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Acute effect of a maximum incremental test until exhaustion on malondialdehyde and antioxidant vitamins in plasma and erythrocytes in athletes

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ABSTRACT

Background: it is well known that moderate or vigorous physical exercise produces an increase in free radicals.

Aim: the aims of this study were to observe changes in malondialdehyde and antioxidant vitamins after a maximum incremental test, and to relate malondialdehyde and antioxidant vitamin values to performance parameters.

Methods: eighty-four male athletes participated in this study. Participants performed a maximum incremental test until exhaustion on a treadmill. Malondialdehyde in plasma and antioxidant vitamins in plasma and erythrocytes were determined before and after the test.

Results: in plasma, there was a decrease in malondialdehyde after the test. In erythrocytes, results showed increases in vitamin C and decreases in vitamin E after the test. Maximal oxygen uptake values were associated positively with vitamin C and negatively with malondialdehyde levels before the test. On the other hand, maximal oxygen uptake, total test time, and total test distance were positively related to the malondialdehyde values obtained after the test.

Conclusions: a maximum incremental test did not produce any changes in plasma vitamins in athletes. However, it increased the levels of vitamin C in erythrocytes and decreased malondialdehyde values in plasma and vitamin E in erythrocytes. The levels of malondialdehyde, vitamin C and vitamin E were related to performance parameters. These results may be linked to the adaptation of antioxidant systems due to regular training.

Keywords: Erythrocyte. Plasma. Vitamins. Free radicals.

RESUMEN

Introducción: es sabido que el ejercicio físico moderado o vigoroso produce un aumento de radicales libres.

Objetivos: los objetivos del estudio fueron: observar los cambios del malondialdehído y las vitaminas antioxidantes después de un test incremental máximo y relacionar los niveles de malondialdehído y vitaminas antioxidantes con parámetros de rendimiento.

Métodos: ochenta y cuatro atletas masculinos participaron en el estudio. Los participantes realizaron un test incremental hasta la extenuación en un tapiz rodante. El malondialdehído en plasma y las vitaminas antioxidantes en plasma y en eritrocitos se determinaron antes y después del test incremental.

Resultados: en el plasma hubo una disminución del malondialdehído después de la prueba incremental. En los eritrocitos, los resultados mostraron un aumento de la vitamina C y un descenso de la vitamina E después de la prueba. El consumo máximo de oxígeno se asoció positivamente con la vitamina C e inversamente con el malondialdehído antes del test. Por otro lado, el consumo máximo de oxígeno, el tiempo total del test y la distancia total durante el test se asociaron positivamente con los valores de malondialdehído obtenidos tras el test máximo.

Conclusión: un test incremental máximo hasta la extenuación no produjo cambios en las vitaminas antioxidantes del plasma. Sin embargo, sí aumentó los niveles de vitamina C en los eritrocitos y redujo los niveles de malondialdehído en el plasma y los de vitamina E en los eritrocitos. Los niveles de malondialdehído, vitamina C y vitamina E se relacionaron con los parámetros de rendimiento. Estos resultados podrían estar relacionados con la adaptación de los sistemas antioxidantes debido al entrenamiento regular.

Palabras clave: Eritrocitos. Plasma. Vitaminas. Radicales libres.

INTRODUCTION

Physical exercise is associated with numerous benefits for the body (1). However, it is also known that moderate or vigorous physical exercise produces an increase in free radicals that may cause damage at the level of cellular lipids, DNA, and protein degradation (2). In this respect, compounds produced during the attack of free radicals on membrane lipids, such as malondialdehyde (MDA), are considered a marker of oxidative stress (3). Oxidative stress is understood as the impaired balance between free radical production and antioxidant systems.

Reactive oxygen species (ROS) are free radicals produced by the body as part of the oxidative metabolism. Increased oxygen consumption, catecholamine release, or excess lactic acid can increase ROS production during exercise (4). Accordingly, the oxidative stress response may depend on parameters such as exercise intensity or duration (5).

Antioxidants form a defense system to protect cells and tissues against oxidative damage. They are present in both the intracellular and extracellular matrix. The antioxidant defense system is divided into enzymatic (endogenous) and non-enzymatic (exogenous) antioxidants (6). Vitamin A, vitamin C, and vitamin E are considered antioxidant (exogenous) vitamins. Diverse strategies are applied by both endogenous and exogenous antioxidants to protect against ROS (7). These include conversion of ROS into less active molecules (i.e., scavenging) and prevention of the transformation of the less harmful ROS into more damaging forms (i.e., conversion of hydrogen peroxide to the hydroxyl radical) (7).

Erythrocytes are continuously exposed to sources of ROS that can damage the erythrocyte membrane and impair its function (8). Erythrocytes are particularly susceptible to oxidative stress due to their role as oxygen transporters and their high content of polyunsaturated fatty acids and transition metals (9). The chronic alteration of erythrocytes, due to oxidative damage, is considered a potent inductor of disease or morbidity. To minimize the effect of ROS,

erythrocytes have an extensive antioxidant system involving both non-enzymatic and enzymatic antioxidants (8).

Although ROS is associated with harmful events, they are necessary for cell development and function. It is known that ROS act as biological stimuli by being messengers in molecular signaling processes and in the modulation of enzyme and gene activation (2). Physiological adaptation to oxidative stress is based on redox signaling processes. When ROS production increases, for example, during physical exercise, redox signaling induces protective mechanisms by positively regulating antioxidant responses (10).

Different studies have observed the acute response to physical exercise in parameters of oxidative stress and antioxidant response. Bloomer et al. (11) observed increases in oxidative stress markers during aerobic and anaerobic exercise. Another study showed increases in plasma antioxidant vitamins and decreases in erythrocyte antioxidant vitamins after a maximum test in cyclists (5). Braakhuis et al. (12) analyzed the relationship between training status and plasma antioxidant levels in rowers. However, to our knowledge, there are no studies that analyze the associations between markers of oxidative stress and antioxidant vitamins in erythrocytes and performance parameters in athletes. Therefore, the aims of the present study were: a) to observe changes in plasma MDA, as a marker of lipid peroxidation, and antioxidant vitamins (A, C, and E) in plasma and erythrocytes after a maximum incremental test until exhaustion; b) to relate the levels of MDA and antioxidant vitamins in plasma and erythrocytes to the performance parameters obtained in the maximum incremental test until exhaustion in athletes.

MATERIALS AND METHODS

Participants

Eighty-four long and middle distance male athletes (23.2 ± 3.25 years) participated in this study. Participants were previously informed

about the purpose of the study and signed their voluntary informed consent. A code was assigned to each participant for the collection and treatment of samples in order to maintain their anonymity. This research was conducted under the Helsinki Declaration ethical guidelines, updated at the World Medical Assembly in Fortaleza in 2013, for research with human participants, and the protocol was approved by the Ethics Committee of the University of Extremadura (ref. number: 52/2012).

All the subjects were participants in national and international championships and had at least five years of training experience (1500 to 5000 m race modalities). The athletes had trained regularly over the previous year with a volume of training of around 89.94 ± 13.11 km/week. During that period, 13.02 ± 5.98 km/week were performed at an intensity above the anaerobic threshold (VT2), and 76.91 ± 17.76 km/week were performed at an intensity below VT2. Pulsometers (Polar M430, Norway) were used to track the training loads and to analyze training routines.

Subjects reported to the laboratory after an overnight fast, and were instructed to abstain from hard training and competition for at least 48 h before testing.

Anthropometric measurements

Body height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 220, Hamburg, Germany), and body weight was measured to the nearest 0.01 kg using calibrated electronic digital scales (Seca 769, Hamburg, Germany) in nude, barefoot condition. Body fat content was estimated from the sum of 6 skinfolds (abdominal, suprailiac, tricipital, subscapularis, thigh, and calf skinfolds). Measurements were made by the same operator, skilled in kinanthropometry techniques, using a Harpenden calliper (Holtain Skinfold Caliper, Crosswell, UK). Anthropometrics were assessed as shown in the kinanthropometry Spanish group (13) (Table I).

Nutritional evaluation

All participants completed a dietary questionnaire to record nutritional intake. The questionnaire consisted of a 3-day, daily nutritional record, filled out on two pre-assigned weekdays and one weekend day. On each day, all participants recorded the amount (in grams or liters) of each food consumed in every meal ingested. Every questionnaire compiled the total amount of each food consumed grouped by meals. Then the nutritional composition of their diets was evaluated using food composition tables (14). None of the participants followed a specific diet, nutritional plan or took specific antioxidant supplementation (Table II).

Maximal exercise test until exhaustion

The incremental test consisted of running on a treadmill (Ergo-Fit 4000, Germany) until exhaustion. The treadmill was equipped with a gas analyzer (Geratherm Respiratory GMBH, Ergostik, Germany) and a pulsometer (Polar M400, Norway) was used to evaluate the heart rate (HR). To guarantee a warm-up phase before the test, all participants ran progressively for 15 min, ending at the initial speed of the test. The subjects completed 5 min at 7 km/h, 5 min at 8 km/h, and 5 min at 9 km/h. Then, the participants started the exercise test. The protocol consisted of running in incremental stages, until voluntary exhaustion, starting at an initial speed of 10 km/h and increasing it by 1 km/h every 400 m, with a stable slope of 1 %. Three criteria were used to determine the maximal parameters in the incremental test: to achieve a plateau in oxygen uptake (VO_2), the respiratory exchange ratio (RER) had to exceed 1, and inability to maintain the speed required. All the tests were performed in the morning (between 10:30 a.m. and 13:30 p.m.) (Table I).

Sample collection

Two draws of 10 mL of venous blood were taken from the antecubital vein of each participant. Plastic syringes fitted with stainless steel needles were used to carry out the blood draws. The first sample was drawn before the exercise test after an overnight fasting period (start time, 08:30 a.m.). The second sample was obtained two minutes after test completion. Once extracted, the samples were collected into a metal-free polypropylene tube (previously washed with diluted nitric acid). After the first blood draw the participants ingested a similar breakfast, which consisted of 250 ml of a 5 % glucose solution drink. Once collected, the blood samples were centrifuged at 2500 revolutions per minute for 10 min at room temperature to separate the serum and red cells. The serum and red cells were aliquoted into an Eppendorf tube (previously washed with diluted nitric acid) and preserved at −80 °C until biochemical analysis.

Determination of hematocrit and hemoglobin

To determine hematocrit (Htc) and hemoglobin (Hb), 200 µL of blood were taken, deposited into a tube, and measured on a Coulter (Coulter AcT, Coulter Electronics LTD, Model 6706319. Northwell Drive, Luton, England). Both Htc and Hb were used to correct the changes in plasma volume (15). These changes were applied to avoid a possible error caused by hemoconcentration during acute exercise.

MDA and antioxidant vitamin determination

This section was similar to the one reported by Muñoz Marín et al. (5). Oxidative stress in plasma was estimated by measurement of MDA as described in Chirico (16), using high-performance liquid chromatography (HPLC) (Spectra SERIES P100/UV 100), and performing a linear calibration with a variation coefficient of 2.3 %. HPLC was used to analyze vitamins A and E with the method described by Shearer (17) using as internal standard α-tocopherol (type of vitamin E) acetate with a coefficient of variation of 3 %. The vitamin C content of the samples was determined by HPLC using the

methods described by Manoharan & Schwille (18). A linear calibration was performed with a variation coefficient of 1.15 %. Erythrocyte parameters were expressed in µg/g Hb, and plasmatic parameters were expressed in µg/mL or µM/mL.

Statistical evaluation

Statistical analyses were carried out with the software IBM SPSS Statistics 20.0 for Windows. The statistical evaluation consisted of an initial Kolmogorov-Smirnov test to examine the distribution of variables, and Levene's test to examine variance homogeneity. The differences between before and after maximum tests were obtained using Wilcoxon's test for non-parametric paired samples. The intra-group effect sizes (ES) were calculated following the guidelines by Fritz, Morris & Richler (19). Threshold values for assessing the magnitudes of standardized effects were 0.20, 0.60, 1.20, and 2.00 for small, moderate, large, and very large, respectively (20). Pearson's correlation coefficient (r) was used to determine relationships between parameters. A simple linear regression model was used to determine the β coefficients and determination coefficients (R^2) in the significant correlations. A $p < 0.05$ was considered statistically significant.

RESULTS

Table III shows the results obtained during the study in weight, Hb, and Htc values.

A decrease in weight ($p < 0.01$) and increases in Hb and Htc were observed after the maximum incremental test ($p < 0.01$).

Table IV shows the changes in MDA and antioxidant vitamins in plasma (P) and erythrocytes (E) after the incremental test.

Decreases in plasma MDA ($p < 0.01$) and erythrocyte vitamin E ($p < 0.05$) were observed after the maximum test. On the other hand, vitamin C concentrations in erythrocytes increased after the test ($p < 0.05$).

Table V shows correlations and simple linear regressions between antioxidant vitamins and MDA—collected before the maximum incremental test—with test performance parameters.

The analysis of the data reveals relationships between weight-related maximal oxygen uptake (VO_2 max) and plasma vitamin C ($r = 0.252$; $\beta = 0.348$; $R^2 = 0.064$), between absolute VO_2 max and plasma vitamin C ($r = 0.219$; $\beta = 0.043$; $R^2 = 0.048$), between absolute VO_2 max and MDA ($r = -0.247$; $\beta = -2.436$; $R^2 = 0.061$), and between total time in the test and vitamin C in erythrocytes ($r = -0.211$; $\beta = -0.047$; $R^2 = 0.045$) ($p < 0.05$).

Table VI shows correlations and simple linear regressions between MDA and antioxidant vitamin parameters—obtained after the maximum test—with the test performance parameters.

There were significant relationships between weight-related VO_2 max and MDA ($r = 0.230$; $\beta = 21.103$; $R^2 = 0.053$), between weight-related VO_2 max and Vitamin E in erythrocytes ($r = -0.218$; $\beta = -0.223$; $R^2 = 0.047$), between total distance in the test and MDA ($r = 0.224$; $\beta = 1507.94$; $R^2 = 0.051$), and between total time in the test and MDA ($r = 0.319$; $\beta = 8.756$; $R^2 = 0.102$) ($p < 0.05$).

Figure 1 shows the linear regressions of the previous significant correlations graphically.

DISCUSSION

The aim of this study was, on the one hand, to observe the changes in MDA and antioxidant vitamins in plasma and erythrocytes after a maximum incremental test and, on the other hand, to relate the levels of MDA and antioxidant vitamins to the performance parameters obtained during the test. To the best of our knowledge, this is the first study to analyze the relationships between erythrocyte antioxidant vitamins and performance parameters in athletes.

Long and middle distance athletes generally run many kilometers a day to achieve successful results. Regular training generates physiological adaptations in muscle metabolism and cardiorespiratory

function (21). Furthermore, regular training involves adaptive processes in biological systems to enable the body to cope with increased physical demand. Athletes show increased amounts of endogenous antioxidant enzymes and increased tolerance to exercise-induced oxidative stress (22). Without optimal antioxidant systems the body would not be able to cope with the amounts of free radicals generated by exercise, and a state of chronic oxidative stress would result.

An important aspect to consider is the diet followed by the participants in the study. Adequate dietary intake of antioxidant vitamins is required to maintain optimal antioxidant status in athletes who undergo regular training (23). In the present study, the intake of vitamins A and C was higher than recommended (800-900 µg/day and 80-90 mg/day respectively). However, vitamin E intake was below the recommended level (15 mg/day) (23). These data may be related to the observed decrease in vitamin E levels in erythrocytes.

One of the main consequences in the analysis of acute responses to physical exercise is water loss through sweating. This change can induce dehydration and hemoconcentration. In this regard, the increase in Htc and Hb and the decrease in body weight after the maximum incremental test indicate hemoconcentration due to fluid loss through sweating (24). To avoid this methodological problem, the equation for hemoconcentration was applied using Hb and Htc (15).

In the present study, a decrease in plasma MDA was observed after the incremental test ($p < 0.01$). These results coincide with those observed by Groussard et al. (25). The previous study showed that MDA concentrations in plasma decreased after a Wingate test and continued to decrease minutes later in the recovery period (25). However, other investigations obtained results that are contradictory to those observed in our study (5). The decrease observed in our study could be explained, on the one hand, by the elimination of plasma MDA during the post-exercise period, possibly due to increased catabolism, excretion, or body redistribution (26). On the

other hand, it could be due to the level of training of the study subjects as they were national and international athletes with training experience. It is known that regular training produces adaptations in the antioxidant system (27). The training status of the athletes generates an optimal antioxidant system which could lead to a higher amount of endogenous antioxidants or lower production of free radicals, decreasing markers of oxidative stress (28).

No significant changes in plasma antioxidant vitamins were observed in our study. Aguiló et al. did not report significant changes in vitamin A after a 171 km cycling stage (29). Similarly, Muñoz-Marín et al. did not observe changes when submaximal exercise (30 minutes duration at 75 % VO_2 max intensity) was evaluated (5). The absence of changes in antioxidant vitamins could be related to adaptation of the antioxidant system due to regular training. Perhaps, the increase in antioxidant enzymes after physical exercise, as in other studies in athletes, may not result in alterations in plasma antioxidant vitamin concentrations (30). Endogenous antioxidants are important for the maintenance of cellular functions. However, under conditions that support high oxidative stress, endogenous antioxidants may not be sufficient and exogenous antioxidants may be needed to maintain cellular functions (31). The reduction of MDA in plasma after the test indicates less oxidative stress. Constant values of plasma antioxidant vitamins may indicate that the endogenous antioxidant system is sufficient to maintain the oxidative state. However, it was not possible to evaluate antioxidant enzymes to confirm this hypothesis.

With respect to the antioxidant vitamins in erythrocytes, increases in vitamin C and decreases in vitamin E were observed after the maximum test ($p < 0.05$). Other authors observed decreases in the concentrations of both vitamins after a maximum incremental test in cyclists (5). As previously mentioned, erythrocytes appear to be more vulnerable to oxidative damage during intense exercise due to their exposure to high amounts of oxygen and their high concentrations of polyunsaturated fatty acids and iron (9). As with plasma vitamins, the

increase in vitamin C in erythrocytes after the test may be related to adaptations of the antioxidant systems due to regular training. It is known that endurance training reduces erythrocyte susceptibility to oxidative stress (32). There is evidence that erythrocytes contribute to maintaining antioxidant levels in the circulation due to their mobility and oxidative scavenger properties (33). At the cellular level, there is an increase in the levels of antioxidant enzymes that constitute the first barrier to fight free radicals, which could favor the exit of vitamin C to the plasma (22). However, the increases in plasma antioxidant enzymes observed in other studies in athletes (34) could decrease the output of vitamin C and increase its intracellular content. This would be related to previous research in athletes (35). The decrease of antioxidant enzymes in erythrocytes could be related to their release into the plasma or to ROS inhibition. The activity of some antioxidant enzymes (glutathione reductase and catalase) is inhibited by increases in the concentration of hydrogen peroxide (36). This suggests a depletion of the reserve of enzymatic antioxidants. In contrast to our study, Muñoz-Marín et al. found higher plasma levels than those found in erythrocytes after the test (5). However, in the previous study, the incremental test until exhaustion was performed on a cycle ergometer unlike the present study, which was performed on a treadmill. The greater involvement of muscle groups on the treadmill and the participants' level of training may explain the differences between the two studies.

Vitamin E is activated in proportion to the level of oxidative stress that cells are subjected to as a result of exercise intensity in order to protect the cell from ROS (37). After a maximum test, erythrocyte levels of vitamin E may decrease because other antioxidant systems are unable to neutralize the oxygen free radicals responsible for lipid peroxidation (5). However, we believe that the decrease in intracellular vitamin E may be related to deficient intake, as indicated when evaluating nutrient intake.

Concerning correlations, different correlations were observed in our study with the parameters obtained before and after the test. Other authors have analyzed the correlations between enzymes and antioxidant vitamins with training and performance parameters in rowers (12). In the previous study, they observed positive correlations between total antioxidant capacity and performance, and negative correlations between years of training and total antioxidant capacity. The authors concluded that the level of training correlates with antioxidant status rather than diet (12). In our study, correlations observed between VO_2 max and plasma vitamin C, and VO_2 max and MDA were positive and negative, respectively ($p < 0.05$). In addition, there was a negative correlation between total time in the test and vitamin C in erythrocytes ($p < 0.05$) as obtained before the maximum incremental test. On the other hand, positive correlations were observed for total time in the test, total distance in the test, and VO_2 max with MDA, and negative correlations between VO_2 max and vitamin E in erythrocytes as measured after the incremental test ($p < 0.05$).

Vitamin C is important for metabolism and the immune system during exercise (38). Vitamin C may eliminate free radicals and increase strength production, leading to increased muscle performance and therefore increased oxygen consumption (39). On the other hand, the correlations observed after the incremental test in MDA values would be related to the increase in free radicals as the test is performed. Under conditions of physiological stress, for example during intense exercise, the production of free radicals increases dramatically, altering the redox state of the muscle and possibly inhibiting contractile muscle function (40). The consequence would be an increase in the occurrence of muscle fatigue. As time, distance, and oxygen consumption increase, the intensity of the test increases, and consequently the production of free radicals increases.

Absence of measurement of endogenous antioxidants, cytokines, and other markers of oxidative stress, and absence of a control group are

among the limitations of this study. The impossibility to find a high number of subjects with similar characteristics to those of athletes is the reason why no control group was included in this study. For future research, it would be interesting to assess the relationships between endogenous and exogenous antioxidants, and to analyze the relationship between inflammation/oxidative markers and antioxidant vitamins in plasma and erythrocytes. Moreover, we encourage researchers to evaluate the same oxidative parameters in platelets, and to quantify non-oxidant vitamins before and after the maximal incremental test as a potential control. Finally, an analysis of these parameters at different moments during a sports season would be interesting.

CONCLUSIONS

A maximum incremental test until exhaustion produced decreases in plasma MDA, decreases in erythrocyte vitamin E, and increases in erythrocyte vitamin C. The incremental test did not cause changes in plasma antioxidant vitamins. The values of plasma vitamin C, plasma MDA, and erythrocyte vitamin E were correlated with distance, time, and VO_2 max as obtained in the maximum incremental test. These results may be related to adaptations of the antioxidant system due to regular training in athletes.

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Table I. Anthropometric characteristics and ergospirometric values

	n = 84
Weight (kg)	65.11 ± 7.14
Height (m)	1.77 ± 0.05
Σ6 skinfolds (mm)	46.57 ± 9.62
Fat (%)	5.38 ± 1.03
HRmax (ppm)	192.75 ± 8.39
VO_2 max (mL/kg/min)	68.20 ± 7.21
VO_2 max (mL/min)	4.40 ± 1.01
Distance (m)	4570.91 ± 725.76
Time (min)	20.72 ± 2.16

HRmax: maximum heart rate; Σ: sum

Table II. Nutritional intake

	n = 84
Energy (kcal/day)	2771.3 ± 432.2
Carbohydrates (g/kg/day)	5.78 ± 0.56
Lipids (g/kg/day)	1.58 ± 0.21
Proteins (g/kg/day)	1.86 ± 0.43
Vitamin A (µg/day)	1145.76 ± 269.34
Vitamin C (mg/day)	87.24 ± 15.63
Vitamin E (mg/day)	8.76 ± 4.35

Table III. Weight, hemoglobin, and hematocrit levels before and after the maximum incremental test

	Before	After	ES
Weight (kg)	65.11 ± 7.14	64.21 ± 7.41*	0.53
Hemoglobin (g/dL)	15.08 ± 1.24	15.89 ± 1.18*	0.65
Hematocrit (%)	45.25 ± 3.76	47.69 ± 3.53*	0.66

ES: effect size; *p < 0.01 for differences between before vs after

Table IV. Plasma concentrations of MDA and plasma (P) and erythrocyte (E) antioxidant vitamins before and after the maximum incremental test

	Before	After	ES
MDA (µM/mL)	0.726 ± 0.10	0.613 ± 0.07†	0.75
P-Vitamin C (µg/mL)	16.45 ± 5.22	16.93 ± 8.95	0.04
P-Vitamin E (µg/mL)	8.01 ± 10.62	8.61 ± 13.43	0.05
P-Vitamin A (µg/mL)	0.138 ± 0.08	0.130 ± 0.06	0.03
E-Vitamin C (µg/g Hb)	10.10 ± 9.78	11.93 ± 8.97*	0.45
E-Vitamin E (µg/g Hb)	10.81 ± 10.14	7.37 ± 7.05*	0.56
E-Vitamin A (µg/g Hb)	0.763 ± 0.57	0.681 ± 0.44	0.16

MDA: malondialdehyde; P: plasma; E: erythrocyte; ES: effect size; *p < 0.05; †p < 0.01 for differences between before vs after

Table V. Correlations and simple linear regressions between MDA and antioxidant vitamin levels before incremental test with ergospirometric parameters

	VO_2 max (mL/kg/min)				VO_2 max (mL/min)			
	r	**R^2**	**β (95 % CI)**	***p***	**r**	**R^2**	**β (95 % CI)**	***p***
P-MDA (μM/mL)	-0.052			0.640	-0.247	0.061	-2.436 (-4.53/-0.33)	0.024
P-Vitamin C (μg/mL)	0.252	0.064	0.348 (0.05/0.64)	0.021	0.219	0.048	0.043 (0.00/0.08)	0.046
P-Vitamin E (μg/mL)	0.003			0.978	-0.059			0.592
P-Vitamin A (μg/mL)	0.014			0.901	-0.042			0.706
E-Vitamin C (μg/g Hb)	0.053			0.633	0.038			0.731
E-Vitamin E (μg/g Hb)	-0.002			0.985	0.090			0.468
E-Vitamin A (μg/g Hb)	0.026			0.816	0.074			0.504
	Distance (m)				**Time (min)**			
	r	**R^2**	**β (95 % CI)**	***p***	**r**	**R^2**	**β (95 % CI)**	***p***
P-MDA (μM/mL)	-0.004			0.971	0.032			0.771
P-Vitamin C (μg/mL)	0.013			0.907	-0.018			0.868
P-Vitamin E (μg/mL)	0.032			0.769	0.005			0.964
P-Vitamin A (μg/mL)	-0.026			0.817	0.060			0.585
E-Vitamin C (μg/g Hb)	-0.087			0.431	-0.211	0.045	-0.047 (-0.09/0.00)	0.048
E-Vitamin E (μg/g Hb)	0.015			0.895	-0.036			0.742
E-Vitamin A (μg/g Hb)	0.055			0.621	0.064			0.561

MDA: malondialdehyde; P: plasma; E: erythrocytes; r: Pearson's coefficient of correlation; β: beta coefficient; CI: confidence interval; R^2: coefficient of determination; *p*: p-value

Table VI. Correlations and simple linear regressions between MDA and antioxidant vitamin levels after incremental test with ergospirometric parameters

	VO_2 max (ml/kg/min)				VO_2 max (ml/min)			
	r	**R^2**	**β (95 % CI)**	***p***	**r**	**R^2**	**β (95 % CI)**	***p***
P-MDA (µM/mL)	0.230	0.053	21.103 (1.48/40.72)	0.035	0.106			0.336
P-Vitamin C (µg/mL)	0.163			0.138	0.171			0.119
P-Vitamin E (µg/mL)	0.000			0.997	0.016			0.883
P-Vitamin A (µg/mL)	0.108			0.327	-0.148			0.179
E-Vitamin C (µg/g Hb)	-0.032			0.773	0.080			0.471
E-Vitamin E (µg/g Hb)	-0.218	0.047	-0.223 (-0.442/-0.003)	0.047	0.098			0.376
E-Vitamin A (µg/g Hb)	-0.143			0.193	-0.160			0.145
	Distance (m)				**Time (min)**			
	r	**R^2**	**β (95 % CI)**	***p***	**r**	**R^2**	**β (95 % CI)**	***p***
P-MDA (µM/mL)	0.224	0.051	1507.94 (64.78/2951.1)	0.041	0.319	0.102	8.756 (3.03/14.4)	0.003
P-Vitamin C (µg/mL)	0.071			0.519	0.109			0.325
P-Vitamin E (µg/mL)	0.012			0.913	0.060			0.586
P-Vitamin A (µg/mL)	0.025			0.824	0.139			0.208
E-Vitamin C (µg/g Hb)	0.090			0.414	0.098			0.375
E-Vitamin E (µg/g Hb)	0.056			0.615	0.050			0.653
E-Vitamin A (µg/g Hb)	-0.068			0.537	-0.125			0.259

MDA: malondialdehyde; P: plasma; E: erythrocytes; r: Pearson's coefficient of correlation; β: beta coefficient; CI: confidence interval; R^2: coefficient of determination; *p*: p-value

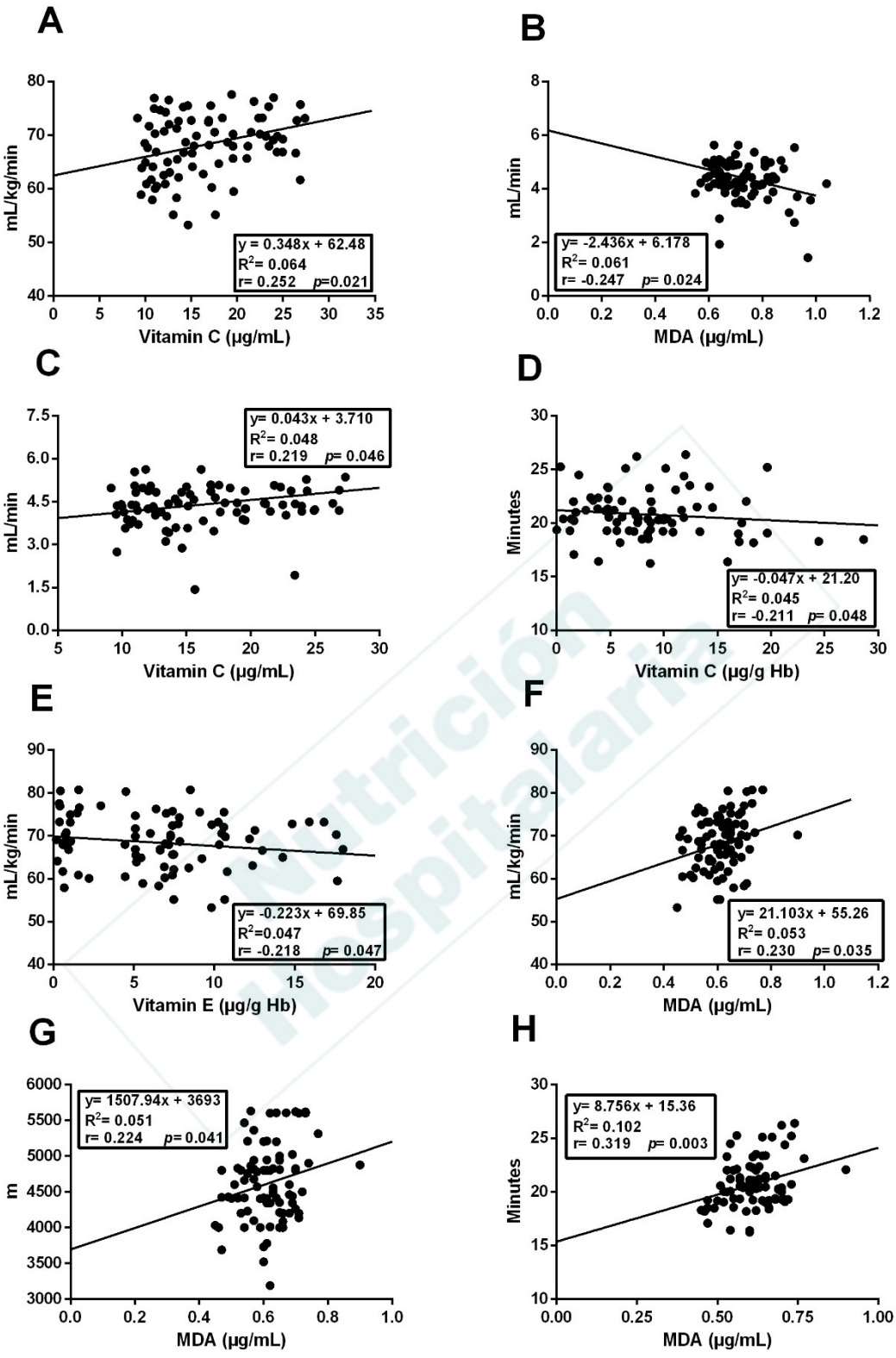

Fig. 1. Linear regressions of significant correlations between performance parameters and the values obtained before the maximum test (A, B, C, and D). Linear regressions between performance parameters and the values obtained after the maximum

test (E, F, G, and H). A: linear regression between pre-test plasma vitamin C levels and VO_2 max; B: linear regression between pre-test plasma MDA levels and VO_2 max; C: linear regression between pre-test plasma vitamin C levels and VO_2 max; D: linear regression between pre-test RBC vitamin C levels and total test time; E: linear regression between post-test RBC vitamin E levels and VO_2 max; F: linear regression between post-test plasma MDA levels and VO_2 max; G: linear regression between post-test plasma MDA levels and total distance in the test; H: linear regression between post-test MDA levels and total test time. MDA: malondialdehyde; RBC: red blood cell.