European Journal of Sport Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tejs20

Redox status and antioxidant response in professional cyclists during training

Roberto Carlos Leonardo-Mendonça[ab], Melquiades Concepción-Huertas[ab], Eduardo Guerra-Hernández[c], Mikel Zabala[d], Germaine Escames[ab] & Darío Acuña-Castroviejo[abe]

[a] Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Granada, Spain

[b] Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain

[c] Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Granada, Spain

[d] Departamento de Educación Física y Deportiva, Facultad de Ciencias del Deporte, Universidad de Granada, Granada, Spain

[e] Unidad de Gestión Clínica de Laboratorios, Hospital Universitario San Cecilio, Granada, Spain

Published online: 07 May 2014.

To cite this article: Roberto Carlos Leonardo-Mendonça, Melquiades Concepción-Huertas, Eduardo Guerra-Hernández, Mikel Zabala, Germaine Escames & Darío Acuña-Castroviejo (2014): Redox status and antioxidant response in professional cyclists during training, European Journal of Sport Science, DOI: 10.1080/17461391.2014.915345

To link to this article: http://dx.doi.org/10.1080/17461391.2014.915345

European Journal of Sport Science, 2014
http://dx.doi.org/10.1080/17461391.2014.915345

Routledge
Taylor & Francis Group

ORIGINAL ARTICLE

Redox status and antioxidant response in professional cyclists during training

ROBERTO CARLOS LEONARDO-MENDONÇA[1,2], MELQUIADES CONCEPCIÓN-HUERTAS[1,2], EDUARDO GUERRA-HERNÁNDEZ[3], MIKEL ZABALA[4], GERMAINE ESCAMES[1,2], & DARÍO ACUÑA-CASTROVIEJO[1,2,5]

[1]*Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Granada, Spain,* [2]*Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain,* [3]*Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Granada, Spain,* [4]*Departamento de Educación Física y Deportiva, Facultad de Ciencias del Deporte, Universidad de Granada, Granada, Spain,* [5]*Unidad de Gestión Clínica de Laboratorios, Hospital Universitario San Cecilio, Granada, Spain*

Abstract
The aim of this study was to investigate whether different phases of training affect oxidative stress and antioxidant defences in professional cyclists. Ten professional cyclists, aged 21.8 ± 2.5 years, were enrolled in the study. They were classified into two groups of five athletes each one with similar nutritional intake excepting for the overload of vitamin C (1000 mg day^{-1}) and E (400 mg day^{-1}) supplementation in one of them. The cyclists of both groups performed the same exercise design, consisting of hard, tapering and recovery training periods. Total antioxidant capacity (TAC) of the diet, plasma oxygen radical absorption capacity (ORAC), lipid peroxidation (LPO), DNA damage (8-OHdG) and erythrocyte glutathione disulfide/glutathione ratio ($GSSG:GSH^{-1}$) were measured. During the intense exercise trainings, the cyclists without vitamin supplements had the TAC of diet significantly lower than the supplemented group. Plasma ORAC, LPO and 8-OHdG were similar in both groups of athletes. Athletes with supplements had a basal $LPO:ORAC^{-1}$ ratio lower than that without supplements, but this ratio converged to the same level at the end of the training in both groups of cyclists. Both groups of cyclists showed similar changes in $GSSG:GSH^{-1}$ ratio and in GSSG and GSH levels along the study. The data suggest that well-trained athletes with suitable ultra-endurance training volume and intensity do not require antioxidant vitamin supplements to adapt their endogenous antioxidant defenses to exercise-induced ROS.

Keywords: *Training, nutrition, exercise, stress, physiology*

Introduction

The benefits of non-exhaustive training exercise in health promotion and disease prevention are well established. During heavy physical exertion, however, the oxygen flux to active skeletal muscles multiplies by two orders of magnitude, increasing the oxygen consumption and enhancing production of reactive oxygen species (ROS). An imbalance between the generation of ROS and antioxidant defence capacity of the cell is closely associated with natural ageing and multiple disease processes (Fisher-Wellman & Bloomer, 2009). High concentrations of ROS are hazardous for living organisms, and they damage all major cellular constituents. At moderate concentrations, however, ROS play an important role in the regulation of cell functions, serving as survival and repair signals (Nils-Georg, 2007).

In biologic systems, defence mechanisms against oxidative stress depend primarily on an orchestrated synergism between both endogenous and dietary antioxidants (Pepe, Balci, Revan, Akalin, & Kurtoglu, 2009). Some of the non-enzymatic antioxidants, which are not synthesised by the body, must be obtained exogenously by the diet, primarily from ingestion of fruit and vegetables. These include vitamins A, C, and E and bioflavonoids. These compounds are able to scavenge various free radicals

Correspondence: Darío Acuña-Castroviejo, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Avda. del Conocimiento s/n, 18100-Armilla, Granada, Spain. E-mail: dacuna@ugr.es

by electron donation. Vitamin C may help strengthen immune defence, while vitamin E could enhance energy balance at high altitude (Gross, Baum & Hoppeler, 2011). However, studies evaluating the efficiency of these dietary antioxidants in reducing exercise-induced muscle injury have yielded contradictory results (Nils-Georg, 2007).

There are many studies exploring the effect of the exercise type (aerobic or anaerobic), intensity and duration and the consumption of dietary antioxidants on the redox status of the athletes. Generally, these studies considered exercise-induced ROS detrimental to physiological function, including decreased performance and immune function, and increased fatigue (Fisher-Wellman & Bloomer, 2009). Other studies have observed an adaptation in the body's antioxidant defence system as a result of aerobic and anaerobic exercise training (Concepcion-Huertas et al., 2013). The mechanisms behind these adaptive processes are not well understood, but professional athletes are normally more protected to exercise-induced ROS than untrained subjects. Thus, it seems that training facilitates the efficiency of the endogenous antioxidant defences. Nevertheless, the generation of ROS during training can overwhelm the antioxidant capacity of the body, causing proteins, lipids and DNA damage (Gómez-Cabrera, Domenech, & Viña, 2008). These findings led to the use of high doses of antioxidant supplements to prevent the excess of exercise-induced ROS in athletes. Beneficial effects of this widespread practice on muscle function are, however, uncommon (Higashida, Kim, Higuchi, Holloszy & Han, 2011). In contrast, there are a growing number of studies showing the deleterious effects of antioxidant treatment in athletes (Gómez-Cabrera et al., 2008).

There is not much information, however, regarding how different schedules of training influence oxidative stress in professional cyclists, a class of highly trained athletes. The aim of this study was, therefore, to investigate whether different phases of training and antioxidant supplements affect oxidative stress and antioxidant defences in professional cyclists.

Methods

Subjects

Ten Caucasian male professional road cyclists, aged 21.8 ± 2.5 years and weighing 70 ± 5 kg, were enrolled in the study. Those athletes with the same training and competition level were strictly selected from a professional cyclist team. Only cyclists who had the same training schedules, similar competition level and similar physical performances at the time of the study were enrolled. The study was approved by the Ethics Committee of the Granada's University and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Dietary intake and experimental groups

To estimate the average energy and nutritional intake, participants recorded their dietary intake during three consecutive days weekly, i.e. during days 1–3, 8–10, 15–17 and 23–25. A trained nutritionist gave detailed verbal instructions to the athletes about proper dietary recordings. A full description of foods and fluids consumed was requested, including the brand names of packaged food, cooking or processing methods and food items and ingredients added during preparation. Participants estimated the amount of foods or fluids consumed by referring to the weight or volume information provided on food packages or by using standardised household measures. Dietary information was converted to energy and nutrients with the software DIAL (Alce Ingenieria, Madrid, Spain). This programme was supplemented with information for composite dishes, commercial foods and sports foods whenever reliable nutritional composition data could be obtained. Nutritional intake was checked with the dietary reference intake (DRI) of "Food and Nutrition Board's, Institute of Medicine, 2000" (World Health Organization [WHO], 2003).

The athletes were grouped into two subgroups of five subjects each one. Both subgroups had the same nutritional intake composition except for vitamin supplementation in one of them. Supplementation consisted in a daily intake of 1000 mg of vitamin C and 400 mg of vitamin E at breakfast time, from one month before the study and until the end of it.

Samples

Blood samples (10 ml) were collected from the antecubital vein between 7–8 am and after 8–10 hours of fasting. A total of four blood samples (S1–S4) were taken during the period of training and competition as indicated in Figure 1A. The first (S1), corresponding to the control, was taken at day 7, i.e. just before beginning the second phase of training. The second sample (S2) was taken at day 13, before the third phase of training. The third blood sample (S3) was taken at day 17, one day before the competition day. The fourth blood sample (S4) was taken at day 25, seven days after the competition, and at the end of the recovery period.

Training design

The study was conducted through five different phases of training (Figure 1A). The first phase corresponds to a control period of seven days of rest

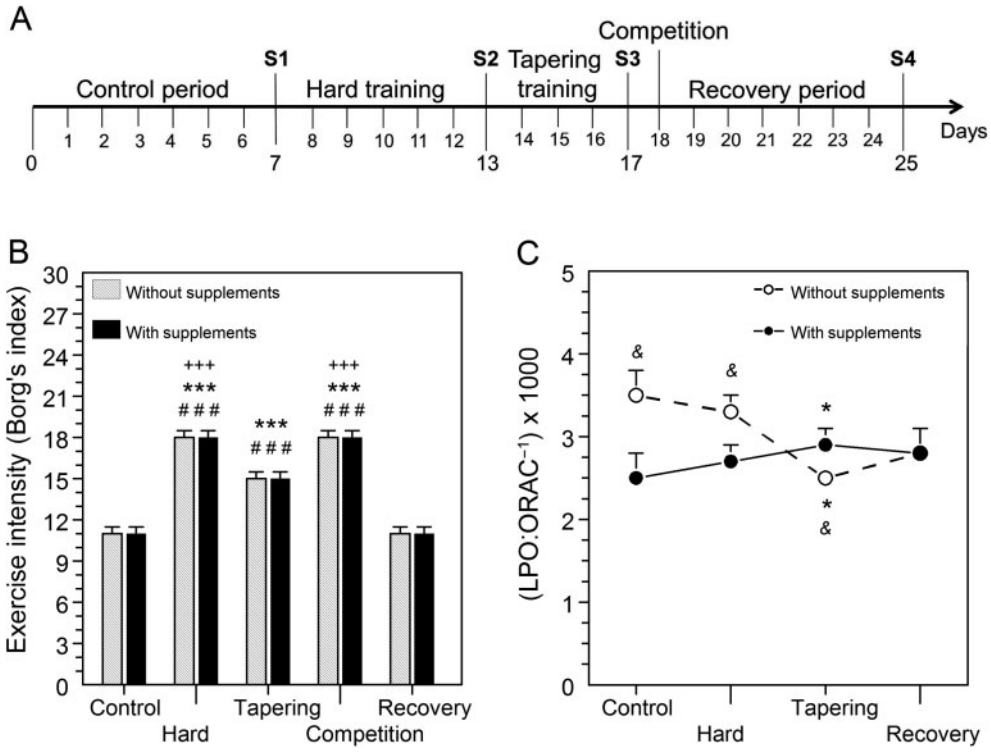

Figure 1. Diagram depicting the chronological schedule of the study (A). Exercise intensity of athletes along the different training phases of the study in accordance with Borg's scale (B). Evolution of LPO:ORAC^{-1} ratio in the athletes (C). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. control without supplements; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. control with supplements; $^{+++}P < 0.001$ vs. tapering. $^{\&}P < 0.05$ vs. athletes with supplements.

(days 0–6). In the second phase, the cyclists developed a hard training of six days (days 7–12) consisting in a load of intense exercise on the road bike with an average of 130 ± 25 km daily, at maximum altitude of 1200 m and an average temperature of 30 ± 5°C. During the third phase, the cyclists had a tapering training of four days (days 13–16) consisting in short loads training of intense exercise with an average of 50 ± 10 km daily on a flat road at a maximum altitude of 800 m with mean temperature of 30 ± 5°C. The fourth phase corresponded to the one-day competition (day 18) of the Spanish Cycling Cup, involving 130 km at a maximum altitude of 1600 m and temperature average of 31 ± 5°C. The fifth phase corresponded to a 7-day recovery period (days 18–24).

Exercise intensity measurement

The rate of perceived exertion (RPE) method was used to quantify the exercise intensity determined by the athletes (Borg, & Dahlstrom, 1962). The RPE scale is commonly used in both team or individual sports, and it ranges the exercise intensity from 6 to 20 points.

Total antioxidant capacity of the dietary intake

To estimate the average of the total antioxidant capacity (TAC) of the diet, subjects made a record of food consumption throughout the study period, where they indicated the amount of food and supplements consumed every day. The TAC of the diet was transformed into mmol day^{-1} of Trolox equivalents (TE) according to the American board of oxygen radicals absorption capacity of foods elsewhere published (Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, & USDepartment of Agriculture, 2007). Due to the absence of values for beer and coffee in this table, these values were obtained from other studies (Rufián-Henares & Morales, 2007). The values for vitamins C and E were analysed in the laboratory.

Measurement of plasma vitamins C and E

Plasma levels of vitamins C and E were analysed by high-pressure liquid chromatography with electrochemical detection (HPLC-ED; Battino, Leone, & Bompadre, 2004; Li & Franke, 2009). Plasma vitamins C and E were purified with an Acquity

UHPLC BEH system using a 2.1 × 50 mm C18 1.7 μm column (Waters Co., Milford, MA), and their levels are measured in a Waters Xevo TQ-S tandem mass spectrometer (Waters Co., Milford, MA). The vitamin concentration was expressed in μmol l^{-1}.

Determination of the plasma oxygen radical absorption capacity (ORAC)

The ORAC values of plasma were measured following a procedure elsewhere published (Prior et al., 2003). The fluorescence of the samples was measured in a thermostatised (37°C) plate reader spectrofluorometer (Polarstar Optima, BMG Lab technologies, Ortenberg, Germany), with excitation and emission wavelengths of 490 nm and 545 nm, respectively. The ORAC concentration was expressed in μmol l^{-1} of TE.

Determination of DNA damage in plasma

The commercial kit Highly Sensitive 8-OHdG Check ELISA (Institute for the Control of Aging, Nikken SEIL Co., Ltd. Shizuoca, Japan) was used to determine the concentration of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in plasma (Okamoto & Ochi, 1992). The concentration of 8-OHdG was expressed in μg l^{-1}.

Determination of glutathione and glutathione disulfide levels in erythrocytes

Both glutathione (GSH) and glutathione disulfide (GSSG) levels in erythrocytes were spectrofluorometrically measured using o-ophthalaldehyde (Hissin & Hilf, 1976). The fluorescence of GSH and GSSG samples was measured at 350-nm excitation and 420-nm emissions in a plate-reader spectrofluorometer (Bio-Tek Instruments, Inc., Winooski, USA). The concentrations of GSH and GSSG were expressed as μmol g^{-1} Hb. Haemoglobin (Hb) was measured by the cianometahemoglobin method.

Determination of lipid peroxidation (LPO) in plasma

The commercial LPO assay kit was used to determine both malondialdhehyde and 4-hydroxyalkenals (Bioxytech LPO-586 assay kit, Oxis Research, Portland, OR, USA). LPO concentration is expressed in μmol l^{-1} (Esterbauer & Cheeseman, 1990).

Statistical analysis

All statistical analyses were performed using Graphpad Prism 5.0. Data was analysed for their normal distribution with the Shapiro–Wilk test. Repeated measures of analysis of the variance (ANOVA) was used to check for significant differences between training phases and among groups in each training phase. Differences were considered statistically significant at P values <0.05. Values are expressed as means ± SE.

Results

The different phases of training are depicted in Figure 1A. The changes in exercise intensity perception along the study were monitored according to Borg's scale (Borg & Dahlstrom, 1962), as shown in Figure 1B. The exercise intensity of hard, tapering and competition phases was significantly higher than in the control and recovery periods. As planned, the tapering training intensity was significantly lower than in hard and competition phase.

The nutritional intake did not change significantly between each training period of the study. Table I shows the mean of all days of the nutritional intake of the two groups of participants during the study. No significant differences were found in energy intake, macro-nutrients, fibre and lipid profile among groups with and without vitamin supplements. The supplements of vitamins C (1000 mg day^{-1}) and E (400 mg day^{-1}) are also indicated.

The TAC of the diet, ORAC, vitamins C and E, 8-OHdG and LPO values are shown in Table II. Subjects with vitamin supplements had higher TAC of diet values than those without supplements during all periods of training ($F_{(1,6)}$ = 28.3: P < 0.05). No significant differences were found among training periods. The ORAC values of cyclists without vitamin supplements rose significantly after the tapering training and remained higher during the recovery period compared with the control group. There were no significant changes in the ORAC plasma values of the athletes with supplements along the study. Moreover, supplemented and non-supplemented groups of cyclists showed no differences in their ORAC values. The levels of vitamins C and E were higher in the supplemented group. Plasma LPO values were similar in all groups, and they were unrelated to the vitamin supplements. Also, the values of 8-OHdG did not differ significantly between athletes with and without vitamin supplements, although the former showed lower values in all training periods.

The relation between plasma LPO and ORAC is shown in Figure 1C. The athletes without vitamin supplements showed higher control values of LPO compared with supplemented athletes. Nevertheless, the former showed a significant reduction in the LPO:$ORAC^{-1}$ ratio along the study, reaching the lowest values during tapering. In turn, athletes supplemented with vitamins increased the LPO:$ORAC^{-1}$ ratio with the highest values during tapering. At this time, LPO:$ORAC^{-1}$ ratio was significantly higher in

Table I. Nutritional intake composition in the two groups of athletes

	Without supplements	With supplements
Energy intake (kcal)	4500 ± 314	4520 ± 326
Proteins (g)	172 ± 12.5	180 ± 13.4
Proteins (% EI)	15 ± 1.1	15 ± 1.5
Carbohydrates (g)	518 ± 30	521 ± 30
Carbohydrates (% EI)	53 ± 3.2	53 ± 3.5
Fiber (g)	39.3 ± 4.4	35,8 ± 4.1
Fat (g)	155 ± 10.7	149 ± 12.1
Fat (% EI)	32 ± 2.2	32 ± 2.5
Saturated fat (g)	62 ± 6.3	63 ± 7.2
Monounsaturated fat (g)	63 ± 7.2	65 ± 9.4
Polyunsaturated fat (g)	16 ± 2.1	16 ± 2.5
Cholesterol (mg)	290 ± 42.6	295 ± 38.4
Vitamin B1 (mg)	4.2 ± 0.6	4.1 ± 0.7
Vitamin B2 (mg)	4.4 ± 0.7	4.2 ± 0.8
Eq. Niacin (mg)	75.8 ± 10.4	76.6 ± 9.1
Vitamin B6 (mg)	5.8 ± 0.8	5.3 ± 0.8
Folic Acid (μg)	665 ± 65.7	657 ± 59.5
Vitamin B12 (μg)	12.5 ± 1.3	14.1 ± 1.1
Vitamin C (mg)	300 ± 41.8	1280 ± 39.0*
Vitamin A (RE μg)	1964 ± 180.9	2032 ± 233.4
Pantothenic acid (mg)	7.4 ± 0.9	7.9 ± 1.4
Biotin (μg)	31 ± 5.7	29 ± 14.6
Vitamin D (μg)	10.1 ± 1.5	11.3 ± 1.7
Vitamin E (mg)	9.5 ± 1.3	408.5 ± 2.5*
Calcium (mg)	1890 ± 202	1979 ± 208
Iron (mg)	34 ± 5.0	33 ± 6.1
Iodine (μg)	113 ± 13.0	116 ± 12.7
Magnesium (mg)	498 ± 43	513 ± 40
Zinc (mg)	14,6 ± 1.6	15.1 ± 1.8
Selenium (mg)	226 ± 15.3	236 ± 13.7
Sodium (mg)	4750 ± 393	4633 ± 380
Potassium (mg)	4439 ± 464	4225 ± 440
Phosphorus (mg)	2328 ± 184	2268 ± 181
Fluoride (μg)	282 ± 32.0	268 ± 36.2

EI, energy intake; RE, retinol equivalents.
*$P < 0.001$ vs. without supplements.

supplemented than in non-supplemented athletes ($F_{(1,6)} = 22.5$: $P < 0.05$).

Figure 2 shows the values of GSH, GSSG, GSSG:GSH^{-1} ratio and total glutathione (GSH + GSSG) in athletes with and without vitamin supplements. Both groups show a significant GSSG rise during hard and tapering training periods compared with control values ($P < 0.05$), decreasing at the recovery phase. The levels of GSH also increased in both groups of athletes with hard training ($P < 0.05$), and this elevation persisted in athletes without supplements during the tapering and recovery periods. However, GSH levels drop during the tapering period in the supplemented group. Similar behaviour was observed for total glutathione levels. The GSSG:GSH^{-1} ratio tends to decrease along the study in athletes without vitamin supplements, with the lowest levels reached during the recovery period ($P < 0.05$), and increased in supplemented athletes during the tapering period ($P < 0.05$).

Discussion

The energy profile of the dietary intake of cyclists enrolled in this study was close to that recommended by the World Health Organization (WHO, 2003) for males aged 19–30 years and similar to that reported elsewhere in cyclists (Serrano et al., 2010), canoeist (Teixeira, Valente, Casal, Marques, & Moreira, 2009a) and in Spanish university students who play sports regularly (Leonardo-Mendonça, Sospedra, Sanchis, Mañes, & Soriano, 2012; Sánchez Oliver & Guerra Hernández, 2004;). Following the recommendation of the American College of Sports and Medicine (2009), athletes do not need a diet substantially different from that recommended in the Dietary Guidelines for the general population. The lipid profile was similar to other studies in athletes (Teixeira, Valente, Casal, Marques, & Moreira, 2009b) and university students. The daily average consumption of micronutrients in the athletes of the study covered the Dietary Recommended Intake (Sánchez Oliver & Guerra Hernández, 2004). In the supplemented group, the levels of vitamins C and E were more than 9 and 20 times the DRI, respectively.

The TAC of the diet of cyclists without supplements of vitamins C and E was 40% higher than the normal average for the Spanish population (Martínez Álvares & Izquierdo Pulido, 2005). The diet TAC values were similar within each group of cyclists along the study. Nevertheless, diet TAC values increased up to 15 times in the athletes with supplemented intake of vitamins C and E. Supplemented athletes had 30% and 25% higher plasma levels of vitamins C and E, respectively, than non-supplemented athletes. These values were comparable to that obtained in basketball players supplemented with similar amounts of vitamins C and E (Naziroglu et al., 2010) and cyclist (Yfanti et al., 2012).

Considering diet TAC values and plasma vitamin C and E levels, we would expect that plasma ORAC might have the same behaviour, justified by the increased use of vitamins during training (Schröder, Navarro, Mora, Galiano, & Tramullas, 2001; Teixeira et al., 2009b) or by an increase in plasma vitamin C and E in supplemented athletes (Gómez-Cabrera et al., 2008). Nevertheless, no significant difference in plasma ORAC values between both groups of athletes was found. This apparent paradox may reflect different adaptive mechanisms to exercise. Intense exercise can activate the body's antioxidant defences increasing the ORAC in serum of well-trained athletes (Margonis et al., 2007), but not in over-trained athletes (Palazzetti, Richard, Favier, &

Table II. Redox parameters in the two groups of athletes during the study

	Without supplements				With supplements			
	Control	Hard	Tapering	Recovery	Control	Hard	Tapering	Recovery
TAC (mmol TE day^{-1})	14.5 ± 4.4	15.4 ± 4.4	13.9 ± 6.3	13.8 ± 3.4	247.2 ± 66***	249.5 ± 63***	251.1 ± 64***	243.0 ± 71***
ORAC (µmol TE l^{-1})	2373 ± 141	2517 ± 294	3108 ± 366*	3097 ± 248*	2489 ± 213	2492 ± 143	2945 ± 172	2626 ± 302
Vitamin C (µmol l^{-1})	30.5 ± 2.6	31.2 ± 2.4	32.7 ± 1.9	30.8 ± 2.2	42.5 ± 3.0***	40.5 ± 2.3***	41.0 ± 0.6***	40.3 ± 1.9***
Vitamin E (µmol l^{-1})	12.1 ± 1.2	13.2 ± 1.6	12.6 ± 1.4	12.3 ± 1.4	15.7 ± 1.1***	16.2 ± 1.6***	15.1 ± 1.9***	16.5 ± 1.9***
LPO (µmol l^{-1})	8.9 ± 1.4	8.4 ± 1.2	6.7 ± 0.8	7.5 ± 1.0	6.0 ± 0.6	6.6 ± 0.4	8.6 ± 0.5**	7.3 ± 0.8
8-OHdG (µg l^{-1})	0.28 ± 0.03	0.32 ± 0.06	0.32 ± 0.06	0.29 ± 0.06	0.24 ± 0.05	0.25 ± 0.04	0.26 ± 0.06	0.24 ± 0.06

*P < 0.05 vs. control without supplements; **P < 0.05 vs. control with supplements; ***P < 0.05 vs. subjects without supplements.

Margaritis, 2003). Many studies in literature indicate an intensity–response relationship between adaptations of antioxidant enzymes and response to ultra-endurance exercises. Adequate volume and intensities of ultra-endurance training confer protection against the increase in free radicals damage. However, these effects are reduced in athletes supplemented with vitamins C and E (Gómez-Cabrera et al., 2008).

The lack of plasma antioxidant changes in response to vitamin supplements was also reflected in LPO levels, a sensitive marker of oxidative damage to cell membranes. Supplementation with vitamins C and E seemed to reduce initial LPO values, but did not prevent its increase after intensive training periods (Teixeira et al., 2009b). Nevertheless, there are variable results for resting LPO levels in the literature, depending on differences in type, training and nutritional status of the athletes and timing of blood sampling (Bloomer, Canale, Blankenship, & Fisher-Wellman, 2010). So we used the LPO:ORAC^{-1} ratio to further check the antioxidant effects on plasma redox status, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants (Schröder, Navarro, Mora, Galiano, & Tramullas, 2001). Basal LPO:ORAC^{-1} ratio reveals that supplemented athletes presented a better balance between oxidants and antioxidants. This balance, however, decreased significantly during intense training periods. On the other hand, the group without supplements improved significantly the LPO:ORAC^{-1} ratio after tapering training. Then, the endogenous antioxidant response acquired by athletes during training seems to be more important than the antioxidant benefit due to vitamin supplementation (Dawson et al., 2002).

Plasma 8-OHdG, a marker used for DNA damage, was not modified significantly by C and E supplementation, supporting previous data (Tsakiris, Partimos, & Schulpis, 2006). Other studies showed that daily supplementation with 400 mg of vitamin E and 1 g of vitamin C did not affect plasma 8-OHdG levels in trained subjects at rest (Bloomer et al., 2006). Similar findings were reported in basketball players after consuming 200 mg of vitamin E for one month (Tsakiris et al., 2006), or after exercise of short duration in trained subjects (Nieman et al., 2004). Almar et al. (2002), however, found a significant increase in 8-OHdG, but only during the first week of a 3-week race in professional cyclists that were not supplemented with antioxidants, a finding explained by an adaptive response to exercise-induced oxidative stress. Overall, the data further support that chronic exogenous antioxidant supplementation did not modify the endogenous redox status of trained subjects significantly.

GSH is particularly important in the mitochondria in defending against both physiologically and

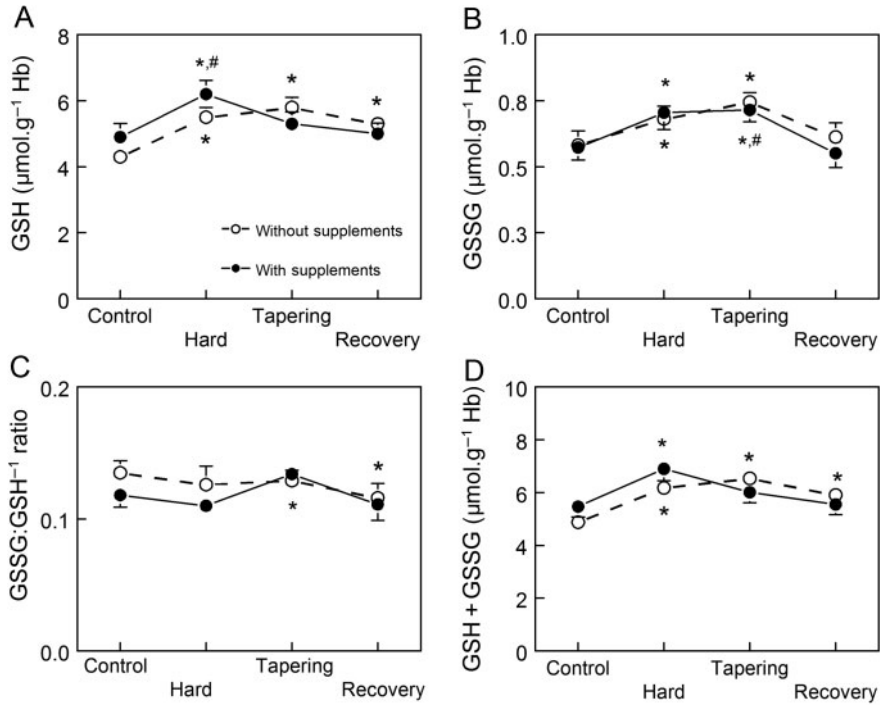

Figure 2. Erythrocyte content of glutathione (GSH), glutathione disulfide (GSSG), glutathione disulfide.glutathione^{-1} ratio (GSSG:GSH^{-1}) and total glutathione (GSH + GSSG) in athletes with and without vitamins supplementation. $^{*}P < 0.05$ vs. control without supplements; $^{\#}P < 0.05$ vs. control with supplements.

pathologically generated oxidative stress (Lu, 2013). The GSSG:GSH^{-1} ratio in erythrocytes reflects the intracellular redox status of the skeletal muscle fibres (Chahbouni et al., 2011). In our study, the changes in the GSSG:GSH^{-1} ratio were unrelated to the antioxidant vitamin intake, suggesting that skeletal muscles of well-training athletes do not need further antioxidant supply to counteract the oxidative stress induced by exercise. The severe oxidative stress caused by intensive training showed a transient increase of GSSG levels during these training periods, without further modification by vitamin supplementation. Interestingly, GSH increased in both groups after the hard training period, preventing a major shift in the redox equilibrium, but only the athletes without supplements were able to prevent the posterior loss of GSH. Thus, our data suggest that vitamins C and E supplementation seem to affect the regulation of GSH synthesis and redox signalling (Tong, Lib, Lippi, & Tian, 2012). The increase of total glutathione pool in the cell may suggest that during elevated intensity exercise, there may be an increase of the GSH synthesis to compensate for the peak of oxidative stress, either through the activation of glutathione synthase or gamma-glutamylcysteine synthase enzymes (Lu, 2013).

There is an agreement in the field that intense exercise may increase the redox status. But in well-trained subjects, periods of appropriate training are able to yield beneficial adaptation of the antioxidant defenses in response to exercise (Concepcion-Huertas et al., 2013). This condition was reflected in our study because the athletes without antioxidant supplements improved the relation between ORAC and LPO, GSSG:GSH^{-1} ratio and GSH. Moreover, vitamins C and E supplementation only tend to improve the redox status before intensive training and tend to reduce DNA damage during training. Although vitamin supplements can be useful in some circumstances, their chronic intake may not yield additional benefits but reduce the exercise adaptations and efficiency of athletes. In this regard, one of the main problems with antioxidant supplementation is that ROS cannot be easily targeted and scavenged in biological systems. Moreover, large antioxidant doses could interfere with the signalling functions of ROS (McGinley, Shafat, & Donnelly, 2009). If athletes do not have a vitamin deficiency, the endogenous antioxidant defences that they are able to induce by exercise are far more important than any antioxidant benefit that they might gain from chronic exogenous vitamin intake (Halliwell, 2012). Nevertheless, further research with a larger number of athletes, studied under different timing and exercise intensity schedules, and including measures of physical performance, will clarify the beneficial versus harmful effects of antioxidant vitamin supplements in these conditions.

Conclusions

Well-trained athletes with adequate ultra-endurance training intensities and volume are able to adapt themselves to exercise-induced ROS production and, thus, prevent the hyperoxidative state that normally follows high-intensity training. However, chronic exogenous antioxidant supplementation could influence the athlete's redox balance, reducing their capability to adapt their endogenous antioxidant defences to exercise. This condition may negatively interfere with training efficiency. The intake of vitamins C and E as an attempt to improve redox status and antioxidant capacity response may delay the adaptation of athletes to training.

Acknowledgements

The authors thank the athletes from CIDI Cyclist Team, Granada, Spain, and its team manager Servando Velarde, for their participation and collaboration in the study. This paper constitutes part of the RC Leonardo-Mendonça's Doctoral Thesis performed in the Biomedicine Doctorate Program of the University of Granada.

Funding

This study was supported in part by grants from the Instituto de Salud Carlos III [grant number RD12/0043/0005] and [grant number PI12/00002]; from the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía, Spain (CTS-101), and from the European Regional Development Fund (ERDF).

References

Almar, M., Villa, J. G., Cuevas, M. J., Rodríguez-Marroyo, J. A., Avila, C., & González-Gallego, J. (2002). Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. *Free Radical Research*, *36*, 247–253. doi:10.1080/10715760290019255

American College of Sports Medicine, American Dietetic Association, & Dietitians of Canada. (2009). Nutrition and athletic performance. *Medicine & Science in Sports & Exercise*, *41*, 709–731. doi:10.1249/MSS.0b013e31890eb86

Battino, M., Leone, L., & Bompadre, S. (2004). High-performance liquid chromatography–EC assay of mitochondrial coenzyme Q9, coenzyme Q9H2, coenzyme Q10, coenzyme Q10H2, and vitamin E with a simplified on-line solid-phase extraction. *Methods Enzymology*, *378*, 156–162.

Borg, G., & Dahlstrom, H. (1962). A pilot study of perceived exertion and physical working capacity. *Acta Societatis Medicorum Upsaliensis*, *67*, 21–27.

Bloomer, R. J., Canale, R. E., Blankenship, M. M., & Fisher-Wellman, K. H. (2010). Effect of Ambrotose AO® on resting and exercise-induced antioxidant capacity and oxidative stress in healthy adults. *Nutrition Journal*, *9*, 1–17. doi:10.1186/1475-2891-9-49

Bloomer, R. J., Goldfarb, A. H., & McKenzie, M. J. (2006). Oxidative stress response to aerobic exercise: Comparison of antioxidant supplements. *Medicine & Science in Sports & Exercise*, *38*, 1098–1105. doi:10.1249/01.mss.0000222839.51144.3e

Chahbouni, M., Escames, G., López, L. C., Sevilla, B., Doerrier, C., Muñoz-Hoyos, A., … Acuña-Castriviejo, D. (2011). Melatonin treatment counteracts the hyperoxidative status in erythrocytes of patients suffering from Duchenne muscular dystrophy. *Clinical Biochemistry*, *44*(10–11), 853–858. doi:10.1016/j.clinbiochem.2011.04.001

Concepcion-Huertas, M., Chirosa, L. J., De Haro, T., Chirosa, I. J., Romero, V., Aguilar-Martinez, D., … Acuña-Castriviejo, D. (2013). Changes in the redox status and inflammatory response in handball players during one-year of competition and training. *Journal of Sports Sciences*, *31*, 1197–1207. doi:10.1080/02640414.2013.773404

Dawson, B., Henry, G. J., Goodman, C., Gillam, I., Beilby, J. R., Ching, S., … Kakulus, B. A. (2002). Effect of vitamin C and E supplementation on biochemical and ultrastructural indices of muscle damage after a 21 km run. *International Journal of Sports Medicine*, *23*(1), 10–15. doi:10.1055/s-2002-19273

Esterbauer, H., & Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymology*, *186*, 407–421. doi:10.1016/0076-6879(90)86134-H

Fisher-Wellman, K., & Bloomer, R. J. (2009). Acute exercise and oxidative stress: A 30 year history. *Dynamic Medicine*, *8*, 1–25. doi:10.1186/1476-5918-8-1

Gómez-Cabrera, M. C., Domenech, E., & Viña, J. (2008). Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. *Free Radical Biology & Medicine*, *44*, 126–131. doi:10.1016/j.freeradbiomed.2007.02.001

Gross, M., Baum, O., & Hoppeler, H. (2011). Antioxidant supplementation and endurance training: Win or loss? *European Journal of Sport Science*, *11*(1), 27–32. doi:10.1080/17461391003699088

Halliwell, B. (2012). Free radicals and antioxidants: Updating a personal view. *Nutrition Reviews*, *70*, 257–265. doi:10.1111/j.1753-4887.2012.00476.x

Higashida, K., Kim, S. H., Higuchi, M., Holloszy, J. O., & Han, D. (2011). Normal adaptations to exercise despite protection against oxidative stress. *American Journal of Physiology Endocrinology and Metabolism*, *301*, E779–E784. doi:10.1152/ajpendo.00655.2010

Hissin, P. J., & Hilf, R. (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Analytical Biochemistry*, *74*, 214–226. doi:10.1016/0003-2697(76)90326-2

Leonardo-Mendonça, R. C., Sospedra, I., Sanchis, I., Mañes, J., & Soriano, J. M. (2012). Comparison of the somatotype, nutritional assessment and food intake among university sport and sedentary students. *Medicina Clinica*, *139*, 54–60. doi:10.1016/j.medcli.2011.03.034

Li, X., & Franke, A. (2009). Fast HPLC–ECD analysis of ascorbic acid, dehydroascorbic acid and uric acid. *Journal of Agricultural and Food Chemistry*, *877*, 853–856. doi:10.1016/j.jchromb.2009.02.008

Lu, S. (2013). Glutathione synthesis. *Biochimica et Biophysica Acta*, *1830*, 3143–3153. doi:10.1016/j.bbagen.2012.09.008

Margonis, K., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Douroudos, I., Chatzinikolaou, A., … Kouretas, D. (2007). Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. *Free Radical Biology & Medicine*, *43*, 901–910. doi:10.1016/j.freeradbiomed.2007.05.022

Martínez Álvares, J., & Izquierdo Pulido, M. (2005). *La capacidad antioxidante de la dieta española, la "rueda de los alimentos" antioxidantes* [The antioxidant capacity of the Spanish diet, the "food wheel"]. Madrid: Sociedad Española de Dietética y Ciencias de la Alimentación, pp. 1–24.

McGinley, C., Shafat, A., & Donnelly, E. (2009). Does antioxidant vitamin supplementation protect against muscle damage? *Sports*

Medicine, *39*, 1011–1032. doi:10.2165/11317890-000000000-00000

Naziroglu, M., Kilinç, F., Uguz, A., Çelik, Ö., Bal, R., Butterworth, P., & Baydar, M. (2010). Oral vitamin C and E combination modulates blood lipid peroxidation and antioxidant vitamin levels in maximal exercising basketball players. *Cell Biochemistry and Function*, *28*, 300–305. doi:10.1002/cbf.1657

Nieman, D. C., Henson, D. A. Mcanulty, S. R., Mcanulty, L. S., Morrow J. D., Ahmed, A., & Heward. C. B. (2004). Vitamin E and immunity after the Kona triathlon world championship. *Medicine & Science in Sports & Exercise*, *36*, 1328–1335. doi:10.1249/01.MSS.0000135778.57355.CA

Nils-Georg, A. (2007). Antioxidants: How good are they? *Scandinavian Journal of Food and Nutrition*, *51*(2), 47. doi:10.1080/17482970701420924

Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, & U.S. Department of Agriculture. (2007). *Oxygen radical absorbance capacity (ORAC) of selected foods*. Beltsville, MD: Department of Agriculture.

Okamoto, S., & Ochi, H. (1992). *Chemical Abstract, 129859a. Japanese Patent No. 3,091,974*. Fukuroi: Japan Institute for the Control of Aging.

Palazzetti, S., Richard, M. J., Favier, A., & Margaritis, I. (2003). Overloaded training increases exercise-induced oxidative stress and damage. *Canadian Journal of Applied Physiology*, *28*, 588–604. doi:10.1139/h03-045

Pepe, H., Balci, S. S., Revan, S., Akalin, P. P., & Kurtoglu, F. (2009). Comparison of oxidative stress and antioxidant capacity before and after running exercises in both sexes. *Gender Medicine*, *6*, 587–595. doi:10.1016/j.genm.2009.10.001

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., … Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, *51*, 3273–3279. doi:10.1021/jf0262256

Rufián-Henares, J. A., & Morales, F. J. (2007). Effect of in vitro enzymatic digestion on antioxidant activity of coffee melanoidins and fractions. *Journal of Agricultural and Food Chemistry*, *55*, 10016–10021. doi:10.1021/jf0718291

Sánchez Oliver, A., & Guerra Hernández, E. (2004). Estudio nutricional de la dieta de alumnos de la Facultad de Ciencias de la Actividad Física y el Deporte de Granada [Nutritional study of the student's diet of the faculty of physical education and sport of Granada]. *Nutrición Clínica*, *24*, 74–81.

Schröder, H., Navarro, E., Mora, J., Galiano, D., & Tramullas A. (2001). Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *International Journal of Sports Medicine*, *40*, 178–184.

Serrano, E., Venegas, C., Escames, G., Sánchez-Muñoz, C., Zabala, M., Puertas, A., … Acuña-Castroviejo, D. (2010). Antioxidant defence and inflammatory response in professional road cyclists during a 4-day competition. *Journal of Sports Sciences*, *28*, 1047–1056. doi:10.1080/02640414.2010.484067

Teixeira, V., Valente, H., Casal, S., Marques, F., & Moreira, P. (2009a). Antioxidant status, oxidative stress, and damage in elite trained kayakers and canoeists and sedentary controls. *International Journal of Sport Nutrition and Exercise Metabolism*, *19*, 443–456.

Teixeira, V., Valente, H., Casal, S., Marques, F., & Moreira, P. (2009b). Antioxidant status, oxidative stress, and damage in elite trained kayakers after 1 year of training and competition in 2 seasons. *Applied Physiology, Nutrition, and Metabolism*, *34*, 716–724. doi:10.1139/H09-062

Tong, T. K., Lib, H., Lippi, G., & Tian, Y. (2012). Serum oxidant and antioxidant status in adolescents undergoing professional endurance sports training. *Oxidative Medicien and Cellular Longevity*, *2012*, 1–7. doi:10.1155/2012/741239

Tsakiris, T., Partimos, N., & Schulpis, K. H. (2006). Alfa-tocoferol supplementation reduces the elevated 8-hydroxy-2-deoxyguanosine blood levers induced by training in basketball players. *Clinical Chemistry and Laboratory Medicine*, *44*, 1004–1008.

World Health Organization. (2003). *Diet, nutrition and the prevention of chronic diseases*. Geneva: Author.

Yfanti, C., Fisher, C., Nielsen, S., Akerström, T., Nielsen, A., Veskoukis, A., … Pedersen, B. (2012). Role of vitamin C and E supplementation on IL6 in response to training. *Journal of Applied Physiology*, *112*, 990–1000. doi:10.1152/japplphysiol.01027.2010