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ORIGINAL CONTRIBUTION

Low vitamin C values are linked with decreased physical performance and increased oxidative stress: reversal by vitamin C supplementation

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Abstract
Purpose It has been suggested that part of the failure of antioxidant supplementation to reduce oxidative stress and promote health is that it has been administered in humans with normal levels of antioxidants.
Methods To test this hypothesis, we screened 100 males for vitamin C baseline values in blood. Subsequently, the 10 individuals with the lowest and the 10 with the highest vitamin C values were assigned in two groups. Using a placebo-controlled crossover design, the 20 selected subjects performed aerobic exercise to exhaustion (oxidant stimulus) before and after vitamin C supplementation for 30 days.
Results The low vitamin C group had lower VO_2max values than the high vitamin C group. Vitamin C supplementation in this group marginally increased VO_2max. Baseline concentration of F_2-isoprostanes and protein carbonyls was higher in the low vitamin C group compared to the high vitamin C group. Vitamin C supplementation decreased the baseline concentration of F_2-isoprostanes and protein carbonyls in both groups, yet the decrease was greater in the low vitamin C group. Before vitamin C supplementation, F_2-isoprostanes and protein carbonyls were increased to a greater extent after exercise in the high vitamin C group compared to the low vitamin C group. Interestingly, after vitamin C supplementation, this difference was narrowed.
Conclusion We show for the first time that low vitamin C concentration is linked with decreased physical performance and increased oxidative stress and that vitamin C supplementation decreases oxidative stress and might increase exercise performance only in those with low initial concentration of vitamin C.

Keywords Antioxidants · Exercise · Oxidative stress · Physical performance · Supplementation

Introduction

One of the current issues in nutrition and redox biology is the controversy in the literature regarding the true effectiveness of antioxidants to change redox homeostasis. Indeed, a number of studies have reported a failure of antioxidant supplementation to reduce oxidative stress [1] and affect health and disease (e.g., mortality, cancer, or physical performance) [2–4]. It has been suggested that part of the failure of antioxidant supplements to decrease oxidative stress (herein considered as a decrease in free radical production and/or a decrease in levels of oxidant biomarkers [5]) and promote health is the fact that antioxidant supplements have been administered in humans with normal levels of oxidative stress biomarkers [6]. In fact, it has been hypothesized in review papers by eminent researchers that the beneficial effects of antioxidant supplementation are restricted in individuals with high baseline levels of oxidative stress and/or low antioxidants [7–15].

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Table 1 Anthropometric, macronutrient, and vitamin C intake in the low and high vitamin C status groups (mean ± SD). Independent *t* tests were performed

	Low vitamin C ($n = 10$)		High vitamin C ($n = 10$)	
	Placebo	Vitamin C	Placebo	Vitamin C
Age (years)	21.7 ± 2.5		21.8 ± 2.1	
Height (cm)	175.6 ± 4.8		174.6 ± 4.7	
Weight (kg)	76.3 ± 5.1	77.3 ± 6.0	79.1 ± 6.8	78.8 ± 6.4
Energy (kcal/day)	2,567 ± 198	2,465 ± 157	2,512 ± 140	2,557 ± 172
Carbohydrates (% energy)	45.4 ± 12.6	49.2 ± 9.8	50.6 ± 11.5	52.7 ± 12.1
Fat (% energy)	38.2 ± 9.5	35.8 ± 8.2	32.1 ± 7.9	33.4 ± 8.2
Proteins (% energy)	16.4 ± 8.2	15.0 ± 8.0	17.3 ± 7.6	13.9 ± 6.7
Vitamin C (mg)	31 ± 11	35 ± 8	167 ± 25[¶]	164 ± 17[¶]

[¶] Significant difference between low and high vitamin C groups

To experimentally test the validity of the hypothesis that initial concentration of antioxidants is a determining factor implicated in the effectiveness of supplementation, we screened one hundred males for vitamin C baseline values in blood. Subsequently, the 10 individuals with the lowest and the 10 with the highest vitamin C values were assigned in two groups. Both groups performed aerobic exercise to exhaustion (an oxidant stimulus) before and after vitamin C supplementation. Based on this experimental design, we aimed to examine whether baseline values of vitamin C determine the efficacy of antioxidant supplementation in reducing oxidative stress and thus promoting physical performance.

Materials and methods

Participants

One hundred, recreationally trained healthy males expressed interest to voluntarily participate in the study. Blood collection was performed between 08:00 and 9:00 h, following 12-h overnight fast. For the measurement of vitamin C, a blood sample was drawn from a forearm vein, collected in EDTA tubes and centrifuged immediately at 1,370 g for 10 min at 4 °C, and the plasma was collected. Plasma samples were stored at −80 °C and thawed only once before analysis. Vitamin C concentration was assessed spectrophotometrically in plasma after derivatization with 2,4-dinitrophenylhydrazine [16]. EDTA tubes and metaphosphoric acid were used in order to minimize ex vivo oxidation of vitamin C [17, 18]. Moreover, the reactions run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogens [16].

Three out of the 100 individuals were excluded from the study because they found to suffer from hypovitaminosis (i.e., vitamin C concentration below 23 μmol/L [19]). This was done in order to ensure that we would not include individuals with subclinical symptoms of hypovitaminosis C and/or vitamin C deficiency. Furthermore, since our study is focused on redox responses to exercise, we wanted to locate individuals who might have disturbed redox homeostasis directly due to low vitamin C values and not due to a possible health complication linked to hypovitaminosis C. Then, of the 97 participants with normal vitamin C values, the 10 with the lowest values (35 ± 8 μmol/L) and the 10 with the highest values (78 ± 11 μmol/L) were assigned to either the low or the high vitamin C group, respectively (Table 1). It is worth mentioning that two participants within the 10 with the lowest vitamin C values and one participant within the 10 with the highest vitamin C values declined to continue in the study and were replaced by the next ranked individuals. Measurements of vitamin C concentration for the participants of both groups were performed before and after supplementation as well as pre- and immediately post-exercise. None of the subjects had any muscle disease or a history of musculoskeletal injury of the lower limbs that could limit their ability to perform the exercise sessions. Volunteers were instructed to abstain from any strenuous exercise during their participation in the study (except of the exercise sessions performed during the experimental procedures). They were also asked to abstain from alcohol consumption for 2 days before the blood sampling and caffeine the day of the testing. Subjects did not receive medication/nutrient supplements known to influence the variables measured during their participation in the study. Volunteers were provided with a written set of instructions for monitoring dietary consumption and a record sheet for recording food intake the 2 days before each exercise session. An informed written consent was obtained for all participants, after they were informed of all risks, discomforts, and benefits involved in the study. The procedures were in accordance with the Helsinki declaration of 1975, as revised in 2000, and approval was received from the Ethics Committee of the local university.

Study design

An overview of the study design is shown in Fig. 1. All measurements were performed between 08:00 and 11:00 h

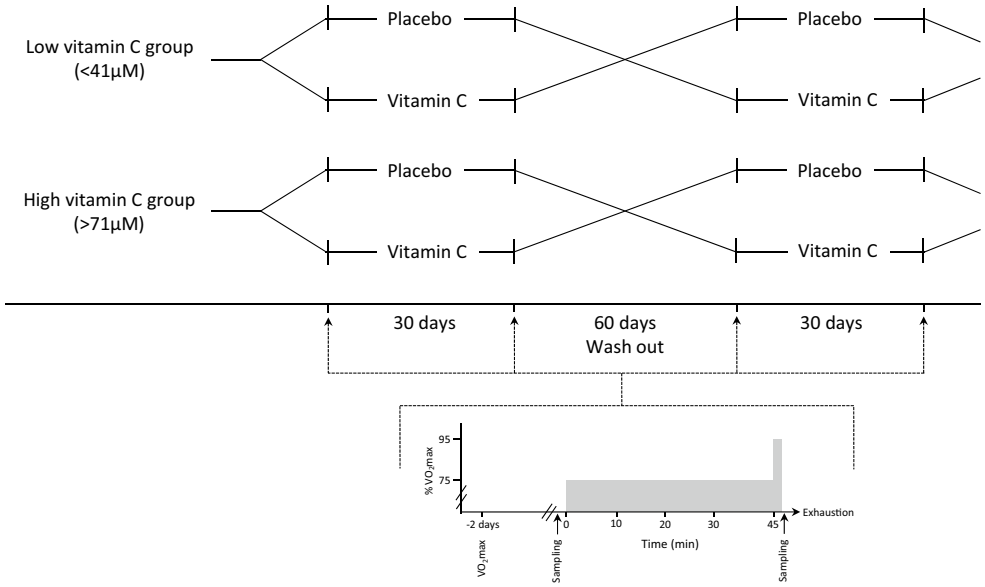

Fig. 1 Placebo-controlled crossover design of the study

after overnight fasting. Initially, to examine whether resting blood vitamin C concentration affects aerobic performance, VO_2max was assessed (using incremental cycling test to volitional exhaustion) and was compared in both the low and the high vitamin C groups (Monark, Vansbro, Sweden). More specifically, the protocol started with a 50 W load at 50 rpm and increased by 10 W every 2 min until volitional fatigue. The test was terminated when three of the following four criteria VO_2max were met: (1) volitional fatigue, (2) a lower than 2 mL/kg/min increase in VO_2 despite an increase in workload, (3) a respiratory exchange ratio greater than or equal to 1.10, and (4) heart rate within 10 bpm of the predicted maximal heart rate (220–age). Respiratory gas variables were measured using a metabolic cart (Quark b2, Cosmed, Italy), which was calibrated before each test using standard gases of known concentration. The VO_2max assessment was used as a reference value to calculate the workload at the relative intensity of each subject and ensured that all subjects would cycle at similar relative intensity during the following aerobic exercise sessions.

Following VO_2max assessment, subjects within both the low and the high vitamin C groups received either placebo or vitamin C supplementation, in a double-blind randomized crossover fashion (Fig. 1). To test whether vitamin C supplementation decreases exercise-induced oxidative stress, subjects in both the low and the high vitamin C groups performed an acute exhaustive exercise protocol (an oxidant stimulus), before and after vitamin C or placebo supplementation for 30 days. Pre- and immediately post-exercise selected redox biomarkers were measured before and after the 30-day placebo or vitamin C supplementation. In addition, to test whether vitamin C supplementation improves aerobic performance, a second VO_2max test was performed after the 30-day supplementation regimen and was compared with the initial VO_2max test performed before supplementation. This intervention scheme was repeated after a 60-day washout period using a placebo-controlled crossover design.

The acute exhaustive exercise session took place 2 days after the VO_2max test and was performed on the same cycle ergometer at an intensity corresponding to 70–75 % of the subject's VO_2max for 45 min at 50 rpm. Following the 45 min cycling, the intensity of the cycle ergometer was increased to elicit 95 % VO_2max, and exercise was terminated at exhaustion. Fatigue was defined as an inability of the subject to maintain the required rpm or when the subject stopped voluntarily. Expired gas samples were obtained every 10 min to ensure the prescribed exercise intensity. The placebo group daily received orally three lactose tablets, and the antioxidant group received orally three vitamin C tablets (each tablet contained 333 mg of vitamin C; Lamberts Health Care Ltd, Kent, United Kingdom). All participants were instructed to receive the capsules every 8 h in order to achieve high concentration of vitamin C throughout the 24 h [20]. Each individual received the capsules pre-packed in daily doses labeled with the day of consumption. Blood samples were drawn pre- and post-exercise of each session for the collection of plasma and erythrocytes, while urine was collected on the spot at the same time points. Individuals were asked to follow and record their diet for 3 days before each exercise session. Each volunteer was provided with a written set of instructions for monitoring dietary consumption and a record sheet for recording food intake.

Collection and handling of body fluids

Blood sample was drawn from a forearm vein, collected in EDTA tubes and centrifuged immediately at 1,370 g for 10 min at 4 °C, and the plasma was collected. The packed erythrocytes were lysed with 1:1 (v/v) distilled water, inverted vigorously, and centrifuged at 4,000 g for 15 min at 4 °C. Urine spot samples were collected in a container. For standardizing the dilution of urine, creatinine levels were measured using a kit (Fisher Diagnostics, Middletown, USA). Body fluid samples were stored at −80 °C and thawed only once before analysis.

Assays

A competitive immunoassay was used for the quantitation of F_2-isoprostanes in urine (Cayman Chemical, Charlotte, USA). Urine was purified using the solid phase extraction cartridges. The purification and the subsequent ELISA were performed following the manufacturer's recommendations.

Protein carbonyls were determined adding 50 μL of 20 % TCA to 50 μL of plasma (diluted 1:10). This mixture was incubated in an ice bath for 15 min and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded, and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (in 2.5 N HCl) for the sample or 500 μL of 2.5 N HCL for the blank was added in the pellet. The samples were incubated in the dark at room temperature for 1 h, with intermittent vortexing every 15 min, and were centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded, and 1 mL of 10 % TCA was added, vortexed, and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded, and 1 mL of ethanol–ethyl acetate (1:1 v/v) was added, vortexed, and centrifuged at 15,000 g for 5 min at 4 °C. This washing step was repeated twice. The supernatant was discarded, and 1 mL of 5 M urea (pH 2.3) was added, vortexed, and incubated at 37 °C for 15 min. The samples were centrifuged at 15,000 g for 3 min at 4 °C, and the absorbance was read at 375 nm. Calculation of protein carbonyl concentration was based on the molar extinction coefficient of dinitrophenylhydrazine. The intra-assay coefficient of variation for proteins carbonyls measured in plasma was 5.4 %.

Erythrocyte glutathione (GSH) activity was determined spectrophotometrically, adding 20 μL of erythrocyte lysate treated with 5 % TCA mixed with 660 μL of 67 mM sodium potassium phosphate (pH 8.0) and 330 μL of 1 mM 5,5′- dithiobis-2 nitrobenzoate. The samples were incubated in the dark at room temperature for 45 min, and the absorbance was read at 412 nm. GSH concentration was calculated on the basis of calibration curves constructed using commercial standards. The intra-assay coefficient of variation for GSH was 4.2 %.

Statistical analysis

The distribution of all dependent variables was examined by Shapiro–Wilk test and was found not to differ significantly from normality. Independent *t* tests were carried out for comparing the anthropometric, performance, and nutritional characteristics between the low and the high vitamin C concentration groups. Two-way ANOVA [group (high and low vitamin C) × time (pre- and post-exercise)] followed by simple main effect analysis was used for VO_2max. Three-way ANOVA [group (high and low vitamin C) × supplementation (placebo and vitamin C) × time (pre- and post-exercise)] with repeated measurements on "time" followed by simple main effect analysis was used for redox biomarkers. The α-level was set at 0.05. Data are presented as mean ± SD. SPSS version 20 was used for all analyses (SPSS Inc, Chicago, IL).

Results

There were no significant differences in anthropometric characteristics and macronutrient intake between the subjects of the low and the high vitamin C group. Vitamin C intake was lower in the low vitamin C group compared with the high vitamin C group (Table 1). The low vitamin C group had lower VO_2max values than the high vitamin C group. Vitamin C supplementation in this group marginally non-significantly increased VO_2max ($p = 0.079$) (Fig. 2). However, vitamin C supplementation did not affect VO_2max in the high vitamin C group. As expected, vitamin C concentration was higher at all-time points in the high compared to the low vitamin C group. Vitamin C supplementation increased vitamin C values in both groups, while the increase was greater in the low vitamin C group (Table 2). Exercise did not affect vitamin C concentration in both groups neither at baseline nor after placebo/vitamin C supplementation (Table 2).

Baseline concentration of F_2-isoprostanes and protein carbonyls was higher in the low vitamin C group compared with the high vitamin C group (Table 2). Vitamin C supplementation decreased the baseline concentration of F_2-isoprostanes and protein carbonyls in both the low and high vitamin C groups, yet the decrease was greater in the low vitamin C group. Before vitamin C supplementation, F_2-isoprostanes and protein carbonyls increased to a greater extent following exercise in the high vitamin C group compared to the low vitamin C group. Interestingly, after vitamin C supplementation, this difference was narrowed (and was not significant), yet the percent increase in F_2-isoprostanes and protein carbonyls was still greater in the high vitamin C group after exercise (Table 2; Fig. 3a, b). Overall, after supplementation, the post-exercise increase

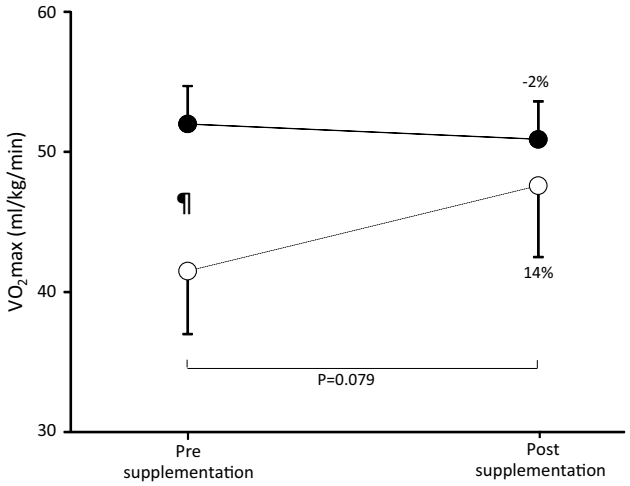

Fig. 2 Effect of placebo vs vitamin C supplementation on exercise performance pre- and post-exercise in the low (open circles) and the high (closed circles) vitamin C status groups ($n = 10$ for each group). A two-way ANOVA (group × time) followed by simple main effect analysis was performed. ¶ Indicates significant difference at the same time point between the two groups

in F_2-isoprostanes and protein carbonyls was greater compared to the respective post-exercise increase prior to supplementation in both the low and high vitamin C groups. Baseline concentration of GSH was not different between the low and the high vitamin C groups (Table 2). Exercise decreased GSH values in both low and high vitamin C groups irrespectively of the supplementation (Table 2; Fig. 3c). It is worth mentioning that part of the data from Table 2 is also presented in Fig. 3 in order to illustrate the dependence of exercise-induced oxidative stress on initial vitamin C values.

Discussion

To our knowledge, this is the first study that assessed redox responses and physical performance to vitamin C supplementation between humans with low and high baseline vitamin C concentration. We found higher resting levels of oxidative stress and decreased exercise performance in the individuals with low baseline values of vitamin C compared to those with high vitamin C values. Vitamin C supplementation decreased oxidative stress and marginally non-significantly improved exercise performance only in individuals with poor initial vitamin C status. We also observed that the initial values of redox biomarkers are important predictors of the responses to exercise. Indeed, we noticed that individuals with low baseline values of vitamin C (and consequently higher levels of oxidant biomarkers) were more resistant to redox alterations and oxidative stress when exposed to an oxidant stimulus (i.e., exercise) compared to subjects with high baseline values of vitamin C. A novel finding of this study (derived from the methodological choice of excluding individuals with vitamin C values below 23 μM) is that you do not need to suffer from hypovitaminosis C or vitamin C deficiency to be benefit from vitamin C supplementation as regards to redox status and physical performance.

At rest before supplementation, we found higher concentration of F_2-isoprostanes and protein carbonyls in individuals with poor vitamin C status (below 41 μmol/L) compared to individuals with high concentration of vitamin C (above 71 μmol/L). Vitamin C supplementation decreased the levels of oxidative stress biomarkers at rest essentially only in individuals with low initial concentration of vitamin C, whereas only few and minimal effects appeared in individuals with initially high vitamin C concentration. Similarly, individuals with low concentration of vitamin C had lower aerobic capacity than individuals with high concentration of vitamin C. More importantly, only the low vitamin C group showed signs of improvement in exercise performance after antioxidant supplementation. This agreement between biochemical and physiological data suggests that normal vitamin C concentration is required for a "healthy" redox status and proper organismal function. Considering that the vitamin C values of participants were experimentally manipulated at study entry allows us to infer a cause–effect relationship among vitamin C concentration, oxidative stress, and performance. Noteworthy, the differences observed between the group with the high and the low vitamin C levels would have probably been even more pronounced if we had included the three participants detected with hypovitaminosis C.

In the present investigation, 1 month of vitamin C supplementation was sufficient to almost restore the baseline vitamin C concentration, redox status, and potentially exercise performance in the low vitamin C group. A striking disagreement exists among studies regarding the influence of vitamin C supplementation on redox status and physical performance. Indeed, several studies have reported that vitamin C supplementation attenuates oxidative stress [21, 22], others have reported that it induces a pro-oxidant effect [23, 24] and others have reported that it does not affect redox status [25, 26]. Likewise, several studies have indicated that vitamin C supplementation induces a positive effect [27], a negative effect [28, 29], or no effect [26, 30] on exercise performance. Certainly, there are many possible reasons for this divergence regarding the effects of vitamin C supplementation on redox status and exercise performance: (1) dose of vitamin C administered, (2) animal species used, (3) training models applied, (4) training status of the participants, (5) training endpoints measured, (6) oxidative stress biomarkers determined, and (7) biological

Table 2 Effect of placebo vs vitamin C supplementation on redox biomarkers pre- and post-exercise in low and high vitamin C status groups ($n = 10$ for each group) (mean ± SD)

	Placebo supplementation				Vitamin C supplementation				Main effects and interactions						
	1st exercise bout		2nd exercise bout		1st exercise bout		2nd exercise bout								
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	T	G	S	T × G	T × S	G × S	T × G×S
Vitamin C (μmol/L)															
Low vitamin C	33.9 ± 4.3[¶]	34.5 ± 3.5[¶]	35.8 ± 5.7[¶]	35.4 ± 6.0[¶]	33.3 ± 4.3[¶]	35.5 ± 4.2[¶]	49.4 ± 8.6[¶#]	51.5 ± 8.0[¶#]	0.044	<.001	<.001	0.006	0.481	0.156	<.001
High vitamin C	78.5 ± 4.5	79.5 ± 5.5	76.9 ± 4.3	78.3 ± 3.8	80.9 ± 3.5	83.4 ± 4.4	89.9 ± 9.5[#]	92.0 ± 8.5[#]							
F_2-Isoprostanes (pg/mg cr.)															
Low vitamin C	690 ± 176	777 ± 163*	742 ± 178[¶]	806 ± 185*	714 ± 135[¶]	773 ± 143*	587 ± 107[#]	792 ± 131*	<.001	0.025	0.308	0.003	<.001	0.845	0.256
High vitamin C	640 ± 134	744 ± 135*	602 ± 142	730 ± 123*	573 ± 119	759 ± 175*	513 ± 124[#]	769 ± 065*							
Carbonyls (nmol/mg pr.)															
Low vitamin C	0.62 ± 0.16	0.80 ± 0.15*	0.68 ± 0.13[¶]	0.79 ± 0.12*	0.66 ± 0.18[¶]	0.82 ± 0.17*	0.51 ± 0.16[¶#]	0.84 ± 0.13*	<.001	0.014	0.075	<.001	0.226	0.198	0.005
High vitamin C	0.54 ± 0.12	0.88 ± 0.10*	0.52 ± 0.14	0.85 ± 0.17*	0.46 ± 0.12	0.72 ± 0.16*	0.41 ± 0.12[#]	0.78 ± 0.15*							
Glutathione (μmol/g Hb)															
Low vitamin C	3.20 ± 0.31	2.64 ± 0.35*	3.37 ± 0.30	2.56 ± 0.30*	3.28 ± 0.24	2.74 ± 0.28*	3.14 ± 0.31	2.53 ± 0.28*	<.001	0.185	0.918	0.165	0.558	0.684	<.069
High vitamin C	3.48 ± 0.55	2.66 ± 0.55*	3.17 ± 0.18	2.70 ± 0.37*	3.51 ± 0.47	2.55 ± 0.37*	3.42 ± 0.44	2.67 ± 0.27*							

A three-way ANOVA (group × supplementation × time) followed by simple main effect analysis was performed

T: Main effect of time; G: Main effect of group; S: Main effect of supplementation; T × G: 2-way interaction for time and group; T × S: 2-way interaction for time and supplementation; G × S: 2-way interaction for group and supplementation; T × G×S: 3-way interaction for time, group and supplementation; and NM: not measured

[¶] Significant difference between low and high vitamin C groups at the same time point

[#]Significant difference between the first and the second exercise bout in the same group

*Significant difference from the pre-exercise value in the same group and in the same exercise bout

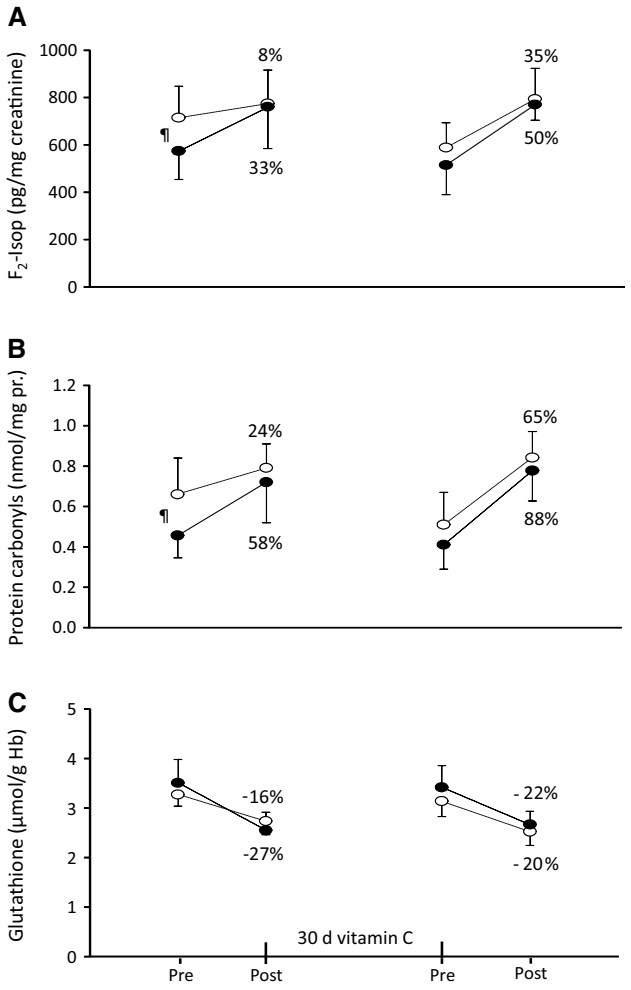

Fig. 3 Effect of exercise on F_2-isoprostanes (**a**), protein carbonyls (**b**) and glutathione (**c**) values in the low (*open circles*) and high (*closed circles*) vitamin C status groups ($n = 10$ for each group). The post-exercise percent changes before and after vitamin C supplementation are depicted. A two-way ANOVA (group × time) followed by simple main effect analysis was performed. All the post-exercise values were significantly different from the pre-exercise values. ¶ Indicates significant difference at the same time point between the two groups

matrices in which the biomarkers were determined. However, based on the present data, we believe that probably the most important reason for this disagreement in the literature may be the indiscriminate use of participants. We suggest future studies to employ individuals with either decreased levels of antioxidants and/or increased levels of oxidant biomarkers.

Our group has recently reported that the initial values of redox biomarkers are important predictors of exercise responses, that is, individuals with lower initial values in the oxidant biomarkers tended to exhibit greater percent increases in the biomarkers after exercise and vice versa [31]. Our previous observation was verified in the present study where participants in the high vitamin C group (exhibiting lower initial values of lipid and protein oxidation) showed greater increases in F_2-isoprostanes and protein carbonyls after the oxidant stimulus (i.e., exercise). Moreover, the experimental design used in this study allowed us to investigate whether the initial values also affected the response to an antioxidant stimulus (i.e., vitamin C supplementation). We found that the initial values of oxidant biomarker are also important determinants of their response to vitamin C supplementation in the opposite direction though. Specifically, we found that the participants in the low vitamin C group (exhibiting higher initial values of lipid and protein oxidation) showed the greater decreases in F_2-isoprostanes and protein carbonyls after the antioxidant stimulus (i.e., vitamin C supplementation). What is probably more interesting is that after the antioxidant supplementation both groups responded with a higher degree of exercise-induced oxidative stress than before the supplementation. Using the current interpretative framework, most researchers would conclude that vitamin C supplementation exerted a "pro-oxidant effect" considering that "exacerbated" the exercise-induced oxidative stress. However, based on our findings, this greater oxidative stress response after exercise more likely indicates that the antioxidant stimulus was effective to sufficiently decrease the oxidant biomarkers values to such an extent allowing greater increases in biomarkers after exercise. This "Janus face" effect of antioxidant supplementation in resting and exercise-induced oxidative stress signifies the limitations of the current framework used to interpret alterations in redox homeostasis. We suggest that researchers should be careful when finding a greater response to an oxidant stimulus (such as exercise) after a period of antioxidant supplementation, since this finding may more probably indicate an "antioxidant" effect and not a "pro-oxidant effect" of the antioxidant supplement.

Given the conventional wisdom that free radicals are harmful, many athletes supplement their diet with vitamin C and other antioxidants to "protect" against the "negative" consequences of exercise [9]. In contrast, the current scientific wisdom supports negative or no effect of vitamin C and/or antioxidant supplementation on exercise performance [26, 28, 32–35]. Our data somehow reconcile these two conflicting truths by revealing a group of individuals not benefited (those with sufficient vitamin C) and another group of individuals benefited (those with poor vitamin C) by exactly the same type and dosage of antioxidant supplementation. This finding suggests that future studies should consider enrolling individuals with low vitamin C concentration and/or hypovitaminosis in order to increase the possibility of finding an effect after antioxidant administration. In conclusion, our data show that low vitamin C values are linked with decreased physical performance and

increased oxidative stress and that vitamin C supplementation decreases oxidative stress and might increase exercise performance only in those with low initial concentration of vitamin C. Therefore, the popular habit of consuming antioxidants during exercise training seems worthwhile only for those who are deficient in vitamin C.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards An informed written consent was obtained for all participants, after they were informed of all risks, discomforts, and benefits involved in the study. The procedures were in accordance with the Helsinki declaration of 1975, as revised in 2000, and approval was received from the Ethics Committee of the local university.

References

1. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M (2003) Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr 22:18–35
2. Bjelakovic G, Nikolova D, Gluud C (2014) Antioxidant supplements and mortality. Curr Opin Clin Nutr Metab Care 17:40–44
3. Jiang L, Yang KH, Tian JH, Guan QL, Yao N, Cao N, Mi DH, Wu J, Ma B, Yang SH (2010) Efficacy of antioxidant vitamins and selenium supplement in prostate cancer prevention: a meta-analysis of randomized controlled trials. Nutr Cancer 62:719–727
4. Nikolaidis MG, Kerksick CM, Lamprecht M, McAnulty SR (2012) Does vitamin C and E supplementation impair the favorable adaptations of regular exercise? Oxid Med Cell Longev 2012:707941
5. Nikolaidis MG, Kyparos A, Spanou C, Paschalis V, Theodorou AA, Vrabas IS (2012) Redox biology of exercise: an integrative and comparative consideration of some overlooked issues. J Exp Biol 215:1615–1625
6. Halliwell B (2009) The wanderings of a free radical. Free Radic Biol Med 46:531–542
7. Bernard D, Christophe A, Delanghe J, Langlois M, De Buyzere M, Comhaire F (2003) The effect of supplementation with an antioxidant preparation on LDL-oxidation is determined by haptoglobin polymorphism. Redox Rep 8:41–46
8. Block G, Jensen CD, Morrow JD, Holland N, Norkus EP, Milne GL, Hudes M, Dalvi TB, Crawford PB, Fung EB, Schumacher L, Harmatz P (2008) The effect of vitamins C and E on biomarkers of oxidative stress depends on baseline level. Free Radic Biol Med 45:377–384
9. Braakhuis AJ (2012) Effect of vitamin C supplements on physical performance. Curr Sports Med Rep 11:180–184
10. Duffield-Lillico AJ, Slate EH, Reid ME, Turnbull BW, Wilkins PA, Combs GF Jr, Park HK, Gross EG, Graham GF, Stratton MS, Marshall JR, Clark LC (2003) Selenium supplementation and secondary prevention of nonmelanoma skin cancer in a randomized trial. J Natl Cancer Inst 95:1477–1481
11. Halliwell B (1999) Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutr Rev 57:104–113
12. Halliwell B (1999) Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 31:261–272
13. Hercberg S (2006) The SU.VI.MAX study, a randomized, placebo-controlled trial on the effects of antioxidant vitamins and minerals on health. Ann Pharm Fr 64:397–401
14. Lykkesfeldt J, Poulsen HE (2010) Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. Br J Nutr 103:1251–1259
15. Steinhubl SR (2008) Why have antioxidants failed in clinical trials? Am J Cardiol 101:14D–19D
16. Omaye ST, Turnbull JD, Sauberlich HE (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 62:3–11
17. Karlsen A, Blomhoff R, Gundersen TE (2005) High-throughput analysis of vitamin C in human plasma with the use of HPLC with monolithic column and UV-detection. J Chromatogr B Analyt Technol Biomed Life Sci 824:132–138
18. Lykkesfeldt J (2012) Ascorbate and dehydroascorbic acid as biomarkers of oxidative stress: validity of clinical data depends on vacutainer system used. Nutr Res 32:66–69
19. Jacob RA (1990) Assessment of human vitamin C status. J Nutr 120(Suppl 11):1480–1485
20. Nikolaidis MG, Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Vrabas IS (2014) Common questions and tentative answers on how to assess oxidative stress after antioxidant supplementation and exercise. CRC Press, New York
21. Close GL, Ashton T, Cable T, Doran D, Holloway C, McArdle F, MacLaren DP (2006) Ascorbic acid supplementation does not attenuate post-exercise muscle soreness following muscle-damaging exercise but may delay the recovery process. Br J Nutr 95:976–981
22. Moller P, Viscovich M, Lykkesfeldt J, Loft S, Jensen A, Poulsen HE (2004) Vitamin C supplementation decreases oxidative DNA damage in mononuclear blood cells of smokers. Eur J Nutr 43:267–274
23. Elhaimeur F, Courderot-Masuyer C, Nicod L, Guyon C, Richert L, Berthelot A (2002) Dietary vitamin C supplementation decreases blood pressure in DOCA-salt hypertensive male Sprague-Dawley rats and this is associated with increased liver oxidative stress. Mol Cell Biochem 237:77–83
24. Premkumar K, Bowlus CL (2004) Ascorbic acid does not increase the oxidative stress induced by dietary iron in C3H mice. J Nutr 134:435–438
25. Kelly RP, Poo Yeo K, Isaac HB, Lee CY, Huang SH, Teng L, Halliwell B, Wise SD (2008) Lack of effect of acute oral ingestion of vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects. Free Radic Res 42:514–522
26. Theodorou AA, Nikolaidis MG, Paschalis V, Koutsias S, Panayiotou G, Fatouros IG, Koutedakis Y, Jamurtas AZ (2011) No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training. Am J Clin Nutr 93:1373–1383
27. Rossman MJ, Garten RS, Groot HJ, Reese V, Zhao J, Amann M, Richardson RS (2013) Ascorbate infusion increases skeletal muscle fatigue resistance in patients with chronic obstructive pulmonary disease. Am J Physiol Regul Integr Comp Physiol 305:R1163–R1170
28. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J, Vina J (2008) Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87:142–149
29. Marshall RJ, Scott KC, Hill RC, Lewis DD, Sundstrom D, Jones GL, Harper J (2002) Supplemental vitamin C appears to slow racing greyhounds. J Nutr 132:1616S–1621S

30. Roberts LA, Beattie K, Close GL, Morton JP (2011) Vitamin C consumption does not impair training-induced improvements in exercise performance. Int J Sports Physiol Perform 6:58–69
31. Margaritelis NV, Kyparos A, Paschalis V, Theodorou AA, Panayiotou G, Zafeiridis A, Dipla K, Nikolaidis MG, Vrabas IS (2014) Reductive stress after exercise: the issue of redox individuality. Redox Biol 2:520–528
32. Higashida K, Kim SH, Higuchi M, Holloszy JO, Han DH (2011) Normal adaptations to exercise despite protection against oxidative stress. Am J Physiol Endocrinol Metab 301:E779–E784
33. Paulsen G, Cumming KT, Holden G, Hallen J, Ronnestad BR, Sveen O, Skaug A, Paur I, Bastani NE, Ostgaard HN, Buer C, Midttun M, Freuchen F, Wiig H, Ulseth ET, Garthe I, Blomhoff R, Benestad HB, Raastad T (2014) Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: a double-blind, randomised, controlled trial. J Physiol 592:1887–1901
34. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci USA 106:8665–8670
35. Yfanti C, Akerstrom T, Nielsen S, Nielsen AR, Mounier R, Mortensen OH, Lykkesfeldt J, Rose AJ, Fischer CP, Pedersen BK (2010) Antioxidant supplementation does not alter endurance training adaptation. Med Sci Sports Exerc 42:1388–1395