

Effect of α -lipoic acid supplementation on oxidative stress of heart and liver tissues of endurance-trained mice submitted to an exhaustive exercise

Efeito da suplementação de ácido α -lipóico no estresse oxidativo de tecidos cardíaco e hepático de camundongos treinados em endurance submetidos a um exercício exaustivo

Letícia Santana Wolf¹ , Álisson de Carvalho Gonçalves² , Ruan Carlos Macedo de Moraes³ , Ana Carolina Nunes Rodrigues⁴ , Susana Merino⁵ , Guilherme Vannucchi Portari⁶ 

1. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
2. Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Urutaí, Urutaí, GO, Brazil
3. Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil
4. Instituto de Ciências da Saúde, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil
5. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
6. Instituto de Ciências da Saúde, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil

ABSTRACT

Introduction: High intensity exercise causes an increase in reactive oxygen species production, which can be harmful to the health and function of several organ tissues. **Objective:** To analyze the effect of supplementation with α -lipoic acid against the oxidative stress in the heart and liver of endurance-trained mice submitted to the exhaustive endurance exercise bout. **Methods:** Thirty-two male mice were submitted to 6-week endurance swimming training, and divided in two groups according to supplementation protocol, α -lipoic acid or vehicle, during the last two weeks. The last training session was destined to the exhaustive exercise bout. It was analyzed the lipid peroxidation, oxidative damage to proteins and antioxidant marker in liver and heart immediately (0h) and four-hours (4h) after the exhaustive exercise in both groups. **Results:** The heart of supplemented animals showed a lower protein damage and higher levels of antioxidant in 0h and 4h. In the liver, lipid peroxidation was higher in supplemented animals in 0h but did not differ 4h after the exhaustion. The liver of supplemented animals showed higher levels of carbonylated protein in both 0h and 4h. **Conclusion:** The α -lipoic acid supplementation is an efficient antioxidant to the heart of trained mice submitted to exhaustive exercise but is unnecessary to avoid exhaustion-induced oxidative stress in the liver.

Keywords: physical exercise; oxidative stress, lipoic acid.

RESUMO

Introdução: O exercício de alta intensidade promove um aumento na produção de espécies reativas, o que pode ser prejudicial para a saúde e função de diversos órgãos. **Objetivo:** Analisar o efeito da suplementação com ácido α -lipóico contra o estresse oxidativo no coração e no fígado de camundongos treinados em endurance submetidos a uma sessão de exercício exaustivo. **Métodos:** Trinta e dois camundongos machos foram submetidos a 6 semanas de treinamento em natação. Os animais foram divididos em dois grupos de acordo com a suplementação, ácido α -lipóico ou veículo, oferecida durante as duas últimas semanas. A última sessão do treinamento foi destinada ao exercício de exaustão. Foram analisadas a peroxidação lipídica, dano oxidativo às proteínas e marcador antioxidante no fígado e coração imediatamente (0h) e quatro horas (4h) após o exercício exaustivo nos dois grupos. **Resultados:** O coração dos animais suplementados apresentou menor dano proteico e maiores níveis de antioxidante nas 0h e 4h. No fígado, a peroxidação lipídica foi maior nos animais suplementados em 0h, mas não diferiu 4h após a exaustão. O fígado dos animais suplementados apresentou níveis mais altos de proteína carbonilada nas 0h e nas 4h. **Conclusão:** A suplementação com ácido α -lipóico é um antioxidante eficiente para o coração de camundongos treinados submetidos a exercício exaustivo, mas é desnecessário para evitar o estresse oxidativo hepático induzido pelo esforço exaustivo.

Palavras-chave: exercício físico; estresse oxidativo; antioxidante.

Received: June 15, 2020; Accepted: May 4, 2021.

Correspondence: Guilherme Vannucchi Portari, Av. Getúlio Guaritá, 159/121 Abadia, 38025-440 Uberaba MG, guilherme.portari@uftm.edu.br

Introduction

The aerobic metabolism is the main reactive oxygen species (ROS) endogenous source. The oxidative phosphorylation produces a large number of superoxide anions, which can form other reactive species, like as hydrogen peroxide and hydroxyl radical [1]. However, in physiological conditions, the ROS are neutralized by a complex endogenous antioxidant system, which is composed of enzymatic and non-enzymatic antioxidants. The antioxidant molecules are extremely important to avoid oxidative damage to cell components, such as proteins, nucleic acids, and lipids [2,3].

High intensity or strenuous physical exercise bouts cause an expressive increase in ROS production [1]. Besides exercise is a potential stimulus to trigger ROS production, the scientific literature has shown that an adequate physical exercise-training program has a protective effect against oxidative damage, since it enhanced the antioxidant system [4]. However, it has been shown that high production of ROS and impairment in the antioxidant system can limit the exercise adaptation and performance [1,5]. Chronic exposition to high ROS levels can decrease significantly the activity of enzymatic antioxidant system (superoxide dismutase, catalase, glutathione peroxidase, etc.) and the concentration of non-enzymatic antioxidant (coenzyme Q10, glutathione, vitamin C and E, lipoic acid, etc.), impairing the cellular function by triggering damage, apoptosis, and necrosis [2].

The oxidative damage from exercise in trained muscle has been largely studied since the muscle is the most recruited organ during an exercise bout. However, other organs have an increase in their activity during and immediately after an exercise bout, especially heart and liver [6,7]. Studies have shown negative alterations in the redox status of the heart and liver after strenuous exercise [8,9]. Fortunately, it has been studied nutritional strategies able to prevent and/or reduces oxidative damage, and consequently, reduces physical stress, muscle pain, and impairments to sports performance [10]. Some studies have shown that the intake of exogenous antioxidant molecules has positive effects against exercise-induced oxidative damage [8,11].

The α -lipoic acid is cofactor to mitochondrial enzymes involved in energy metabolism also, play a role in ROS neutralization and metal chelation [12]. The α -lipoic acid has been considered universal antioxidant since it acts both in the aqueous phase and in the membrane, act in synergy with other antioxidants (such as glutathione, vitamin C and vitamin E). Also, it can recycle other antioxidants such as ascorbic acid, glutathione (GSH) and vitamin E [12,13]. Studies have shown that α -lipoic acid supplementation can improve antioxidant defense and reduces oxidative damage in muscle tissue after an exercise bout [14,15].

Thus, the present study aimed to analyze the effect of supplementation with α -lipoic acid against oxidative stress in the heart and liver of endurance-trained mice submitted to the exhaustive endurance exercise bout.

Methods

Animals

Thirty-two 6-week-old male Swiss mice (*Mus musculus*) were placed in two experimental groups according to the intervention: 1) Vehicle group (VEH) (n = 16): animals submitted to exercise training and which did not receive supplementation; 2) Supplemented group (SUP) (n=16): animals submitted to exercise training and which received supplementation. The animals were housed in plastic cages, in an inverted circadian cycle (12-h dark/light), in 22 ± 1 °C and $55 \pm 5\%$ humidity with feeding and tap water ad libitum, in the animal facility of the Laboratory of Experimental Nutrition of Federal University of Triangulo Mineiro (UFTM). The experiment protocol had the previous authorization of the Ethics Committee of Animal Use of UFTM, under protocol number 219/12.

Supplementation protocol

The α -lipoic acid (Zhejiang Chemicals, China) was diluted (1 mg/mL) in a vehicle solution (10% dimethyl sulfoxide (DMSO) in soybean oil). The solution was administered by gavage (100 mg/kg/day) during the last seven days of the exercise training protocol, in the animals of the SUP. The VEH received the same volume of the vehicle solution during the last seven days of the exercise training protocol.

Exercise training protocol

The exercise training protocol was applied according to proposed by Sampaio-Barros *et al.* [16]. The first week was destined for acclimation to the exercise training protocol. The animals were submitted to swimming training for 5-minutes on the first day, 15-min in the second, 30-min in the third day, 45-min, and 60-min in the fourth and fifth days, respectively. Then, the animals were daily submitted to a swimming session, 5 days per week, 60-min per session, during 6-weeks. The training session was applied in groups of 5 animals aimed to increase the intensity of exercise [16]. Animals swam in a plastic container of 22 cm diameter and 60 cm height, with a tap water depth of 40 cm, maintained at 32 °C (± 1 °C) controlled by the heater with an automatic thermostat (HOPAR SA-333 Zhong Shan, China).

The last session was dedicated to the exhaustive exercise bout. The animals were submitted to an individual swimming session with a metal load corresponding to 10% of bodyweight attached to the proximal portion of the tail. Animals were considered to be exhausted when they were unable to support the snout in the surface of the water for 8 seconds [17].

Sample collection and preparation

The animals were euthanized by decapitation after being anesthetized with ketamine (80 mg/kg) and (5mg/kg) xylazine immediately after (0h) the exhaustive exercise (n = 8) and four-hours after (4h) the exhaustive exercise (n = 8). After the

euthanasia confirmation, the liver and heart were immediately excised, washed in saline solution, and immediately frosted in liquid nitrogen. The organs were kept at -20°C until the time of analyzes. Aliquots of heart and liver tissue were homogenized with 25 mM phosphate buffer pH 7.4 (1:100 w/vol) immediately before the analyses start.

Determination of carbonylated proteins

The concentration of carbonylated proteins was determined by the method proposed by Odetti *et al.* [18]. Homogenate tissue (100 μL) was vigorously mixed with 100 μL 20% tricarboxylic acid (TCA) and centrifugated for 10 minutes at 3500 rpm. The supernatant was discarded and 500 μL of 10mM 2,4-dinitrophenylhydrazine (DNPH), diluted in 2M HCl, was added to precipitate. The solution was incubated for 1 hour at room temperature in the dark, with shaking every 15 min. Then, it was added 1 mL of ethanol-ethyl acetate (1:1 vol/vol) was added and the solution was centrifugated for 10 min at 3500 rpm at 4°C . Ethanol-ethyl acetate was removed and the pellet was suspended in 2 mL of 6M guanidine. The protein-guanidine solution was kept in a water bath at 34°C for 15 min. The final solution was read in spectrophotometry set at 370 nm, and the coefficient molar extinction rate 22,000 $\text{M}^{-1}\text{cm}^{-1}$ was used to calculate the carbonyl content.

Determination of lipid peroxidation

The lipid peroxidation was measured by the concentration of thiobarbituric reactive substances (TBARS) according to Buege and Austi [19]. It was added 1mL of TCA-TBA-HCL reagent to 500 μL of tissue homogenate. The solution was kept for 15 minutes in boiling water (100°C). After cooling, the solution was centrifuged for 10 minutes at 10000g. The absorbance of the supernatant was read at 535nm. The TBARS concentration was determined using an equation of the calibration curve obtained by a similar reaction using commercial malondialdehyde solution.

Determination of non-protein thiols

The non-protein thiols concentration was measured by colorimetric method, using the reaction of sulfhydryl group with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). Tissue homogenate was deproteinized by adding 10% TCA. It was added 200 μL of 0.2 M Tris -0.02M EDTA, 300 μL of DTNB, and 1.6 mL of methanol to 100 μL of tissue homogenate supernatant. The solution was incubated for 15-min at room temperature and in dark. Then, the absorbance was read with a spectrophotometer set at a wavelength of 412 nm. The non-protein thiols concentration was determined using an equation of the calibration curve obtained by a similar reaction using commercial GSH solution [20].

Statistical analysis

The data are presented as mean \pm standard deviation. The results were analyzed using the software SPSS 20.0. To check the variances' equality and data distribu-

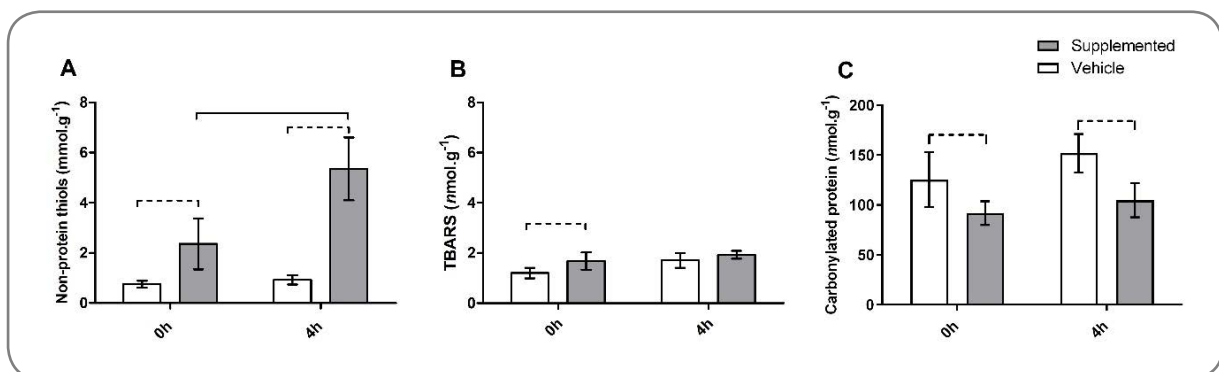
tion, Levene's test and Shapiro-Wilk test, respectively, were applied. The data were compared by the analyzes of variance (ANOVA) two-way and Tukey's post hoc . A significance level of 95% ($p < 0.05$) was adopted.

Results

The heart tissue of the VEH group showed a lower concentration of non-protein thiols in 0h-time compared to 4h. Similarly, the heart of supplemented animals showed a higher concentration of non-protein thiols in 4h than in 0h-time. The SUP group showed a higher concentration of non-protein thiols than the VEH in both experimental periods (0h, and 4h) (Figure 1A).

The heart tissue of the SUP group showed higher TBARS concentration compared to the VEH group in 0h-time. However, TBARS concentration was not different between the groups in 4h-time. The TBARS concentration in the heart tissue of both the groups did not alter through the experimental time (0h to 4h) (Figure 1B).

In 0h and 4-time, the heart tissue of the SUP group showed lower carbonylated protein concentration than the VEH group. Both the SUP and VEH group did not show any difference between the experimental periods (Figure 1C).



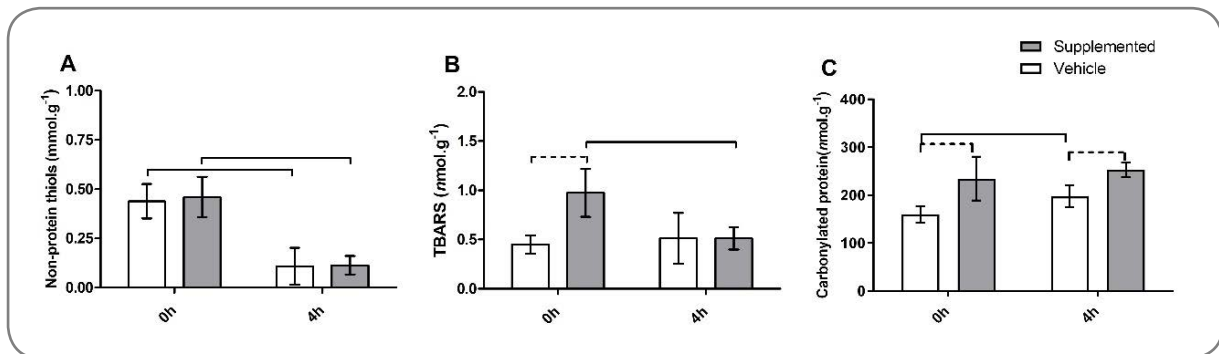
A = Non-protein thiols; B = Thiobarbituric acid reactive substances TBARS; C = Carbonylated protein. White bars represent the VEH group. Grey bars represent the SUP group. Dotted connector indicates an intergroup variation statistically significant ($p < 0.05$) at same time (SUP vs. VEH). Whole connector indicates an intragroup variation statistically significant ($p < 0.05$) at different times (0h vs. 4h)

Figure 1 - Oxidative stress markers in heart tissue

In the liver, the concentration of the non-protein thiols in 0h-time was higher than in 4h-time in both the SUP and VEH groups. There was no difference between groups in both experimental times (0h and 4h) (Figure 2A).

In supplemented animals, the TBARS concentration in liver tissue showed a significant reduction 4 hours after the exhaustive exercise. The hepatic TBARS concentration of the VEH group did not differ through the experimental time. Immediately after exhaustive exercise bout (0h), the TBARS concentration showed to be higher in the SUP than in the VEH group. However, there was no difference between the groups 4-hours after the exhaustive exercise (Figure 2B).

The concentration of carbonylated protein of the SUP group was higher than VEH in both experimental times (0h, and 4h). The liver of the non-supplemented animals showed higher protein carbonyl content in 4h compared to 0h-time. It was not finding any difference in carbonylated protein concentration between the times 0h and 4h (Figure 2C).



A = Non-protein thiols; B = Thiobarbituric acid reactive substances TBARS; C = Carbonylated protein. White bars represent the VEH group. Grey bars represent the SUP group. Dotted connector indicates an intergroup variation statistically significant ($p < 0.05$) at same time (SUP vs. VEH). Whole connector indicates an intragroup variation statistically significant ($p < 0.05$) at different times (0h vs. 4h)

Figure 2 - Oxidative stress markers in liver tissue

Discussion

The present study aimed to evaluate how the α -lipoic acid supplementation affects the exhaustive exercise-induced oxidative stress in the heart and liver of endurance-trained animals after. It was found positive alterations in oxidative stress markers in the heart tissue of the supplemented animals, especially four hours after the exhaustive exercise. However, the α -lipoic supplementation seems to be related to increases in the oxidative damage in the liver.

ROS play an essential role in cell signaling and function [21]. The increase in the ROS concentration in the myocardium results in effects on its structure and functions, such as stimulation of cardiac hypertrophy and apoptosis of cardiomyocytes, thus contributing to cardiac remodeling [22]. Since the improvement in heart efficiency is one of the most important exercise adaptations, the presence of moderate ROS levels seems to be necessary. However, the excessive ROS attack on macromolecules can be harmful to cell function and can lead it to apoptosis [23]. Thus, the balance between antioxidant capacity and pro-oxidant activity must be considered to the maintenance of the cardiac health and sports performance of individuals engaged in exercise training programs.

During an exhaustive effort, the heart workout is highly required. The myocardium energy demand is mainly supplied by the aerobic metabolism [24]. Thus, the heart tissue is submitted to intense reactive species attack, since the mitochondrial metabolism is the main source of ROS. However, studies have shown no increases in oxidative damage in the heart of endurance-trained animals [25,26]. The present study also did not find any increase in oxidative damage markers in heart tissue four

hours after the exhaustion compared to the results obtained immediately after the exhaustive effort.

The α -lipoic acid could have mitigated the harmful effects of exhaustion-induced oxidative damage in the heart proteins. The heart tissue of supplemented animals showed a lower concentration of carbonylated protein, which indicates less oxidative damage to proteins. This could be related to the higher antioxidant concentration in the supplemented animal. However, similar results could not be observed in the lipid peroxidation marker of heart tissue. Despite the TBARS concentration in supplemented animals to be slightly higher than non-supplemented animals immediately after the effort, the values did not differ four hours later. It seems that the swimming exhaustive exercise was not enough stimulus to induce lipid peroxidation in the heart tissue of the trained mice. In fact, it was shown that exhaustive endurance exercise was able to alter some oxidative stress markers in the heart tissue of endurance-trained rats, but not the lipid peroxidation marker [27].

The non-protein thiols, which are antioxidant molecules, showed in higher levels in the myocardium of supplemented animals in both the experimental times (0h and 4h). The α -lipoic acid has a known potential effect to recovery and/or improve other antioxidant molecules and mechanisms, that can be related to high antioxidant concentration, especially GSH [12], the main representative of non-protein thiols [28].

Although skeletal-muscles and myocardium are widely used during a strenuous and prolonged effort, the liver work also has a significant increase in this situation [6]. Some works have shown increases in oxidative damage markers in liver tissue after exhaustive endurance exercise [6,29]. Several studies have shown the antioxidants effects of α -lipoic acid in the liver [30-32]. An experimental study found that the administration of α -lipoic acid was effective in reducing lipid peroxidation and preserving glutathione peroxidase activity and GSH concentration in the liver of rats subjected to toxic doses acetaminophen [31]. In non-trained rats, it was showed that α -lipoic acid supplementation is able to protect liver cells against oxidative lipid damage promoted by strenuous exercise bout [30].

In the present study, it was observed an expressive reduction in antioxidant concentration in four hours after the exhaustive exercise in both groups. Interestingly, the antioxidant concentration in 0h and 4h was not different between the groups. These data indicate that despite the α -lipoic acid play an important role in antioxidants recycling [12], it is not able to increase the concentration of non-protein thiols immediately after an exhaustive endurance bout in the liver tissue of trained mice. In addition, the supplementation protocol failed to maintain the antioxidant concentration throughout the four hours following the exhaustion.

It is important to highlight that the concentration of non-protein thiols suffered a significant reduction four hours after the exhaustive effort, in both the experimental groups. This indicates that changes in oxidative stress markers continue to change for a few hours after the exhaustion bout. Several studies [33-35] have shown

that exercise-induced oxidative stress can be observed for a long time after exercise. In humans, it was shown that the antioxidant capacity was lower 24 hours after than immediately after a strenuous exercise bout [33].

There was a modest increase in the oxidative damage to proteins in the liver of the VEH group. However, the concentration of carbonylated proteins maintained higher in SUP groups in both the experimental periods. This can be related to the antioxidant mechanism of α -lipoic acid. Lipoic acid and dihydrolipoic acid (DHLA) (produced from α -lipoic acid) are reactive to thiol protein compounds [12]. In addition, DHLA is able to accelerate iron-dependent hydroxyl radical generation and lipid peroxidation [13]. Possibly, the same result was not observed in heart because iron concentration in hepatic tissue is expressively higher than in myocardium [36]. Moreover, the liver cells have a high capacity for uptake and accumulation of α -lipoic acid metabolites, such as DHLA and lipoate [37].

Immediately after the exercise, the liver of non-supplemented animals suffered less lipid peroxidation. The TBARS concentration in the liver showed a modest decrease 4 hours after the effort only in supplemented animals, but the result was not different than in the non-supplemented animals. Thus, α -lipoic acid seems to not affect the exhaustive exercise-induced oxidative stress in the liver tissue of trained mice. The mice's training level could be related to the inefficiency of the supplementation protocol proposed in this study. Navarro *et al.* [38] showed that moderate exercise training, per se, decreases the oxidative stress in the liver of middle-age mice. Other study [39] found that endurance training promotes liver adaptations, which can attenuate exhaustive exercise-induced oxidative stress, becoming the antioxidant supplementation an unnecessary strategy against oxidative stress in the liver.

Conclusion

The α -lipoic acid supplementation is effective to increase the antioxidant capacity and to reduce the oxidative damage in the heart tissue of trained mice after an exhaustive effort. However, the α -lipoic acid cannot maintain the antioxidant levels in liver tissue and it was related to an increase in oxidative damage. Thus, the α -lipoic acid supplementation is an effective strategy to avoid the exhaustion-induced oxidative stress in the heart of trained mice, but does not in the liver tissue.

Potential conflict of interest

No potential conflicts of interest relevant to this article have been reported.

Financing source

There were no external funding sources for this study.

Author contributions

Conception and design of the research: Santana LF, Merino S, Portari GV. **Data collection:** Santana LF, Merino S, Gonçalves AC, Rodrigues ACN, Moraes RCM. **Data analysis and interpretation:** Gonçalves AC, Rodrigues ACN, Moraes RCM, Portari GV. **Statistical analysis:** Gonçalves AC, Moraes RCM. **Writing of the manuscript:** Santana LF, Gonçalves AC, Moraes RCM. **Critical review of the manuscript for important intellectual content:** Portari GV.

References

1. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev* 2008;88(4):1243-76. doi: 10.1152/physrev.00031.2007
2. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol* 2013;51:15-25. doi: 10.1016/j.fct.2012.09.021
3. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 2006;8(9-10):1865-79. doi: 10.1089/ars.2006.8.1865
4. Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, et al. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget* 2018;9(24):17181. doi: 10.18632/oncotarget.24729
5. Reid MB. Redox interventions to increase exercise performance. *J Physiol* 2016;594(18):5125-33. doi: 10.1113/jp270653
6. Huang C-C, Lin W-T, Hsu F-L, Tsai P-W, Hou C-C. Metabolomics investigation of exercise-modulated changes in metabolism in rat liver after exhaustive and endurance exercises. *Eur J Appl Physiol* 2010;108(3):557-66. doi: 10.1007/s00421-009-1247-7
7. Oláh A, Németh BT, Mátyás C, Horváth EM, Hidi L, Birtalan E, et al. Cardiac effects of acute exhaustive exercise in a rat model. *Int J Cardiol* 2015;182:258-66. doi: 10.1016/j.ijcard.2014.12.045
8. Carvalho AG, Moreira EJS, Portari GV. Benfotiamine supplementation prevents oxidative stress in anterior tibialis muscle and heart. *J Integr Med* 2019; 17(6):423-429. doi: 10.1016/j.joim.2019.07.001
9. Yan F, Wang B, Zhang Y. Polysaccharides from *Cordyceps sinensis* mycelium ameliorate exhaustive swimming exercise-induced oxidative stress. *Pharm Biol* 2014;52(2):157-61. doi: 10.3109/13880209.2013.820197
10. Pingitore A, Lima GPP, Mastorci F, Quinones A, Iervasi G, Vassalle C. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition* 2015;31(7-8):916-22. doi: 10.1016/j.nut.2015.02.005
11. Abadi A, Crane JD, Ogborn D, Hettinga B, Akhtar M, Stokl A, et al. Supplementation with α -lipoic acid, CoQ10, and vitamin E augments running performance and mitochondrial function in female mice. *PLoS One*. 2013;8(4):e60722. doi: 10.1371/journal.pone.0060722
12. Moini H, Packer L, Saris N-EL. Antioxidant and prooxidant activities of α -lipoic acid and dihydrolipoic acid. *Toxicol Appl Pharmacol* 2002;182(1):84-90. doi: 10.1006/taap.2002.9437
13. Scott BC, Aruoma OI, Evans PJ, O'Neill C, Van Der Vliet A, Cross CE, et al. Lipoic and dihydrolipoic acids as antioxidants. A critical evaluation. *Free Radic Res* 1994;20(2):119-33. doi: 10.3109/10715769409147509
14. Chae C-H, Shin C-H, Kim H-T. The combination of α -lipoic acid supplementation and aerobic exercise inhibits lipid peroxidation in rat skeletal muscles. *Nutr Res* 2008;28(6):399-405. doi: 10.1016/j.nutres.2008.02.010
15. Kinnunen S, Oksala N, Hyypä S, Sen CK, Radak Z, Laaksonen DE, et al. α -Lipoic acid modulates thiol antioxidant defences and attenuates exercise-induced oxidative stress in standardbred trotters. *Free Radic Res* 2009;43(8):697-705. doi: 10.1080/10715760903037673
16. Sampaio-Barros MM, Farias-Silva E, Grassi-Kassisse DM, Spadari-Bratfisch RC. Effect of swimming session duration and repetition on metabolic markers in rats. *Stress* 2003;6(2):127-132. doi: 10.1080/1025389031000110169
17. Voltarelli FA, Gobatto CA, De Mello MA. Determination of anaerobic threshold in rats using the lactate minimum test. *Braz J Med Biol Res* 2002;35(11):1389-94. doi: 10.1590/s0100-879x2002001100018
18. Odetti P, Giribaldi S, Gurreri G, Aragno I, Dapino D, Pronzato MA, et al. Protein oxidation in hemodialysis and kidney transplantation. *Metabolism* 1996;45(11):1319-22. doi: 10.1016/s0026-0495(96)90108-0
19. Buege J, Aust S. Microsomal lipid peroxidation. *Methods Enzymol* 1978;52:302-10. doi: 10.1016/s0076-6879(78)52032-6
20. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968;25:192-205. doi: 10.1016/0003-2697(68)90092-4
21. Hancock J, Desikan R, Neill S. Role of reactive oxygen species in cell signaling pathways. *Biochem Soc Trans* 2001;29(2):345-9. doi: 10.1042/bst0290345
22. Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. *Hypertension* 2007;49(2):241-8. doi: 10.1161/01.hyp.0000254415.31362.a7
23. Biary N, Xie C, Kauffman J, Akar FG. Biophysical properties and functional consequences of reactive oxygen species (ROS) induced ROS release in intact myocardium. *J Physiol* 2011;589(21):5167-79. doi: 10.1113/jphysiol.2011.214239
24. Khouri EM, Gregg DE, Rayford CR. Effect of exercise on cardiac output, left coronary flow and myocardial metabolism in the unanesthetized dog. *Circ Res* 1965;17(5):427-37. doi: 10.1161/01.res.17.5.427
25. Frankiewicz-Józko A, Faff J, Sieradzian-Gabelska B. Changes in concentrations of tissue free radical marker and serum creatine kinase during the post-exercise period in rats. *Eur J Appl Physiol* 1996;74(5):470-4. doi: 10.1007/s004210050101
26. Stanojevic D, Jakovljevic V, Barudzic N, Zivkovic V, Srejovic I, Ilic KP, et al. Overtraining does not induce oxidative stress and inflammation in blood and heart of rats. *Physiol Res* 2016;65(1). doi: 10.33549/physiolres.933058
27. Gul M, Demircan B, Taysi S, Oztasan N, Gumustekin K, Siktar E, et al. Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. *Comp Biochem Physiol A Mol Integr Physiol*

2006;143(2):239-45. doi: 10.1016/j.cbpa.2005.12.001

28. Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. *Biochem Pharmacol* 2002;64(5-6):1019-26. doi: 10.1016/S0006-2952(02)01172-3

29. Korivi M, Hou C-W, Huang C-Y, Lee S-D, Hsu M-F, Yu S-H, et al. Ginsenoside-Rg1 protects the liver against exhaustive exercise-induced oxidative stress in rats. *Evid Based Complement Alternat Med* 2012;2012. doi: 10.1155/2012/932165

30. Khanna S, Atalay M, Laaksonen DE, Gul M, Roy S, Sen CK. α -Lipoic acid supplementation: tissue glutathione homeostasis at rest and after exercise. *J Appl Physiol* 1999;86(4):1191-6. doi: 10.1152/jappl.1999.86.4.1191

31. Abdel-Zaher AO, Abdel-Hady RH, Mahmoud MM, Farrag MM. The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. *Toxicology* 2008;243(3):261-70. doi: 10.1016/j.tox.2007.10.010

32. Maritim A, Sanders R, Watkins Iii J. Effects of α -lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. *J Nutr Biochem* 2003;14(5):288-94. doi: 10.1016/S0955-2863(03)00036-6

33. Neubauer O, Koenig D, Kern N, Nics L, Wagner K-H. No indications of persistent oxidative stress in response to an ironman triathlon. *Med Sci Sports Exerc* 2008;40(12):2119-28. doi: 10.1016/S0162-0908(09)79565-0

34. Meihua S. The time effect of DNA damage and oxidative stress on mice liver cells induced by exercise fatigue. In: *Education Management, Education Theory and Education Application*. Springer 2011:661-7. doi: 10.1007/978-3-642-24772-9-96

35. Gonçalves AC, Rodrigues LR, Terra MP, Sasaki JE, Portari GV. Exercício aeróbio exaustivo aumenta o estresse oxidativo em corredores fundistas treinados. *Rev Bras Prescrição Fisiol Exerc* 2019;13(83):493-500.

36. Petry CD, Eaton MA, Wobken JD, Mills MM, Johnson DE, Georgieff MK. Iron deficiency of liver, heart, and brain in newborn infants of diabetic mothers. *J Pediatr* 1992;121(1):109-114. doi: 10.1016/S0022-3476(05)82554-5

37. Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihm BH. α -Lipoic acid in liver metabolism and disease. *Free Radic Biol Medic* 1998;24(6):1023-39. doi: 10.1016/S0891-5849(97)00371-7

38. Navarro A, Gomez C, López-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Am J Physiol-Regul Integr Comp Physiol* 2004;286(3):R505-11. doi: 10.1152/ajpregu.00208.2003

39. Taysi S, Oztasan N, Efe H, Polat M, Gumustekin K, Siktar E, et al. Endurance training attenuates the oxidative stress due to acute exhaustive exercise in rat liver. *Acta Physiol Hung* 2008;95(4):337-47. doi: 10.1556/aphysiol.95.2008.4.2