THE ACUTE EFFECT OF RAST TEST ON OXIDATIVE STRESS AND MUSCLE DAMAGE MARKERS IN YOUNG ATHLETES

O EFEITO AGUDO DO RAST TEST SOBRE O ESTRESSE OXIDATIVO E OS MARCADORES DE DANOS MUSCULARES EM ATLETAS JOVENS

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RESUMO

Poucos estudos abordam os efeitos dos exercícios de alta intensidade e curta duração como o Running Anaerobic Sprint Test (Rast test), o que pode favorecer o adequado controle das sessões de treino e, consequentemente, o desempenho atlético. O objetivo deste estudo foi investigar o efeito agudo do Rast Test sobre o estresse oxidativo e danos musculares em jovens atletas. Participaram 09 atletas jovens, com idade entre 15 e 18 anos. O Rast Test determinou o PAN-pico, PAN média, mínimo PAN (534,8 \pm 138,9; 714,6 \pm 102,2; 285,2 \pm 285,2 Watts, respectivamente) e FI (7,0 \pm 1.5 Watts / seg) dos atletas corredores, resultando em um aumento da peroxidação lipídica analisadas pelo TBARS (pré: $00:48 \pm 0.1$ nmolEq MDA.mL vs pós: $0,66 \pm 0,0$ nmolEq MDA.mL), da atividade antioxidante da glutationa peroxidase (pré: $165,8 \pm 87.7$ mmol / min / mg vs pós: 297.4 mmol \pm 624,4 / min / mg) (p <0,05), e aumento das concentrações séricas de lactato desidrogenase (pré: $326,0 \pm 72,65$ U / L e pós: $758,72 \pm 135,09$ U / L) e creatina quinase (pré: $278,1 \pm 78,64$ U / L e pós: $983,62 \pm 339,49$ U/ L) (p <0,05). Conclui-se que o Rast Test promove estresse oxidativo e danos musculares em jovens atletas.

Palavras-chave: Exercício de alta intensidade. Desempenho atlético. Estresse oxidativo. Dano muscular.

ABSTRACT

Few studies about the effects investigated of high intensity and short duration exercises such as Rast Test, which may favor the adequate control of training sessions and, consequently, athletic performance. The objective of this study was to investigate the acute effect of Rast Test on oxidative stress and muscle damage in young athletes. Participating were 09 young athletes, aged between 15 and 18 years. Rest Test determined PAN-peak, mean PAN, minimum PAN (534.8 \pm 138.9, 714.6 \pm 102.2, 285.2 \pm 285.2 Watts, respectively) and FI (7.0 \pm 1.5 Watts / sec) of the runner athletes, resulting in an increase of the lipid peroxidation analized by TBARS (pre: 0.48 \pm 0.1 nmolEq MDA.mL vs post: 0.66 \pm 0.0 nmolEq MDA. mL), antioxidant activity of glutathione (p <0.05), and increased serum lactate dehydrogenase concentrations (pre: 326.0 mmol / \pm 72.65U / L and powders: 758.72 \pm 135.09 U / L) and creatine kinase (pre: 278.1 \pm 78.64 U / L and powders: 983.62 \pm 339.49 U / L) <0.05). It is concluded that Rast Test promotes oxidative stress and muscle damage in young athletes.

Keywords: High intensity exercise. Athletic performance. Oxidative stress. Muscle damage.

Introduction

The Running Aerobic Sprint Test (Rast Test) is a high-intensity, short-length exercise protocol that has been widely used to estimate anaerobic fitness, being important for the quantification of exercise intensities and training prescription for athletes¹. In addition, the Rast Test can be a relevant tool to improve performance in short-length races, mainly in track and field, which requires a prolonged maintenance of high amounts of energy supply².

Short high-intensity exercises cause an imbalance in cell homeostasis due to increased oxidative stress resulting from a higher production of reactive oxygen species (ROS)³. An imbalance between ROS production and antioxidant bioavailability, which leads to a higher ROS production and lower antioxidant defense, causes a metabolic situation characterized as oxidative stress, which is associated with damages in the phospholipids of cell membranes, oxidation of thiol compounds, enzymatic cofactors, proteins, nucleotides and DNA, besides muscle metabolic dysfunctions and increase of their respective damage markers^{4,5}.

Page 2 of 10 Santos et al.

During intense muscle activity, the energy demanded may rise to thirty-five times in relation to rest; therefore, there is a pronounced increase in oxygen intake, mostly as a consequence of increased muscle work. Some studies reveal that higher ROS are directly linked to initial mechanisms of muscle injuries, resulting in the increase of intracellular proteins such as creatine kinase (CK), lactate dehydrogenase (LDH), troponin I, myoglobin and myosin, lipid peroxidation of the cell membrane, as well as increased inflammatory process after exercise^{4,6,7}.

Rannou et al.⁸, Lima et al.⁹ and Vendrusculo et al.¹⁰ analyzed CK and LDH serum concentrations after exercise and found them to be good indicators for increased cell permeability resulting from muscle damage.

Moreover, exercise promotes mechanisms related to oxidative stress, with the latter being caused by an imbalance between the production of ROS and the fight against them by antioxidant enzymes; depending on the intensity and length of the exercise performed, there may be fatigue, performance decrease and muscle damage due to oxidative injuries involving lipids, nucleic acids and cellular proteins^{11,12}.

Studies have investigated the effects of high-intensity, short-length exercises on the activity of antioxidant enzymes^{13,14} and indirect muscle injury markers in young individuals as an alternative to either mitigate the oxidative stress caused by this type of exercise or, possibly, enhance athletic performance¹⁰. There is a gap in the literature as to the effect of the Rast Test on oxidative stress and on indirect muscle damage markers in young athletes, as well as possible correlations between these physiological markers with performance in said test.

Broadly speaking, the hypothesis is that there would be an increase in these physiological variables after the test and that they would not correlate with damage to the phospholipids of cell membranes, especially for the anaerobic nature of the test. Thus, the objective of this study was to analyze the acute effect of the Rast Test on oxidative stress and muscle damage in young athletes.

Methods

Participants

The sample was composed of nine young healthy participants aged between 15 and 18 years old, who had been practicing track and field for at least one year. All volunteers were informed about the risks and benefits involved in this study and signed a free and informed consent form, and so did their respective legal guardians. According to exclusion criteria, the volunteers could not be sedentary, as per analysis through the IPAC, short version, have chronic diseases that interfered with the performance of maximal exertion, have osteomyoarticular injuries, be tobacco smokers, so as to not influence the variables collected, and disagree with the free and informed consent form, besides failing to return it duly signed by their legal guardian. The study was approved by the Ethics Committee of the Federal University of Sergipe (research protocol No 643.484/2014).

Table 1. Volunteers anthropometric characteristics (mean \pm SD)

Weight (kg)	Height (cm)	BMI (Kg/m^2)	WHR	%G
59.2 ± 11.4	1.7 ± 0.1	19.6 ± 2.5	0.84 ± 0.08	12.6 ± 4.0

Source: The authors

Procedures

The volunteers were instructed not to ingest alcoholic beverages, coffee, medication, neither exercise for 24 hours before the experimental procedures. The foods recommended for them to keep an adequate abstinence, with minimal influence on the variables to be collected, were: chocolate and products made from cocoa, guarana powder, black teas (maté, iced tea, energy drinks), cola- and guarana-based sodas, and coffee ¹⁵. For control of the athletes' food consumption, a 24h recall was carried out on three non-consecutive days, following the recommendations of the International Society of Sports Nutrition (ISSN)¹⁶.

Anthropometric assessment procedures and the anaerobic fitness test were executed in this sequence; in the previous week, a familiarization with the maximal exertion test (Rast) was performed. All procedures involved in the tests and blood collection were conducted at the Physical Education Department of the Federal University of Sergipe (UFS). As for biochemical analyses, they were done in two laboratories of the UFS's Physiology Department, namely: Heart Biophysics Laboratory [Laboratorio de Biofisica do Coração] (LBC), for CK, LDH and GPx analyses, and the Natural Products Chemistry and Biochemistry Laboratory [Laboratório de Química de Produtos Naturais e Bioquímica] (LQPNB), for TBARS analysis.

Prior to the physical exercise protocol, all volunteers underwent an anthropometric assessment, in which height was verified with the aid of a Sanny® stadiometer, and weight was checked on a portable digital scale with capacity of 150kg and precision of 100g. To measure abdominal, waist and hip circumferences, a Sanny® measuring tape was used. Fat percentage was verified through Lohman's protocol¹⁷, and triceps and subscapular skinfolds measures were taken with a compass of the same brand.

Rast Test Protocol

The physical exercise protocol executed was the Running Anaerobic Sprint Test (Rast Test), whose purpose is to assess the anaerobic fitness of those who perform it. Although it has a fixed, pre-established running distance of 35 m, which disfavors the athletic specificity of different sports modalities, this protocol is widely used to evaluate athletes, mainly for being of easy application¹⁸.

Before the test, all volunteers did a warm-up for approximately five minutes, made up of light-intensity running and 10m sprints. After warm-up, the general instructions of the test were reinforced, and possible doubts were clarified so that the participants could properly perform the test.

The Rast Test consisted of performing six 35m sprints with 10s intervals in between. The elapsed time was recorded for each sprint. The breaks and start of each sprint were duly informed by sound stimuli. Time was recorded as the run started, and immediately stopped when the volunteer moved past the 35th meter. For proper control of sprint time and break recording, three digital stopwatches were used (Flax technology®, timex iroman g85, USA) by three assessors. The 35m distance was measured with a measuring tape, and the extremes of the course were demarcated by cones to facilitate visualization and a proper time recording. The parameters analyzed by the Rast Test were: maximal power (the most powerful sprint), medium power (mean of power generated in the six sprints, minimal power, Fatigue Index 1 (in Watts/sec) and Fatigue Index 2 (in %).

Biochemical Analysis

Blood was collected in the beginning and right after the Rast Test, in an air-conditioned room, by four duly-trained and qualified nursing technicians. The participants remained seated, and local antisepsis was done using alcohol 70% and cotton. For serum

Page 4 of 10 Santos et al.

sourcing, blood was collected by venous puncture through vacuum collection system and contained in a tube (Injex 4ml vacuum tube), without anticoagulant. Thus, 4ml of blood were collected, immediately separated into 2ml tubes, quickly placed inside a polystyrene box in gel, and taken to the LBC at the Physiology department. To obtain the serum, the blood was centrifuged at 4000 rpm, under temperature of 10°C, for 10 minutes.

Creatine kinase (CK) and lactate dehydrogenase (LDH) were the enzymes used as tissue injury biomarkers in this study. For quantitative determination of CK and LDH in serum, CK-Nac Liquiform and LDH Liquiform commercial kits were used, in which working reagents presented 1000 μL of specific reagents of the kits, and only 20 μL of the serum. Reading was done in quartz cuvettes thermostated at 37±0.2°C in UV/VIS spectrophotometer with absorbance at 340nm.

The determination of the oxidative stress caused by the short high-intensity exercise was assessed through lipoperoxidative quantification by the thiobarbituric acid test (Tbars), in accordance with Lapenna's method ^{19,20}.

GPx determination was carried out through the BioAssay Systems QuantiChrom™ Glutathione Assay Kit, specifically designed to accurately measure reduced glutathione in biological samples. The serum was diluted 20x, mixed with the kit's working reagents and incubated 25 minutes at room temperature. Reading was done in UV/VIS spectrophotometer with absorbance at 412nm.

Statistical Analysis

Data were expressed as mean±SD. Student's t test for paired data was used for comparison of each biochemical variable before and after the test. Pearson's correlation was applied to all variables, with 5% level of significance. The software used for data analysis was Graph Pad Prism, version 5.0, San Diego, USA.

Results

Data are presented descriptively as mean±SD and sequentially organized in tables and figures according to anthropometric characteristics, Rast Test performance results, as well as to muscle damage and oxidative stress values. The anthropometric characteristics of the studied sample are displayed in Table 1.

Table 2 shows the runners' absolute values, peak power, medium power, minimal power, fatigue index 1 and fatigue index 2, obtained by the maximal anaerobic test.

Table 2. Mean values of peak power (peak AnP), medium power (medium AnP), minimal power (min AnP) and fatigue indexes (FI) of the nine runners, obtained in the Rast Test.

Peak AnP (Watts)	Medium	AnP	Min AnP	FI
	(Watts)		(Watts)	(Watts/seg)
534.8 ± 138.9	714.6 ± 102.2		285.2 ± 285.2	7.0 ± 1.5

Source: The authors

Concentrations of LDH (pre: 326.0 ± 72.65 U/L and post: 758.72 ± 135.09 U/L) and CK (pre: 278.1 ± 78.64 U/L and post: 983.62 ± 339.49 U/L) increased after the Rast test (p \leq 0.05) and are illustrated in Figure 1.

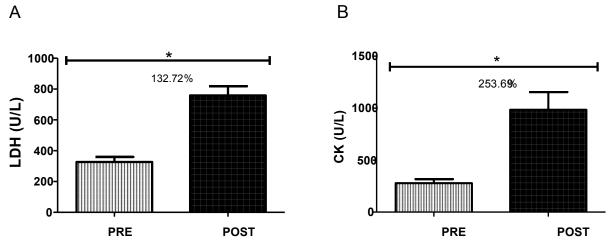


Figure 1. Muscle damage markers before and after the Rast Test

Note: Panel (a) presents LDH plasmatic concentration. Panel (B) presents CK. *Statistically significant difference in all values (p<0.05) in relation to moment Pre

Source: The authors

Concerning the antioxidant enzyme, the protocol promoted a significant increase in the activity of glutathione after the test (pre: 165.84 ± 87.73 mmol/min/mg and post: 624.38 ± 297.38 mmol/min/mg; Figure 2A); there was also increase in the tissue oxidative stress assessed by TBARS in the post-test (pre: $0.48\pm0,12$ nmolEq MDA. mL and post: $0.66\pm0,0$ nmolEq MDA. mL; Figure 2B).

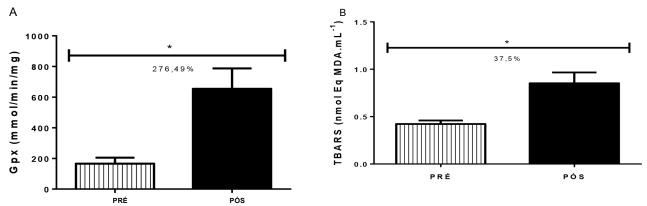


Figure 2. Oxidative stress markers before and after the Rast Test

Note: Panel (A) presents the enzymatic activity of GPx. Panel (B) presents the plasmatic concentration of TBARS.

*Statistically significant differences in all values (p<0.05) in relation to moment Pre

Source: The authors

Finally, in order to verify associations between the variables studied, a correlational matrix was done, which is displayed in Table 3. Most of the variables presented in Table 2 and Figures 1 and 2 showed correlated moderately and highly.

Page 6 of 10 Santos et al.

Table 3. Correlations between peak power (peak AnP), medium power (medium AnP), minimal power (min AnP) and fatigue index (FI) values with LDH, CK, GPx and TBARS

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	LDH	CK	GPX	TBARS
Peak AnP	0.60*	0.14	0.84*	-0.94
Medium AnP	0.49*	0.03	0.82*	-0.95
Min AnP	-0.71	0.48*	0.68*	-0.69
FI	0.90*	0.48*	0.68*	-0.69

Note: *p<0.05 for correlation between Rast Test variables, muscle damage indirect marker enzymes [CK, LDH] and oxidative stress markers [TBARS, GPx].

Source: The authors

Discussion

The overall objective of the present study was to investigate the effect of the Rast Test on biochemical markers: muscle damage and oxidative stress in young athletes. According to the main results, the test was sufficient to affect LDH, CK, GPx and TBARS serum levels, with correlations between them and some anaerobic fitness variables (peak AnP, medium AnP, min AnP and FI1) (Table 3).

Anaerobic fitness is a determinant performance factor in races that require a prolonged maintenance of great power and anaerobic capacity for energy supply², being an essential component for good performance in some sports modalities, because, in certain moments, maximal anaerobic effort is required²¹⁻²³.

In this context, anaerobic ATP resynthesis must happen quickly and efficiently so as to prevent fatigue and keep muscle contraction contributing to the athlete's performance²⁴. It is worth highlighting that maximal muscle power and anaerobic capacity are highly dependent on age, sex, morphological characteristics and physical conditioning level² therefore, determining anaerobic fitness becomes necessary for training adequacy.

The results obtained through the Rast Test (Table 2) allowed assessing maximal or peak power, which, bioenergetically, reflects the efficiency of resynthesizing ATP via ATP-CP. The fatigue index was determined as well, which interferes with the maintenance condition in which the individual can resynthesize ATP through anaerobic metabolism. The determination of these variables allows for a safe and adequate physical exercise prescription, controlling training variables and respecting the athletes' biological individuality^{8,22}.

Zagatto et al.²³, assessing 17 moderately active individuals on a 400m track through the Rast Test, observed the following values: peak AnP (695.4±107.4W), medium AnP (555.2±77.30W) and FI (36.01±8.79%), which are higher than those found in the present investigation (Table 2). The results of the athletes' power in the present study are considered weak compared to that of professional athletes, which may be attributed to the conditioning level of the individuals assessed. On the other hand, the fatigue indexes (Table 2) are deemed acceptable. According to Bangsbo table¹⁸, for fatigue index classification, 6.97 to 8.90 is considered good; it can thus be concluded that the fatigue indexes of the participants fit this classification.

Additionally, the Rast Test significantly increased CK and LDH serum levels (Figure 1), corroborating with the findings of other studies²³⁻²⁵, that is, signaling muscle damage resulting from the maximal anaerobic test. Considering that the Rast Test is a maximal intermittent effort, the energy required to execute it comes primarily from anaerobic sources; thus, it is capable of raising the serum levels of muscle damage marker enzymes^{3,23}.

In a different study, Clarkson and Tremblay²⁵ observed a significant increase of 11.22% in CK plasmatic concentration, and 13.16% in LDH concentration after the Wingate test. Thus, high-intensity, short-length exercises elevate lipid peroxidation and concentrations of muscle damage marker enzymes, being indicative of oxidative stress^{4,8,26,27}.

According to the results of this study (Figures 1 and 2B), high LDH and CK serum levels were found after the exercise, as well as for TBARS, similarly to another study²⁰ that found high lipid peroxidation levels after acute exercise; however, a great GPx increase was also found in the present study. This increase indicates that the "balance" between antioxidants and oxidants after the exercise was favorable to body defense, in a movement to prevent oxidative stress.

This study used a high-intensity, short-length exercise protocol; after the test, there was an increase in the young athletes' oxidative stress, since TBARS values were significantly higher in relation to the pre-exercise period (Figure 2B). Sureda et al.²⁸ detected increased malondialdehyde in lymphocytes after a single intense exercise session, which is also considered a lipid peroxidation marker and signals oxidative stress.

Exercise-induced changes in antioxidant levels have been investigated, but their importance in determining oxidative stress is more fragile compared to lipid peroxidation, for instance. However, as per our results, not only TBARS, but glutathione peroxidase (GPx) as well increased after the Rast Test (Figure 2A), just as described by other authors that also reported increased activity of antioxidant enzymes right after the Rast Test in young football players²⁹.

According to Deminice et al.³⁰, significant increase was found for oxidative stress marker MDA in the plasma (1.53±0.19µmol/L-1), and a significant increase in the activity of antioxidant enzyme GPx (57.5±5.3U/gHb-1) soon after the Rast Test, corroborating with the results found in our study and confirming, once again, that the protocol raises the levels of oxidative stress markers, "challenging" the antioxidant system. Furthermore, studies by Margaritis et al.³¹ state that the better the VO2max of triathletes, the higher the activity of antioxidant enzyme GPx, protecting the body against cell membrane damage.

Correlating the anaerobic parameters of the Rast Test (peak AnP, med AnP, min AnP and FI1) with the muscle damage markers (LDH, CK) and oxidative stress (GPx) (Table 3), associations between each other were detected, mainly as to oxidative stress. According to Gladden³², accelerated anaerobic glucose increases the activity of enzymes LDH and PFK, the saturation of proton-pump mechanisms, phosphorylase due to a higher concentration of calcium, inorganic phosphate and adenosine monophosphate³³, as well as peripheral vasoconstriction, decreasing oxygenation in several tissues and attenuating the binding of H+ to O^{27,34}. This physiological response may elucidate the reason for the inverse correlation between powers and TBARS (Table 3), that is, individuals with higher power values (better anerobic fitness) present lower TBARS values; thus, those runners that were more aerobically prepared (lower anaerobic fitness) presented higher TBARS values.

According to Finkel and Holbrook³⁴, the most efficient way to raise the amount of antioxidants in the body would be by inducing the oxidative stress itself, stimulating cellular antioxidant mechanisms and elevating the resistance to injuries induced by high-intensity physical exercise^{34,35}. Moreover, Leeuwenburgh et al.³² argues that exercise-induced oxidative stress may trigger adaptations in specific tissues as a response to training. These adaptations relate to a series of systems, of which the most important ones are enzymatic systems, composed of superoxide dismutase, catalase and glutathione peroxidase.

Nevertheless, Palazzetti et al.³⁶ described that athletes in training overload conditions present higher lipid peroxidation indexes, which are assessed by the level of reactive substances such as thiobarbituric acid (Tbars), CK-MB and plasmatic myoglobin (muscle

Page 8 of 10 Santos et al.

injury markers), in addition to a decrease in the GSH-GSSG ratio (reduced glutathione:glutathione disulphide ratio), clearly indicating that this overload impairs antioxidant defense mechanisms related to exercise-induced responses. In this sense, to comprehend the overload imposed to the body after maximal training and anaerobic tests allow for proper adjustments as to rest and subsequent training sessions, so that there is no antioxidant defense impairment, performance loss and possible injury³⁷.

Complementarily, it is important to observe some indicators mentioned by Silva et al. 38, which may affect an athlete's performance, such as growth aspects, biological maturing, acquired prior training level, effort perceived during sessions, and goals to be achieved, minimizing any health impairment. Different strategies to increase the antioxidant capacity and decrease muscle injury have been used in studies, such as supplementation, diet restrictions and medicines 4. A strategy mentioned by Finkel and Holbrook 34 as more efficient to raise the endogenous amount of antioxidants may be a higher induction of the oxidative stress itself, as has been commented, since it would stimulate cellular antioxidant mechanisms and increase resistance to exercise-induced injuries 9,39. This highlights the need for attention to rest in between sessions, as well as to variables related to training, such as volume and intensity, which should be prescribed in a gradual and individualized way 5, thus improving all of the athletes' performance-related aspects, considering that the Rast Test values found in the present study allowed for high levels of power and fatigue resistance, and emphasizing lactic and alactic anaerobic training.

Conclusions

In conclusion, the Rast Test promoted oxidative stress and muscle damage in young athletes, with significant increase in the enzymatic activity of Glutathione Peroxidase (GPx), as well as significant increase in concentrations of lipid peroxidative markers (TBARS) and muscle damage markers (LDH and CK) in young athletes. Thus, the Rast Test, for being characterized as a high-intensity exercise, and the increased concentrations of these enzymes are a good parameter for assessment of oxidative stress induced by this type of exercise.

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Page 10 of 10 Santos et al.

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