Journal of Complementary Medicine And Alternative Healthcare
ISSN: 2572-1232

Juniper Publishers key to the Researchers

Case Report
Volume 11 Issue 1- May 2020
DOI: 10.19080/JCMAH.2020.11.555810

J Complement Med Alt Healthcare

Bioanalytical Approach-Ageing, Exercise and Oxidative Stress

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Submission: May 07, 2020; **Published:** May 27, 2020

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Abstract

Abstract: Exercise interferes with the ageing, causing changes in markers of the antioxidant system, such as nitric oxide (NO) and uric acid.

Aim: The objective of this study was to evaluate if regular exercise affects the ageing process by causing changes in the antioxidant markers, nitric oxide and uric acid, in individuals aged ≥ 65 years, subjected to an exercise plan.

Methods: The study involved 12 participants distributed in an experimental and control group. The experimental group performed exercises guided by a physiotherapist during 12 weeks. Three blood samples were collected from participants: T0, at the start of the study; T1, after 12 weeks of exercise and T2, 12 weeks after finishing the exercise. NO and uric acid were quantified with commercial kits. The statistics were performed using SPSS and the ANOVA and Student t-tests were applied.

Result: In the experimental group, the mean values of NO showed an increase between T0 and T1, with a slight decrease between T1 and T2. Similar effects were observed for the uric acid concentration in the experimental group, with statistical significance from T0 to T1.

Conclusion: After exercise, there was an increase in blood uric acid and NO levels, highlighting the importance of moderate and controlled exercise practice. Therefore, exercise seems relevant and beneficial in activating antioxidant mechanisms.

Keywords: Ageing; Exercise; Nitric oxide; Uric acid; Ageing process; Signalling pathway; Postponement; Biological process

Introduction

Ageing is an active, irreversible biological process, including morphological, functional, biochemical and physiological changes. These changes have an impact on the health and quality of life of the elderly, leading to a progressive functional loss of the organism. In the process of ageing, there is a decrease in the metabolic rate caused by the reduction of energy exchanges, which is related to the presence and action of free radicals [1]. The accumulation of free radicals in the body leads to a phenomenon called oxidative stress. This process entails a disruption of the redox signalling pathway, which is fundamental to the aerobic system and metabolism. Free radicals are produced as part of the normal physiology and in disease. Oxidative stress is involved in a variety of chronic and degenerative diseases, such as arthritis, autoimmune disorders, cardiovascular and neurodegenerative diseases, as well as in cancer, inflammatory conditions, ageing and even following intense physical exercise [2-4]. The fundamental aspect in the prevention of oxidative stress is the correlation between the ability to inhibit or delay the onset of, for example, cancerous cells and the antioxidant activity of substances able to block the free radicals. The same principle accounts for the postponement of cellular ageing [5]. Ageing is associated with

a functional decline and performing physical exercise can play a role in the reduction and prevention of ageing [6]. Although living organisms have a biochemical defence mechanism which protects them against potentially harmful radicals, certain events of oxidative stress cannot be inhibited by these mechanisms. One example is exercise: it initiates oxidative stress, however, this activation is important to induce an activation of the antioxidative mechanisms. Training can, therefore, have a positive or a negative effect on oxidative stress, depending on the frequency, intensity, duration and exercise type practised as well as on the initial fitness levels of the individual [7]. In general, the physical activities should promote the production of antioxidative molecules to such a level, that the oxidative stress caused by the exercises is neutralised. Ideally, the produced anti-oxidant levels are even higher, thereby also reducing oxidative species induced by other events (for example inflammation, cancer, ageing) [1,8].

A common approach to assessing oxidative stress in biological systems involves the measurement of the increase or decrease of a redox-sensitive molecule that responds to this kind of stress. Usually, reliable markers are chemically unique and easily detectable. In addition, the marker should be rapidly increased during periods of oxidative stress and immediately decreased when the event has passed. Nitric oxide (NO), uric acid and enzymes are examples of such markers. In addition, these markers can be rapidly altered during oxidative stress periods, taking into account the surrounding environment [9,10]. Biological antioxidants of low molecular weight have the ability to prevent oxidative damage by direct and indirect interactions with reactive oxygen species (ROS) [8,11]. Uric acid is a secreted form of a derivate from the purine catabolism. These derivates are components of cellular molecules involved in the energy household, such as adenosine triphosphate (ATP), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Uric acid is produced in the liver and secreted in the urine. The anti-oxidative capacity of uric acid is the protection of DNA and lipids from ROS and reactive nitrogen species (RNS). Enhanced levels of uric acid are often found in individuals with atherosclerosis, where the inhibition of oxidative stress is of utmost importance [5,6]. NO is a highly reactive mediator shown to play different roles in a variety of different biological processes and in ageing. NO is a free radical of high biological importance. An increase in the levels of NO can lead to stress responses, whereby NO reacts with other radicals, such as the superoxide anion. NO synthase (NOS), when activated, produces NO and L-citrulline from L-arginine using nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen. There are tissue-specific constitutive isoforms of NOS: neuronal (nNOS) and endothelial (eNOS). The other form is the inducible type of NOS (iNOS), which is found to be expressed in various cell types and is induced under pathological conditions [12,14]. According to some studies, exercise promotes an increase in NO concentrations, thereby improving endothelial function with increased oxygen consumption [12,15]. The main objective of the presented study was, to evaluate if exercise performed by aged individuals (≥ 65 years of age) still affects the levels of the antioxidants NO and uric acid.

Material and Methods

The study cohort was composed of 12 individuals (control group n=6; experimental group n=6) with a mean age of 84 years who lived in a home care situation. The sole inclusion criteria for participation in the study was the age of the individuals (≥ 65 years of age). The exclusion criteria were the presence of a physical disability that prevented the accomplishment of the exercise plan or the presence of a marked cognitive deficit.

The exercise plan was adapted from the HIFE Program [16]. This plan involved strength training exercises of the lower limbs, of static and dynamic balance and gait. The intensity was defined individually, and each participant was encouraged by the physiotherapist to perform the exercises in a progressive way in order to reach a high intensity. The exercises were performed for 12 weeks, one session per week. The individuals were evaluated at three different time points: T0 (before starting the exercise plan), T1 (immediately after finishing the exercise program) and T2 (12 weeks after finishing the exercise). In addition to the assessment, the Late-Life Function and Disability Instrument (LLFDI) scale was adapted and validated for Portuguese culture by Cavalheiro and coauthors (2003), for the measurement of physical function and disability and the Six Item Cognitive Test Commitment (6 CIT) to assess cognitive ability [17]. The project was presented to each of the participants. All signed an informed consent form before participating in the study. Work was carried out in accordance with the principles of the Helsinki Declaration. A venous blood sample was taken by venipuncture at 3 different time points (T0, T1, T2) and collected in test tubes without supplements. All the samples were centrifuged 1800*g* at 4°C for 10min, followed by separation of the serum and stored at -23°C. For quantification of NO and uric acid the following kits were used: Total Nitric Oxide and Nitrate/Nitrite Assay (R&D Systems Europe, Abingdon, United Kingdom) and Liquick Cor-UA (Cormay, Łomianki, Poland), respectively. For NO the spectrophotometric reading was performed at 540 nm using a *ThermoScientific Multiskan GO* (Japan). The uric acid levels were detected at 546nm using a *Tokyo Boeki Prestige 24i* (Diamond Diagnostic, Japan). The techniques were performed according to the manufacturers' instructions. All statistical analyses were performed using the statistical package IBM SPSS Statistics 22.0. After testing, the data was statistically analyzed by the use of ANOVA and Student's t-tests. The results are presented as the mean ± standard deviation (SD) and were considered statistically significant at $p < 0.05$.

Results

After analysing demographic data, the experimental group had an average of 85.0±2.4 years and the control group had an average of 83.0±3.9 years at the beginning of the study. Both groups consisted mainly of female subjects (5 females and 1 male). Concerning the existence of chronic pathologies, all

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participants reported having dyslipidemias, respiratory or cardiac insufficiencies, pathologies of the central nervous systems and Diabetes Mellitus. With respect to the use of medication, all individuals in the experimental group and 83.3% of the control group reported taking at least 4 tablets per day.

Quantification of uric acid

The uric acid measurements showed that differences occurred between the values measured at the different time points in both the control and the experimental group. After exercise, in the experimental group, a mean value of 6.20±1.78 mg/dL was detected at T1, compared to 5.47±1.64 mg/dL at T0 and a concentration of 6.03±2.0 mg/dL at T2. The control group had a mean value of 5.80±1.0 mg/dL at T1 compared to 5.44±0.95mg/dL at T0 and finally 5.34±1.15 mg/dL at T2 to uric acid (Figure 1). Using the ANOVA statistical test, we did not obtain significant differences between the various time points (T0, T1 and T2), in the experimental and control group. However, when applying the Student's t-test between two time points, the experimental group revealed a statistically significant increase (p = 0.03) from T0 to T1 (Figure 1).

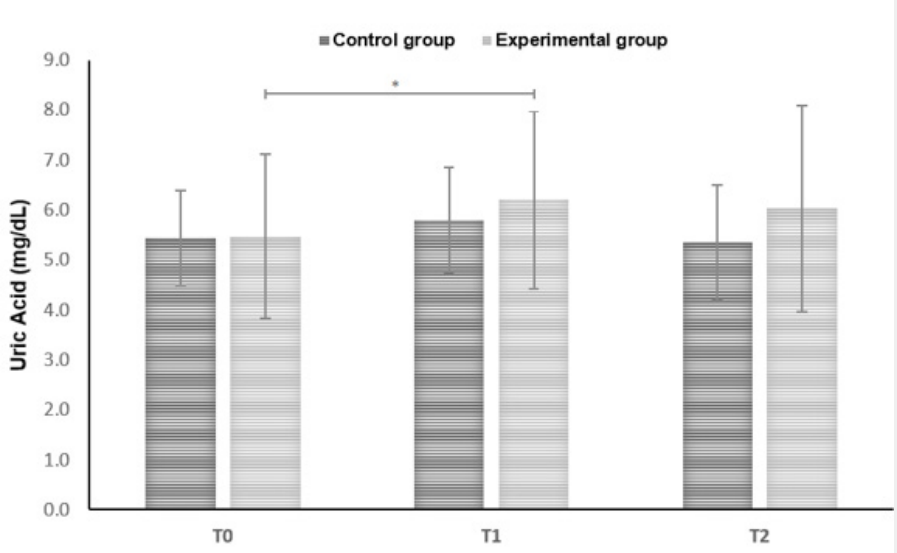

Figure 1 : Uric acid quantification at T0, T1 and T2 in the control and experimental groups. T0-at the start of the study; T1 - after 12 weeks of exercise; T2 - after 12 weeks of exercise followed by 12 weeks without this exercise. The results are reported in mg/dL. *p <0.05.

Quantification of NO

The NO data showed that there was no statistically significant difference between the different time points analyzed in the experimental group. This was also due to the large variation of NO in the serum samples. At T1, a mean value of 22.86±12.48 µmol/L was detected after the intervention. At T0 the NO levels were 12.72±1.55 µmol/L and at T2 these were 15.28±3.55 µmol/L. The NO concerning the control group; at T1 was 21.67±12.52 µmol/L compared to T0 with 18.84±15.0 µmol/L and 29.98±8.52 µmol/L at T2. There was no statistical difference between the 2 groups, nor between the time points analyzed.

Discussion

The theory about the action of free radicals as the main cause of the ageing process was proposed by Harman in 1955. This theory is currently being confirmed by a large number of scientific publications [18]. Therefore, the biological ageing of humans is associated with an increase in free radicals that can cause oxidative changes in proteins, lipids and DNA. Due to the increased oxidative stress, exercise is constantly used as an oxidizing stimulus, which is under normal circumstances directly counterbalanced by antioxidative measures. The health benefits related to regular exercise are widely described in the literature. Some of the major mechanisms are related to the prevention of DNA damage involved in various degenerative diseases and in the ageing process. Thus, individuals who practice moderate exercise tend to exhibit an increase in antioxidant markers compared to non-practitioners. Therefore, the human body adapts to training intensities through a defense network that regulates and protects against oxidative reactions to ensure survival [3,7,19]. The present study utilized 3 different time points (T0, T1 and T2), in order to investigate whether the performance of an intervention plan with several exercises training strength, endurance, balance, flexibility and gait for 12 weeks was able to induce the production of antioxidant species [16]. The uric acid analysis showed an increase in the average value from T0 to T1 and a tendency to decrease from T1 to T2. This observation was similar for both groups (experimental and control), which was unexpected, as the control group was not instructed to change their behavior. This may simply be associated

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with feeding by purine metabolism [5,6]. However, the effect on the experimental group was significantly more pronounced, indicating that exercise induces the formation of antioxidant uric acid and shows that it is a good marker for detecting antioxidant effects in humans. In the present study, the results of the NO levels revealed slight changes in the mean values of T0 to T1 and of T1 to T2, but without statistical significance and the strongest increase was observed in the control group and not in the exercise group. Moreover, the analysis at T0 already indicated a difference between the two study groups, which was not observed for the uric acid measurements. There are several factors that may have affected these results, such as the effects of the seasons on activities in general, differences or alterations in the intake of medication or also difficulties in the analysis of NO as a marker, since it is unstable and can react with oxygen in the medium transforming into another compound. Therefore, NO might not be the most suitable marker to detect antioxidative effects in humans. Recent studies have shown that exercise increases the production and availability of NO in the body. Thus, exercise can, among others, induce positive improvements of the cardiovascular, hepatic, skeletal and muscle systems. Souza et al (2009) observed in rats that swim training for 8 weeks promoted an increase in plasma nitrite and, consequently, an increase in endothelial function and performance [20]. Many studies state that the participation of the elderly in physical activity programs is an independent way to reduce and prevent functional decline associated with ageing [6,7] Nelson et al (2007) indicated the need to choose the type and amount of exercise to improve the quality of life of elderly individuals, taking into consideration the modality, duration, frequency, intensity and progression [21]. Individuals who undergo intense and prolonged exercises, exhaustive training and very high frequency may exceed the capacity of the endogenous antioxidant system, thereby promoting severe muscular injuries, with consequent induction of inflammatory processes and oxidative stress [4,22]. Thus, it is important to emphasize that while the exercise plan stimulates the antioxidant activity, there is a need for a longer intervention of the physiotherapy team, so that the values of the markers can be more precise, and the project reproduction and markers, especially uric acid, may be utilized with a larger sample for a better evaluation.

Conclusion

This study is one of the first to detect the effects of exercise on antioxidants in the serum of elderly people, showing the importance of life-long physical activities adapted to personal needs. The data confirms that oxidative stress is a factor that has a great impact on the health and quality of life on humans. Strategies, such as the practice of moderate and controlled exercise, should be instituted as an important factor in the activation of antioxidant mechanisms. This results in a later adaptation of the organism, increasing the efficiency against ageing.

Acknowledgments

The authors thank the departments of Biomedical Laboratory Sciences and Physiotherapy of the Coimbra Health School. This study was supported by Labinsaúde. Project co-financed by the QREN under the "Programa Mais Centro" of the Coordination Commission of the Central Region and the European Union through the European Regional Development Fund. AV is financed by national funds through FCT - Foundation for Science and Technology, I.P., within the scope of the projects UIDB/04292/2020 – MARE - Marine and Environmental Sciences Centre.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. De Oliveira MA, Assunção MS, Meneses YPSF (2015) Influence of Physical Exercise on the Aging Process and Human Oxidative Stress, FIEP Bulletin 85 (Special Edition), Article I.
2. Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health, International Journal of Biomedical Science 4(2): 89-96.
3. Bottino DA, Lopes FG, Oliveira FJ, De Mecenas ADS, Clapauch R, et al. (2015) Relationship between biomarkers of inflammation, oxidative stress and endothelial/microcirculatory function in successful aging versus healthy youth: a transversal study, BMC Geriatrics 15: 41.
4. Porter C, Reidy PT, Bhattarai N, Sidossis LS, Rasmussen BB (2015) Resistance Exercise Training Alters Mitochondrial Function in Human Skeletal Muscle, Medicine and science in sports and exercise 47(9): 1922-1931.
5. Adonis L, García EZ (2002) Aging and oxidative stress. Cuban Journal of Biomedical Investigations 21: 178-185.
6. Maciel MG, Física EDE, Antipoff FH (2010) Physical activity and functionality of the elderly, Motriz 16(4): 1024-1032.
7. He F, Li J, Liu Z, Chuang C, Yang, W, et al. (2016) Redox Mechanism of reactive oxygen species in exercise, Frontiers in Physiology 7: 1-10.
8. Fisher-Wellman K, Bloomer RJ (2009) Acute exercise and oxidative stress: a 30-year history, Dynamic Medicine 8: 1-25.
9. Kandola K, Bowman AM (2015) Birch Machin A (Oxidative stress a key emerging impact factor in health, ageing, lifestyle and aesthetics, International Journal of Cosmetic Science 37: 1-8.
10. Powers SK, Jackson MJ (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production, Physiology Reviews 88(4): 1243-1276.
11. Lilian L, Eliza M, Sato O, Santos DM (2004) Free Radicals and Skin Aging, Bonaerean Pharmaceutical Acta 23: 418-424.
12. Urszula K, Domek Ł, Strosznajder JB (2010) Cyclic GMP and Nitric Oxide Synthase in Aging and Alzheimer's Disease, Molecular Neurobiology 41(2-3): 129-137.
13. Jensen FB (2009) The role of nitrite in nitric oxide homeostasis: A comparative perspective, Biochimica et Biophysica Acta – Bioenergetics 1787: 841-848.

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14. Förstermann U, Sessa WC (2012) Nitric oxide synthases: Regulation and function, European Heart Journal 33(7): 829-837.

15. Owusu BY, Stapley R, Honavar J, Patel RP (2013) Effects of erythrocyte aging on nitric oxide and nitrite metabolism, Antioxidants & Redox Signaling 19(11): 1198-1208.

16. Littbrand H, Rosendahl E, Lindelöf N, Lundin Olsson L, Gustafson Y, et al. (2006) A high-intensity functional weight-bearing exercise program for older people dependent in activities of daily living and living in residential care facilities: Evaluation of the applicability with focus on cognitive function, Physical Therapy 86(4): 489-498.

17. Cavalheiro LM, Castanheira P, Gonçalves RS, Ferreira PL (2013) Measuring physical functioning in the elderly population: adaptation and validation of the late-life function and disability instrument (LLFDI) to the Portuguese culture, Qual Life Res 22: 61.

18. Harman D (1955) Aging: a theory based on the free radical and radiation chemistry, Unclassified Health and Biology 11(3): 298-300.

19. Ceci R, Beltran Valls MR, Duranti G, Dimauro I, Quaranta F, et al. (2014) Oxidative stress responses to a graded maximal exercise test in older adults following explosive-type resistance training, Redox biology 2: 65-72.

20. Souza HC, De Araújo JE, Martins Pinge MC, Cozza IC, et al. (2009) Nitric oxide synthesis blockade reduced the baroreflex sensitivity in trained rats, Autonomic Neuroscience 150: 38-44.

21. Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO (2007) Physical Activity and Public Health in Older Adults: Recommendation From the American College of Sports Medicine and the American Heart Association, Public Health Common 116: 1094-1105.

22. Palazzetti S, Richard MJ, Favier A, Margaritis I (2003) Overloaded training increases exercise-induced oxidative stress and damage, Canadian Journal of Applied Physiology 28(4): 588-604.

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