

## Acute aerobic exercise does not cause DNA damage in trained individuals after a running session

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ORIGINAL ARTICLE

### ABSTRACT

Performing long-duration exercise may increase oxygen consumption, which may result in redox imbalance inducing a frame of oxidative stress. The aim of the present study was to analyze the effects of EROS after a training session on the DNA of human lymphocytes and biochemical parameters (TBARS, triglycerides, proteins, glucose, albumin, and urea). A cross-sectional, descriptive and analytical study of 18 Caucasian individuals, males, between 18 and 35 years of age, underwent a continuous aerobic training session (60-minute run with intensity comprised between 70% and 75% of the maximum heart rate. The participants had a mean age of  $21.78 \pm 3.82$  years; body mass  $72.04 \pm 6.35$  kg; Height  $1.79 \pm 0.05$  m, BMI of  $22.37$  kg/cm<sup>2</sup>  $\pm 1.70$ , fat mass  $8.43 \pm 3.97$  %. Results showed a statistically significant increase in total protein, glucose and triglyceride levels post-exercise, while albumin decreased significantly post-exercise, whereas other biochemical parameters did not present significant change. DNA damage analysis showed no significant effects. We conclude that aerobic training in the conditions herein did not induce damage due to oxidative stress in the studied structures, probably because the individuals were well-trained and previously adapted to the conditions of exercise.

*Keywords:* oxidative stress, comet assay, micronucleus test, run

### INTRODUCTION

The imbalance between free radical production and body antioxidant capacity is defined as oxidative stress. The production of EROS and oxidative stress have been associated with the etiology of the overtraining syndrome and several deleterious situations applied to the sport (Finkel & Holbrook, 2000; Petry, Alvarenga, Cruzat, & Tirapegui, 2010). Research relating oxidative stress and aerobic physical exercises seek to better clarify what happens organically when there is an imbalance between oxidant production and the antioxidant system. Oxidants can cause several damages, including damage to DNA, lipids, and proteins, which are linked to a number of conditions such as fatigue, injuries among others (Schafer, & Buettner, 2001; Terra, Silva, Pinto, & Dutra, 2012; Villañoa et al., 2015). Aerobic exercises elevate the

consumption of O<sub>2</sub>, especially at the level of the active muscle fiber, when compared to the state of rest. While essential for the synthesis of energy, oxidative metabolism, when stimulated, can also trigger actions that can bring damage to cells. About 2.5 to 5% of the O<sub>2</sub> consumed can be diverted into the mitochondrial respiratory chain, being an important source of EROS. These deviations cause this percentage of O<sub>2</sub> consumed to be reduced in a univalent manner, leading to EROS synthesis (Petry, Alvarenga, Cruzat, & Tirapegui, 2010).

Studies have shown that frequent high-intensity, aerobic physical exercises such as marathons, ultra-marathons, and triathlon may lead to increased EROS production and consequently increased lipid peroxidation (PL) and DNA damage (Finkel & Holbrook, 2000; Petry, Alvarenga, Cruzat, & Tirapegui, 2010;

Manuscript received at December 3<sup>rd</sup> 2018; Accepted at August 1<sup>st</sup> 2019

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Villañoa et al., 2015). On the other hand, continuous practice with progressive intensities can induce adaptive responses by increasing the efficiency of endogenous antioxidant systems. In addition, it has been shown that physical exercise when performed in the long run has beneficial effects, such as reduction of cardiovascular disease, cancer and diabetes (Finkel & Holbrook, 2000; Petry, Alvarenga, Cruzat, & Tirapegui, 2010; Villañoa et al., 2015). In order to monitor effects of exercise intensity and volume, research has focused more on responses to its effects on the endocrine, cardiorespiratory, neuromuscular, skeletal, digestive (nutrition and bioenergetics), and biomechanical systems in system cells, in the production of cytokines, in signaling pathways involved in the immune response, and few have taken into account what its effects on DNA (ACSM, 2009). However, the inconsistency of results still persists in the literature, and further studies including other biomarkers, such as glucose and triglycerides, are warranted. Based on the above, the aim of the present study was to analyze the effects of an aerobic running session on human lymphocyte DNA damage, through the Comet and micronucleus (MN) test.

## METHODS

### Design and participants

This is a cross-sectional, descriptive, and analytical study of 18 individuals between 18 and 35 years of age undergoing continuous aerobic training (60-minute run with intensity between 70% and 75% of the FCM).

All participants were males with a mean age of  $21.7 \pm 3.8$  years; body mass  $72.0 \pm 6.3$  kg; Height  $1.79 \pm 0.05$  m, Caucasian, Body Mass Index (BMI)  $22.3 \text{ kg/cm}^2 \pm 1.70$ , fat mass  $8.43 \pm 3.97$  %. All anthropometric measures were obtained according to the guidelines established in the adult SAPAF 4.06 software (Guedes, 1999).

All subjects were regular practitioners of physical activity, with at least two years of regular and systematized exercised for at least three times a week, non-smokers, and who did not use medication at the time of the study. They were not consumers of alcoholic beverages and had a percentage of fat, BMI within the normal range for their age.

The work was developed according to declarations and guidelines on research involving human beings: the Nuremberg Code, Declaration of Helsinki and Resolution No. 196 of October 10<sup>th</sup> of 1996 from the National Health Council, as well as approved and regulated by the Institution's Ethics Committee Executing agency approved under No. of Opinion 201/2012.

### Experimental Protocol

Participants were instructed not to eat antioxidant foods such as fruits, fruit juices, chimarrão (traditional drink from Rio Grande do Sul made from mate plant containing antioxidant properties), vegetables, and any type of supplementation that contained antioxidants. These requirements were controlled in the 24 hours prior to the evaluations, through the application of nutritional written individual records.

The individuals underwent a continuous aerobic training session (60-minute run) on an athletic track, with an effort intensity between 70-75% of the maximum heart rate controlled according to ZTA Aerobic training) corresponding to each age group (Tanaka, Monahan, & Seals, 2001).

Samples of blood were collected at rest, shortly after the end of the training session, and 5 hours after the training. Five mL of venous blood was collected aseptically in the antecubital fossa of the volunteers. Blood samples were packed in a testing tube for biochemical analysis of DNA damage and metabolism markers. DNA damage analysis was performed using the comet test where 100 randomly selected cells were analyzed for each blood sample. The cells were classified into five classes of damage (no damage = 0, maximum damage = 4) (Villela, de Oliveira, da Silva, & Henriques, 2006), thus constituting a DNA damage index as described by Singh, McCoy, Tice, and Schneider (1998). For the micronucleus test, the blood samples were processed as described by Schmidt (1975) and Sturbelle et al. (2010).

To measure lipid damage, the samples were evaluated by the degree of lipid peroxidation, as described by Esterbauer and Cheesman (1991). The biochemical analysis for metabolic markers

was based on the determination of triglycerides (Trinder - Labtest® method), glucose (oxidase - Labtest method), Urea (test - Labtest method), total proteins (Biureto - Labtest method), according to manufacturer specifications. After the technical execution, analyte concentrations were determined on Biosystems BTS 350® semiautomatic biochemical analyzer.

### Statistical analysis

The results are presented in measures of central tendency and measures of dispersion (deviation and standard deviation). The assumptions of normality (Shapiro-Wilk test)

and homoscedasticity (Levene's test) were verified. Confirming the assumptions, the analysis of variance (One Way ANOVA) was applied, followed by Tukey's post hoc test to evaluate changes induced by training in the dependent variables. Statistical significance was set at 95%.

### RESULTS

The data referring to the effects of exercise performed on the biochemical parameters used in the analysis of subjects tested at rest, post-training, and 5 hours after the training session are described in table 1.

Table 1

Mean ( $\pm$  standard deviation) of biochemical analysis at rest, post-training, and 5 hours after the resistance training session. Results expressed as mean, standard deviation. ANOVA test results are shown (F, p,  $\mu^2$ ).

	Rest	Post-training	5 hours Post-training	F	P	$\mu^2$
TBARS (nmol)	0.29 $\pm$ 0.10	0.27 $\pm$ 0.10	0.25 $\pm$ 0.03	0.876	0.424	0.043
Proteins (g/dL)	7.60 $\pm$ 0.82	8.05 $\pm$ 0.57	8.45 $\pm$ 0.89 *	3.704	0.035	0.183
Glucose (mg/dL)	82.72 $\pm$ 14.75	114.33 $\pm$ 21.07**	97.84 $\pm$ 25.40	5.678	0.008	0.256
Triglycerides (mg/dL)	63.89 $\pm$ 26.43	78.53 $\pm$ 39.24	129.25 $\pm$ 94.87 *	3.478	0.043	0.174
Albumin (g/dL)	3.40 $\pm$ 0.38	3.47 $\pm$ 0.13	2.82 $\pm$ 0.46 ¥	11.982	0.000	0.421
Urea (mg/dL)	96.02 $\pm$ 7.07	97.58 $\pm$ 6.93	96.59 $\pm$ 6.79	0.136	0.873	0.008

TBARS are expressed relative to protein. \* different from rest in the post-hoc analysis (p<0.05); \*\* different from rest in the post-hoc analysis (p<0.01); ¥ different from rest and from post-training in the post-hoc analysis (p<0.01)

Table 2

Analysis of the degree of DNA damage, ID (damage index) by the comet and micronucleus test at rest, post-training, and 5 hours after the resistance training session. Results expressed as mean  $\pm$  standard deviation.

	Rest	Post-training	5 hours Post-training	F	P	$\mu^2$
Damage Degree 0	88.2 $\pm$ 1.7	88 $\pm$ 1.7	88.7 $\pm$ 1.8	0.671	0.516	0.028
Damage Degree 1	11 $\pm$ 1.15	11.1 $\pm$ 1.6	10.2 $\pm$ 1.5	1.078	0.348	0.044
Damage Degree 2	0.6 $\pm$ 0.7	0.7 $\pm$ 0.8	0.6 $\pm$ 0.5	0.169	0.845	0.070
Damage Degree 3	0.1 $\pm$ 0.3	0.1 $\pm$ 0.3	0.3 $\pm$ 0.5	2.118	0.132	0.083
Damage Degree 4	-	-	-	-	-	-
Index of Damage	12.6 $\pm$ 2.1	13 $\pm$ 2.2	12.6 $\pm$ 2.7	0.122	0.885	0.005
Micronucleus	2.7 $\pm$ 1.1	2.6 $\pm$ 0.9	2.0 $\pm$ 1.07	1.870	0.165	0.740

Results show a 13% reduction in TBARS concentration, however, statistical analysis shows that this reduction was not statistically significant, indicating that the protocol applied in the proposed conditions did not induce relevant lipid damage in the individuals that were analyzed.

Lymphocyte DNA damage was analyzed by the comet test, where we counted 100 cells and established the percentage of degrees 1,2,3,4 and damage index of each individual (see table 2).

The measure of DNA damage assessed in the comet test has increasing order (grade 0 - least damage to grade 4 - greater damage). The mean grade zero damage showed 88.22% of its preserved cells. In the degrees of damage 1, 2, and 3, damage index (ID) and the presence of micronuclei showed no significant difference. This indicates that the aerobic training protocol in the analyzed population was not able to induce genotoxicity via EROS.

## DISCUSSION

Aerobic activities ranging from 70% to 89% of maximum heart rate are considered high intensity (Pollock et al., 1998; Haskell et al., 2007). The exercise protocol performed in the present study is considered to be predominantly aerobic and of high intensity.

The results herein show that the protocol applied did not induce a scenario of DNA damage, as analyzed by the comet and micronucleus test nor of lipid damage, as measured by TBARS. The no evidence of lipid damage agrees with the results by Selman et al. (2002), who studied the effects of running on various biomarkers of oxidative stress in the skeletal muscle and heart of rats.

Diaz et al. (2011), investigated the response to oxidative stress at a short period of submaximal exercise in a group of healthy young adults who completed a treadmill test (modified Bruce protocol for 75-80% of their reserve heart rate) and concluded that there were no significant changes in the lipid damage index measured by TBARS and protein damage as measured by post-exercise protein carbonylation. Plasma TBARS concentrations were similar at all times when compared to pre-exercise levels. On the other hand, a study in swimming-trained rats showed a significant increase in blood TBARS concentrations in relation to the sedentary control group (Prada et al., 2004). That study analysed biomarkers of aerobic conditioning and oxidative stress in the intensity corresponding to the anaerobic threshold. In another study, nine untrained male subjects aged 19 to 21 participated in a 12-week training program. The training program consisted of running at 80% of FCM, 60 minutes a day, five days a week. This work also showed that there was a significant increase in lipid peroxidation, indicating a major EROS attack (Miyazaki et al., 2001). Schneider et al. (2009) did not observe significant differences in TBARS and protein damage in 11 triathletes, measured before and after a half ironman competition. The authors sought to assess whether physical training can partially prevent the formation of free radicals during exercise and increase antioxidant defenses by

measuring blood biochemical parameters of oxidative stress.

In relation to protein, a significant increase in post-training compared to pre-training was observed in this study. The proteins have many reactive sites, that is, the proteins can be the reactive target of the EROS that are formed in its vicinity, being a vulnerable and important target. There is only significance in the EROS injury to the protein if there is an accumulation or if the lesion concentrates on the particular sites of particular proteins (Barreiros, David, & David, 2006; Schneider et al., 2009).

The analysis of energy metabolism of athletes measured by the concentration of glucose, triglycerides, proteins, albumin and urea, the latter as an indicator of proteolysis, is related to the intensity and duration of the exercise, which are determinants of the type of energy substrate used (dos Santos, 2007). Analysis of the protein metabolism of athletes typically shows a statistically significant increase in total protein levels, with no significant difference in the amount of urea. The progressive elevation of protein levels can be explained by a possible adaptive microleakage, since the muscle cells may undergo disruption and their protein-rich cytoplasmic content leak into the plasma. The determination of urea can be considered an indicator of proteolysis since it is originated as the final product of ammonia metabolism, which in turn increases in hypercatabolic conditions. In the present study, the combined analysis of total protein and urea results do not show evidence of a proteolytic framework that could imply loss of athletic performance and/or fatigue. This indicates that the exercise protocol applied herein may be safe to avoid a hypercatabolic process. Another possible explanation for increases in protein in the blood could be the loss of plasma volume (dehydration) due to the exercise itself.

In this study, a statistically significant increase in blood glucose measured immediately after the training session was evident. Others have also reported this post-exertional increase in glucose, suggesting the maintenance of glycaemia by the process of muscle glycogenolysis (Ling & Rönn, 2014; Souza,

Oliveira, & Pereira, 2005). The elevation observed 5 hours after the training session is due to the energy replacement through feeding since the test was performed in the morning and the last analysis was done afternoon. The glucose response depends on whether the exercise is aerobic or anaerobic, but also dependent on the level of intensity of the exercise. Some studies have concluded that running interval exercise increases blood glucose levels and others supplement by stating that in high-intensity exercise, glucose is the main source of energy (dos Santos, 2007). In contrast, other studies comparing aerobic (running) and anaerobic (bodybuilding) exercises have shown that glycemic behavior in anaerobic and aerobic exercises tends to increase in anaerobic exercises and to fall into aerobic exercises (Porpino, Agnoleti, & Silva, 2010). Da Costa Santos et al. (2009) and Beck et al. (2013) showed that aerobic exercise of 20-min in the treadmill, at an intensity of 70 to 80% of maximum heart rate, causes a glycemic reduction when compared to its initial levels.

Vieira Júnior et al. (2013) studying the effect of aerobic swimming training (NRT) on glycogen content and cell growth in skeletal muscle of rats showed a reduction in TBARS levels, combined with a muscle glycogen content and protein / DNA ratios in the trained group. Altogether, these data confirm that aerobic training improved antioxidant defense, which may be associated with increased glycogen content in skeletal muscle. These results indicate that regular physical exercise is an important strategy to combat the supraphysiological production of EROS since it increased the activity of antioxidant enzymes and reduced the lipid peroxidation in the skeletal muscle.

As for triglycerides, the results of the present study show a post-workout increase, but with a significant difference between measurements performed before training and 5 hours after the training session. This elevation may have to do with food intake and not necessarily directly related to the training. Indeed, one limitation of the study was that subjects had a meal (lunch) about 2h after the completion of exercise and hence before the 5h post-training measurement.

Previous studies presented different results from those in the present study, that is, they showed a decrease in the amount of post-exercise triglycerides (Gill et al., 2006; Miyashita & Tokuyama, 2008; Schlierf et al. 1988).

DNA damage analysis measured by the comet test did not show any grade four damage (4), suggesting that the protocol applied was not sufficient to induce a DNA damage process, as well as suggesting an antioxidant adaptation of the athletes caused by their trained status. A possible explanation for the lack of damage may lie in the fact that in the case of athletes who train regularly, the antioxidant defense mechanisms are adapted to neutralize the oxidant species that induce DNA damage. The magnitude of these adaptations may be dependent on the training intensity and on the physical fitness of the subjects, i.e., a high  $VO_2\text{max}$ , as suggested by Soares et al. (2013), which further showed that younger individuals have smaller DNA breaks and higher  $VO_2\text{max}$  being transient damage due to the acute effects of daily physical activity. Hence the training protocol herein can be considered safe in terms of its genotoxicity (Vasconcellos et al., 2007; Villañoa et al., 2015).

Analysis of the results for micronucleus (Silva & Jasiulionis, 2014) also shows that the training protocol applied did not modify the pattern of cellular damage significantly compared to rest. The measurement of DNA damage by the two methods (comet and micronucleus) can increase the reliability of training prescription and can become a biomarker to quantify intensity, volume, frequency and duration in a physical training process, as well as to prevent overtraining. It should be noted that the damage measured 5-hour post-exercise was not significant and, theoretically, another exercise session could be performed if necessary, at least within the duration and intensities that were studied. A possible explanation for these results is that the intensity was not able to generate significant damage or an adaptation of the antioxidant status of the athletes occurred (or a combination of the two). Hence, it is recommended for future studies to perform cell damage analyzes following a sequence of two workouts on the same day.

### CONCLUSION

The aerobic training protocol did not induce lipid damage (TBARS) and DNA, as given either by the Comet or micronucleus tests, possibly because of the population, who were runners with more than two years of training experience. Hence, the results herein conform that regular and systematic exercise can minimize the effect of oxidative stress and chronic complications from exposure to the free radical formation, even with the absence of a previous antioxidant-based meal. It is noteworthy that the damage measurement 5 hours after exercise was non-significant, showing that another exercise session can be performed on the same day (at least within the duration and intensities studied). However, it is recommended for future studies to perform cell damage analysis following a sequence of two workouts on the same day.

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#### Acknowledgments:

To Graziela Krabbe for the statistical analysis and to Márcio T. Leiria for translation and revision.

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#### Conflict of interests:

Nothing to declare.

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#### Funding:

Nothing to declare.

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