RESEARCH ARTICLE

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Evaluation of the oxidative profile in leukocytes of jiu jtisu athletes

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Abstract: Oxidative stress is a process where there is an increase in reactive species, both oxygen and nitrogen. This can happen at both systemic and mitochondrial levels. One of the physiological conditions currently associated with oxidative stress is high-performance physical activity. In this way, it is possible to relate that this problem impacts on sports competitions, especially in Jiu-Jitsu, a growing sport in the world and which demands intense physical effort. Thus, the objective was to evaluate the level of mitochondrial and systemic oxidation in leukocytes of jiu-jitsu athletes. 20 adult men were selected for the control group and 13 athletes for the experimental group. A blood collection was performed to perform MTT and chemiluminescence assays for an analysis of mitochondrial and systemic oxidation, respectively. In addition, two reactive nitrogen species were measured: nitric oxide and peroxynitrite. The evaluation of the leukocyte reducing capacity by MTT showed that the athletes presented a greater reducing environment compared to the control. On the other hand, the chemiluminescence evaluation showed that the athletes' leukocytes showed greater systemic oxidation. There was an increase in both reactive nitrogen species in alteta leukocytes. Given this, it was possible to notice that the athletes presented greater systemic oxidative stress, but with mitochondria with better adaptive capacity to the cell's metabolic demands.

Keywords: Oxidative stress, Mitochondria, Free radicals, Jiu Jitsu

1. Introduction

Currently, there is an increase in fans practicing the sport of Jiu-Jitsu. In recent years, a diverse public has been observed looking to practice sports in gyms, fight training centers, as well as other establishments where sports practices are promoted (Nicolini et al., 2021).

Therefore, jiu-jitsu competitors, due to their exhaustive training routine, increase the oxidative load that already occurs naturally at the cellular level. This occurs due to the high degree of physical effort required in preparing for the sport and during tournaments. The most common injuries are sprains and strains in the fingers, neck, knee and ankle, as well as bruises from a fall, shoulder and wrist. Such damage occurs mainly in the training environment, where athletes are forced to perform to the maximum in tournaments and competitions, both in terms of muscular effort and physical conditioning and at a biochemical level (Fonseca et al., 2022).

Organelles are important physiological microstructures of cells. Among them, mitochondria stand out for their broad functionality, as they are involved in cellular respiration - with the ultimate objective of generating energy (ATP) -, intracellular regulation of Ca2+, production and elimination of reactive oxygen species, regulation of apoptotic cell death and activation of the caspase family (Miranda et al., 2024).

Free radicals are species defined as oxidants, which can be reactive oxygen species (ROS) or nitrogen species (RNS). The accumulation of these promotes dysfunction in the cell's redox control and signaling. This biochemical process is called oxidative stress, since there is an imbalance between the action of antioxidants and oxidants (Houldsworth, 2024). This metabolic dysfunction has a great physiological impact, since these species degrade biogenic substances, such as DNA, lipids and proteins, which in turn can lead to tissue damage and a greater predisposition to various

diseases. It is important to highlight that mitochondria are the largest generator of these species, but not the only one. These molecules can be formed by solar radiation, consumption of cigarettes and alcoholic beverages and even errors in the metabolism itself (Chaudhary et al., 2023). Therefore, the objective of the research is to biochemically evaluate how high-performance jiu-jitsu changes the oxidative profile of its athletes, as studies in this area are scarce in the literature.

2. Materials and Methods

2.1. Participants

33 healthy men aged between 20 and 40 years were selected. Samples from the control group were provided in partnership by the IVC Health Laboratory (n=20). The athletes' samples were provided in partnership with Gracie Barra - Escola de Jiu-Jitsu (n=13). Both locations are located in the city of Belo Horizonte, State of Minas Gerais, Brazil. The project was approved by the Ethics and Research Committee of the Federal University of Minas Gerais (CAAE: 33842420.4.0000.5149). Regarding the exclusion criteria, there are: smokers, carriers of infection, inflammation, lipoproliferative disorders, arteriosclerosis, heart failure and use of medications that directly influence immunological function, in addition to the use of anabolic steroids. Those who were able to participate will be required to sign a Free and Informed Consent Form (TCLE).

2.2. Obtaining leukocytes

In short, 4ml of heparinized blood was added to 3ml of Leucopaque ® gradient in glass tubes. After centrifugation at 400g for 15 minutes, two distinct phases separated by two interphase rings were obtained. The plasma corresponding to the first phase formed after centrifugation was discarded. The leukocytes were placed in a siliconized tube, which had its volume filled with phosphate buffer PBS (pH 7.3).

2.3. Cell viability

Leukocyte viability was assessed using the trypan blue assay. It is a compound that can cross the plasma membrane of dead cells, turning them blue. 100 µL of 0.3% trypan blue solution was added to 100 µL of cells. Next, 10 µL was transferred to the Neubauer Chamber, where the cells were counted. The calculation used to evaluate the percentage of cell viability was: Number of unstained cells/number of total cells x 100.

2.4 Mitochondrial oxidation

To evaluate mitochondrial oxidation, the MTT test (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) will be performed. MTT, when incubated with living cells, has its substrate broken down by mitochondrial enzymes called succinate dehydrogenases, transforming a yellow compound into a violet compound: Formazan. First, 200µL of leukocytes were placed in 96-well cell culture plates at a concentration of $5x10^3$ cells/well. Then 10µL of the MTT solution was added to the wells and the plate was placed in an oven at 37°C for 1 hour. Then, 100µL of DMSO was added to dissolve the generated formazan crystals. The absorbance was read at 570 nm on the microplate reader.

2.5 Reactive oxygen species analysis

For the quantitative assessment of ROS, the luminol-dependent chemiluminescence assay was used. For this, $1x10^6$ cells were added to a tube along with 500µL of luminol (10^{-4}M) and 100µL of PBS buffer. The reading was performed immediately in 10-minute runs with Relative Light Units/minute (RLU/min) values.

2.6 Reactive nitrogen species analysis

Nitric oxide (NO) was evaluated by the production of nitrite, according to the GRIESS reaction (Griess, 1897) while peroxynitrite (ONOO-) was evaluated by the HUGHES & NICKLIN method (Hughes & Nicklin, 1968). Therefore, after obtaining the leukocytes, the cells were centrifuged at 400g for 10 minutes:

- 100µl of supernatant was placed in 96-well plates and 100µl of Griess solution was added. This solution is formed by two reagents, in a 1:1 ratio. Reagent A (1% sulfanilamine in 2.5% phosphoric acid) and Reagent B (0.1% naphthylenediamine in 2.5% phosphoric acid). The plate content was analyzed by a microplate reader at a wavelength of 540 nm. Nitrite concentration was calculated by linear regression using a standard curve obtained from a 1 mM sodium nitrite solution;
- The pellet was resuspended in 100 µL of MilliQ water and vortexed for 1 minute. Such content

was added to a 96-well plate and measured on a spectrometer at 302 nm.

2.6 Statistical analysis

The results were expressed as mean ± standard deviation. All data were analyzed using the GraphPad Prism 7.0 software using the ANOVA test followed by the Student's t test, with $p<0.05$ considered significant.

3. Results and Discussion

The profile description of the athletes selected for the study is shown in Table 1. As can be seen, there were no significant differences between the age and Muscle Mass Index (MMI) of the athletes in the different ranges. For this reason, all athletes were grouped into a single experimental group. This measure was taken to increase the number of individuals and make the data more robust. The mean age and MMI of the control group were 32.3 ± 6 years of age and MMI = 26.4 ± 2, respectively. Afterwards, total leukocytes from each donor were isolated and cell viability was assessed. As seen in Figure 1, viability remained above 90% in both groups. This finding was already expected, since no additional treatments were carried out on these cells and the experiments were carried out on the same day that the donors' blood was collected.

In relation to total leukocytes, literature findings show that there is an increase in monocytes post-fight, but that they decrease after 24 hours. These data refer to the presence of a transient inflammatory response observed after intense exertion in which there is a change in the innate immune response (represented by leukocytes) as well as in the acquired immune system (antibodies), but which returns to baseline levels a few hours after exertion (Coswig et al., 2013a; Coswig et al., 2013b; Lopes et al., 2018).

As previously mentioned, mitochondria are the largest but not the only source of reactive species. Therefore, the mitochondrial and systemic oxidation profile of leukocytes were analyzed. The data observed in Figure 2A shows a very interesting profile. The athletes' mitochondria had a greater reducing (i.e., antioxidant) capacity than the control group's mitochondria. Figure 2B shows that in a systemic way, the athletes' cells present greater oxidation when compared to the cells obtained from control donors.

It is known that physical exercise is a powerful stimulus that leads to metabolic and physiological adaptations, inducing changes in the remodeling and plasticity of muscle fibers, which are the result of a complex relationship or several regulatory and signaling factors. Studies suggest that the production of reactive species can promote mitochondrial biogenesis, increased antioxidant defenses and muscle hypertrophy. The role that ROS play, for example in jiu jtisu athletes, is based on the idea that these reactive species are capable of affecting protein function, enzymatic activity and gene transcription. In other words, the increase in ROS production would also be a stimulus for a higher rate of renewal and production of mitochondria, leading to an antioxidant response (Ferraro et al., 2014; Gomez-Cabrera et al., 2021). With regard to mitochondria, the term "mitohormesis" refers to exposure to a concentration of stress that could improve mitochondrial function and that contributes to the adaptive hormonal response, which in this case would be a benefit to mitochondria and athletes' performance (Merry & Ristow, 2016).

It is known that Reactive Species are of two types: oxygen species (ROS, which were quantified in the chemiluminescence assay) and nitrogen species (RNS). Among the latter, two can be highlighted: nitric oxide (NO) and peroxynitrite (ONOO-).

Table 1. Profile description of the jiu jitsu athletes participating in this study.

Range	Number of athletes	Mean of age (years old)	Mean of MMI
White	2	31 ± 2.45	24.69 ± 2.17
Blue	3	30 ± 7.59	25.95 ± 4.08
Purple	2	33.5 ± 5.68	25.07 ± 1.78
Brown	2	28.5 ± 6.36	25.51 ± 1.16
Black	4	34.25 ± 3.53	26.31 ± 1.44

Being MMI (Muscular Mass Index).

The profile of the control group was 32.3 ± 6 years of age and MMI= 26.4 ± 2 (n=20 control group and n=13 athlete group).

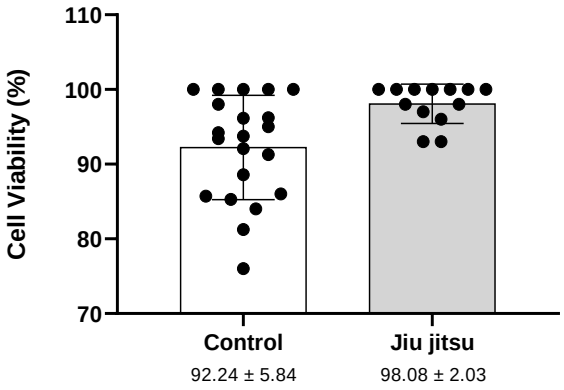

Figure 1. Cellular viability in leukocytes. Viability was assessed by the Trypan Blue assay. There was no significant difference between the groups, with viability being greater than 90% in all of them. n (control)= 20 and n (athletes)= 13.

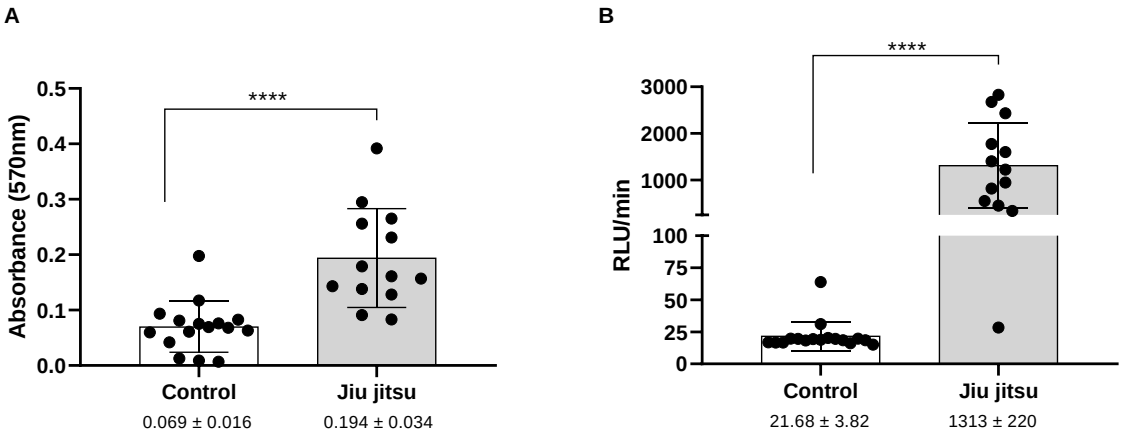

Figure 2. Assessment of mitochondrial and systemic oxidative stress in leukocytes. (A) The reducing capacity of leukocytes was expressed by the absorbance of MTT at 570nm. (B) ROS generation was expressed in Relative Light Units/minute (RLU/min) for 10 minutes in the chemiluminescence assay. The result was significant by ANOVA analysis and Student's t post-test. Being ****p<0.0001. n (control)= 20 and n (athletes)= 13.

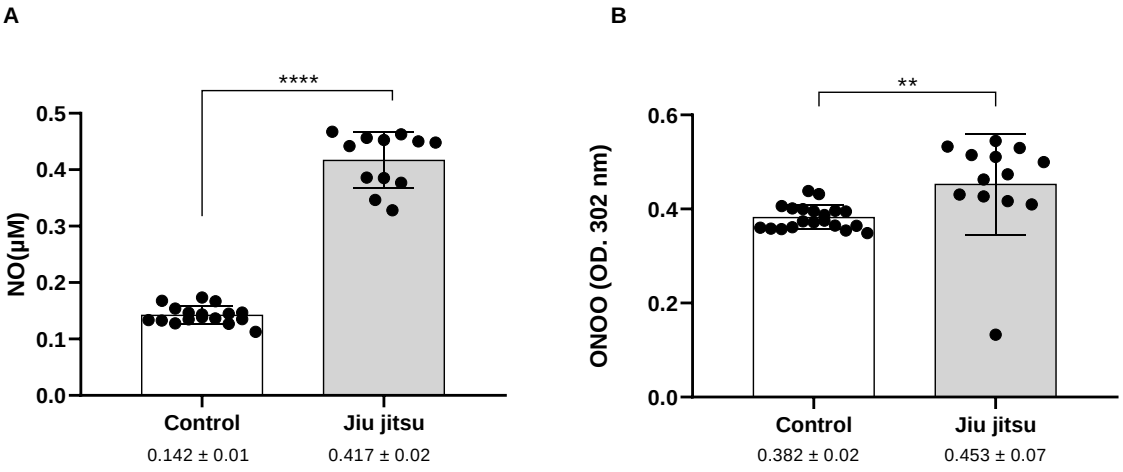

Figure 3. Assessment of the production of Reactive Nitrogen Species in leukocytes. (A) NO generation was expressed in µM. (B) ONOO- generation was expressed in optical density measured at 302nm. The result was significant by ANOVA analysis and Student's t post-test. With **p<0.01 and ****p<0.0001. n (control)= 20 and n (athletes)= 13.

The results regarding the quantification of these two reactive species are shown in Figure 3. It is observed that there was an increase in NO and ONOO- in both leukocytes isolated from the athletes when compared to the respective control groups.

According to Agricola & Guillo (2020), NO acts in the activation of mitochondrial biogenesis in skeletal muscle, thus improving breathing, and directly interferes with the supply of nutrients and oxygen to the myocyte through the regulation of blood flow. Prolonged exposure to NO triggers mitochondrial biogenesis. These findings are in line with what was observed in our study, as there was an increase in NO and an improvement in mitochondrial activity. Furthermore, studies have shown that NO production is largely influenced by predominant nerve stimulation, in which parasympathetic activity stimulates production, whereas sympathetic activity is associated with low production of NO, Predominantly sympathetic stimulation tends to reduce endothelial NO concentrations and even cause an increase in blood pressure (Rossi et al., 2009).

An interesting finding was the increase in both RNS in the athletes' leukocytes. To discuss this, the direct relationship between the production of NO and ONOO- must be highlighted. An increase in ONOO- can directly favor the production of NO. NO, despite being classified as RNS, is a more stable molecule, less harmful to cells and with beneficial actions in exercise, as already mentioned. The literature on this shows that, with the increase in nitrogen radical species (ONOO-) and oxygen in groups such as these athletes, there may be the formation of NO as a compensatory mechanism and eventually reduce the data caused by reactive molecules (Pall, 2013; Reid, 2016). This was indeed observed in our study. Therefore, our hypothesis is that the practice of jiu jtisu, despite leading to an increase in oxidative stress, favors antioxidant defense mechanisms, observed by the increase in NO and mitochondrial antioxidant capacity. More studies are necessary to evaluate other reactive species, as well as the products of cellular damage and bichemical aspects of this growing sport in the world.

4. Conclusion

According to the data observed, it is possible to see that the athletes' leukocytes presented greater systemic oxidative stress, but with mitochondria with better reducing capacity. Although the data initially seem conflicting, the hypothesis is that, although high energy demand leads to an increase in oxidative stress, physical exercise increases mitochondrial biogenesis and improves the quality of mitochondria - a process observed in people who practice regular physical activity. However, mitochondrial stimulation appears to be insufficient in combating systemic stress. This highlights how more research is needed to characterize the biochemical aspects of athletes not only in this but also in other high-performance sports, an area with few studies seen to date.

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Author's contribution & Statement

Brenda Evangelista Peixoto (methodology, validation, formal analysis investigation, term and conceptualization). Carolina Martins Santos (methodology, validation, formal analysis investigation, term and conceptualization). Glaucy Rodrigues de Araújo (methodology supervision). Miriam Martins Chaves (resources, supervision, funding acquisition). Filipe Nogueira Franco (writing - review & editing, supervision and project administration).

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Ethics Approval Statement

The study followed compliance with ethical standards, being approved by the Ethics Committee of the Institution (CAAE: 33842420.4.0000.5149).

Informed Consent

All individuals selected to participate in our study gave consent by signing a "Free and Informed Consent Form". In addition, the authors declare that there was no conflict of interest.

Conflict of Interest

The authors declare that there was no conflict of interest.

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