of 12 marathoners, who ran for 2.5 h on treadmills after receiving either vitamin C or placebo supplementation, revealed no differences in the pattern of changes in hormonal and immune values (including natural killer cell activity, mitogen-stimulated lymphocyte proliferation, granulocyte/monocyte phagocytosis and oxidative burst activity, leukocyte subsets, and IL-6) (Nieman et al. 1997). Krause et al. (2001) reported no effects of vitamin C supplementation (2000 mg/day for 1 week) on neutrophil phagocytosis and bactericidal ability following a competitive biathlon (16 km uphill cycling and 2 km uphill running). However, that study did not include a placebo supplement, and subjects were not randomized to treatment conditions. In another study, male runners ingested 500 mg vitamin C and 400 mg vitamin E or placebo for 14 days before and 7 days after running downhill for 90 min on treadmills (Petersen et al. 2001). Despite a substantial elevation in plasma measures of vitamin C and E, no group differences were measured for the pattern of change in cytokines and 13 lymphocyte subsets.

The results of this study revealed no correlation between serum cortisol and post-race sIgA parameters, but post-race serum cortisol was positively correlated with serum vitamin C. These findings do not support the hypothesis that vitamin C supplementation attenuates the exercise-induced increase in cortisol levels. These findings are in disagreement with the results of a study of 29 ultramarathon runners competing in the Comrades 90-km race. In that study, post-race serum cortisol measures were negatively correlated with vitamin C, and the pattern of change in cortisol was significantly lower in the vitamin C compared to placebo groups (Nieman et al. 2000; Peters et al. 2001). There are several possible explanations for the conflicting results. In the Comrades study, subjects were not randomized to treatment groups, and carbohydrate intake during the race was not controlled. This is in contrast to methods followed in the present study (Nieman et al. 2000; Peters et al. 2001). Carbohydrate compared to placebo beverage ingestion has a marked effect on post-exercise changes in stress hormones, including cortisol, and immunity. (Nehlsen-Cannarella et al. 1997; Nieman 2000; Nieman et al. 1998, 2001b).

In conclusion, our data indicate that vitamin C supplementation does not serve as a countermeasure to post-race oxidative and sIgA changes in carbohydrate-fed ultramarathon runners. The decreases in sIgA concentration, secretion rate, and sIgA protein concentration following heavy exertion appear to be stereotypical and not affected by vitamin C supplementation. Statistical correlations suggest that oxidative stress has little influence on the mucosal immune changes that take place during or following a competitive ultramarathon race. Despite vitamin C supplementation, the decrease in sIgA in this study was large, suggesting that continued research is necessary for other potential countermeasures that may have significant implications for ultra-athletes.

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References

Anderson R, Van Wyk H (1980) The effect of ascorbate ingestion on levels of salivary IgA in adult volunteers. Am J Clin Nutr 33:6–7

Anderson R, Oosthuizen R, Maritz R, Van Rensburg AJ (1980) The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. Am J Clin Nutr 33:71–76

Ashton T, Young IS, Peters JR, Jones E, Jackson SK, Davies B, Rowlands CC (1999) Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. J Appl Physiol 87:2032–2036

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Campbell JD, Cole M, Bunditrutavorn B, Vella AT (1999) Ascorbic acid is a potent inhibitor of various forms of T cell apoptosis. Cell Immunol 194:1–5

Chen CY, Huang YL, Lin TH (1998) Association between oxidative stress and cytokine production in nickel-treated rats. Arch Biochem Biophys 356:127–132

Cupps TR, Fauci AS (1982) Corticosteroid mediated immunoregulation in man. Immunol Rev 65:133–155

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247–248

Enwonwu CO, Sawiris P, Chanaud N (1995) Effect of marginal ascorbic acid deficiency on saliva level of cortisol in the guinea pig. Arch Oral Biol 40:737–742

Franci O, Amici A, Margarit R, Merendino N, Piccolella E (1996) Influence of thermal and dietary stress on immune response of rabbits. J Anim Sci 74:1523–1529

Gleeson M (2000) Mucosal immunity and respiratory illness in elite athletes. Int J Sports Sci 21 [Suppl. 1]:S33–S43

Gleeson M, Hall ST, McDonald WA, Flanagan AJ, Clancy RL (1999a) Salivary IgA subclasses and infection risk in elite swimmers. Immunol Cell Biol 77:351–355

Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA, Clancy RL (1999b) Salivary IgA levels and infection risk in elite swimmers. Med Sci Sports Exerc 31:67–73

Gleeson M, McDonald WA, Pyne DB, Clancy RL, Cripps AW, Francis JL, Fricker PA (2000) Immune status and respiratory illness for elite swimmers during a 12-week training cycle. Int J Sports Med 21:302–307

Grimble RF (1997) Effect of antioxidative vitamins on immune function with clinical applications. Int J Vitam Nutr Res 67:312–320

Hughes DA (1999) Effects of dietary antioxidants on the immune function of middle-aged adults. Proc Nutr Soc 58:79–84

Jacob RA, Burri BJ (1996) Oxidative damage and defense. Am J Clin Nutr 63 [Suppl.]:985S–990S

Kodama M, Kodama T, Murakami M, Kodama M (1994) Vitamin C infusion treatment enhances cortisol production of the adrenal via the pituitary ACTH route. In Vivo 8:1079–1085

Krause R, Patruta S, Daxbock F, Fladerer P, Biegelmayer C, Wenisch C (2001) Effect of vitamin C on neutrophil function after high-intensity exercise. Eur J Clin Invest 31:258–263

Krzywkowski K, Petersen EW, Ostrowski K, Link-Amster H, Boza J, Halkjaer-Kristensen J, Pedersen BK (2001) The effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. J Appl Physiol 91:832–838

Laney PH, Levy JA, Kipp DE (1990) Plasma cortisol and adrenal ascorbic acid levels after ACTH treatment with a high intake of ascorbic acid in the guinea pig. Ann Nutr Metab 34:85–92

Levine M, Wang Y, Sebastian P, Morrow J (2001) A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci USA 19(17):9842–9846

Mackinnon L (1996) Immunoglobulin antibody and exercise. Exerc Immunol Rev 2:1–35

Mackinnon LT, Hooper S (1994) Mucosal (secretory) immune system response to exercise of varying intensity and during overtraining. Int J Sports Med 15 [Suppl. 3]:S179–S183

Mackinnon L, Chick TW, Van As A, Toamasi TB (1987) The effect of exercise on secretory and natural immunity. Adv Exp Med Biol 216A:869–876

McDowell SL, Chaloa K, Housh TJ, Tharp GD, Johnson GO (1991) The effect of exercise intensity and duration on salivary immunoglobulin A. Eur J Appl Physiol 63:108–111

Morrow JD (2000)The isoprostanes: their quantification as an index of oxidant stress status in vivo. Dru Metab Rev 32:377–385

Müns G, Liesen H, Riedel H, Bergmann K-Ch (1989) Einfluß von langstreckenlauf auf den IgA-gehalt in nasensekret und speichel. Deutsch Z Sportmed 40:63–65

Mylonas C, Kouretas D (1999) Lipid peroxidation and tissue damage. In Vivo 13:295–309

Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Bailey E, Warren BJ, Davis JM (1997) Carbohydrate and the cytokine response to 2.5 hours of running. J Appl Physiol 82:1662–1667

Nehlsen-Cannarella SL, Nieman DC, Fagoaga OR, Kelln WJ, Henson DA, Shannon M, Davis JM (2000) Saliva immunoglobulins in elite women rowers. Eur J Appl Physiol 81:222–228

Nieman DC (2000) Carbohydrates and the immune response to prolonged exertion. In: Nieman DC, Pedersen BK (eds) Nutrition and exercise immunology. CRC, Boca Raton, Fla., pp 25–42

Nieman DC, Nehlsen-Cannarella SL (1991) The effect of acute and chronic exercise on immunoglobulins. Sports Med 11(3):183–201

Nieman DC, Henson DA, Butterworth DE, Warren BJ, Davis JM, Fagoaga OR, Nehlsen-Cannarella SL (1997) Vitamin C supplementation does not alter the immune response to 2.5 hours of running. Int J Sport Nutr 7(3):173–184

Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F, Butterworth DE (1998) Influence of mode and carbohydrate on the cytokine response to heavy exertion. Med Sci Sports Exerc 30:671–678

Nieman DC, Peters EM, Henson DA, Nevines EI, Thompson MM (2000) Influence of vitamin C supplementation on cytokine changes following an ultramarathon. J Interferon Cytokine Res 20:1029–1035

Nieman DC, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis M, Nehlsen-Cannarella SL (2001a) Changes in salivary IgA following a competitive marathon race. Int J Sports Med 22:1–7

Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM, Kaminsky DE, Shute M. (2001b) Cytokine changes after a marathon race. J Appl Physiol 91:109–114

Nieman DC, Henson DA, Mcanulty SR, McAnulty L, Swick NS, Utter AC, Vinci DM, Opiela SJ, Morrow JD (2002) Influence of vitamin C supplementation on oxidative and immune changes following an ultramarathon. J Appl Physiol 92:1970–1977

Niess AM, Sommer M, Schneider M, Angres C, Tschositsch K, Golly IC, Battenfeld N, Northoff H, Biesalski HK, Dickhuth HH, Fehrenbach E (2000) Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of RRR-α-tocopherol supplementation. Antiox Redox Signal 2:113–126

Peters EM, Anderson R, Theron AJ (2001) Attenuation of the increase in circulating cortisol and enhancement of the acute phase protein response in vitamin C supplemented ultramarathon runners. Int J Sports Med 22:120–126

Petersen EW, Ostrowski K, Ibfelt T, Richelle M, Offord E, Halkjaer-Kristensen J, Pedersen BK (2001) Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. Am J Physiol 280:C1570–C1575

Piercy RJ, Hinchcliff KW, DiSilvestro RA, Reinhart GA, Baskin CR, Hayek MG, Burr JR, Swenson RA (2000) Effect of dietary supplements containing antioxidants on attenuation of muscle damage in exercising sled dogs. Am J Vet Res 61:1438–1445

Redmann A, Mobius K, Hiller HH, Oelkers W, Bahr V (1995) Ascorbate depletion prevents Aldosterone stimulation by sodium deficiency in the guinea pig. Eur J Endocrinol 133:499–506

Rokitzki L, Logemann E, Sagredos AN, Murphy M, Wezel-Roth W, Keul J (1994) Lipid peroxidation and antioxidative vitamins under extreme endurance stress. Acta Physiol Scand 151:149–158

Sanchez-Quesada JL, Jorba O, Payes A, Otal C, Serra-Grima R, Gonzalez-Sastre F, Ordonez-Llanos J (1998) Ascorbic acid inhibits the increase in low-density lipoprotein (LDL) susceptibility to oxidation and the proportion of electronegative LDL induced by intense aerobic exercise. Coron Art Dis 9:249–255

Sen CK, Roy S (2001) Antioxidant regulation of cell adhesion. Med Sci Sports Exerc 33:377–381

Steerenberg PA, van Asperen IA, van Nieuw Amerongen A, Biewenga A, Mol D, Medema GJ (1997) Salivary levels of immunoglobulin A in triathletes. Eur J Oral Sci 105:305–309

Tanaka M, Muto N, Gohda E, Yamamoto I (1994) Enhancement by ascorbic acid 2-glucoside or repeated additions of ascorbate of mitogen-induced IgM and IgG productions by human peripheral blood lymphocytes. Jpn J Pharmacol 66(4):451–456

Thompson D, Williams C, Kingsley M, Nicholas CW, Lakomy HKA, McArdle F, Jackson MJ (2001) Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation. Int J Sports Med 22:68–75

Tomasi TB, Trudeau FB, Czerwinski D, Erredge S (1982) Immune parameters in athletes before and after strenuous exercise. J Clin Immunol 2:173–178

Vider J, Lehtmaa J, Kullisaar T, Vihalemm T, Zilmer K, Kairane C, Landor A, Karu T, Zilmer M (2001) Acute immune response in respect to exercise-induced oxidative stress. Pathophysiology 7:263–270

White A, Estrada M, Walker K, Wisnia P, Filgueira G, Valdes F, Araneda O, Behn C, Martinez R (2001) Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp Biochem Physiol A Mol Integr Physiol 128:99–104