Effect of the intense anaerobic exercise on nitric oxide and malondialdehyde in studies of oxidative stress

Ana Valado, Leonel Pereira, Paula C. Tavares and Carlos Fontes Ribeiro

***Abstract*—** The physical exercise is considered beneficial contributing for physical, psychological and social wellbeing and balance of the individual, being able to delay the aging process. The physical exercise unchains a physiological stress situation, to which, the sympathetic nervous system activity answers activating adaptation mechanisms. The availability of oxygen and the nitric oxide release, provide the formation of reactive oxygen species (ROS), related with the origin of cellular and tissue injuries. In order to evaluate the effect of the exercise we selected a set of sixteen healthy young individuals, voluntary, that they had constituted two distinct groups: the athletes, constituted by *Futsal* athletes; and the control group, formed for individuals that did not practice any type of sport with regularity. The main objective of this work was to investigate if the acute and intense exercise originates, in both the groups, in the production and release of NO and in the production of free oxygen radicals. With this purpose the Wingate test is used (supramaximum anaerobic test executed in 30 seconds). After that, the concentrations of blood lactate, platelet and plasmatic nitric oxide and the plasmatic malondialdehyde (MDA) had been determined. All the determination had been made in two blood samples: one harvested before the exercise and the other 15 minutes after the Wingate test; with the exception of lactates, which was executed 5 minutes after the test. The innovation of the present study showed in the plasmatic malondialdehyde levels, which revealed in the athletes a significant reduction, in rest and after exercise, relatively to the control group. A significant reduction in the blood lactate concentration was verified in the athletes, after exercise, in relation to the control. On the other hand, the concentrations of total intra-platelet nitrites and released for the platelet, presented in the athletes a significant increase, in rest and after exercise, relatively to the control. The differences are related with the physical training, seeming to stimulate the adaptation mechanisms and the antioxidation defenses of the athletes, conferring bigger cardiovascular protection and enhanced protection against physical and oxidative stress, comparatively to the individuals that did not practice sport with regularity. Thus, in young individuals, seems to us that the regular physical activity and the intense exercise develop a physiological adaptation, such that, after a maximum acute exercise, has pointers of an enhanced cardiovascular protection and against oxidative stress.

***Keywords*—** Anaerobic-exercise, MDA, nitric-oxide, nitrites, oxidative-stress, ROS.

I. Introduction

THE physical exercise carried through according to some principles and rules is considered beneficial, not only for the health, as it also seems to intervene with the aging process, delaying it [1], [2].The beginning of the exercise unchains in the individual stimulation to the level of the sympathetic nervous system that leads to the release of vasoconstrictor substances. Continuing the exercise an adaptation of the cardiovascular system is verified, with increase of the cardiac rhythm and cardiac force, increase of the arterial pressure, adaptation of the respiratory system, increase of the sanguineous flow, increment of the metabolism, rise of the glucose concentration in the blood, increase of glycolysis in the liver and muscle. All these factors, in set, contribute for a good performance of the physical exercise [3]. The presence of oxygen, although indispensable, can become dangerous, promoting oxidative stress. The increase of the volume of oxygen favors the production of reactive oxygen species (ROS), unchaining of oxidative stress, with all the baleful consequences [4], [5]. The ROS increase can compromise the antioxidant (chemical and enzymatic) defense available in the organism. Thus, it is suggested an implementation of alimentary habits with antioxidant supplements [6], [7] and its association with regular physical activity [6].

Related studies showed that the organism present high nitric oxide (NO) and reactive nitrogen species (RNS) concentrations during exercise [5]. The duration and the intensity of the exercise are important factors in the origin of oxidative stress [7]-[9]. The lipid peroxidation index, indicator of oxidative stress potential, is evaluated by the malondialdehyde levels [5], [7], [10].

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Table I - Age and anthropometric characteristics of the different groups (athletes and control)

Group	n	Age (years old)	Corporal mass (kg)	Height (cm)	ICM (Kg/m^2)
Athletes	9	18,7 ± 0,2 [18,0 - 19,0]	62,9 ± 2,9 [54,0 - 84,5]	174,0 ± 1,9 [165,0 - 184,5]	20,8 ± 0,8 [18,1 - 27,0]
Control	7	19,4 ± 0,3 [19,0 - 21,0]	66,7 ± 3,3 [54,0 - 80,5]	173,0 ± 1,3 [167,7 - 177,2]	22,3 ± 0,9 [18,6 - 25,6]

The data are presented as mean ± standard error; ICM - Index of corporal mass

II. Material and Methods

A. Sample Selection and General Procedures

In this study 16 voluntary, of the masculine sex, healthful individuals, with ages between 18 and 21 years old, was analyzed. The population was divided in two groups: athletes, consisting of 9 players of *Futsal* (indoor soccer) and control, formed for 7 individuals that did not practice sport with regularity.

After presentation and explanation of the work protocol the study with anthropometric measurements was initiated. The anaerobic exercise was evaluated by the Wingate test [11] and the harvest of peripheral blood (Fig. 1) in ACD was carried according Pollock and collaborators [12], in two moments: rest and exercise, respectively, before and 15 minutes after the Wingate test, for quantification of plasmatic and platelet NO, and MDA.

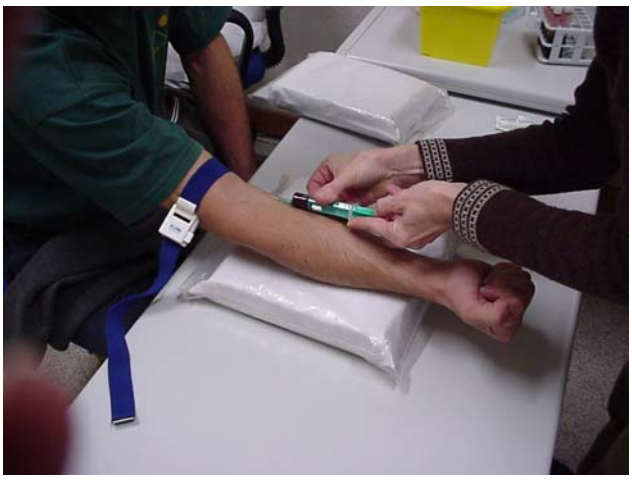


Fig.1 harvest of peripheral blood

B. Wingate Test

The Wingate test is a 30 seconds sprint (supramaximum anaerobic test), made in cycloergometer (Fig. 2) (MONARK 824 E), according the methodology described by Inbar and collaborators [11].

Fig.2 cycloergometer

C. Determination of the Blood Lactate Concentration

The blood was collected (Fig. 3) in rest and 5 minutes after the exercise. For the determinations we followed the indications of the commercial kit (Lactate, Dr. LANGE Cuvette Test, LKM 140), based in enzymatic method "LOX-PAP", according to Böning and collaborators [13]. The results were presented in mmol/L of lactate.

D. Platelet NO Quantification

The nitric oxide has a short time of life. In the oxygen presence, the NO is oxidized quickly in nitrites and nitrates, for what the concentration of nitrites and nitrates are habitually used as index of the NO production [14].

Platelets are an excellent experimental model, because reflect the endothelial alterations, for the similarities with the

Table II – Performance indicators in the Wingate test in *Futsal* athletes (n = 9) and control group (n = 7)

Performance indicators	*Athletes*	*Control*
Maximum Power (W)	646,9 ± 0,8	686,7 ± 48,4
Maximum Power relativa (W Kg^{-1})	10,4 ± 0,3	10,3 ± 0,4
Average Power (W)	543,3 ± 18,3	561,7 ± 33,4
Average Power relativa (W Kg^{-1})	8,7 ± 0,2	8,4 ± 0,2
Fatigue Index (%)	47,8 ± 2,4	54,0 ± 1,4

The data are presented as mean ± standard error

endothelium and smooth muscle cells. To the platelet faction, obtained through blood samples fractionization, was applied the methodology followed by Pollock and collaborators [12].

The intra-platelet nitrites and nitrites release quantification was carried according to the *Griess* method [15]. The results were presented in mmol/10^9 platelets.

E. Platelets Counting

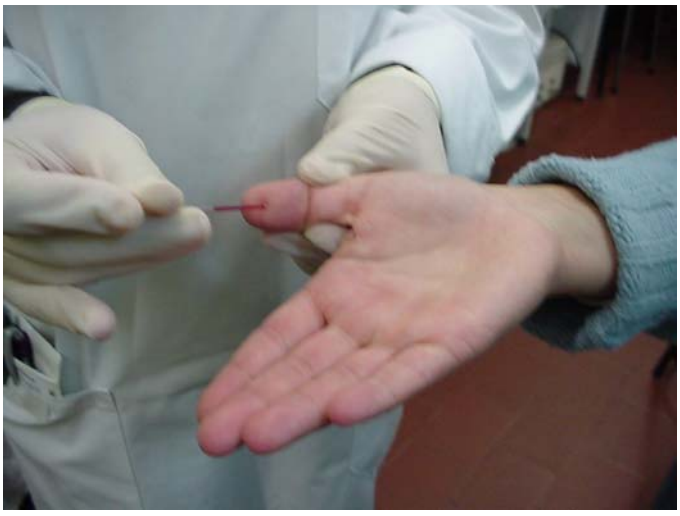
For the platelets counting a half-automatic accountant Cell Counter AI 134 was used.

F. Plasmatic NO Quantification

The plasmatic nitrites and nitrates concentration was determined with the Nitralyzer™ II kit [16] and the nitrites were quantified by the *Griess* method [15]. The results were presented in μM.

G. Malondialdehyde (MDA) Quantification

Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation [17]. The laboratorial methods



for the quantification of malondialdehyde, modified by Proença [18], were applied in this work. The results were presented in μM.

Fig.3 harvest of hair blood

H. Statistical Analysis

The results were presented in the form of mean ± standard error; the maximum and minimum values were presented between square brackets. Data were analyzed using the analysis of variance (ANOVA), having considered statistical significant the values of $p < 0.05$.

III. Results And Discussion

A. Anthropometric Characteristics

Table I presents the age and the anthropometric characteristics of the elements of both groups (athletes and control).

B. Wingate Test

Table II show the performance indicators in the *Wingate Test*. The type of physical exercise, the used energetic way, the adaptation mechanisms and the oxygen bioavailability are important in the physical exercise performance. With the Wingate test we evaluated the performance indicators, having gotten similar results in the two groups.

The fatigue index presented a tendency to diminish in the athletes, reflecting the absence of training in the control group, or one better adaptation of the athletes to the exercise [19].

C. Blood Lactate Concentration

Relatively to the blood lactate concentration in rest the athletes presented 1.8 ± 0.3 mmol/L, while the control group presented 2.0 ± 0.2 mmol/L; in the evaluation 5 minutes after the exercise, the athletes presented 8.7 ± 1.0 mmol/L, while the control group presented 13.8 ± 0.6 mmol/L, having been significant ($p < 0.05$) the lactate reduction in the athletes, after the exercise, in relation to the control.

The lactate concentration, five minutes after the anaerobic exercise, was significantly lower in the athletes than in control group, what evidences different training levels [1]. Lower values could be related with the reduction of muscular glycogenolysis or with the increase of the lactate removal

Table III – Intra-platelet nitrites, released platelet nitrites, plasmatic nitrites plus nitrates and MDA concentrations.

Group	*Intra-platelet nitrites* ***(nmol/10^9 platelets)***	*Realised platelet nitrites* ***(nmol/10^9 platelets)***	*Plasmatic nitrites + nitrates* ***(μM)***	*MDA* ***(μM)***
Athletes				
Rest	11,2±1,9 *	11,4±1,2 *	17,1±1,2	0,75±0,03 *
After 15 minutes	10,2±1,7 *	10,6±1,5 *	15,7±3,7	0,81±0,05 *
Control				
Rest	6,6±0,8	8,0±1,1	13,8±2,0	1,03±0,09
After 15 minutes	5,8±0,7	4,9±0,8	15,7±2,3	1,04±0,08

The concentrations were determined in rest and 15 minutes after the anaerobic exercise. The data are presented as mean ± standard error; (*) $p < 0.05$ in relation to the respective control.

capacity [20].

Is also important the elevation of the cardiac debt [1], for lactate arrive quickly to liver, to occur gluconeogenesis and the consequent production of ATP, giving continuity to the physical exercise [19]. These results suggest also, a bigger capacity of the athletes' recovery, with a bigger oxidation of lactates, an increase of the renal elimination or a bigger metabolic transformation in glucose.

D. Other Parameters

The other parameters (intra-platelet nitrites, nitrites release, plasmatic nitrites and nitrates), determined in rest and 15 minutes after the Wingate test, was presented in Table III.

The increase of the vasoconstrictor agents is compensated with a bigger production and release of vasodilator substances, as NO [21], [22]. Platelets also synthesizes NO, increasing the efficiency of the reply mechanism, with the production and the release of this vasodilator agent [19].

The platelet nitrite content (reflecting the amount of NO) is more significant in the athletes than in the control group (in rest), suggesting the existence of an adaptation mechanism to the exercise. This tendency remains after the exercise, with a bigger concentration of inter-platelet nitrites in the athletes [19]. In control group, the platelets suffer an aggregation and break; therefore they do not support the attrition with the walls of blood vessels, in consequence of its morphologic and functional characteristics, when being activated by the exercise. According Tozzi-Ciancarelli and collaborators [8] the intense exercise increases the platelet aggregation.

The nitrites diminish with the exercise, because they compensate the vasodilatation. Relatively to the amount of realized platelet nitrites, we verified a significant increase of the values in the athletes, in both situations (rest and after exercise), comparatively to the control (see Table III). However, a minor reduction in the nitrites release was observed in athletes after exercise, relatively to the values presented in rest. Thus, the results suggest that the nitrites production and release seem to contribute for an efficient performance of the physical exercise [19].

Relatively to the plasmatic NO concentrations, had an identical trend to the registered in the platelets, explained for the contribution of NO in the vasodilatation, originating a reduction of the peripheral vascular resistance, facilitating the sanguineous flow. However, in the control we registered an increase trend of the nitrites + nitrates concentration after the exercise, explained for the possible NO conversion in peroxynitrite. The increase of the concentration of nitrites + nitrates can be related with the increase of free radicals of oxygen leading the lipid peroxidation mechanisms.

In this way, the quantification of MDA was pertinent, considered a good biomarker of oxidative stress [5], [10], [17]. The gotten values of MDA are superior in the control, relatively to the athletes in rest and after exercise. The increase of the concentrations of plasmatic MDA in the control could be justified by the elevation of the plasmatic nitrites + nitrates, for the reduction of the antioxidant defenses, inducing probably, oxidative stress. However, the increased basal values can reflect the absence of adaptation mechanisms originated by the absence of trainings in the control group. The athletes present significant alterations, with reduction of the MDA concentration, comparatively to the control in both the situations.

Thus, the results seem to confirm the hypothesis of that the exercise is beneficial, since it unchains adaptation mechanisms, with protector effect, inducing the reduction of oxidative stress.

IV. Conclusion

The observed alterations, although little significant, can be

attributed to the conditions of the sport practice. Therefore, in agreement with what is described, the intensity, the duration and the frequency are factors that influence the level of oxidative stress.

Thus, our results are concordant with other studies [23], being able to strengthen the idea of that the regular physical exercise, improves the capacity of the organism in the prevention of the toxic effect of lipid peroxidation.

We can conclude also that the physical exercise did not develop more the glycolytic way in the metabolism, but increased the capacity activation/reply of other systems: bigger cardiocirculatory capacity and bigger protection against physical and oxidative stress.

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