Open Access

OBM Integrative and Complementary Medicine

LIDSEN Publishing Inc.

Original Research

Effect of Hydrotherapy and Physical Activity on Uric Acid and Nitric Oxide Concentrations in Serum Samples

Ana Valado [1, 2, 3, †, *], Márcia Morais [1, †], Stephanie Fortes [1], Joana Rosado [4, 5], João Paulo Figueiredo [6], Armando Caseiro [1, 2]

1. Polytechnic of Coimbra, Coimbra Health School, Biomedical Laboratory Sciences, Rua 5 de Outubro - SM Bispo, 3045-043 Coimbra, Portugal; E-Mails: valado@estesc.ipc.pt; marciad.morais1@gmail.com; fannylima97@gmail.com; armandocaseiro@estesc.ipc.pt; ORCID ID: 0000-0002-0157-6648; 0000-0001-7844-0506
2. Laboratory for Applied Health Research (LabinSaúde), Rua 5 de Outubro - SM Bispo, Apartado 7006, 3045-043 Coimbra, Portugal
3. Research Centre for Natural Resources Environment and Society (CERNAS), Polytechnic Institute of Coimbra, Bencanta, 3045-601 Coimbra, Portugal
4. Polytechnic of Coimbra, Coimbra Health School, Physiotherapy, Rua 5 de Outubro - SM Bispo, Apartado 7006, 3045-043 Coimbra, Portugal; E-Mail: joanarosado@caritascoimbra.pt
5. Center Rainha Santa Isabel Cáritas Diocesana de Coimbra, Coimbra, 3030-382, Portugal
6. Polytechnic of Coimbra, Coimbra Health School, Medical, Social and Human Sciences, Rua 5 de Outubro - SM Bispo, Apartado 7006, 3045-043 Coimbra, Portugal; E-Mail: jpfigueiredo@estesc.ipc.pt

† These authors contributed equally to this work.

* **Correspondence:** Ana Valado; E-Mail: valado@estesc.ipc.pt; ORCID ID: 0000-0002-0157-6648

Academic Editor: Pedro Morouco

Special Issue: The Impacts of Physical Activity, Exercise, and Sports on Human Health

OBM Integrative and Complementary Medicine
2024, volume 9, issue 2
doi:10.21926/obm.icm.2402028

Received: November 20, 2023
Accepted: May 16, 2024
Published: May 21, 2024

© 2024 by the author. This is an open access article distributed under the conditions of the Creative Commons by Attribution License, which permits unrestricted use, distribution, and reproduction in any medium or format, provided the original work is correctly cited.

Abstract

Hydrotherapy is highly recommended for older adults because it offers warmth and support, so people with pain or reduced mobility and balance can move and exercise more freely compared to regular physical activity. The high plasma antioxidant capacity of uric acid in neutralizing oxidative species and the importance of nitric oxide (NO) in vasodilation and inflammatory response are relevant in older adults. This work aimed to evaluate hydrotherapy's effect on uric acid and NO concentrations in a population aged ≥ 60 years before and after hydrotherapy. A randomized study with 37 individuals was divided into two groups: the experimental group, which was evaluated at two time points: T0-before treatment and T1-after 15-hydrotherapy sessions, and the control group, which did not practice any exercise. The determinations of uric acid and NO levels in serum were carried out by spectrophotometry. Statistics were carried out with SPSS. The experimental group showed a significant increase in the concentration of uric acid in T1 compared to T0. NO results did not show significant differences between T0 and T1. Regular hydrotherapy increases the concentration of serum uric acid, potentially beneficial in the performance of antioxidant functions, reducing oxidative stress, delaying aging, and improving the well-being of older adults.

Keywords

Hydrotherapy; aging; oxidative stress; uric acid; nitric oxide

1. Introduction

Aging is a natural biological process that consists of the decline in functional capacity associated with changes in mobility, autonomy, balance, and health in older adults. It can contribute to physical disability and, in some situations, promote social isolation [1]. Aging increases the risk of neurodegenerative diseases, diabetes, obesity, and atherosclerosis [2]. This process is guided by molecules and metabolic pathways involved in the general degeneration of physiological functions [3], with oxidative stress described as one of the determinants in the development of diseases in older adults [4].

The exact mechanism of aging caused by oxidative stress is still unclear. The increase in reactive oxygen and nitrogen species (RONS) levels probably leads to cellular senescence, a physiological mechanism that interrupts cell proliferation in response to damage that occurs during replication. Senescent cells acquire an irreversible senescence-associated secretory phenotype involving secretion of soluble factors such as (growth factors, interleukins, and chemokines), insoluble protein components/extracellular matrix, and degradative enzymes such as matrix metalloproteases [5].

Therefore, oxidative stress constitutes a risk factor for aging, and its increase contributes to developing mechanisms that disturb redox balance [6]. The adaptive response to oxidative stress decreases with aging [7], and in redox imbalance, there is the formation of free radicals [8], which are reactive chemical species, with an unpaired electron in the external orbit and synthesized in aerobic processes [9]. The main forms of radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as nitric oxide (NO) and its metabolites [10]. Uncontrolled ROS production is implicated in vascular injury [11], gradually decreasing cellular and tissue function over time [12].

In response to oxidants, antioxidants appear, which can be enzymatic, non-enzymatic, uric acid and albumin [9]. Uric acid, the end product of purine catabolism, is excreted mainly by the kidneys. Xanthine oxidase is responsible for producing uric acid, a potent antioxidant, responsible for approximately 60% of plasma antioxidant capacity and DNA protection in the repair of any nuclear area exposed to oxidation [13]. Uric acid has demonstrated its ability to scavenge reactive radicals resulting from harmful processes such as hemoglobin auto-oxidation or peroxide production by macrophages that protect the erythrocyte membrane from lipid peroxidation [14].

Nitric oxide (NO) is synthesized from the amino acid L-arginine and oxygen by the enzymatic action of nitric oxide synthase (NOS), inducible (iNOS), neuronal (nNOS) and endothelial (eNOS) [9]. NO synthesis is similar, with distribution in various cell types and different activation mechanisms. nNOS and eNOS are expressed in neuronal and endothelial cells, while iNOS is expressed in non-vascular endothelial cells (macrophages, fibroblasts, and hepatocytes). NO is a signaling radical with a short half-life and endogenous production [15], highly diffusible in different biological and molecular pathways: neurotransmission, vasodilation, and response to infections [16]. It also regulates the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, and angiogenesis [17]. An imbalance in NO production is associated with inflammatory and degenerative diseases, and reducing its bioavailability increases the production of ROS [18].

The high disability revealed by older adults can be minimized by implementing exercise programs that improve physical capacity and reduce accumulated stress [1, 19]. Practicing exercises performed in water, such as hydrotherapy, is one of the most recommended due to the low load and mechanical stress induced on joints and supporting muscles. This activity is recommended for rehabilitation and therapeutic procedures and is included in exercise programs to improve muscular fitness in adults and older adults with movement limitations [20]. Particularly in elderly people with disabilities, hydrotherapy reduces pain and increases safety when performing exercises [21]. The healing properties of hydrotherapy are based on mechanical and thermal effects. The prolonged application of heat and pressure exerted by water carries fundamental impulses to stimulate the immune system, reduce stress, strengthen circulation and digestion, stimulate blood flow, and decrease sensitivity to pain [22]. Hydrotherapy offers beneficial benefits in rehabilitating patients with the most diverse pathologies. Water reduces the impact of joint movement, reducing the risk of injury in patients with joint or degenerative diseases; it can be used to assist the movements of weak and paralyzed muscles, as resistance to gain muscle strength; balance and proprioception training is stimulated with instability in the water; the water temperature relaxes muscles, reduces pain and facilitates stretching exercises, helping to improve muscle tone; in water the patient loses fear, facilitating gait and balance training [23, 24].

Hydrotherapy stimulates the production of antioxidants, essential molecules in the aging process. The objective of the present study was to evaluate the concentrations of uric acid and nitric oxide before and after the practice of hydrotherapy applied to a group of older adults.

2. Materials and Methods

2.1 Sample Characterization

The study consisted of 37 participants of both sexes, aged 60 years or older, divided into an experimental group (EG) and a control group (CG). All participants are users of a physiotherapy rehabilitation center in central Portugal. The EG participants were previously evaluated by a physician and instructed to perform hydrotherapy sessions under the guidance of a physiotherapist. Participants only performed hydrotherapy sessions during the study as a physiotherapeutic treatment [25]. All participants started hydrotherapy treatment in the present study. After a medical prescription for hydrotherapy, participants were contacted by the researcher, the project was explained, and only after consent did they join the study and follow the protocol.

For laboratory evaluation of the 27 individuals in the EG, peripheral blood samples were collected at two-time points (T0) before the hydrotherapy sessions and (T1) after 15 sessions at the end of the prescription. The CG participants performed only one venous blood collection. These participants did not practice hydrotherapy.

Age equal to or greater than 60 years and absence of movement limitations were considered inclusion criteria. The impossibility of practicing movements and lack of balance were assumed as exclusion criteria [25].

For the sample estimation regarding the two groups (EG and CG), we took into account the following elements: a confidence level of 95%, for a random error of 5%, a test power of 80% (1-B), and an effect size of 0.80 (Cohen's reference values), requiring 42 participants: 21 for the EG and 21 for the CG [26, 27]. We gathered 27 participants in the EG, but in the CG, there were dropouts, which accounted for only 10 participants. Nevertheless, the participants in the CG were from the same rehabilitation center as the EG and were well-matched (see Results).

2.2 Hydrotherapy Sessions

The treatment consisted of 15 hydrotherapy sessions twice a week, with adequate exercise for 30 min in a therapeutic pool. Each hydrotherapy session had a maximum of 8 participants. All performed similar therapeutic exercises. The intensity was adjusted to the participant's capacity. The hydrotherapy sessions had a sequence of movements composed of three moments: 5 min warm-up to produce neuromuscular activation, 15 min aerobic exercise (exercise intensity was defined at a mean of 60% of their maximum heart rate on the Borg Scale Rating of Perceived Exertion) and 5 min relaxation (calm down, dynamic stretching and normalize respiratory pattern) and critical, always in the presence of a physiotherapist. The Aquatic Exercise Association carried out the exercise plan, which recommends an aquatic exercise protocol for specific populations, implemented in a pool with a temperature adjusted to 33 ± 1˚C and the water depth at the level of the xiphoid process [20].

2.3 Sample Collection and Treatment

All blood collections were performed between 8 am and 10 am. Blood samples were collected from the veins of the participants’ median cubital fossa. Samples were always collected at the hydrotherapy site before or after the last session. Participants were warned not to perform physical activity before harvesting.

In the EG, all participants performed two venous blood collections before the start of treatment, moment (T0), and at the end of the 15 hydrotherapy sessions, moment (T1). The GC performed only one collection. Peripheral blood samples were collected in tubes without anticoagulant and centrifuged at 2000 g for 10 min at 4°C. Then, the serum was separated into the cryotubes and preserved at -80°C.

After the blood collection, in all samples, the parameters under study were evaluated in duplicate, and good laboratory practices were maintained [25].

2.4 Quantification of Uric Acid

The quantification of uric acid in serum samples was performed in the Tokyo Boeki Prestige 24i (Diamond Diagnostic, Japan) automated analyzer, using the Kit LQ UA (Cormay SA Wiosenna 22.05-092, Lomianki, Poland), with the enzymatic colorimetric method of uricase and peroxidase, using spectrophotometry and reading at 546 nm.

2.5 Quantification of Nitric Oxide

As an initial procedure, the samples were deproteinized, which consisted of transferring 200 µL of sample to a molecular filter of 10 kDa, then centrifuged at 4°C for 30 min at 14000 g, using the Heraeus, Labofuge 300 (Thermo Scientific centrifuge, United Kingdom).

The quantified NO in serum samples was quantified using the Total Nitric Oxide and Nitrate/Nitrite kit (R & D Systems, UK & Europe) and following the kit's description. The test evaluated the concentration of nitric oxide based on the enzymatic conversion of nitrate to nitrite by the action of the enzyme nitrate reductase. Nitrites were detected by the Griess reaction with a spectrophotometric reading at 540 nm on the equipment Multiskan GO (Thermo Scientific, Japan).

2.6 Statistical Analysis

The program IMB SPSS Statistics, version 25.0, was used for statistical treatment, applying a descriptive analysis followed by the t-Student test. The unpaired t-test was applied for comparisons between CG and EG, and the paired t-test was used for comparisons within EG at time 0 and time 1. Values are presented as mean ± standard deviation. The differences/correlation between the groups were considered statistically significant for p values < 0.05 with a 95% confidence interval.

The principles of the Declaration of Helsinki were present in the study. It was approved by the Ethics Committee of the Polytechnic Institute of Coimbra (45/2018). All participants were volunteers, provided their sociodemographic information, had information about the project, and signed the informed consent form before participating.

3. Results

3.1 Characterization of Participants

The total number of study participants was divided into an EG formed of 27 individuals with a mean age of 68.3 ± 5.2 years and a CG with 10 individuals with a mean age of 70.5 ± 6.6 years (Table

1). From a biological point of view, the groups were very balanced despite the age being higher and significant (p = 0.018) in CG. Most participants were female and had chronic inflammatory and cardiac diseases, characteristics of the age group under study. All participants manifested age-related comorbidities and were on medication.

Table 1 Patient's sociodemographic characterization.

		Experimental Group n = 27	Control Group n = 10
Age (years)		68.3 ± 5.2	70.5 ± 6.6
Min/Max		60/82	67/89
Sex	Female	n = 24	n = 8
	Male	n = 3	n = 2

Min - minimum; Max - maximum; Results expressed as mean ± standard deviation.

3.2 Quantification of Uric Acid

The concentration of uric acid at the initial moment (T0) in the EG registered an average value of 4.63 ± 1.16 mg/dL, and in the CG, 4.63 ± 1.0 mg/dL, without statistically significant differences (p = 0.824) between the groups. When uric acid levels were reassessed after the hydrotherapy intervention period, in the EG was observed a substantial increase from 4.63 ± 1.16 mg/dL in T0 to 5.02 ± 1.29 mg/dL in T1 (p = 0.003), see Figure 1.

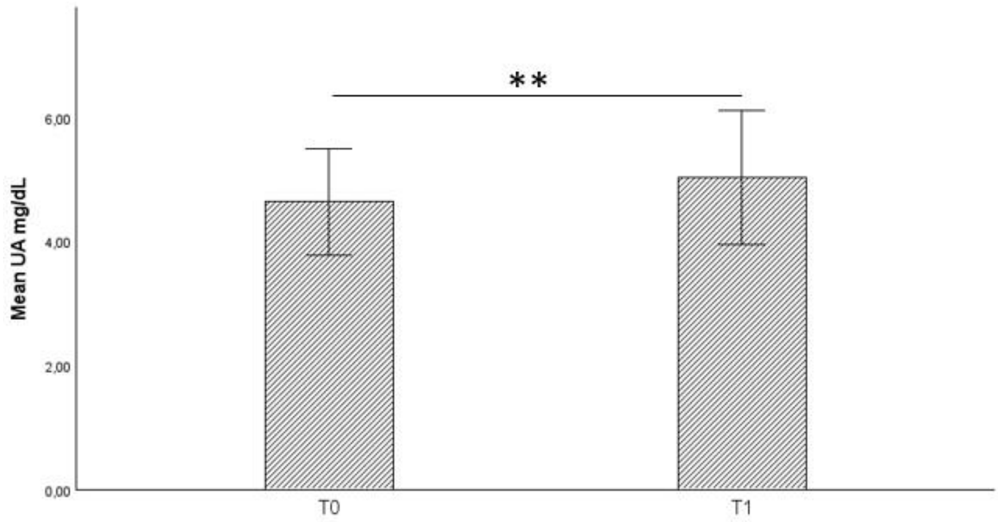

Figure 1 Concentration of uric acid in T0 and T1 in the experimental group. T0: before hydrotherapy treatment; T1: after 15 hydrotherapy sessions. The results are presented in mg/dL; ** p = 0.003.

3.3 Quantification of Nitric Oxide

The concentration of NO presented in the groups in T0 was in the EG 10.63 ± 6.76 µmol/L and the CG 13.78 ± 10.59 µmol/L, showing no statistically significant differences (p = 0.321) between groups. In the EG, when concentrations were compared, T0: 10.63 ± 6.76 µmol/L and T1: 11.94 ± 6.40 µmol/L, there were also no significant differences.

4. Discussion

The present study analyzed the impact of hydrotherapy in relation to the concentrations of uric acid and NO in older adults to understand the relationship between aging and physical exercise. The World Health Organization has revealed that lack of exercise is among the top four causes of mortality worldwide and is one of the leading public health problems [1]. Preserving good health with advancing age is one of the best ways to guarantee the quality of life of a population, particularly in older adults, when there are usually functional deficiencies and a lower degree of autonomy due to the occurrence of degenerative processes [28]. It is essential to invest in strategies that reduce physical inactivity and encourage the increase of physical activity programs to promote health and prevent various age-related diseases such as heart disorders, osteoporosis, and hypertension [1]. According to Martinez-Velilla and collaborators, an individualized and multicompetent intervention, including low-intensity resistance training exercises performed during a short period, significantly benefits older adults' health and can help reversing functional decline, cognitive status and well-being [29]. Felcar et al. recommend hydrotherapy to this population because it takes place in the water, consequently requires less muscle strength, and is a safe practice (always supervised). They also showed that aerobic exercise improves adaptation to oxidative stress, increasing the levels of antioxidants to combat excess oxidative species [30]. Similar observations were made by Gomes et al. in 2017, describing that regular exercise increases the formation of ROS and can induce beneficial adaptations by stimulating the regulation of cellular antioxidant systems and stimulation of oxidative damage repair systems [31]. Exercise can act as a potential non-pharmacological strategy for treating conditions such as hypertension because of its benefits on oxidative stress [32]. In 2016, Korsager and co-authors showed that aerobic exercise proved effective in significantly reducing ROS and the occurrence of these reactive species associated with diseases such as hypertension [32]. Also, Pisoschi and Pop 2015 observed that damage induced by free radicals in oxidative stress contributes to the pathogenesis and pathophysiology of chronic pathologies, such as neurodegenerative, cardiovascular, inflammatory and cancer. Promoting exercise can help combat oxidative stress and consequently reduce the variety of associated diseases [9]. Oxidative stress plays an essential role in forming atherosclerotic lesions by overproduction of ROS. Endothelial and smooth muscle cells produce oxidant substances, leading to the proliferation of immune cells in the arterial wall, the release of cytokines, the induction of inflammatory processes, and additional ROS [33].

Based on the importance of antioxidant markers, especially in the older adult population, in association with the practice of physical exercise, our study consisted at two time points (T0 and T1) that allowed us to evaluate the concentrations of uric acid and NO before and after the practice of hydrotherapy. Uric acid concentration increased after exercise. A study supports these results with 30 healthy adults of both sexes based on laboratory and physical exams to screen kidney and liver changes in gastrointestinal, pulmonary, or oncological diseases. The participants performed activities such as football, basketball, swimming, and cycling and showed increased uric acid afterward. In these modalities, the concentration reached its maximum value one hour after the end of the exercise [34].

Regarding NO, Guizoni et al., in a study with mice, reported that aerobic exercise training has beneficial vascular effects and reduces oxidative stress by increasing the bioavailability of NO. The information collected in this study suggested exercise as a potential therapy for preventing certain diseases based on the increase in NO bioavailability [35]. Another study [36] argued that physical exercise is a physiological inducer of NO production since the increase in blood flow and blood vessel distortion induced by the muscular contraction stimulates eNOS and nNOS [36]. The quantification of NO did not show differences after hydrotherapy. Considering the age of the participants, this result can be explained by an insufficient adaptive response to oxidative stress [7]. Also, being a population where the inflammatory process and degeneration are present, an imbalance in NO production is compatible, as well as the reduction of its bioavailability by peroxynitrite generation [18]. According to a study carried out in 2022, the regular practice of hydrotherapy seems to work as a stimulus to the antioxidant enzyme system, with increased activity of the enzyme glutathione peroxidase and glutathione reductase, critical in the aging process, reducing oxidant species [25]. Contrary to what occurs after intense exercise sessions, chronic and moderate exercise is associated with reduced levels of oxidative stress markers and increased enzymatic and non-enzymatic antioxidant capacity in young and older adults [37].

In the recent past, aquatic therapy has been a facilitator for older adults, taking into account the particularity of water as support, resistance, and assistance [38]. The aquatic exercise program benefits muscle coordination, flexibility, and increased exercise capacity [39]. For older adults, aquatic physical therapy is safe and can increase physical activity with reduced risk of injuries and falls during activities [39, 40]. Aquatic therapy appears to be a safe and suitable exercise as training to reduce future falls in the older adult population. However, more evidence is needed to support the benefits [41]. According to Turner et al. in 2018, hydrotherapy is an important training and rehabilitation method for improving the older adults' postural control, mobility and well-being, especially in groups. The training program offers exercises of different levels and increasing complexity for better adaptation and progression of the individual. Thus, hydrotherapy offers a successful alternative method of balance training in preventing falls and injuries in the geriatric population [40].

The authors recognize some limitations of the study, such as the small number of participants in the hydrotherapy sessions, which prevented the organization into subgroups associating comorbidities, age and medication. Alternatively, a single group was formed. Also the fact that the average age was higher in the CG, but as the groups from a biological point of view were very similar, there does not seem to be any reason to suppose that this affected the comparisons between CG and EG for uric acid and nitric oxide, because the participants were well matched in other respects. It should be noted that the relevant results of the study focused on comparisons within the EG between T0 and T1. Another important factor is the time needed for the hydrotherapy intervention, which should be extended. However, with the present study it was possible to demonstrate that the practice of exercises, especially hydrotherapy exercises, is favorable in adapting antioxidant mechanisms. The beneficial characteristics that hydrotherapy presents in terms of muscle relaxation and temperature facilitate movement. It is recommended that the study be replicated, allowing us to reinforce the lack of studies in this modality, which seems to us to be very promising for older adults.

The authors acknowledge the added value of replicating the present study while maintaining the hydrotherapy exercise plan and increasing the number of participants. The beneficial characteristics that hydrotherapy presents in terms of muscle relaxation and temperature facilitate movement. They also reinforce the lack of studies in this modality, which seems promising.

5. Conclusions

The study suggests that the regular practice of hydrotherapy increases the concentration of serum uric acid, potentially beneficial in the performance of antioxidant functions reducing oxidative stress. The relationship between exercise and oxidative stress remains highly complex, depending on the mode, intensity, and duration of training and individual susceptibility to injury.

Acknowledgments

The authors would like to thank all the participants of the study. The author AV thank the Portuguese Foundation for Science and Technology (FCT) for the financial support to the Research Centre for Natural Resources, Environment and Society - CERNAS (UIDB/00681/2020; DOI: 10.54499/UIDP/00681/2020).

Author Contributions

Conception and design of the study, A.V., M.M.; performed data analysis and interpretation, A.V., M.M. Participants selection, sample collection and laboratory treatment A.V., M.M., S.F., A.C., support in hydrotherapy, J.R. and statistical analysis, J.P.F. writing-review and editing, A.V., M.M., A.C. All authors have read and agreed to the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Scolese M, Abi Rached CD, De Liberal MM. The physical exercise as a strategy of reduce costs with medicine for aged. Int J Prev Med. 2019; 10: 153.
2. Panfoli I, Candiano G, Malova M, De Angelis L, Cardiello V, Buonocore G, et al. Oxidative stress as a primary risk factor for brain damage in preterm newborns. Front Pediatr. 2018; 6: 369.
3. Gradinaru D, Margina D, Borsa C, Ionescu C, Ilie M, Costache M, et al. Adiponectin: Possible link between metabolic stress and oxidative stress in the elderly. Aging Clin Exp Res. 2017; 29: 621-629.
4. Bullone M, Lavoie JP. The contribution of oxidative stress and inflamm-aging in human and equine asthma. Int J Mol Sci. 2017; 18: 2612.
5. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018; 13: 757-772.
6. Sánchez-Flores M, Marcos-Pérez D, Costa S, Teixeira JP, Bonassi S, Pásaro E, et al. Oxidative stress, genomic features and DNA repair in frail elderly: A systematic review. Ageing Res Rev. 2017; 37: 1-15.
7. Zhang H, Davies KJ, Forman HJ. Oxidative stress response and Nrf2 signaling in aging. Free Radic Biol Med. 2015; 88: 314-336.
8. Sulaiman MA, Al-Farsi YM, Al-Khaduri MM, Saleh J, Waly MI. Polycystic ovarian syndrome is linked to increased oxidative stress in Omani women. Int J Womens Health. 2018; 10: 763-771.

9. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015; 97: 55-74.
10. Tan BL, Norhaizan ME, Liew WP. Nutrients and oxidative stress: Friend or foe? Oxid Med Cell Longev. 2018; 2018: 9719584.
11. Kattoor AJ, Pothineni NV, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. Curr Atheroscler Rep. 2017; 19: 42.
12. Liu T, Zhong S, Liao X, Chen J, He T, Lai S, et al. A meta-analysis of oxidative stress markers in depression. PLoS One. 2015; 10: e0138904.
13. Roumeliotis S, Roumeliotis A, Dounousi E, Eleftheriadis T, Liakopoulos V. Dietary antioxidant supplements and uric acid in chronic kidney disease: A review. Nutrients. 2019; 11: 1911.
14. Mikami T, Sorimachi M. Uric acid contributes greatly to hepatic antioxidant capacity besides protein. Physiol Res. 2017; 66: 1001-1007.
15. Alimoradi H, Greish K, Gamble AB, Giles GI. Controlled delivery of nitric oxide for cancer therapy. Pharm Nanotechnol. 2019; 7: 279-303.
16. Fionda C, Abruzzese MP, Santoni A, Cippitelli M. Immunoregulatory and effector activities of nitric oxide and reactive nitrogen species in cancer. Curr Med Chem. 2016; 23: 2618-2636.
17. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: A key modulator in neurodegenerative diseases. Molecules. 2019; 24: 1583.
18. Deng Y, Jia F, Chen S, Shen Z, Jin Q, Fu G, et al. Nitric oxide as an all-rounder for enhanced photodynamic therapy: Hypoxia relief, glutathione depletion and reactive nitrogen species generation. Biomaterials. 2018; 187: 55-65.
19. Sica A, Vitiello P, Ronchi A, Casale B, Calogero A, Sagnelli E, et al. Primary cutaneous anaplastic large cell lymphoma (pcALCL) in the elderly and the importance of sport activity training. Int J Environ Res Public Health. 2020; 17: 839.
20. Bocalini DS, Bergamin M, Evangelista AL, Rica RL, Junior FL, Junior AF, et al. Post-exercise hypotension and heart rate variability response after water-and land-ergometry exercise in hypertensive patients. PLoS One. 2017; 12: e0180216.
21. Simas V, Hing W, Pope R, Climstein M. Effects of water-based exercise on bone health of middle-aged and older adults: A systematic review and meta-analysis. Open Access J Sports Med. 2017; 8: 39-60.
22. Comella NF, Matilla MF, Cuesta JA. Have complementary therapies demonstrated effectiveness in rheumatoid arthritis? Reumatol Clin. 2016; 12: 151-157.
23. Almassmoum SM, Balahmar EA, Almutairi ST, Albuainain G, Ahmad R, Naqvi AA. Current clinical status of hydrotherapy; an evidence based retrospective six-years (2012-2017) systemic review. Bali Med J. 2018; 7: 578-586.
24. Torres-Ronda L, i del Alcázar XS. The properties of water and their applications for training. J Hum Kinet. 2014; 44: 237-248.
25. Valado A, Fortes S, Morais M, Barreira R, Figueiredo JP, Caseiro A. Impact of hydrotherapy on antioxidant enzyme activity in an elderly population. Geriatrics. 2022; 7: 64.
26. Borenstein M, Rothstein H, Cohen J. IBM SPSS sample power version 3. Chicago, IL: SPSS Inc.; 2008.
27. Carneiro AV. Cálculo da dimensão da amostra em estudos clínicos: Princípios metodológicos básicos. Rev Port Cardiol. 2003; 22: 1513-1521.

28. Oliveira AL, Silva JT, Lima MP. Aging and health: Self-efficacy for self-direction in health scale. Rev Saude Publica. 2016; 50: 40.
29. Martínez-Velilla N, Casas-Herrero A, Zambom-Ferraresi F, de Asteasu ML, Lucia A, Galbete A, et al. Effect of exercise intervention on functional decline in very elderly patients during acute hospitalization: A randomized clinical trial. JAMA Intern Med. 2019; 179: 28-36.
30. Felcar JM, Probst VS, De Carvalho DR, Merli MF, Mesquita R, Vidotto LS, et al. Effects of exercise training in water and on land in patients with COPD: A randomised clinical trial. Physiotherapy. 2018; 104: 408-416.
31. Gomes MJ, Martinez PF, Pagan LU, Damatto RL, Cezar MD, Lima AR, et al. Skeletal muscle aging: Influence of oxidative stress and physical exercise. Oncotarget. 2017; 8: 20428-20440.
32. Larsen MK, Matchkov VV. Hypertension and physical exercise: The role of oxidative stress. Medicina. 2016; 52: 19-27.
33. Poznyak AV, Grechko AV, Orekhova VA, Chegodaev YS, Wu WK, Orekhov AN. Oxidative stress and antioxidants in atherosclerosis development and treatment. Biology. 2020; 9: 60.
34. Kandar R, Stramova X, Drábková PE, Krenkova J. A monitoring of allantoin, uric acid, and malondialdehyde levels in plasma and erythrocytes after ten minutes of running activity. Physiol Res. 2014; 63: 753-762.
35. Guizoni DM, Dorighello GG, Oliveira HC, Delbin MA, Krieger MH, Davel AP. Aerobic exercise training protects against endothelial dysfunction by increasing nitric oxide and hydrogen peroxide production in LDL receptor-deficient mice. J Transl Med. 2016; 14: 213.
36. Galdino GS, Duarte ID, Perez AC. Central release of nitric oxide mediates antinociception induced by aerobic exercise. Braz J Med Biol Res. 2014; 48: 790-797.
37. Joseph AM, Adhihetty PJ, Leeuwenburgh C. Beneficial effects of exercise on age-related mitochondrial dysfunction and oxidative stress in skeletal muscle. J Physiol. 2016; 594: 5105-5123.
38. Carayannopoulos AG, Han A, Burdenko IN. The benefits of combining water and land-based therapy. J Exerc Rehabil. 2020; 16: 20-26.
39. Hale LA, Waters D, Herbison P. A randomized controlled trial to investigate the effects of water-based exercise to improve falls risk and physical function in older adults with lower-extremity osteoarthritis. Arch Phys Med Rehabil. 2012; 93: 27-34.
40. Turner AJ, Chander H, Knight AC. Falls in geriatric populations and hydrotherapy as an intervention: A brief review. Geriatrics. 2018; 3: 71.
41. Shariat A, Ghayour Najafabadi M, Ghannadi S, Nakhostin-Ansari A, Hakakzadeh A, Shaw BS, et al. Effects of aquatic therapy on balance in older adults: A systematic review and meta-analysis. Eur Geriatr Med. 2022; 13: 381-393.