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# Association between Parameters Related to Oxidative Stress and Trace Minerals in Athletes

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**Abstract:** The aim of the present study was to evaluate the basal concentrations of malondialdehyde (MDA) nonenzymatic antioxidants, such as ascorbic acid,  $\alpha$ -tocopherol, and retinol in plasma or erythrocytes, and the plasma concentrations of 16 trace minerals in endurance athletes from Extremadura (Spain). In addition, we aimed to assess the possible relationships between some parameters related to cellular oxidative stress with plasma concentrations of some trace minerals. Sixty-two national long-distance men athletes participated in this study. The parameters related to oxidative stress and antioxidant activity were analyzed through high pressure liquid chromatography (HPLC), and trace minerals analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS). We found that plasma MDA was positively correlated with selenium and rubidium. Plasma ascorbic acid was positively correlated with manganese and negatively correlated with cobalt and cadmium. Erythrocyte ascorbic acid was related to arsenic and cesium. Plasma  $\alpha$ -tocopherol correlated with copper and manganese negatively and positively with arsenic. Erythrocyte  $\alpha$ -tocopherol was positively related to copper, rubidium, and lithium. The findings show that athletes with a high degree of training should monitor their intake and concentrations of  $\alpha$ -tocopherol for its fundamental role of neutralizing the excess of reactive oxygen species produced by exercise and the prooxidant effects of several minerals such as arsenic, copper, and lithium.

**Keywords:** oxidative stress; trace minerals; exercise; ascorbic acid; vitamin E; retinol

## 1. Introduction

The presence of reactive oxygen species (ROS) is natural in living organisms, and the amount in which they occur is of special interest. Endurance training has been observed to increase the production of ROS [1]. Increased oxygen consumption, catecholamine release, excess lactic acid, enzyme activity such as xanthine oxidase, and the generation of free radicals by mitochondria are sources that can increase ROS production during exercise [2]. The major ROS generators are located in the blood during exercise and can be erythrocytes (mainly due to their amount) and leukocytes, due to the drastic activation during exercise [3].

Traditionally, physical activity has been associated with beneficial effects on the body; however, parameters such as intensity or duration of physical activity seem to be related to oxidative damage [4,5]. High training loads in endurance athletes imply the possibility of excessive production of free radicals [6];

even during recovery from physical exercise, blood cells themselves can produce significant amounts of ROS. Excessive production of free radicals can cause damage to DNA or tissues such as lipids and proteins [7]. In addition, contractile dysfunction related to oxidative damage and muscular fatigue can occur when ROS levels exceed the capacity of the antioxidant system during irregular or strenuous and sustained exercise [8,9], thus making diagnosis vital. Malondialdehyde (MDA) is an end product derived from the lipid peroxidation produced by free radicals, which is why it is one of the most widely used markers for the detection of oxidative stress [10]. Research has found increases in MDA after a stable stress test at 60% and 90% of  $\text{VO}_2$  max and even at the end of an incremental test on a cycle ergometer with sedentary and moderately trained subjects [5,11].

Although exercise leads to an increase in oxidative stress, the same exercise stimulus seems necessary to allow for the upregulation of endogenous antioxidant defenses according to the hormesis theory [12]. Alterations in the antioxidant systems can be used as an indicator of the oxidative stress imposed on the tissue. Thus, the evaluation of redox changes in nonenzymatic antioxidant substances such as ascorbic acid (AA),  $\alpha$ -tocopherol (ATC), and retinol (RT) is used to measure oxidative stress [13], as well as the activity of certain antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione reductase (GR) [14].

Trace minerals (TMs) are elements that are necessary for the human body due to the large number of biological functions in which they could be directly or indirectly involved during different physiological processes that can be altered as adaptation mechanisms to endurance exercise [15]. Essential trace minerals such as copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals [16]. Thus, Cu and Zn are part of the SOD enzyme, and their plasma levels have been reported to be related to immune response, inflammation, and oxidative stress in the human body [17]. Se is a cofactor of GPx that is incorporated in different selenoproteins that protect us from the free radicals generated in cellular metabolism [18]. However, other TMs such as iron (Fe), cadmium (Cd), or lead (Pb) enable the production of oxygen free radicals, behaving as cellular prooxidants [19].

In a previous study, our research group evaluated the possible differences between highly trained endurance athletes and subjects who do not perform regular physical activity, in parameters related to oxidative stress [5]. The study reported similar concentrations of antioxidant vitamins before performing an incremental test to exhaustion, with decreases in plasma and in red blood cell concentrations of vitamins A and E with increased vitamin C at the end in untrained subjects, and a decrease of vitamin C in erythrocytes in trained athletes. We also analyzed the differences between athletes and nonathletes in trace minerals and have found significant differences in the plasma concentrations of some of them [20–22], such as higher plasma concentrations of molybdenum (Mo), Mn, and rubidium (Rb), but there were lower Se concentrations in athletes compared to the untrained.

The main objective of this present study is to see the possible correlations between the trace minerals studied and the oxidative stress parameters in order to examine the positive or negative influence of plasma concentrations of trace minerals in relation to oxidative stress.

## 2. Materials and Methods

### 2.1. Participants

Sixty-two national long-distance high-level men athletes (age:  $23.21 \pm 3.89$  years old) participated in this study. The athletes lived in the same region and had been performing physical training regularly during the previous 5 years, recording a weekly average distance between 85–125 km, doing 15%–25% above the anaerobic threshold and 75%–85% aerobically weekly. The athletes had not been taking vitamins, minerals, or other supplements.

All subjects participated voluntarily; they were informed about the aim of the study and signed the informed consent. Approval for this project was received from the University of Extremadura Ethics Committee according to the latest version of the Helsinki declaration for human research.

Subjects reported to the laboratory after an overnight fast and were instructed to abstain from hard training for at least 72 h before testing. Each test was conducted at the same time (09:00 a.m.). The sections described below follow the model of Maynar et al. [20] used in other studies.

## 2.2. Nutritional Evaluation

All participants were instructed to complete a 3-day diet record, including 1 weekend day and 2 weekdays on the provided nutritional questionnaire; each participant weighed and indicated the amount in grams of each food consumed. The nutritional composition of the athletes' dietary intakes was obtained using several food composition tables [23–25].

## 2.3. Incremental Test until Exhaustion

An incremental test until exhaustion was carried out to verify that the athletes were highly trained in endurance exercise. The participants performed the test on a treadmill (Powerjoc, UK) equipped with a gas analyzer (Metamax, Cortex Biophysik, GmbH, Germany) until voluntary exhaustion was reached, which was indicated through a direct measurement of maximal oxygen uptake ( $\text{VO}_2$  max). During the incremental test,  $\text{VO}_2$  max was determined according to the following criteria: the respiratory exchange ratio (RER) had to exceed 1, and the stabilization in oxygen uptake ( $\text{VO}_2$ ) together with an increment in carbon dioxide ( $\text{CO}_2$ ) elimination, in the RER, and in the ventilatory volume (VE) are induced by the increases in the test velocity. Before each test, athletes performed a moderate-intensity warm-up for 10 min at 10 km/h. The test began at a speed of 10 km/h with a stable slope of 1% and within the recommended parameters [26]; the running speed was then increased by 1 km/h every 400 m until voluntary exhaustion.

## 2.4. Blood Samples

Before the test, 10 mL of blood was drawn from an antecubital vein in each subject. Then, blood samples were transferred into a metal-free polypropylene unheparinized tube with ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. Samples were immediately centrifuged at 3000 rpm for 15 min at room temperature ( $23 \pm 1$  °C) to separate plasma from erythrocytes (P-selecta, MEDITRONIC). Plasma was placed into sterile tubes and stored at  $-20$  °C until assayed. Erythrocytes were washed three times with NaCl 0.9% (wt/v); they were then reconstituted with distilled water in the same volume as plasma and stored at  $-20$  °C until use. The plasma was aliquoted into an Eppendorf tube and was conserved at  $-80$  °C until further analysis.

## 2.5. Analytical Determination

### 2.5.1. Malondialdehyde Determination

Plasma MDA was calculated as described by Chirico [27], analyzed by high pressure liquid chromatography (HPLC) with the Spectra SERIES P100/UV 100 to perform linear calibration ( $r = 0.99$ ). HPLC is very sensitive in detecting changes in MDA concentrations induced by physical exercise. MDA analysis performed by HPLC is the most appropriate technique for detection of MDA in physical exercise due to its precision [28].

### 2.5.2. Nonenzymatic Antioxidant Determination

High pressure liquid chromatography (HPLC) was used for the determination of vitamin C according to the technique described by Manoharan and Schwillie [29]. First, 100  $\mu\text{L}$  of 10% perchloric acid mixed with 1% metaphosphoric acid were added to 200  $\mu\text{L}$  of plasma or erythrocytes, stirred in a vortex for 30 s and stored in a refrigerator for 20 min. Subsequently, 200  $\mu\text{L}$  of mobile phase were added and centrifuged at 12,000 rpm for 2 min, and then 20 mL of supernatant were collected and injected for vitamin C determination by HPLC. The column used was a C18 that was 11 cm long with a 4.7 mm internal diameter; for the mobile phase, we used ammonium phosphate 20 mM: 0.015%

metaphosphoric at a flow of 1 mL/min. The detection was performed at a wavelength of 240 nm. The concentration in  $\mu\text{g/mL}$  of vitamin C was calculated using the sample dilution factor and a straight line constructed with commercial ascorbic acid.

HPLC was used in the same way as previously described by Lim [30] for the determination of vitamin E. An internal standard of 100  $\mu\text{L}$  of  $\alpha$ -tocopherol acetate in ethanol (50 mg/L) was added to 200  $\mu\text{L}$  of plasma or erythrocytes and vortexed for 30 s. Subsequently, 200  $\mu\text{L}$  of n-hexane were added and vortexed again for another 30 s, and then centrifuged for 10 min to centrifuge at 12,000 rpm for 5 min. Once the centrifugation was finished, the upper layer was removed and dried in a stream of  $\text{N}_2$  at 37 °C. Immediately before measuring by HPLC, it was reconstituted in 100  $\mu\text{L}$  of ethanol, with 20  $\mu\text{L}$  injected for vitamin E determination. The column used was a Brownlee OD-MP that was 10 cm long and with an internal diameter of 4.6 mm, and dichloromethane in 7% methanol (v/v) at a flow of 1 mL/min was used as mobile phase. The detection was performed at a wavelength of 292 nm. The concentration in  $\mu\text{g/mL}$  of vitamins A and E was calculated by comparing areas with the internal standard.

### 2.5.3. Trace Mineral Determination

Trace minerals were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) following the method previously described by Maynar et al. [21]. Decomposition of the organic matrix was performed by heating for 10 h at 90 °C after the addition of 0.8 mL  $\text{HNO}_3$  and 0.4 mL  $\text{H}_2\text{O}_2$  to 1 mL of plasma. The samples were then dried at 200 °C on a hot plate. Sample reconstitution was carried out by adding 0.5 mL of nitric acid, 10  $\mu\text{L}$  of In ( $10 \text{ mg}\cdot\text{L}^{-1}$ ) as the internal standard, and ultrapure water to complete the 10 mL. Reagent blanks, element standards, and certified reference material (Seronorm, lot 0511545, Sero AS, Billingstand, Norway) were prepared in the same way and used for accuracy testing. Before the analysis, the commercial control materials were diluted according to the recommendation of the manufacturer.

Digested solutions were assayed by an ICP-MS using an Nexion model 300D (PerkinElmer, Inc., Shelton, CT, USA) equipped with a triple quadrupole mass detector in addition to a reaction cell/collision that allows for operation in three modes: without reaction gas (STD), by kinetic energy discrimination with helium as the collision gas (KED), and in reaction mode with ammonia as the reaction gas (DRC).

### 2.6. Statistical Evaluations

Statistical analyses were performed with IBM SPSS Statistics 21.0 (IBM Co., Armonk, NY, USA). The results are expressed as  $\bar{x} \pm s$ , where  $\bar{x}$  represents mean values and  $s$  is the standard deviation. The normal distribution of the variables was assessed using the Shapiro–Wilks test. Pearson's correlation coefficient ( $r$ ) was used to determine whether there were significant relationships between the basal mineral concentrations in plasma and basal markers of oxidative stress and the nonenzymatic antioxidants measured in both plasma and erythrocytes. A  $p \leq 0.05$  was considered statistically significant.

## 3. Results

Table 1 shows the cardiorespiratory characteristics of the athletes in the study, namely body weight ( $64.97 \pm 7.36 \text{ kg}$ ), muscle weight ( $32.02 \pm 3.96 \text{ kg}$ ), accompanied by low fat weight ( $5.35 \pm 1.01 \text{ kg}$ ); equally, the cardiorespiratory values show characteristic  $\text{VO}_2 \text{ max}$  ( $67.55 \pm 4.11 \text{ mL/min/kg}$ ) values of high-level endurance athletes [31]. Information on the energy, macronutrient, vitamins, and TM intake from the basal diet of athletes is shown in Table 2.

**Table 1.** Cardiorespiratory values of the athletes.

Parameters	Athletes	Range
VO <sub>2</sub> max rel. (mL/min/kg)	67.55 ± 4.11	55.15–77.03
RER max	1.05 ± 0.05	1–1.15
HR max (beats/min)	193.5 ± 7.91	180–200

**Table 2.** Food intake of energy, macroelements, vitamins, and trace minerals in athletes.

Parameters (Reference Daily Intake) (n = 62)	Intake
Energy (kcal/d)	2876.47 ± 657.33
Protein (g/d)	117.16 ± 28.83
Lipids (g/d)	105.98 ± 48.40
Carbohydrate (g/d)	346.98 ± 92.82
Retinol (RT) (900 µg/d)	781.34 ± 520.47
Ascorbic acid (AA) (90 mg/d)	108.16 ± 71.01
α-tocopherol (ATC) (15 mg/d)	4.34 ± 2.58
Co (200–300 µg/d)	295.88 ± 215.28
Cu (2000–3000 µg/d)	1675.69 ± 568.4
Mn (2500–5000 µg/d)	3381.29 ± 1440.06
Mo (75–400 µg/d)	309.26 ± 182.06
Se (50–200 µg/d)	76.44 ± 45.04
V (10–70 µg/d)	25.50 ± 29.26
Zn (10–15 mg/d)	11.09 ± 3.74
B (0.75–1.35 mg/d)	1.34 ± 1.48
Li (180–550 µg/d)	366.78 ± 396.86
As (12–300 mg/d)	1691 ± 834.25
Be (< 50 µg/d)	9.72 ± 9.01
Cd (< 70 µg/d)	23.29 ± 15.37
Pb (< 400 µg/d)	209.03 ± 142.64
Rb (1.5–7 mg/d)	3.903 ± 4.786
Sr (1000–2300 µg/d)	1890.84 ± 1784.40

Notes: Co = cobalt; Cu = copper; Mn = manganese; Mo = molybdenum; Se = selenium; V = vanadium; Zn = zinc; B = boron; Li = lithium; As = arsenic; Be = beryllium; Cd = cadmium; Pb = lead; Rb = rubidium; Sr = strontium. Results are means ± SD.

Table 3 shows the values of plasma MDA ( $0.75 \pm 0.12 \mu\text{M/mL}$ ) as an indicator of lipid peroxidation and AA ( $14.49 \pm 4.44$ – $15.71 \pm 16.94 \mu\text{g/mL}$ ), ATC ( $7.24 \pm 7.23$ – $9.60 \pm 8.46 \mu\text{g/mL}$ ), and RT ( $0.14 \pm 0.09$ – $0.77 \pm 0.77 \mu\text{g/mL}$ ) as nonenzymatic antioxidants in plasma and erythrocyte respectively, in the study athletes. Table 4 shows the plasma concentrations of the elements studied, with the ranges in which these elements were present in the athletes.

**Table 3.** Values of MDA and nonenzymatic antioxidants in plasma (P) and erythrocytes (E) of athletes.

Parameters	Athletes	Range
MDA ( $\mu\text{M/mL}$ )	0.75 ± 0.12	0.55–1.04
Ascorbic acid (AA-P) ( $\mu\text{g/mL}$ )	14.49 ± 4.44	9.15–26.38
α-tocopherol (ATC-P) ( $\mu\text{g/mL}$ )	7.24 ± 7.23	0.4–50.5
Retinol (RT-P) ( $\mu\text{g/mL}$ )	0.14 ± 0.09	0–0.64
Ascorbic acid (AA-E) ( $\mu\text{g/mL}$ )	15.71 ± 16.94	1.88–47.47
α-tocopherol (ATC-E) ( $\mu\text{g/mL}$ )	9.60 ± 8.46	0.4–44.16
Retinol (RT-E) ( $\mu\text{g/mL}$ )	0.77 ± 0.77	0.08–3.83

**Table 4.** Plasma concentrations of trace minerals in athletes.

Trace Minerals	Athletes	Range
Co ( $\mu\text{g/L}$ )	$0.68 \pm 0.10$	0.46–0.89
Cu ( $\mu\text{g/L}$ )	$693.14 \pm 133.51$	454.5–936.97
Mn ( $\mu\text{g/L}$ )	$2.06 \pm 1.52$	0.2–5.46
Mo ( $\mu\text{g/L}$ )	$0.62 \pm 0.59$	0.1–3.32
Se ( $\mu\text{g/L}$ )	$96.48 \pm 13.72$	69.78–124.65
V ( $\mu\text{g/L}$ )	$0.29 \pm 0.36$	0–1.78
Zn ( $\mu\text{g/L}$ )	$792.24 \pm 143.87$	539.71–1212.48
B ( $\mu\text{g/L}$ )	$8.63 \pm 11.08$	0–60.66
Li ( $\mu\text{g/L}$ )	$1.38 \pm 0.82$	0.36–4.7
As ( $\mu\text{g/L}$ )	$2.35 \pm 2.69$	0.25–11.97
Be ( $\mu\text{g/L}$ )	$0.07 \pm 0.03$	0–0.14
Cd ( $\mu\text{g/L}$ )	$0.07 \pm 0.05$	0.01–0.22
Pb ( $\mu\text{g/L}$ )	$0.96 \pm 1.06$	0–4.94
Cs ( $\mu\text{g/L}$ )	$0.69 \pm 0.40$	0.31–1.87
Rb ( $\mu\text{g/L}$ )	$138.48 \pm 23.03$	98.82–185.87
Sr ( $\mu\text{g/L}$ )	$26.24 \pm 8.13$	14.83–47.47

Notes: As = arsenic; B = boron; Be = beryllium; Cd = cadmium; Co = cobalt; Cs = cesium; Cu = copper; Li = lithium; Mn = manganese; Mo = molybdenum; Pb = lead; Rb = rubidium; Se = selenium; Sr = strontium; V = vanadium; Zn = zinc.

Table 5 illustrates the Pearson correlation between the parameters related to oxidative stress and plasma trace minerals. The basal plasma concentrations of the oxidative parameters were not significantly associated with plasma molybdenum (Mo), Zn, vanadium (V), boron (B), beryllium (Be), Pb, or strontium (Sr) levels. Positive correlations were found of Se ( $r = 0.288$ ,  $p = 0.046$ ) and rubidium (Rb) ( $r = 0.301$ ,  $p = 0.038$ ) with MDA; arsenic (A) ( $r = 0.514$ ,  $p = 0.000$ ) and cesium (Cs) ( $r = 0.347$ ,  $p = 0.008$ ) with AA-E; Cu ( $r = 0.301$ ,  $p = 0.037$ ), Rb ( $r = 0.325$ ,  $p = 0.024$ ), and lithium (Li) ( $r = 0.601$ ,  $p = 0.000$ ) with ATC-E; and finally, As ( $r = 0.348$ ,  $p = 0.015$ ) with ATC-P. On the other hand, significant negative correlations were found of manganese (Mn) ( $r = -0.324$ ,  $p = 0.024$ ), cobalt (Co) ( $r = -0.416$ ,  $p = 0.003$ ), and cadmium (Cd) ( $r = -0.379$ ,  $p = 0.008$ ) with AA-P, as well as Cu ( $r = -0.317$ ,  $p = 0.028$ ) and Mn ( $r = -0.287$ ,  $p = 0.048$ ) with ATC-P.

**Table 5.** Correlations between serum concentrations of trace minerals in athletes and oxidative stress parameters in plasma (P) and erythrocytes (E).

Trace Minerals	MDA	AA-P	AA-E	ATC-P	ATC-E
Cu	-	-	-	$r = -0.317$ $p = 0.028$	$r = 0.301$ $p = 0.037$
Mn	-	$r = -0.324$ $p = 0.024$	-	$r = -0.287$ $p = 0.048$	-
Se	$r = 0.289$ $p = 0.046$	-	-	-	-
Rb	$r = 0.301$ $p = 0.038$	-	-	-	$r = 0.325$ $p = 0.024$
As	-	-	$r = 0.514$ $p = 0.000$	$r = 0.348$ $p = 0.015$	-
Li	-	-	-	-	$r = 0.601$ $p = 0.000$
Co	-	$r = -0.416$ $p = 0.003$	-	-	-
Cd	-	$r = -0.379$ $p = 0.008$	-	-	-
Cs	-	-	$r = 0.347$ $p = 0.016$	-	-

Notes: AA = ascorbic acid; ATC =  $\alpha$ -tocopherol; As = arsenic; Cd = cadmium; Co = cobalt; Cs = cesium; Cu = copper; Li = lithium; Mn = manganese; Rb = rubidium; Se = selenium;  $r$  = Pearson's coefficient of correlation;  $p$  =  $p$ -value.

## 4. Discussion

In our study energy, the macronutrient, vitamin, and TM intake from the basal diets were appropriate according to the recommended dietary reference intakes [23,32,33]; however, ATC intake was lower than previously reported for other groups of athletes [34]. TM concentrations and vitamins in plasma and erythrocytes were adequate in the athletes, except that plasma concentrations of ATC ( $7.24 \pm 7.23 \mu\text{g/mL}$ ) and RT ( $0.14 \pm 0.09 \mu\text{g/mL}$ ) were lower than previously reported in other endurance athletes ( $26\text{--}27 \mu\text{g/mL}$  and  $0.53\text{--}0.57 \mu\text{g/mL}$ , respectively) [35], which could be due to the greater utilization of these vitamins to reduce ROS production. Plasma Co concentrations were higher ( $0.68 \pm 0.10 \mu\text{g/L}$ ) than reported in other populations (17); however, negative health effects are unlikely to occur at blood Co concentrations below  $300 \mu\text{g/L}$  in healthy individuals [36].

A Pearson correlation was carried out in order to see the possible relationships in endurance athletes between the parameters related to cellular oxidative stress, such as MDA, nonenzymatic antioxidants such as AA, ATC, and RT, and the plasma concentrations of 16 trace minerals and to examine their positive or negative influence in oxidative stress.

### 4.1. Malondialdehyde

MDA is an end product derived from lipid peroxidation produced by free radicals. In our study, MDA showed a positive correlation with plasma Se concentrations ( $r = 0.289$ ,  $p = 0.024$ ); Jablan et al. [37] found an increase in MDA values in runners after performing a marathon. Se is a cofactor of GPx and different selenoproteins that protect us from free radicals [18], and it facilitates the synthesis of antioxidant enzymes like GPx in response to oxidative stress during physical activity. Margaritis et al. [38] reported that highly trained subjects adopted a high Se diet naturally, which allows a high Se status to be reached in order to ensure its antioxidant activity.

MDA was also related to Rb ( $r = 0.301$ ,  $p = 0.038$ ), which is not currently studied as a TM since its functions are not fully known. Roberts et al. [39] found that this element can easily be exchanged with potassium (K) in chemical reactions such as the activity of the enzyme Na/K ATPase, which according to Nielsen [40] is involved in the energy production process in humans, in the stabilization of the membrane potential of the resting cell, and the regulation of cell volume. Likewise, during the resistance exercise, there was an increase in MDA caused by ROS [2] that induced an alteration in the Na/P ATPase [41]. This could be a result of an increase in the concentrations of Rb in an attempt to favor the entry of K into the cell, since the alteration of the Na/K pump produces an increase in the concentrations of intracellular Na that would cause cell rupture due to the osmotic pressure [42].

### 4.2. Ascorbic Acid

#### 4.2.1. Plasma

We found a high negative correlation between AA plasma concentrations and plasma Co levels ( $r = -0.416$ ,  $p = 0.003$ ). This could be due to the increase in AA to counter the prooxidant action of Co that our athletes presented, which was at a concentration above the reference values. In this respect, Liu et al. [43] affirmed that the AA could prevent cytotoxicity from reducing the level of ROS produced by the activity of the cobalt nanoparticles in the patients with metal-on-metal hip prostheses. In other findings, Yildirim et al. [44] suggested that cobalt therapy may prove effective in improving the impaired antioxidant status during the early stages of diabetes, and AA supplementation can boost the effectiveness of cobalt action. More studies are necessary to understand these functions.

A negative correlation with Mn was also found ( $r = -0.324$ ,  $p = 0.024$ ) with AA plasma. Animal studies have found that plasma Mn concentrations were significantly lower when ascorbate was added to the diet [45]; in humans, these data could be more evident due to their inability to produce vitamin C. In athletes, enough plasma concentrations of AA would ensure a good state of antioxidation and where Mn, an essential mineral in the antioxidant enzyme manganese-superoxide dismutase (Mn-SOD), decreases its concentration.

We also found an inverse correlation between AA plasma with Cd concentrations ( $r = -0.379$ ,  $p = 0.008$ ). Watjen et al. [46] indicated that Cd is incapable of generating free radicals by itself; however, it indirectly favors the formation of ROS. It seems that it can replace Fe and Cu in several plasma and membrane proteins, which would lead to an increase in the free amount of these minerals involved in oxidative stress through the Fenton reaction. The Cu and Fe displacement by Cd can explain the toxicity induced by it, since Cu, which was displaced from its point of attachment, is able to catalyze the breakdown of hydrogen peroxide via the Fenton reaction [47].

Several studies [48–50] have shown that AA reduces the levels of ROS and MDA induced by Cd toxicity, inhibiting the oxidation of DNA, lipids, and proteins. This correlation could be related to the increase in the production of ROS induced by cadmium, which would try to be controlled using AA plasma as a fundamental antioxidant.

#### 4.2.2. Erythrocytes

Ascorbic acid in erythrocytes correlated strongly with As ( $r = 0.514$ ,  $p = 0.000$ ), such that subjects with higher concentrations of erythrocyte AA would compensate for the increase in the ROS produced by the prooxidative action of As to protect the life of the erythrocyte [51]. Chang et al. [49] concluded that the oxidative stress caused by As reducing GSH levels in rats was reversed by the action of AA; their data were corroborated by a later study in which Bera et al. [47] observed that L-ascorbate reduced oxidative stress and the cytotoxicity induced by As in hepatocytes. More studies in humans are necessary to understand this function.

There was also a positive correlation between AA erythrocytes with Cs ( $r = 0.347$ ,  $p = 0.016$ ), which is related to a possible response against the possible cellular toxicity of Cs. Like with Rb, relationships have been reported between Cs and K, and these relationships suggest that Cs could have the ability to act as a substitute for K [40], which would increase its concentration to maintain adequate osmotic pressure in the cell. This would occur as a consequence of the alteration caused by ROS in the Na/K ATPase related to the increase in red cell AA concentrations to compensate for ROS [42].

### 4.3. $\alpha$ -Tocopherol (ATC)

#### 4.3.1. Plasma

We found an interesting relationship between the concentration of ATC and plasma Cu concentrations ( $r = -0.317$ ,  $p = 0.028$ ). This could be related to the prooxidant activity exerted by Cu in the blood; for this reason, the body would use ATC to normalize the status, consequently decreasing its plasma concentrations as a protective effect against liver damage caused by Cu [51]. ATC may strengthen the antioxidant defense system by inhibiting protein oxidation and by enhancing the activity of antioxidant enzymes [52].

Plasma ATC also showed a negative correlation with Mn ( $r = -0.287$ ,  $p = 0.048$ ). Mn is an essential component of the mitochondrial antioxidant scavenging enzyme Mn-SOD [53]. Thus, the dietary deficiency of this micronutrient results in decreased activity of the enzyme and resistance to lipid peroxidation [54]. Further, the administration of higher doses of ATC is associated with the increased activity and expression of Mn-SOD in rats [55]. Therefore, the correlation found could be related to the capacity of Mn as an antioxidant through Mn-SOD.

The activity of key antioxidant enzymes is altered by deficiencies in Mn and vitamin E. Moreover, the glycosylation of hemoglobin is elevated in vitamin E deficiencies in diabetic rats, a fact that highlights the increase in oxidative stress in vitamin E deficient states [56].

We also found a correlation between ATC plasma with As concentrations ( $r = 0.348$ ,  $p = 0.015$ ). Although there is evidence that As exposure has a toxic effect on the nervous system, studies are scarce [57]. Recently, the study by Sharma et al. [58] indicated a positive role of ATC as an antioxidant against As toxicity in the brains of mice. In addition, Mohanta et al. [59] also observed how vitamin E supplementation relieved the toxic effects caused by As by preventing the depletion of the activity



of antioxidant enzymes in cells. Therefore, this correlation could be related to the antitoxic effects of vitamin E against As.

#### 4.3.2. Erythrocyte

Erythrocyte ATC was positively correlated with plasma Cu ( $r = 0.301$ ,  $p = 0.037$ ); it could be due to the prooxidant effect of this element on the body and the protection of ROS by vitamin E in plasma and erythrocytes [60,61]. Vitamin E protects the body against copper-induced damage due to its antioxidant activity [62], although Hajiani et al. [55] suggested that Cu/Zn-SOD activity and expression does not change dramatically in response to vitamin E. Cinar et al. [63] showed that Cu intake caused the oxidative stress, and the combination of vitamin C and the addition of vitamin E could alleviate the damaging effects of Cu, as evidenced by the decrease in lipid peroxidation and liver enzymes, however, more research studies in humans are needed.

We also found a positive correlation between erythrocyte ATC ( $r = 0.601$ ,  $p = 0.000$ ) and Li. We know that Li accumulates in erythrocytes; therefore, we think that these greater ATC deposits in erythrocytes could be determined by an increased presence of Li. In this respect, Toplan et al. [64] indicated that treatment with Li carbonate in rats resulted in increased oxidative damage and in the osmotic fragility of the erythrocyte membrane. This is why the erythrocyte would compensate for these Li effects (mainly to its membranes) by increasing its erythrocytic ATC levels. More studies in humans are necessary to understand these functions.

Finally, a positive correlation was found between erythrocyte ATC and Rb ( $r = 0.325$ ,  $p = 0.024$ ); as previously mentioned, the alteration caused in the enzyme Na/K ATPase as a result of ROS [42] could cause an increase in Rb to maintain an adequate osmotic pressure in the cell and act in synergy with ATC in erythrocytes, in an attempt to counteract the ROS to maintain an adequate Na/P ATPase function. With respect to retinol, no correlations were found with the plasma concentrations of analyzed TMs.

More studies in humans are necessary to understand these functions.

## 5. Conclusions

To conclude, we found that athletes with a high degree of training present lower plasma ATC and retinol concentrations. They exhibit interesting positive and negative correlations between TMs and oxidative stress markers such as MDA and the nonenzymatic antioxidants such as AA and ATC in plasma or erythrocytes. These findings may help to develop specific supplementation regimens for endurance athletes.

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