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ORIGINAL CONTRIBUTION

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Response of blood cell antioxidant enzyme defences to antioxidant diet supplementation and to intense exercise

Summary *Background* Exhaustive exercise induces oxidative stress. The cellular antioxidant defence systems have demonstrated great adaptation to chronic exercise. *Aim* To establish the influence of the antioxidant diet supplementation on the erythrocyte and lymphocyte antioxidant enzyme activities in athletes at basal and post-exercise levels. *Methods* Fifteen amateur trained male athletes were randomly distributed in two groups: control and antioxidant supplemented (90 days' diet supplementation with 500 mg/day vitamin E and 30 mg/day β-carotene, and the last 15 days also with 1 g/day vitamin C). The study was double blind. Maximal and submaximal exercise tests were performed after three months of diet supplementation. The study was developed during the training and competition season. *Results* The sportsmen of the supplemented group presented significantly higher plasmatic final levels of vitamin C, vitamin E and β-carotene. Erythrocyte glutathione reductase activity significantly decreased in the placebo group but was maintained in the supplemented group after the three months studied. The erythrocyte superoxide dismutase activity increased after the training/competition period in the placebo group. Lymphocyte catalase and glutathione peroxidase activities increased significantly in the supplemented group after the supplementation period but were maintained in the placebo group. No effects of the antioxidant supplementation were observed in the erythrocyte antioxidant enzyme response to the exercise tests. The antioxidant supplementation induced a better adaptation of lymphocyte catalase after submaximal test. *Conclusions* Lymphocytes showed higher sensibility to antioxidant supplementation, improving the response of antioxidant enzymes to training and to acute exercise. In erythrocytes the training adaptations were more important than the antioxidant supplementation effects.

Key words lymphocyte – erythrocyte – training – oxidative stress – antioxidants

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Introduction

Exhaustive exercise induces oxidative stress and it may impair immune response, increasing athletes' susceptibility to upper respiratory tract infections [1]. Exercise-related immunological acute changes include signs of inflammation, such as release of cytokines [2], activation of immunocompetent cell lines [3], neutrophil priming for acute phase response [3, 4], higher oxidized glutathione levels and lower antioxidant enzyme levels in neutrophils [5] and the induction of acute phase proteins [6].

The effect of diet-supplementation with several antioxidants such as vitamins E and C and other nutrients like glutamine on exercised-induced acute changes in immune cell function have been reported [7, 8]. Moreover, a negative correlation between oxidative DNA

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damage and glutathione or ascorbate levels has also been proved in human lymphocytes [9]. However, few data are available about the effects of the antioxidant nutrient diet-supplementation and the competition season on the basal antioxidant enzyme activities in lymphocytes and erythrocytes.

Erythrocytes produce high amounts of ROS as a consequence of increased oxygen transport during exercise. Training increases the erythrocyte antioxidant enzyme defences in sedentary people [10, 11], and well trained subjects present higher erythrocyte antioxidant enzyme activities than sedentary individuals [12, 13]. The diet antioxidant supplementation and their availability to react with ROS could modify the antioxidant enzyme adaptations in erythrocytes because acute changes in antioxidant enzyme activities are influenced by the ROS production in the erythrocyte [14].

We have previously observed enhanced basal antioxidant enzyme activities in neutrophils after three months of vitamin E, C and β-carotene supplementation [15]. The aims of this study were i) to establish the influence of the antioxidant diet supplementation during the competition season on the activities of erythrocyte and lymphocyte antioxidant enzymes in amateur trained athletes; and ii) to evaluate the effects of antioxidant supplementation on the response of erythrocyte and lymphocyte antioxidant enzyme activities to acute bouts of exercise. Maximal and prolonged submaximal exercise tests have been used. The maximal exercise test allowed us to measure the effects of a short exercise where the highest intensity was achieved. However, the submaximal exercise test allowed us to study the possible adaptations of the enzymatic defences to the increased oxidative stress because of its longer duration.

Materials and methods

Subjects and protocol

Fifteen trained male amateur athletes volunteered to participate in this study. They were all endurance athletes: three cyclists and twelve sportsmen used to participating in duathlon-like competitions. They trained 14 ± 1 h each week. Their age was 23.3 ± 2.0 years, height 168 ± 3 cm, weight 70.8 ± 1.2 kg and body mass index 24.5 ± 1.3 kg/m^2.

The fifteen sportsmen were randomly and double blinded treated with either an antioxidant nutrient cocktail (consisting of 250 mg of vitamin E, 15 mg of β-carotene) or placebo (lactose). The eight sportsmen of the supplemented group (which includes two cyclists) took two antioxidant cocktail capsules per day for 90 days; the last 15 days they took two additional capsules per day, each containing 500 mg of vitamin C in order to attain synergistic effects of the antioxidant supplementation. The seven sportsmen of the placebo group (including one cyclist) took the same capsules as the supplemented group, which instead contained a placebo (lactose). The consumption of the supplements was periodically controlled. During this study all sportsmen continued their training sessions and participated in several competitions. After the three months of supplementation all the athletes performed a maximal and a submaximal prolonged exercise test. Subjects were instructed to refrain from strenuous exercise the day before the exercise tests. Determinations of hematological parameters and enzymatic activities were made before and after the three months of supplementation and, also, before and after each exercise test performed at the end of the study.

To ensure that the observations represent differences due to the antioxidant supplement rather than to changes in the sportsmen's dietary intake, the usual dietary habits of each participant in the study were assessed using a 3-day 24-hour recall at the beginning of the study and before the maximal test performed after the three months of supplementation. No significant differences between placebo and supplemented groups and no changes in the diet with time were found (results not shown).

Subjects were informed of the purpose of this study before giving their written consent to participate. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Ethical Committee of 'Hospital Son Dureta').

Exercise tests, VO_2 max and maximal heart rate determinations

After the three months of competitions and antioxidant supplementation, two exercise tests were performed on an electromagnetic reduction cycloergometer (Ergometrics 900, MedGraphics™, St Paul, MN, USA): the maximal exercise test and the submaximal prolonged exercise test. During the maximal exercise test the VO_2 and the heart rate were continuously monitored and the VO_2 max and maximal heart rate were measured. The VO_2 was obtained using an automated breath by breath system (CPX; Medical Graphics). The heart rate was continuously monitored by using an electrocardiograph. The instruments were calibrated before each test. This maximal test started at 50 W and the subjects' work rate was increased by 30 W every three minutes. The test ended when increased work did not increase or decrease oxygen consumption; this value was the VO_2 max. Maximal heart rate was determined by using the heart rate recorded during the test. During this maximal exercise test, the sportsmen did not drink anything.

The submaximal prolonged exercise test was carried out one week after the maximal test. The cycloergome-

ter resistance was adjusted so that the athletes worked at 80 % of their maximal capacity of oxygen consumption. This test lasted for 1 h 30 min and all subjects drank 500 ml of spring water. All the tests were performed in overnight fasted subjects, at the same time, in the same room and with the same temperature and humidity conditions.

Erythrocyte and lymphocyte purification and quantification

Blood samples were obtained from the antecubital vein of sportsmen after overnight fasting in suitable vacutainers with EDTA as the anticoagulant and were used to purify erythrocytes and lymphocytes following an adaptation of the method described by Boyum [16]. Blood was centrifuged at 900 g, at 4 °C for 30 min after carefully introducing onto Ficoll in a proportion of 1.5:1. The lymphocyte layer was carefully removed. The plasma and the ficoll phase were discarded. The erythrocyte phase at the bottom was washed twice with 10 ml of PBS and was finally reconstituted with distilled water in the same volume as plasma. The lymphocyte slurry was washed twice with isotonic PBS and centrifuged for ten minutes at 1000 g and 4 °C. Finally, lymphocytes were lysed with distilled water. Erythrocyte and lymphocyte numbers were determined in an automatic flow cytometer analyzer Technicon H2 (Bayer) VCS system [17].

Enzymatic determinations

We determined the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase in erythrocytes and lymphocytes. All activities were determined with a Shimadzu UV-2100 spectrophotometer at 37 °C immediately after sample collection and cell purification.

Catalase activity was measured by the spectrophotometric method of Aebi [18] based on the decomposition of H_2O_2. Glutathione reductase activity was measured by a modification of the Goldberg and Spooner [19] spectrophotometric method. Glutathione peroxidase (GPx) activity was measured by an adaptation of the spectrophotometric method of Flohé and Gunzler using H_2O_2 as the substrate [20]. GPer activity was carried out in erythrocytes as for the GPx, but the substrate was cumene hydroperoxide. SOD activity was measured by an adaptation of the method of McCord and Fridovich [21].

Plasma vitamin determinations

Plasma was obtained after centrifugation of blood samples (collected separately in vacutainers containing heparin as the anticoagulant) at 1000 g, 4 °C for 30 min. For vitamin C determination, plasma was deproteinized with TCA. Plasma and deproteinized plasma were stored at –80 °C until use. The plasma levels of vitamin C, vitamin E and β-carotene were determined by HPLC methods as described previously [15].

Statistical analysis

Statistical analysis was carried out by using a statistical package for social sciences (SPSS 9 for windows). Results are expressed as means ± s. e. m. and $p < 0.05$ was considered statistically significant. All the data were tested for homogeneity of variance. When the effects of the antioxidant supplementation (S) during a training period (T) were tested, the data were analyzed by repeated measurements analysis of variance (ANOVA) with antioxidant supplement/placebo as the categorical variable. The effects of the antioxidant supplementation on the antioxidant enzyme response to the exercise tests were tested by a two-way ANOVA with antioxidant diet supplementation (S) and the exercise tests (E) as ANOVA factors. The sets of data in which there were significant effects were tested by the ANOVA one-way test. The final plasmatic antioxidant levels were compared using a "t student" test for unpaired data.

Results

No differences between the baseline maximal heart rate and VO_2 max measurements were found. The maximal heart rate was maintained after the three months of study in both the control (initial: 182 ± 5 pulses/min; final: 182 ± 5 pulses/min) and the supplemented (initial: 179 ± 4 pulses/min; final: 182 ± 4 pulses/min) groups. No effects of antioxidant diet-supplementation were observed on the VO_2 max of sportsmen. However, the competition season significantly increased the VO_2 max in the control (initial: 60.1 ± 3.1 mL/kg·min; final: 67.3 ± 1.5 mL/kg·min) and in the supplemented (initial: 64.7 ± 2.0 mL/kg·min; final: 71.3 ± 2.2 mL/kg·min) groups.

There were no differences between groups in the basal levels of vitamin C (placebo: 10.5 ± 0.2 μg/mL; supplemented: 10.1 ± 0.1 μg/mL), vitamin E (placebo: 25.4 ± 1.9 μg/mL; supplemented: 26.0 ± 1.3 μg/mL) and β-carotene (placebo: 149 ± 25 μg/L; supplemented: 143 ± 24 μg/L) [15]. As it is shown in Fig. 1, plasma vitamin E, β-carotene and vitamin C concentrations in the antioxidant-supplemented group after three months of antioxidant supplementation were, respec-

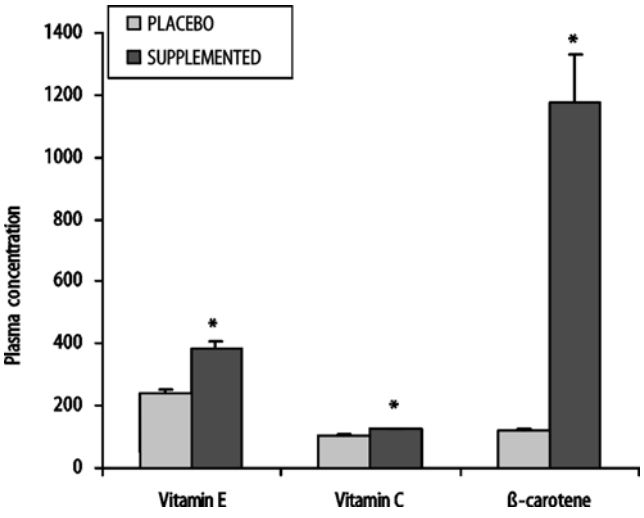

Fig. 1 Plasmatic antioxidant levels after supplementation in placebo and supplemented groups. The results are the mean ± s. e. m. of eight subjects in the supplemented group and seven subjects in the placebo group. * Indicates significant differences control vs. supplemented. Plasma concentration units: vitamin E (µg/cL); ascorbate (µg/cL); β-carotene (µg/L)

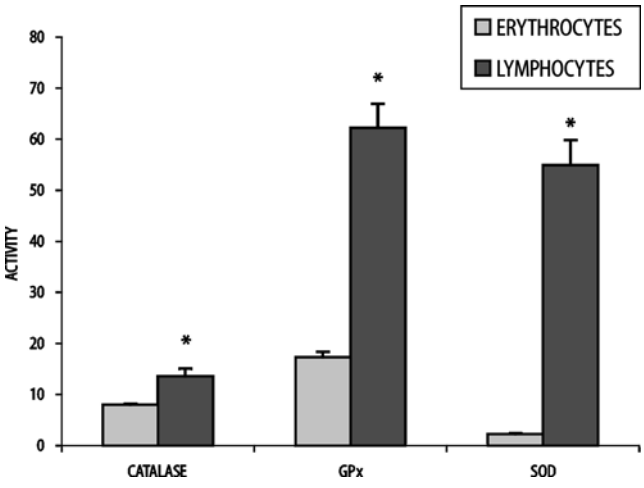

Fig. 2 Basal antioxidant enzyme activities in erythrocytes and lymphocytes. The results are the mean ± s. e. m. of the eight subjects belonging to the supplemented group and the seven subjects belonging to the placebo group. * Indicates significant differences between erythrocyte and lymphocyte activity. Cellular activities: catalase (K/10^9 cells); GPx (nkat/10^9 cells); SOD (pkat/10^9 cells)

tively, 1.6, 10, and 1.2 times higher than those of the placebo group.

Fig. 2 shows the basal antioxidant enzyme activities in erythrocytes and lymphocytes on a cellular basis. The catalase activity was about 1.7 times higher, glutathione peroxidase about 3.5 times higher and SOD about 30 times higher in lymphocytes than in erythrocytes.

Basal erythrocyte number and erythrocyte antioxidant enzyme activities before and after the three months of study are shown in Table 1. No changes were observed in the erythrocyte number. Erythrocyte catalase basal activity was unaffected either by the competition season or by the antioxidant supplementation. Significant differences between initial and final values were observed in both glutathione peroxidase activities in erythrocytes. Glutathione peroxidase determined with hydrogen peroxide as substrate significantly decreased in activity (by about 13.5 %) in the placebo group but was maintained in the supplemented group after the three months studied. The competition season significantly decreased the glutathione peroxidase activity determined with cumene hydroperoxide, although no differences were observed between groups. The erythrocyte

Table 1 Effects of antioxidant supplementation on basal erythrocyte number and erythrocyte antioxidant enzyme activities of sportsmen

	Initial		Final		ANOVA
	Placebo	Supplemented	Placebo	Supplemented	
Erythrocytes 10^6/µL	4.89±0.07	4.99±0.11	4.95±0.08	5.15±0.09	
Catalase K/10^9 cells	8.01±0.22	7.81±0.14	8.10±0.34	7.89±0.19	
GPx nKat/10^9 cells	17.7±1.2	16.4±1.2	14.3±1.9[b]	14.5±0.8	T
Gper nKat/10^9 cells	7.36±0.72	7.58±0.94	6.02±0.59	6.04±0.60	T
G Red nKat/10^9 cells	4.57±0.19	4.07±0.11	3.08±0.26[b]	3.46±0.41	S*T, T
SOD pKat/10^9 cells	1.96±0.09	1.95±0.25	2.52±0.18[b]	2.42±0.24	T

The results are the mean ± s. e. m. of eight subjects in the supplemented group and seven subjects in the placebo group. *GPx* glutathione peroxidase determined with H_2O_2 as substrate; *GPer* glutathione peroxidase determined with cumene hydroperoxide as substrate; *SOD* superoxide dismutase; *T* indicates significant differences before vs. after; *SxT* indicates a significant interaction between supplementation and the training period (ANOVA repeated measures)

[a] Indicates significant differences placebo vs. supplemented; [b] Indicates significant differences initial vs. final

glutathione reductase presented a statistically significant interaction between the supplementation and the training period: this activity was maintained after the three months in the antioxidant diet-supplemented group but decreased about 32 % in the placebo group. Erythrocyte SOD activity showed significant differences before vs. after, increasing significantly, about 29 %, after the competition season. Supplemented and placebo groups behaved similarly, showing an increase over the period of the study. For the placebo group this was statistically significant; and in the two groups combined, the effect was very significant ($p = 0.003$).

Table 2 shows the effects of the competition season and the antioxidant diet supplementation on the lymphocyte number and the antioxidant enzyme basal activities in lymphocytes. The statistical analysis showed significant differences between initial and final lymphocyte catalase activity. This activity in the supplemented group significantly increased about 39 % after the competition season, but the slight increase observed in the placebo group was not statistically significant. Statistically significant effects of antioxidant diet supplementation and competition season were observed in the lymphocyte glutathione peroxidase activity, increasing significantly, about 58 %, only in the supplemented group. The final glutathione peroxidase activity in this supplemented group was significantly higher than in the placebo group. The SOD activity in lymphocytes did not change throughout the study in the two groups, and was unaffected by the diet supplementation.

Table 3 shows the effects of the maximal and the submaximal exercise tests carried out after the three months of competitions and supplementation on the antioxidant enzyme activities in erythrocytes. Both the maximal and the submaximal exercise tests produced no effects on the enzyme activities and on the erythrocyte number.

The effects of the maximal and the submaximal exercise tests performed after the three months of supplementation on the lymphocyte number and on the lymphocyte antioxidant enzyme activities are shown in Table 4. The lymphocyte number was unaffected by both the maximal and the submaximal tests. The maximal exercise test did not produce any effect on the enzymatic activities. When the results of the submaximal test were analyzed, a significant interaction between the supplementation and the competitions period was found in the catalase activity. This activity was maintained after the submaximal test in the placebo group but increased significantly (about 57 %) in the supplemented one after the test.

Discussion

The supplementation with vitamin C, vitamin E and β-carotene produced a real increase of these antioxidant nutrients. However, antioxidant vitamin levels of all sportsmen participating in the study were within the normal range of well-nourished people [22]. The difference between the vitamin E level in the supplemented group and in the placebo is lower than others described in similar experiences with well-nourished adults [23] and similar to others with sportsmen [24]. Probably sportsmen should take a higher amount of vitamin E than normal adults in order to protect themselves against the oxidative stress associated to physical activity [24–26].

β-carotene is the supplemented antioxidant that presented a higher level in plasma after three months of supplementation when it is compared to the final levels in the placebo group. The liposoluble nature of this compound is responsible for the body accumulation. On the other hand, the increase of plasma ascorbate levels in the antioxidant-supplemented group was the lowest of the ones produced by the antioxidant supplementation. The hydrosoluble nature of this vitamin and the existence of

Table 2 Effects of antioxidant supplementation on basal lymphocyte number and lymphocyte antioxidant enzyme activities of sportsmen

	Initial		Final		ANOVA
	Placebo	Supplemented	Placebo	Supplemented	
Lymphocytes $10^3/\mu L$	2.15±0.14	1.96±0.16	2.09±0.12	1.86±0.12	
Catalase K/10^9 cells	12.5±0.9	14.6±2.8	16.4±1.7	20.3±1.9[b]	T
GPx nKat/10^9 cells	58.5±5.6	65.6±7.6	73.3±5.5	104±8[a, b]	S, T
SOD pKat/10^9 cells	58.5±6.8	59.2±7.8	51.5±3.8	61.5±6.8	

The results are the mean ± s. e. m. of eight subjects in the supplemented group and seven subjects in the placebo group. *GPx* glutathione peroxidase determined with H_2O_2 as substrate; *SOD* superoxide dismutase; *T* indicates significant differences before vs. after; *S* indicates significant differences placebo vs. supplemented; *SxT* indicates a significant interaction between supplementation and the training period (ANOVA repeated measures)
[a] Indicates significant differences placebo vs. supplemented; [b] Indicates significant differences initial vs. final

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Table 3 Effect of maximal and submaximal exercise tests on erythrocyte number and on antioxidant enzyme activities

	Placebo		Supplemented		ANOVA		
	Before	After	Before	After	S	E	S*E
Maximal exercise test							
Erythrocytes (10^6/µL)	4.93±0.09	5.07±0.08	5.09±0.11	5.20±0.08			
Catalase (K/10^9 cells)	8.24±0.59	8.31±0.59	7.73±0.34	7.18±0.33[a]	*		
GPx (nkat/10^9 cells)	15.8±0.7	15.0±1.4	15.3±1.3	14.9±1.6			
GPer (nkat/10^9 cells)	6.28±1.10	5.41±1.11	6.14±0.75	6.05±0.79			
G Red (nkat/10^9 cells)	3.18±0.36	2.89±0.36	3.39±0.56	4.01±0.95			
SOD (pkat/10^9 cells)	2.71±0.34	2.24±0.34	2.51±0.38	2.35±0.36			
Submaximal exercise test							
Erythrocytes (10^6/µL)	4.96±0.10	5.19±0.08	5.09±0.10	5.24±0.13			
Catalase (K/10^9 cells)	7.96±0.39	7.45±0.54	7.73±0.34	6.82±0.34			
GPx (nkat/10^9 cells)	12.8±1.6	12.5±1.4	13.6±1.0	13.4±1.0			
GPer (nkat/10^9 cells)	5.77±0.52	5.17±0.46	5.93±1.03	5.54±1.17			
G Red (nkat/10^9 cells)	3.06±0.35	3.18±0.32	3.67±0.71	2.57±0.48			
SOD (pkat/10^9 cells)	2.34±0.12	2.31±0.21	2.28±0.16	2.49±0.34			

The results are the mean ± s. e. m. of eight subjects in the supplemented group and seven subjects in the placebo group. *GPx* glutathione peroxidase determined with H_2O_2 as substrate; *GPer* glutathione peroxidase determined with cumene hydroperoxide as substrate; *SOD* superoxide dismutase. * Indicates significant effects of factor *S*, *E* or the interaction *SxE* (two-way ANOVA). Factor *S* represents supplementation; factor *E* represents the exercise; *SxE* represents the interaction between the two factors
[a] Indicates significant differences placebo vs. supplemented; [b] Indicates significant differences before vs. after

Table 4 Effect of maximal and submaximal exercise tests on lymphocyte number and on antioxidant enzyme activities

	Placebo		Supplemented		ANOVA		
	Before	After	Before	After	S	E	S*E
Maximal exercise test							
Lymphocytes (10^3/µL)	2.13±0.20	2.55±0.32	1.92±0.19	2.15±0.14			
Catalase (K/10^9 cells)	16.8±2.8	17.2±2.9	20.6±2.9	21.0±2.4			
GPx (nkat/10^9 cells)	79.0±8.3	79.4±7.1	98.2±11.3	84.8±9.5			
SOD (pkat/10^9 cells)	49.1±4.5	54.3±5.6	54.2±4.7	53.3±5.4			
Submaximal prolonged exercise test							
Lymphocytes (10^3/µL)	2.05±0.17	2.33±0.16	2.12±0.15	1.98±0.19			
Catalase (K/10^9 cells)	14.4±1.4	14.9±2.4	19.3±1.9	30.3±2.6[a, b]	*	*	*
GPx (nkat/10^9 cells)	66.8±6.7	64.0±7.2	111±9[a]	112±10[a]	*		
SOD (pkat/10^9 cells)	54.1±5.5	46.5±4.3	68.4±9.8	89.1±10.0			

The results are the mean ± s. e. m. of eight subjects in the supplemented group and seven subjects in the placebo group. *GPx* glutathione peroxidase determined with H_2O_2 as substrate; *SOD* superoxide dismutase. * Indicates significant effects of factor *S*, *E* or the interaction *SxE* (two-way ANOVA). Factor *S* represents supplementation; factor *E* represents the exercise; *SxE* represents the interaction between the two factors
[a] Indicates significant differences placebo vs. supplemented; [b] Indicates significant differences before vs. after

homeostatic mechanisms to regulate ascorbate plasma levels [27, 28] could be responsible for this low plasma increase. It is difficult to raise blood levels of vitamin C beyond a certain value [27–29] and they could be attained within 15 days of diet supplementation [29]. These results are similar to others described in the literature [30] and represent a real increase of the sportsmen cellular antioxidant availability.

When this diet intervention was initiated, the sportsmen had high VO_2 max because they were well trained. However this parameter increased about 10% during the competition season, reflecting the adaptation to the

repetitive training and competition sessions developed during this intervention study. The physical performance of athletes attained at the end of the three months was similar in the two groups and in accordance with others [31]; no additional effects of antioxidant diet supplementation were observed on the VO_2 max and the maximal heart rate.

The erythrocyte is the blood cell that presents the lowest enzymatic antioxidant defences. Lymphocytes and erythrocytes present very different basal antioxidant enzyme activities. The catalase vs. SOD activity ratio is very high in erythrocytes and very low in lymphocytes. Catalase is the main enzyme responsible for scavenging H_2O_2 in erythrocytes [32]; however, the relatively higher levels of glutathione peroxidase vs. catalase in lymphocytes than in erythrocytes could indicate a more important participation of glutathione peroxidase in H_2O_2 detoxification in lymphocytes.

Several studies in sedentary people and in athletes have shown different effects of training on the basal activity of erythrocyte antioxidant enzymes [11–13, 33, 34]. Factors such as the initial training status, the training protocols [11, 33] and the nutritional status of sportsmen [34] have been pointed out as conditioning the basal erythrocyte antioxidant enzyme activities. Our results in trained sportsmen during their competition season point towards a basic role of training/competition in increasing the basal activity of erythrocyte SOD. In accordance with the increase observed after a twelve-week aerobic training program [33], SOD activity increased after the three months of training and competition. However, the antioxidant supplementation did not affect this activity at rest. The lack of change in the basal catalase activity in erythrocytes during the study contrasts with previous results [12, 13]. However, these results were obtained in sedentary men after a training program, whereas our subjects were active sportsmen with a high initial erythrocyte catalase activity. The decrease in the basal glutathione peroxidase activities during the competition season has also been observed by others [35]. By contrast, other studies showed increases in this activity with aerobic training [11, 33, 34] or a higher activity in a high-trained group compared with a little-trained group [13].

Increases [12] and decreases [34] in erythrocyte glutathione reductase activity after training have been shown. We point out that the basal activity is maintained in the antioxidant-supplemented group, whereas a decrease is produced after competition season in the placebo group. The decrease in glutathione reductase activity has been related with increased requirements of riboflavin in trained sportsmen [12] because this enzyme is highly dependent on this vitamin. However, under oxidizing conditions, glutathione reductase may form molecular aggregates with a decrease in the activity. These aggregates are dissociable using EDTA or sulphydryl reagents [19]. We added EDTA in the glutathione reductase activity determination. So, it could be suspected that the decrease in the activity could be due to irreversible protein oxidations. In this way, the protective effects of antioxidant diet supplementation could avoid the oxidation of glutathione reductase in the erythrocyte after the three months of competition in the same way as we previously evidenced in the maintenance of this activity in neutrophils [15].

At the moment, little evidence has been found of the possible adaptation of lymphocyte antioxidant defences to training. The major evidence revealed a higher lymphocyte ascorbate concentration in sportsmen after the training period and also compared to the sedentary counter partners [13]. We point out that lymphocyte catalase and glutathione peroxidase activities are higher after the competition season. Glutathione peroxidase activity increased both in the placebo and in the antioxidant supplemented group, but the increase in the antioxidant supplemented group was higher than the increase in the placebo group. It has been established that ROS can regulate the expression of some antioxidant enzymes [36] and we evidenced an effect of antioxidant nutrients on the GPx activity.

In order to determine the influence of the antioxidant supplementation and the training on the response of the antioxidant enzyme activities to oxidative stress we developed two exercise tests. These tests had also been performed before the three months of training and supplementation [37]. The submaximal test performed at the beginning of the study produced decreases in the erythrocyte activities of catalase, glutathione peroxidase and glutathione reductase and an increase in SOD activity [37]. The lack of changes in both groups in the test performed after the supplementation and training period could indicate an adaptation of the enzyme antioxidant defences to the oxidative stress induced by the exercise test. In fact, the increase in erythrocyte SOD activity, as an indicator of a training adaptation, during the three months of study could be enough to avoid the effects of the oxidative stress on the antioxidant enzymes observed in the initial submaximal exercise test. However, as the picture observed in both groups is similar, we can conclude that the antioxidant supplementation did not produce any additional effect on the enzyme adaptations to the oxidative stress induced by an acute exercise.

The maximal exercise test performed before the three months of training and antioxidant supplementation produced a general decrease in the lymphocyte antioxidant enzyme activities [38]. However, when this test was performed at the end of the study, no changes in the antioxidant enzyme activities were observed. The lack of changes in both the placebo and the supplemented group could indicate a positive effect of the training season, with an adaptation to the increased oxidative stress.

The increased basal lymphocyte antioxidant enzyme activities could be enough to avoid the oxidative damage induced by ROS and, thus, the enzyme inactivation observed in the maximal test performed at the beginning of the study [38]. No effects of the antioxidant supplementation have been evidenced in this test. However, we observed that antioxidant supplementation produced an adaptation to the increased oxidative stress during the submaximal test increasing the lymphocyte catalase activity in the supplemented group. In a previous study we reported that lymphocytes showed a great adaptation to increased oxidative stress induced by an exhaustive exercise increasing their antioxidant enzyme activities [39]. However, in this study a specific activation of catalase in the supplemented group has been observed, indicating a possible role of the higher levels of antioxidant nutrients.

In conclusion, we demonstrate the existence of synergistic effects between the training and competition season of the athletes and their antioxidant nutrient intake in order to enhance the basal antioxidant defence in lymphocytes. Moreover, in lymphocytes the antioxidant supplementation improves the response of antioxidant enzymes to an acute bout of exercise. In erythrocytes, the antioxidant supplementation avoids the decrease in the basal glutathione reductase activity. However, the training adaptations are enough to counteract the negative effects of oxidative stress induced by exercise tests and no effects of the antioxidant supplementation have been observed during these exercise tests.

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