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CORRELATION BETWEEN AEROBIC FITNESS AND OXIDATIVE STRESS BIOMARKER IN FUTSAL PLAYERS

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ABSTRACT

The aim of the study was to verify the existence of correlation between level of aerobic fitness and the production of reactive oxygen species against an aerobic workload. The sample was composed of professional futsal players from the state of Sergipe. Variables related to maximal oxygen uptake (VO2max) and the production of reactive oxygen species were analyzed through the quantitative method of thiobarbituric acid reactive substances (TBARS). Data were analyzed using the Pearson correlation test and the student's t-test and significance level of 95% for the dependent variable. The results suggest that the production of reactive oxygen species and free radicals at rest is inversely proportional (r = -0.92) to the athlete's level of aerobic fitness and directly proportional (r = 0.56) during and / or after exercise. However, there was no significant difference (p> 0.05) between TBARS values before and after exercise.

Key words*:* Oxidative stress. VO_2max. Futsal.

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RESUMO

Correlação entre aptidão aeróbia e biomarcador de estresse oxidativo em jogadores de futsal

O objetivo do trabalho surgiu da necessidade de conhecer a existência de correlação entre o nível de aptidão aeróbica e a produção de espécies reativas de oxigênio frente a uma carga de trabalho aeróbica. A amostra foi composta por atletas profissionais de Futsal do Estado de Sergipe. Foram analisadas variáveis referentes ao consumo máximo de oxigênio (VO_2 máx) e a produção de espécies reativas de oxigênio através do método de quantificação de substâncias reativas ao ácido tiobarbitúrico - TBARs. Para a análise dos dados foram utilizados os testes de correlação de “Pearson” e o teste “t” de Student, com grau de significância de 95% para variável dependente. Os resultados sugerem que a produção de espécies reativas de oxigênio e radicais livres em repouso é inversamente proporcional (r = -0,92) ao nível de aptidão aeróbica do atleta e diretamente proporcional (r = 0,56) durante e/ou após o exercício. Entretanto, não houve diferença significativa (p > 0,05) entre os valores de TBARs no pré e pós exercício.

Palavras-chave*:* Estresse Oxidativo. VO_2 máx. Futsal.

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INTRODUCTION

Futsal is a sports practice characterized by intermittent efforts of varying intensities and short duration (Macêdo and collaborators, 2017; Souza and collaborators, 2010; Voser, Da Silva and Voser, 2016).

These characteristics require athletes to have good aerobic fitness (Lima, Silva and Souza, 2005; Menezes and Lopes, 2015) so that in the moments of low intensity during the match, the resynthesis of energy stocks is done more quickly and efficiently, as well as the removal and use of lactate, allowing better performance (Fachineto, Erlo and Martins 2017).

However, the increase in the uptake and use of oxygen (O2) during intense physical exercise can be up to 100 to 200 times its consumption in skeletal muscle fibers (Antunes Neto and collaborators, 2013), where it is directly associated with increased production of several reactive oxygen species (ROS) and free radicals (FR), such as the superoxide anion radical (•O2-), hydroxyl radical (• OH), hydrogen peroxide (H2O2), among others (Halliwell and Whiteman, 2004; Ji, 2008; Souza and collaborators, 2010).

Free radicals are highly reactive atoms or molecules that have unpaired electrons in their last layer of valence (Deus and collaborators, 2017). Thus, it is considered a highly reactive species, able to rapidly react with numerous molecules and acquire stability (Ferreira and Matsubara, 1997).

Literature data report that the high production of FR and ROS is associated with several deleterious actions in the organism, such as: increase in membrane lipid peroxidation levels (Alessio, 1993; Ilhan and collaborators, 2004), protein carbonylation and damage to intracellular DNA (Halliwell and Whiteman, 2004; Koury and Donangelo, 2003). This leads us to believe that FR and ROS are the possible responsible for triggering various lesions in amateur and professional athletes (Ji, 1999, 2008; Hessel and collaborators, 2000).

However, there are numerous protection strategies to minimize the deleterious effects of FR and ROS, including the antioxidant system, which may be endogenous or exogenous (Cruzat and collaborators, 2007). The first system is commonly represented by enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Deus and collaborators, 2017; Ji, 2008).

The second is obtained through diet and are represented by vitamins C (ascorbic acid), E (α-Tocopherol), □-Carotene and some phenolic substances (Bacurau and Rosa, 2004; Clarkson and Thompson, 2000; Ji, 2008; Smolka and collaborators, 2000).

Physical training may improve the athlete's antioxidant system due to the increase in the number of some antioxidant enzymes (Ji, 2008). However, physical exercise may increase the intracellular formation of FR and ROS and promote oxidative stress (Cruzat and collaborators, 2007; Sousa and collaborators, 2010), a phenomenon that can cause innumerable deleterious reactions and cellular disorders (Ji, 2008).

In this sense, the aim of this study was to evaluate important oxidative stress biomarker of a group of professional futsal players through the degree of lipoperoxidation and to correlate the level of oxidative stress to the degree of aerobic fitness.

MATERIAL AND METHODS

The study was approved by the Ethics Research Committee of the Federal University of Sergipe (research protocol No. 643.484 / 2014).

After being informed of the objectives of the study and its possible risks and benefits, the subjects signed the informed consent form (Specific Resolution 196/96 of the National Health Council).

Participants

The sample was composed of six professional male Futsal players belonging to the Real Moitense Club, located in the city of Moita Bonita, state of Sergipe. Participants completed a data sheet and their height and body weight were measured using a Welmy® digital stadiometer, after which the body mass index (BMI) was calculated by dividing weight (in kilograms) by height (in squared meters).

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Procedures

Level aerobic fitness

Aerobic fitness was evaluated through maximal oxygen uptake (VO_2 max) and through the 12-minute Cooper test and was used to quantify exercise intensity during the 40-minute aerobic test. Athletes also underwent a continuous 40-minute run on an official athletics track at intensity of approximately 70% of VO_2 max. Seiko® digital timers and Polar® frequencies were used in tests.

Table 1 - Mean values and standard deviation of anthropometric characteristics and mean value of maximal oxygen consumption.

Age (years)	Height (cm)	Weight (Kg)	Body mass index (kg/m^2)	Vo_2 máx ($mL.kg^{-1}.min^{-2}$)
19,3 ± 2,8	1,70 ± 0,03	68,5 ± 1,52 kg	23,76 ± 0,79	40,3 ± 9,74

Oxidative stress biomarker analysis

5 ml of blood were collected from the branchial vein with heparinized syringe in the pre- and post-test, in a 10-minute interval, respectively. Then, samples were stored in a thermal box at temperature of □ 2°C. Shortly thereafter, to evaluate the degree of oxidative stress by the TBARS method using protocol adapted from Lapenna and collaborators (2001), blood was centrifuged at 800 × g for 15 minutes.

Then, 1 mL of plasma was added to a 2 mL mixture of equal parts of 15% trichloroacetic acid (TCA), 0.25 M hydrochloric acid (HCl) and 0.4% thiobarbituric acid (TBA) plus 2.5 mM butylated hydroxytoluene (BHT) and 0.1 mL of 8.1% sodium dodecyl sulfate (SDS), being heated for 30 minutes at 95°C in an oven.

The pH of the mixture was adjusted to 0.9 with HCl with a digital pH meter. BHT was used to prevent lipid peroxidation during heating. Then, after being cooled to room temperature, 4 ml of n-butanol (Merck) was added and, after homogenization, the mixture was centrifuged at 800 x g for 15 minutes to separate the organic phase. Finally, spectrophotometric reading (Fenton 800 XI) was performed.

For the calibration of the spectrum, the blank used was 1mL of solution formed by equal parts of 15% TCA, 0.25 N HCl, plus 2.5 mM BHT and 0.1% SDS at 8.1%.

Spectrophotometric readings of samples were performed at wavelengths of 532 nm (nanometer). All samples were read in triplicate. To calibrate the TBARS concentration, the molar extinction coefficient of 154 mM-1cm-1 was used (Lapenna and collaborators, 2001).

Statistical

For the statistical treatment of data, means and standard deviation (□SD) were used. To verify the correlation between variable aerobic fitness (VO_2 max) and lipid peroxidation (TBARS), the Pearson correlation test was used. Subsequently, to evaluate the significant difference of TBARS, the paired Student's “t”-test was used, with the aid of the OringinPro 7.5 statistical package. The value of $p < 0.05$ was considered statistically significant.

RESULTS

In figure 1, it was observed that some athletes presented significant levels of lipoperoxidation * $p < 0.05$, while others had reduced lipoperoxidation level ** $p < 0.05$.

In figure 2, we can see that there is no difference in lipoperoxidation between the pre and post exercise moments performed by the athlete’s group.

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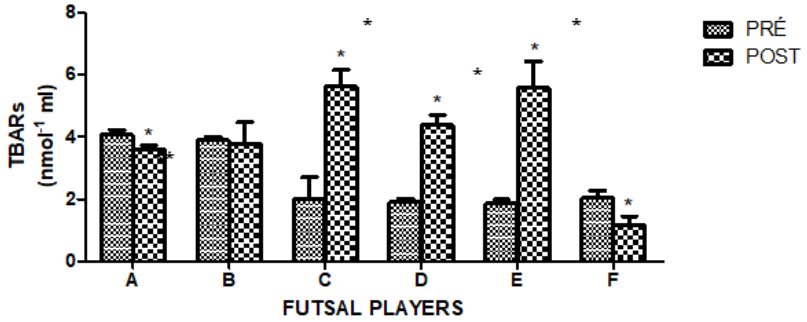

Figure 1 - Lipoperoxidation level of pre and post aerobic exercise of continuous running of 40 minutes duration at 70% of its VO_2 max. Values expressed as mean and standard deviation.

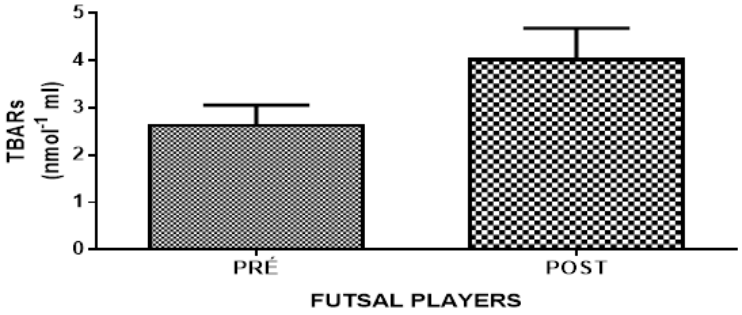

Figure 2 - Lipoperoxidation level of pre and post aerobic exercise of continuous running of 40 minutes duration in athletes group at 70% of its VO_2max. Values expressed as mean and standard deviation.

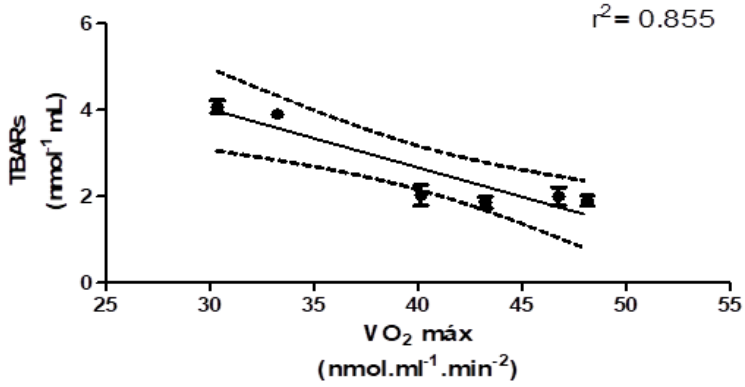

Figure 2 - Correlation between VO_2max values and lipoperoxidation levels of athletes at rest. Data expressed as mean and standard deviation.

In figure 3, it is observed that there is a strong negative correlation (r = -0.92) between the resting lipoperoxidation and the VO_2 max values of the athletes.

This fact leads us to believe that possibly the lower the VO_2 max of the major subject will be its level of lipoperoxidation at rest.

However, in figure 4, we can observe that there is a moderate positive correlation between the variables (r = 0.56). This suggests that, possibly, the higher the individual's VO_2 max, the greater the level of lipoperoxidation after exercise.

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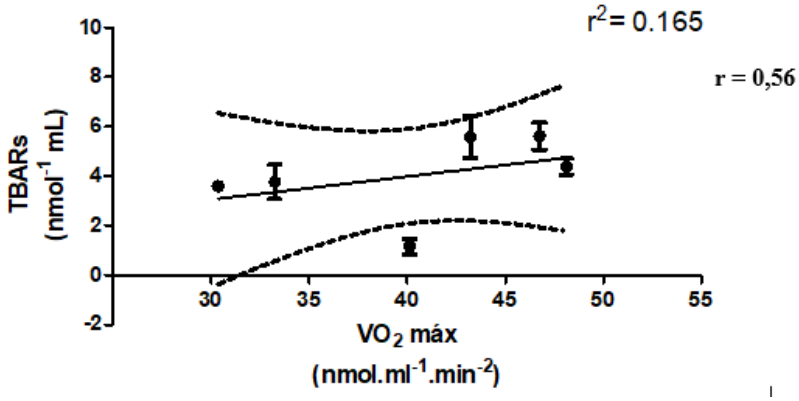

Figure 3 - Correlation between VO_2max values and lipoperoxidation levels of athletes at rest post aerobic exercise of continuous running of 40 minutes duration at 70% of its VO_2 max. Data expressed as mean and standard deviation.

DISCUSSION

The results showed that the mean plasma TBARS values obtained from Futsal players, before and after exercise, did not have significant differences. However, when individually evaluated, some athletes presented significant TBARS values in pre- and post-exercise, indicating that for these, exercise induced high formation of FR and ROS.

This may have occurred, among other factors, due to the conditioning level of athletes not being satisfactory for the performance of exercise with similar characteristics to those performed in this work.

Well-trained subjects have greater antioxidant enzymatic defense conditions in the skeletal muscle capable of neutralizing the action of ROS and FR against possible damage to cellular structures and / or generation of oxidative stress (Cruzat and collaborators, 2007).

This is due to the physiological adaptations acquired through training and / or competition (Cruzat and collaborators, 2010).

Study (Ji, 1999) have shown that individuals or animals adapted to a training protocol have higher levels of antioxidant enzymes and certain non-enzymatic oxidants in the muscle, demonstrating greater resistance to the actions of reactive species and, consequently, oxidative stress induced by physical exercise.

Comparing oxidative stress through lipid peroxidation, Medina and collaborators (2002), submitted 18 male subjects, practitioners of various physical activities, to continuous run for forty minutes at 67.5% of VO_2 max. At the end of the exercise, it was possible to identify that there was no significant difference among subjects regarding lipid peroxidation, that is, subjects did not present oxidative stress after the test.

However, Schneider and Oliveira (2004) evaluated the effects of sports training on the antioxidant defenses and cellular damage of soccer players and found that the levels of TBARS undergo significant changes, increased in plasma and decreased in blood.

Similarly, Fiamoncini and collaborators (2002) studied 18 junior soccer players and evaluated their oxidative stress against an aerobic and anaerobic training load and identified that the levels of TBARS were not significant in both exercises. However, aerobic exercise apparently generated oxidative stress because significant changes in different oxidative stress biomarkers were identified.

For Kouri and Donangelo (2003), this occurs because athletes who practice aerobic activities are more susceptible to increased production of ROS during and after exercise. In addition, during exercise, subjects with high VO_2 max tend to produce more ROS than subjects with lower VO_2 max. This is because, during exercise, high VO_2 max induces greater mobilization of the cellular oxidative system and, consequently, greater increase in mitochondrial electron extravasation (Medina and collaborators, 2002). This fact leads to higher production of ROS as can be observed in figure 4.

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As shown in figure 3, subjects with high VO_2 max during rest had lower tendency to produce ROS than subjects with low VO_2 max due to better adaptation of the mitochondrial and antioxidant oxidative system.

However, Medina and collaborators (2002) did not find significant differences between group with excellent VO_2 max and the below average group. However, the sample was quite different from that evaluated in this study.

That study used athletes of different physical activities, differentiated motor and oxidative specialty, and this study used Futsal players, a very homogeneous sample, allowing a more concise result.

CONCLUSION

The present study allows concluding that physical exercise is a potential agent inducing lipid peroxidation. In addition, as the athlete's aerobic fitness (VO_2 max) increases, greater lipoperoxidation is observed during exercise, as well as lower lipoperoxidation at rest compared to those with reduced VO_2 max.

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