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Antioxidant diet supplementation enhances aerobic performance in amateur sportsmen

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Abstract
The effects of antioxidant diet supplements on blood lactate concentration and on the aerobic and anaerobic thresholds and their adaptations to training were analysed. Fifteen amateur male athletes were randomly assigned to either a placebo group or an antioxidant-supplemented group (90 days supplementation with 500 $mg \cdot day^{-1}$ of vitamin E and 30 $mg \cdot day^{-1}$ of β-carotene, and the last 15 days also with 1 $g \cdot day^{-1}$ of vitamin C). Before and after the antioxidant supplements, the sportsmen performed a maximal exercise test on a cycle ergometer and maximal and submaximal physiological parameters were assessed together with blood lactate concentration. Maximal oxygen uptake ($\dot{V}O_{2max}$), maximal blood lactate concentration, and the maximal workload attained rose significantly in both groups after the 3 months of training. At the end of the study, maximal blood lactate concentration was lower in the group that took supplements than in the placebo group. The percentage of $\dot{V}O_{2max}$ attained at the anaerobic threshold rose significantly in both groups after 3 months of training, although the final value in the supplemented group was higher than that in the placebo group. Antioxidant diet supplements induced lower increases in blood lactate concentration after a maximal exercise test and could improve the efficiency in which aerobic energy is obtained.

Keywords: *Exercise, lactate, oxidative stress, antioxidants*

Introduction

Physical exercise is characterized by an increase in oxygen uptake by the whole body, particularly by muscle, which can be up to 10 or 15 times higher than in regular conditions (Wilmore & Costill, 2004). This increase in oxygen consumption is associated with a rise in the production of reactive oxygen species (Leeuwenburgh and Heinecke, 2001). In fact, about 5% of the oxygen reduced during oxidative phosphorylation in the respiratory chain is converted into superoxide anion (Davies, Quintanilha, Brooks, & Packer, 1982; Jenkins, 1988). The high production of reactive oxygen species (ROS) during exhaustive exercise induces oxidative stress (Viña *et al.*, 2000), increasing the markers of lipid peroxidation in target tissues and blood (Alessio, 1993; Alessio & Goldfarb, 1988; Davies *et al.*, 1982). A linear direct correlation has been observed between the lactate/pyruvate ratio and the oxidized/reduced glutathione ratio (GSSG/GSH) in blood (Sastre *et al.*, 1992). Nevertheless, supramaximal and short-term exercise have recently been reported also to produce oxidative stress (Groussard *et al.*, 2003a, 2003b). Furthermore, recent evidence indicates that high-intensity anaerobic work does result in oxidative damage in both skeletal muscle and blood (Bloomer & Goldfarb, 2004).

The high production of ROS during exhaustive exercise could be responsible for membrane damage, several physiological and biochemical changes (Alessio, 1993; Ji and Fu, 1992; Sastre *et al.*, 1992; Witt, Reznick, Viguie, Starke-Reed, & Packer, 1992), and also for muscular damage leading to a consequent drop in muscular functionality, enzyme release to plasma, histological changes, and muscular soreness (Dekkers, van Doornen, & Kemper, 1996; Kuipers, 1994). It has been noted that oxidative stress could contribute to the development of fatigue. However, more studies are necessary about this topic and the possible mechanisms involved (Powers, DeRuisseau, Quindry, & Hamilton, 2004). Contracting skeletal

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muscle generates ROS that can induce changes in gene expression or cell damage depending upon the pattern of production and the endogenous protective systems (Pattwell & Jackson, 2004). Reactive oxygen species act as a signal to induce adaptive responses, including the maintenance of oxidative homeostasis and the prevention of oxidative damage (Pattwell & Jackson, 2004).

Little research has focused on the effects of antioxidant supplementation on several physiological characteristics such as oxygen consumption ($\dot{V}O_2$), lactate accumulation, and aerobic and anaerobic thresholds. It has been pointed out that diet supplements with antioxidants such as vitamin E and vitamin C do not influence $\dot{V}O_{2max}$ (Sumida, Tanaka, Kitao, & Nakadomo, 1989). However, it has been reported that the blood lactate/pyruvate ratio is directly correlated with the blood GSSG/GSH ratio, which depends on the oxidative stress induced during exercise (Sastre *et al.*, 1992). Hence, the factors that influence oxidative stress could also influence lactate production and affect how the aerobic energy is obtained. Maximal parameters, such as $\dot{V}O_{2max}$, and submaximal parameters, such as the percentage of $\dot{V}O_{2max}$ or the percentage of the maximal workload at fixed lactate concentrations (2 and 4 mM), are used as measures of aerobic performance. The determination of physiological parameters at the 4 mM lactate threshold is interesting because they specify the change from an aerobic to a predominantly anaerobic metabolism. These changes are used as indicators of adaptation to aerobic training.

The aim of this study was to determine the effects of long-term (3 months) diet supplements with an antioxidant cocktail (vitamin E and β-carotene) on lactate concentrations and on the thresholds and their adaptations to training. Vitamin C was also included in the supplementation for the last 15 days because of synergistic effects observed *in vitro* between the three antioxidant nutrients (Bendich, 1989; Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991) and the pattern of appearance of vitamin C in plasma (Linder, 1991).

Methods

Participants and study protocol

Fifteen trained male amateur endurance athletes volunteered to participate in the study. They were all endurance athletes: three cyclists and twelve sportsmen who participated in duathlon-like (running and cycling) competitions. A prior physical examination, including an electrocardiographic evaluation and a blood test (haematological and serum biochemical parameters), ensured that each participant was in good health. Individuals who smoked were excluded from the investigation. Participants were informed of the purpose of this study and the possible risks involved before they provided written consent to participate and to refrain from additional supplementation during the study. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Ethics Committee of 'Hospital Son Dureta').

In a randomized and double-blind fashion, the 15 participants were assigned either to an antioxidant nutrient cocktail (consisting of 250 mg of vitamin E and 15 mg of β-carotene per capsule) or placebo (lactose). The eight participants in the supplemented group took two antioxidant cocktail capsules per day for 90 days; during the last 15 days they took two additional capsules per day, containing 500 mg of vitamin C. The seven participants in the placebo group took similar capsules containing lactose. The consumption of the supplements was periodically controlled. The supplementation increases the plasma antioxidant concentrations of the three antioxidant nutrients supplemented. Plasma vitamin E, β-carotene, and vitamin C concentrations in the antioxidant-supplemented groups were 1.6, 10, and 1.2 times higher respectively than those in the placebo group after nutritional intervention (Tauler, Aguiló, Fuentespina, Tur, & Pons, 2002).

A maximal exercise test was performed by both groups before and after the 3 months of supplementation. Participants reported to the laboratory after a 12-h overnight fast during which they were able to drink mineral water *ad libitum*. During the maximal test, the participants were requested not to drink anything. Participants were instructed to abstain from strenuous exercise for at least 2 days before the exercise tests. Blood samples were obtained before and after each exercise test from the antecubital vein in suitable vacutainers with EDTA as anticoagulant. Participants were seated at rest for at least 15 min before the initial blood samples were taken. The physical characteristics of the participants are shown in Table I. All participants continued their training sessions and participated in several

Table I. Initial physical characteristics of the participants (mean $\pm$ s_x).

	Placebo ($n=8$)	Supplemented ($n=7$)
Age (years)	23.7 $\pm$ 1.7	25.3 $\pm$ 1.7
Height (m)	1.70 $\pm$ 0.03	1.72 $\pm$ 0.02
Body mass (kg)	63.2 $\pm$ 3.6	68.3 $\pm$ 1.9
Training load ($h \cdot week^{-1}$)	15.6 $\pm$ 1.4	15.5 $\pm$ 0.8
BMI	21.9 $\pm$ 1.1	23.1 $\pm$ 0.7

Note: No differences between groups were observed.

competitions during the 3 months of study. All participants adhered to an endurance training programme. Basically, the training sessions were focused on improving aerobic performance (70–75%), the anaerobic threshold (20–25%), and muscular strength (5–10%).

Anthropometry

The height, weight, body mass index (BMI), and skinfold thicknesses (triceps, subscapular, abdominal, and iliac crest) of the participants were measured before and after the 3-month study. Skinfold thicknesses were measured using an Harpenden skinfold caliper. Percentage body fat and the body lean mass were also obtained from the four skinfold thicknesses using the formula of Faulkner (1968).

Dietary intake and physical activity

The dietary habits of the participants were assessed to ensure that the observations made represented differences due to the antioxidant supplement instead of random changes, in particular due to aspects of the sportsmen's dietary intake. These dietary habits were assessed using a 3-day, 24-h recall before each exercise test. To ensure that there were no differences in the evening meals before the tests, the third day of this recall was the day before the tests. A well-trained dietitian verified and quantified the food records. All food items consumed were transformed into nutrients using a self-made computerized program based on the European (Feinberg, Favie, & Ireland-Ripert, 1991) and Spanish (Mataix *et al.*, 1998; Moreiras, Carbajal, Cabrera, & Cuadrado, 1999) Food Composition Tables. The following food characteristics were used: total energy intake (relative to body mass); percentages of energy from carbohydrates, fats, and proteins; and the dietary intake (relative to body mass) of carbohydrates, fats, proteins, fatty acids (saturated fatty acids, mono-unsaturated fatty acids, poly-unsaturated fatty acids), cholesterol, fibre, vitamins (A, B_1, B_2, B_6, B_{12}, C, D, E, niacin, folic acid), and minerals (sodium, potassium, calcium, phosphorus, magnesium, iron, zinc, iodine). The antioxidant cocktail supplement was not included in this dietary analysis.

Maximal exercise tests

Maximal incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergometrics 900, MedGraphics^{TM}, St. Paul, MN). This maximal test was performed after a gradual protocol until exhaustion. Participants were familiarized with the exercise protocol. Participants warmed up for 3 min at 30 W before starting the test. The test began at 50 W and the participants' work rate was increased by 30 W every 3 min. The sparticipants were asked to maintain a pedalling cadence between 50 and 60 rev $\cdot$ min^{-1}. A pedal frequency meter was used to maintain this cadence. Each test finished when the increased work did not increase or decrease the oxygen consumption; this value was recorded as maximal oxygen uptake ($\dot{V}O_{2max}$). A heart rate above 90% of the predicted maximum for age and a respiratory quotient higher than 1.15 were also considered criteria for the maximal exercise end-point. All the tests were performed at the same time, in the same room, and at the same temperature (20°C) and humidity (70%).

Physiological parameters

The maximal parameters were obtained when the criteria for maximal test termination were met. The maximal values for oxygen consumption, heart rate, expiratory volume, respiratory quotient, respiratory frequency, lactate concentration, workload, oxygen pulse, and the ratio $\dot{V}O_2$/workload, as an efficiency measure, were determined.

The submaximal parameters were determined at two fixed blood lactate concentrations (2 and 4 mM) using linear interpolations. The following submaximal parameters were determined at 2 and 4 mM: oxygen consumption ($\dot{V}O_2$), $\dot{V}O_2$ with respect to $\dot{V}O_{2max}$ (%$\dot{V}O_2$), and the workload. The respiratory quotient and the volume of carbon dioxide expired ($\dot{V}CO_2$) were determined at the 4 mM lactate threshold. The $\dot{V}O_2$ between the 2 and the 4 mM lactate thresholds was also determined.

Gas exchange data were obtained using an automated breath-by-breath system (CPX; Medical Graphics). Heart rate was monitored continuously using an electrocardiograph. The instruments were calibrated before each test.

Plasma vitamin determinations

Plasma was obtained from the blood samples after centrifugation at 1000 *g* and stored at −80°C until use. The deep-frozen plasma was thawed and mixed to disperse possible precipitates. The extraction of liposoluble vitamins was carried out using *n*-hexane after deproteinization with ethanol. Vitamin E and *β*-carotene concentrations were determined by high-performance liquid chromatography (HPLC) in the *n*-hexane extract of plasma after drying under nitrogen and re-dissolving in methanol. The mobile phase consisted of 550:370:80 acetonitrile–tetrahydrofuran–H_2O. The chromatograph (Shimadzu) had a diode array detector and the

column was a Nova Pak C18 (3.9 × 150 mm). Vitamin E and β-carotene were determined at 290 nm and 465 nm respectively.

Plasma vitamin C was determined by a HPLC method with electrochemical detection (Dhariwal, Washko, & Levine, 1990; Tsao & Salimi, 1982). Plasma samples were deproteinized with cold perchloric acid (6%). Appropriate volumes of deproteinized plasma, previously diluted 1 : 1 with distilled water, were injected into the HPLC system. The mobile phase consisted of 0.05 M sodium phosphate, 0.05 M sodium acetate, 189 μM dodecyltrimethylammonium chloride, and 36.6 μM tetraoctylammonium bromide in 25/75 methanol/water (v/v), pH 4.8. The HPLC system (Shimadzu) incorporated an electrochemical detector (Waters, Inc.) and a Nova Pak C18 (3.9 × 150 mm) column. The potential of the chromatographic detector was set at 0.7 V versus an Ag/AgCl reference electrode.

Blood lactate analysis

Lactate was determined from capillary blood taken from the ear lobe using an Accusport Analyser (Pinnington and Dawson, 2001). The blood lactate analyser was calibrated before analysis. During the maximal exercise test, capillary blood samples were obtained at each stage. Lactate was also measured 3, 5, and 7 min after completion of the maximal test.

Statistical analysis

A previous sample size analysis was conducted to ensure that the number of sportsmen participating was large enough ($\alpha = 0.05$, $\beta = 0.1 - 0.2$). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, v. 10.0 for Windows). The results are expressed as means and standard errors of the mean (s_x). Statistical significance was set at $P < 0.05$. All the data were tested for normality (Kolmogorov-Smirnov test). The statistical significance of the data was assessed using two-way analysis of variance (ANOVA). The ANOVA factors were antioxidant diet supplementation (S) and training season (T). The sets of data for which there were effects of supplementation, training or a significant interaction between these two factors were tested by one-way ANOVA using the *post-hoc* Least Significant Difference (LSD) test.

Results

Table I shows the initial characteristics of the sportsmen participating in the study. No differences in age, height, body weight or BMI were observed between participants in the placebo and supplemented groups. Furthermore, no differences between groups were found in the number of hours the athletes trained during the study.

No significant differences were observed between the supplemented and placebo groups when the daily energy intake and the caloric profile were analysed both at the beginning and at the end of the study (results not shown).

Table II shows the changes in anthropometry during the 3 months of training and supplementation. No differences were observed between groups at the beginning of the study. Body mass was unaffected in either group, but body composition was modified by the 3 months of training. The percentage of fat decreased significantly in both the placebo (12.6%) and the supplemented (11%) groups after the 3-month study. These changes were due to decreases in triceps and iliac crest skinfold thicknesses. Additionally, after training, lean body mass increased its contribution to overall body mass.

The $\dot{V}O_{2max}$ and maximal workload attained were significantly influenced by training (Table III). Nonetheless, the increases in $\dot{V}O_{2max}$ observed in the placebo and the supplemented groups were not significant. The maximal workload attained on the cycle ergometer rose about 8% in the placebo group

Table II. Anthropometric characteristics of the participants before and after antioxidant supplementation (mean ± s_x).

	Initial		Final		ANOVA		
Variable	Placebo ($n = 8$)	Supplemented ($n = 7$)	Placebo ($n = 8$)	Supplemented ($n = 7$)	S × T	S	T
Body mass (kg)	63.2 ± 3.6	68.3 ± 1.9	63.5 ± 3.4	68.9 ± 1.8			
Skinfolds							
Triceps (mm)	8.3 ± 1.0	7.1 ± 0.9	4.7 ± 0.5#	5.5 ± 0.5			*
Subscapular (mm)	9.9 ± 0.7	10.2 ± 1.0	8.6 ± 0.6	8.6 ± 0.8			
Abdominal (mm)	9.5 ± 1.5	9.2 ± 1.7	7.5 ± 1.0	7.1 ± 0.5			
Iliac crest (mm)	6.9 ± 1.0	7.8 ± 1.2	4.9 ± 0.8	4.1 ± 0.4#			*
Percent body fat	11.1 ± 0.5	10.9 ± 0.7	9.7 ± 0.3#	9.7 ± 0.3#			*

*Significant effects of factor S, T or the interaction S × T (two-way ANOVA). Factor S represents antioxidant diet supplementation and factor T represents the training period. #Significant difference between initial and final values (one-way ANOVA, $P < 0.05$).

Table III. Maximal parameter values of participants before and after antioxidant supplementation (mean $\pm s_x$).

Variable	Initial		Final		ANOVA		
	Placebo ($n=8$)	Supplemented ($n=7$)	Placebo ($n=8$)	Supplemented ($n=7$)	S × T	S	T
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	57.1 ± 2.4	60.3 ± 1.9	62.6 ± 1.4	66.3 ± 2.0			*
Max. heart rate ($beats \cdot min^{-1}$)	182 ± 5	180 ± 4	183 ± 6	184 ± 4			
Max. workload (W)	276 ± 15	282 ± 11	299 ± 9	310 ± 6			*
Max. respiratory quotient	1.19 ± 0.04	1.24 ± 0.04	1.42 ± 0.06$^{\#}$	1.31 ± 0.04			*
Max. respiratory frequency	48.5 ± 2.1	48.4 ± 3	48.1 ± 3	47 ± 2.8			
Max. expiratory volume	134 ± 3	147 ± 4	151 ± 6$^{\#}$	163 ± 3$^{\#\Delta}$		*	*
Max. lactate concentration (mM)	8.8 ± 0.6	8.2 ± 0.5	8.3 ± 0.2	6.5 ± 0.2$^{\#\Delta}$		*	*
Max. O_2 pulse ($ml \cdot beat^{-1}$)	19.9 ± 0.7	22.9 ± 0.9	21.7 ± 0.8	24.8 ± 0.7$^{\Delta}$		*	*
$\dot{V}O_2$/workload	13.2 ± 0.9	14.6 ± 0.7	13.4 ± 0.9	14.3 ± 0.8			

*Significant effects of factor S, T or the interaction S × T (two-way ANOVA). Factor S represents antioxidant diet supplementation and factor T represents the training period. $^{\Delta}$Significant difference between placebo and supplemented groups. $^{\#}$Significant difference between initial and final values (one-way ANOVA, $p < 0.05$).

and 11% in the supplemented one. No changes were observed in maximal heart rate. The respiratory quotient was significantly influenced by the training and competition season, with a significant increase (about 10%) in the placebo group after the 3 months. The training season and supplementation had a significant influence on maximal blood lactate concentration, maximal expiratory volume, and maximal oxygen pulse. Maximal expiratory volume and maximal oxygen pulse were significantly higher, and maximal blood lactate concentration was significantly lower, in the supplemented group than in the placebo group at the end of the nutritional intervention. No changes were observed in the $\dot{V}O_{2max}$/maximal workload ratio.

Figure 1 shows the percentage of $\dot{V}O_{2max}$ at the 2 and 4 mM lactate thresholds (the aerobic and anaerobic threshold respectively) before and after the 3 months of training and supplementation. The percentage of $\dot{V}O_{2max}$ attained at the aerobic threshold was maintained during the study. However, the $\%\dot{V}O_{2max}$ attained at the 4 mM lactate threshold was influenced by both diet supplementation and the 3 months of training, and a statistically significant interaction between the two factors was also observed. This $\%\dot{V}O_{2max}$ at the anaerobic threshold rose about 5.2% in the placebo group and about 14% in the supplemented group. Hence, this value was significantly higher in the supplemented group than in the placebo after the 3 months of antioxidant diet supplementation.

Table IV shows the effects of the 3 months of training and of antioxidant diet supplementation on the submaximal parameters at the anaerobic threshold. The $\dot{V}CO_2$ and workload attained at this 4 mM lactate threshold were increased in both groups after training and diet supplementation, but were higher in the supplemented group than in the placebo group. The respiratory quotient at the anaerobic

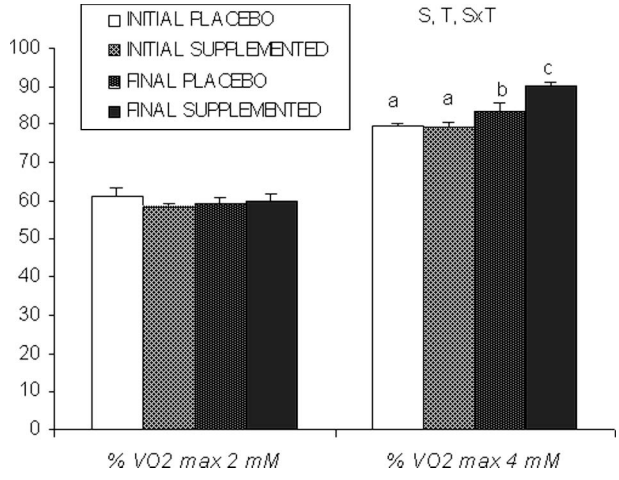

Figure 1. Percent maximal oxygen consumption at the 2 and 4 mM lactate thresholds before and after diet supplementation. The results are the mean and standard error of the mean of eight participants in the placebo group and seven participants in the antioxidant supplemented group. Capital letters indicate the significant effect of factor S, T or the interaction S × T (two-way ANOVA), where S represents antioxidant diet supplementation, T represents the training and competition period, and S × T represents the interaction between the two factors. Different letters indicate significantly different values when a significant S × T interaction was observed.

threshold was significantly affected by the 3 months of training, increasing in both groups after training.

Figure 2 shows the oxygen consumption ranges in the aerobic – anaerobic transition – that is, the oxygen consumption difference between the 2 and 4 mM lactate thresholds. Analysis of variance showed significant effects of both the training season and antioxidant supplementation, with increased values in both the placebo and the supplemented groups. However, the final value in the supplemented group was significantly higher than in the placebo group.

Table IV. Submaximal parameter values of participants before and after antioxidant supplementation at the 4 mM lactate threshold (mean $\pm$ s_x).

	Initial		Final		ANOVA		
	Placebo ($n=8$)	Supplemented ($n=7$)	Placebo ($n=8$)	Supplemented ($n=7$)	S × T	S	T
$\dot{V}CO_2$ (ml · min^{-1})	2735 ± 61	2933 ± 59	3536 ± 243$^{\#}$	3931 ± 147$^{\#\Delta}$		*	*
Respiratory quotient	0.99 ± 0.02	0.96 ± 0.02	1.05 ± 0.02$^{\#}$	1.03 ± 0.03$^{\#}$			*
Workload (W)	210 ± 10	223 ± 6	246 ± 5.6$^{\#}$	265 ± 3$^{\#\Delta}$		*	*
% Max. workload	76.3 ± 1.0	79.6 ± 2	82.7 ± 1.2$^{\#}$	85.6 ± 1.3$^{\#}$		*	*

*Significant effects of factor S, T or the interaction S × T (two-way ANOVA). Factor S represents antioxidant diet supplementation and factor T represents the training period. $^{\Delta}$Significant difference between placebo and supplemented groups. $^{\#}$Significant difference between initial and final values (one-way ANOVA, $P < 0.05$).

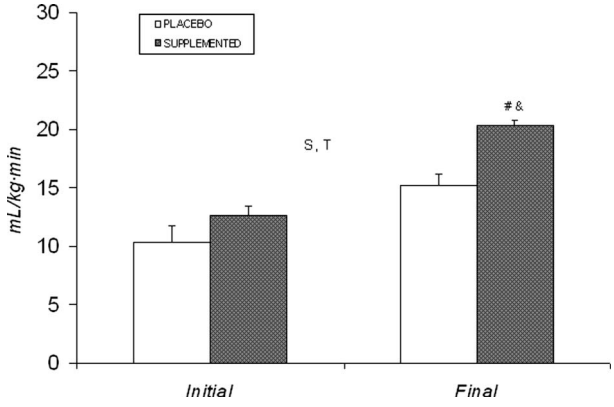

Figure 2. Oxygen consumption ranges in the aerobic–anaerobic transition area in amateur sportsmen before and after diet supplementation. The results are the mean and standard error of the mean of eight participants in the placebo group and seven participants in the antioxidant supplemented group. Capital letters indicate the significant effect of factor S, T or the interaction S × T (two-way ANOVA), where S represents antioxidant diet supplementation, T represents the training and competition period, and S × T represents the interaction between the two factors. $^{\#}$Significant difference between initial and final values. $^{\&}$Significant difference between placebo and supplemented groups.

Discussion

The lack of differences in the diet of the sportsmen in the placebo and the supplemented groups and the similar plasma antioxidant vitamin concentrations in both groups allowed us to study the effects of the antioxidant diet supplementation. The increase in plasma concentrations of vitamins C and E and β-carotene observed after the 3 months of supplementation highlighted the intake of the antioxidant supplements. In a previous study, we reported that neutrophils from sportsmen belonging to the supplemented group presented with a lower oxidative status, as indicated by the lower GSSG/GSH ratio, than neutrophils from sportsmen in the placebo group (Tauler *et al.*, 2002). Thus, the high antioxidant intake in the supplemented group increased the antioxidant concentrations and helped to neutralize the higher production of ROS induced by exhaustive exercise. This result is in line with other studies in which antioxidant supplementation was reported to limit oxidative damage (Tauler *et al.*, 2003). The results for the maximal and submaximal physiological parameters in the two groups of sportsmen allowed us to study the effects of long-term diet supplementation with antioxidant nutrients on performance.

No effects of antioxidant diet supplementation were found on $\dot{V}O_{2max}$ (Sumida *et al.*, 1989). Improvement in performance is currently measured by an increase in $\dot{V}O_{2max}$. Maximal oxygen consumption measures the maximal capability to use oxygen and depends on oxygen transport and uptake (Wilmore & Costill, 2004). Performance measured as $\dot{V}O_{2max}$ was similar in the two groups and increased in both the supplemented and the placebo groups during the training period. It should be noted that the beginning of the study coincided with the beginning of the athletes' training season. After the 3 months, a decrease in the percentage of fat was observed. Body mass, however, did not change and thus it can be concluded that lean body mass increased. This result is consistent with other studies in which an increase in muscular mass was observed after training and the competition season (Wilmore & Costill, 2004). Lean body mass is the main body component that contributes to oxygen consumption. Thus, the increase in the muscular component, which resulted in higher oxygen consumption, could explain the rise in $\dot{V}O_{2max}$. However, lean body mass was unaffected by antioxidant supplementation and supplementation did not increase $\dot{V}O_{2max}$.

We have shown that after 3 months of training, maximal blood lactate concentration in the supplemented group was lower than in the placebo group; the 4 mM lactate threshold was moved to the right (i.e. was attained later). Thus, the increase in oxygen consumption during the aerobic–anaerobic transition was higher in the supplemented group than in

the placebo group. The acidosis produced in this aerobic–anaerobic transition is predominantly buffered by hydrogen carbonate (Beaver, Wasserman, & Whipp, 1986), with a consequent production of CO_2 and H_2O. It also corresponds to the area between the first and second ventilatory thresholds (Jacobs, Sjodin, Kaiser, & Karlsson, 1981; Sjodin & Jacobs, 1981). The CO_2 produced is removed by respiration. This area could be determined by using ventilatory methods and it has been named the ''isocapnic buffering area'' because CO_2 does not increase in this area (Chicharro, Hoyos, & Lucia, 2000). Several studies with runners have analysed the effects of training on the isocapnic buffering area and on hypocapnic hyperventilation. It has been reported that after endurance training, the increase in the second ventilatory threshold is greater than that in the first ventilatory threshold (Oshima, Tanaka, & Miyamoto, 1998). Our results are in line with this observation because the period of training did not result in a change in $\dot{V}O_2$ at a blood lactate concentration of 2 mM, whereas at 4 mM it did increase, with a significantly greater increase in the supplemented group. Thus, at the anaerobic threshold, the participants in the supplemented group were able to undertake more work. The workload attained by the participants in the supplemented group at the anaerobic threshold was higher than that for the placebo group. The higher CO_2 production in the supplemented group at the anaerobic threshold is in line with the enhanced aerobic metabolism in the supplemented group. The higher CO_2 production in the supplemented group could indicate a more effective utilization of oxygen for ATP regeneration. These results suggest that long-term antioxidant supplementation improves the aerobic metabolism, increasing the efficiency of obtaining energy.

Lactate accumulation in blood is dependent on the balance between lactate production by working muscle and lactate removal by liver and other tissues. Endurance training causes an increase in capillary density of the working muscle and mitochondrial adaptations that result in a reduction in lactate formation and an increase in lactate removal. It has been reported that the reduction in lactate production after training is related to a lower plasma epinephrine concentration, which reduces glycogenolysis via α-adrenergic receptor stimulation (Mazzeo *et al.*, 1994). We suggest that these mechanisms could be influenced by the antioxidant supplementation because maximal blood lactate concentrations decreased only in the supplemented group.

The lower maximal blood lactate concentrations in the supplemented group are related to the presence of high antioxidant concentrations. A linear relation between the blood GSSG/GSH ratio and the blood lactate/pyruvate ratio in athletes has been reported (Sastre *et al.*, 1992). Thus, glutathione oxidation and oxidative stress are produced only when blood lactate concentrations are high – that is, when exercise is exhaustive. Indeed, it has been also reported that anaerobic exercise induces oxidative damage (Bloomer and Goldfarb, 2004; Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005). As antioxidant supplementation could decrease oxidative stress in athletes (Sastre *et al.*, 1992), we propose that high antioxidant levels could induce a lower increase in blood lactate concentrations. However, the possible mechanisms underlying this observation are unclear. This result is also in accordance with other studies in which a linear relationship was found between xanthine oxidase activity and lactate concentration (Radak *et al.*, 1995). Xanthine dehydrogenase is converted into xanthine oxidase by several factors, including the action of proteases and the existence of oxidative conditions. The appearance of xanthine oxidase produces an increase in oxygen consumption and in anion superoxide generation (Hellsten, 2000). Taken together with previous findings (Viña *et al.*, 2000), this observation could indicate that xanthine oxidase is the main source of ROS when exercise intensity is close to the anaerobic threshold. In a previous study, we demonstrated that higher levels of ascorbate induced a lower increase in uric acid after exhaustive exercise, indicating a lower conversion of xanthine dehydrogenase into xanthine oxidase (Tauler *et al.*, 2003). The presence of higher concentrations of antioxidants in the supplemented group could prevent the transformation of xanthine dehydrogenase into xanthine oxidase, reducing the use of oxygen by xanthine oxidase. Thus, efficiency in oxygen consumption improved, as shown in the present study. However, the possible role of ROS as mediators of adaptation and damage in skeletal muscle (Pattwell & Jackson, 2004) indicates that further studies are necessary to establish a more accurate dose of the antioxidant nutrient supplied.

In conclusion, antioxidant supplementation induced lower increases in blood lactate concentration after a maximal exercise test and enhanced the aerobic metabolism in amateur sportsmen. Long-term antioxidant supplementation could improve the efficiency in which aerobic energy is obtained.

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