DOI: 10.4025/jphyseduc.v30i1.3044

Original Article

VASCULAR FUNCTION AND NITRITE LEVELS IN ELDERLY WOMEN BEFORE AND AFTER HYDROGYMNASTICS EXERCISES

FUNÇÃO VASCULAR E NÍVEL DE NITRITO EM IDOSAS ANTES E APÓS PROGRAMA DE HIDROGINÁSTICA

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RESUMO
A prática regular de exercício físico pode reduzir o estresse oxidativo e os fatores de risco para doenças cardiovasculares. O objetivo deste estudo foi avaliar nível de nitrito sanguíneo, alterações na resistividade de artérias cerebrais e no colesterol de idosas submetidas a treinamento de hidroginástica. Quarenta mulheres formaram a amostra, com idade de 69.21 ±5.27 anos, randomizadas e separadas em grupo controle e de intervenção. Níveis de Nitrito foram analisados pela reação de Griess, resistência arterial observada em ultrassom Doppler e o colesterol foi analisado pelo método de colorimetria enzimática. O programa de exercício físico durou 16 semanas. Resultou em incremento no NO_2 (p=0.003), redução na resistividade da artéria carótida comum direita (p=0.024), CT (p=0.003) e LDL (p=0.001) no grupo de intervenção. Foi encontrada correlação negativa entre níveis de nitrito e resistividade arterial, assim como entre níveis de nitrito e de colesterol em pós-teste. Sugere-se que o programa de hidroginástica promoveu modificações favoráveis nos níveis de nitrito, na resistividade arterial e no colesterol de mulheres idosas.
Palavras-chave: Oxido nítrico. Dislipidemias. Exercício. Serviços de saúde para idosos

ABSTRACT
The regular practice of physical exercise may reduce the oxidative stress and the risk factors for cardiovascular diseases. This study aims to evaluate serum levels of nitrite and changes in cerebral arterial resistance and cholesterol in elderly women submitted to hydrogymnastic training. The sample was composed of 40 women with overage age of 69.21 ±5.27 years old, separated randomly into an intervention and a control group. Nitrite concentration was analyzed through Griess reaction. The arterial resistivity was observed by Doppler ultrasound, and the cholesterol was determined using colorimetric enzymatic method. The water exercise program lasted 16 weeks. It was seen an increase in NO_2 (p=0.003), reduction in right common carotid artery resistance (p=0.024), TC (p=0.003) and LDL (p=0.001) cholesterol in the WG. There was a negative correlation between NO_3 and RVA, r = -0.221 and p=0.049 and between NO_3 and CT (r = -0,269 and p=0,016) after intervention. The hydrogynmnastic program promoted favorable changes in nitrite levels, arterial resistivity and cholesterol in the elderly women.
Keywords: Nitric oxide. Dyslipidemias. Physical fitness. Health services for the aged.

Introduction

The practice of regular physical exercise may reduce the oxidative stress and the risk factors for cardiovascular diseases. These benefits may be achieved in elderly population, depending on the type of exercise performed, duration, intensity and muscle mass involved[1]. In addition, researches indicate that the mortality rate is inversely proportional to the fitness level of elderly women[2,3].

Several studies have been done on land and in water using aerobic exercise and resistance exercise separately, evaluating free radicals and antioxidants getting inconclusive results, but demonstrating improviment in quality of life in general[4-7]. Interventions involving aerobic exercise on a cycle ergometer and treadmill respectively demonstrated improvements in lipid profile and increase of the levels of NO (nitric oxide)[7] as well as resistance exercises on land presented, besides the increasing release of NO, venous return facilitated in elderly women[8].

However, some studies involving intervention with resisted aquatic exercises showed no improvement in vascular remodeling[9], but improvements were seen in the lipid profile[4,5,10]; as well as it happened afterwards in aquatic training program involving games[11].

Aquatic exercise have a positive impact on human aging due to the resulting massaging effect of water turbulence, facilitation of the body movements, relieve of the impact on joints and improvement of venous return. Water, itself, provides a load that varies according to the movement's speed and amplitude. According to the time of individual adaptation to the aquatic environment, the possibility of movement also increases[4].

Few studies were found involving concurrent aquatic exercise and blood markers to evaluate changes in the health of elderly. Thus, the present study proposed a moderate intensity concurrent aquatic exercise program (aerobic and resistance training), for 16 weeks, with the following goals: 1) Determinate changes caused in serum levels of nitrite (NO_2); 2) Verify changes in cerebral arterial resistance index and in the cholesterol; 3) Determinate the correlation between serum levels of nitrite and cerebral arterial resistance and cholesterol.

Methods

Participants

This was a randomized controlled trial**.** Three hundred elderly women, enrolled in the women's health assistance program in the city of Teresina, Brazil, were invited to participate. In this program they received orientation in monthly meetings with social workers and nursing staff on health care for prevention and control of diabetes and hypertension. The following inclusion criteria were adopted: age between 60 and 80 years, independent in activities of daily living (ADLs), medically able to take part in the intervention and testing protocols, and not having participated in regular systematized physical activity for at least six months. Subjects with any form of transmittable or uncontrolled disease or insulin-dependent, hypertension, those unable to perform functional autonomy tests or undergoing hormonal replacement therapy were excluded from the study.

Randomization occurred by simple random among women who were willing to participate in the study, according to the flow diagram (Figure 1). Registration of Control trial was - NCT01642654. The 40 eligible women were randomly divided into an experimental group (WG) n = 20, submitted to an aquatic exercise program, and a control group (CG) n = 20. The CG was instructed not to perform any regular physical activity during the study period. Figure 1 shows the study design flow chart.

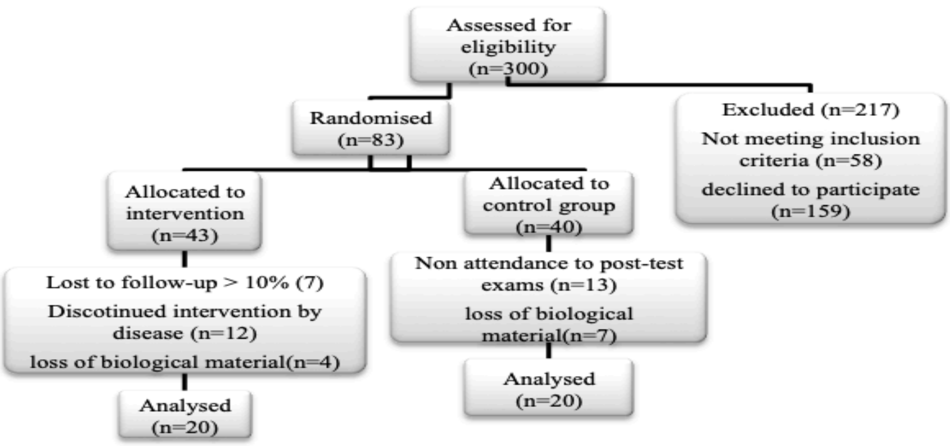

Figure 1. Flow diagram of the progress though the phases of a parallel-randomized trial of two groups

Source: The authors

The research protocol was approved by the Institutional Research Ethics Committee (nº 095/06) and all participants gave their written informed consent.

The power of the sample was calculated from post-hoc analysis of twenty volunteers in each group using MedCal ® software. To obtain this value we considered post-treatment means and standard deviations for both groups, considering anα of 0.05 for all variables. The resulting power was NO_2 = 89%; RCCA (Resistance of the right common carotid artery) = 99%; RVA (Resistance of the right vertebral artery) = 95%; TC (Total cholesterol) = 90%; and LDL (Low Density Lipoprotein) = 99%.

Procedures

Body mass and height were assessed on an anthropometric scale equipped with a stadiometer (Filizola, Brazil); waist and hip circumference were measured with a metal measuring tape (Sanny, Brazil) and blood pressure and resting heart rate with a blood pressure monitor (BP A100 Microlife-China). The sample was assessed before and after the 16-week intervention.

Blood collection and cholesterol

After 12 hours, the cholesterol was evaluated via antecubital blood collection, from which 10 ml was extracted, 5ml separated into heparinized vacutainer tubes to analyze nitric oxide, and 5ml into glass tubes for immediate specialized biochemical analysis using a semi-automated BIOPLUS 2000 analyzer, applying the colorimetric enzymatic method and Labtest kit. Cholesterol reference values were analyzed according to Brazilian Guideline IV on dyslipidemias and atherosclerosis prevention guidelines of the Atherosclerosis Department of the Brazilian Cardiology Society[12]. TC, high density lipoprotein (HDL) and LDL were also assessed.

Measurement of NO from plasma nitrite

For NO analysis, serum was separated from the erythrocyte mass. The material was centrifuged in a SIGMA 4k15 centrifuge at 3500 rpm and 18° for 10 minutes (LANEX laboratory– UFPI); the serum was then pipetted and frozen at -80°C for evaluation after retesting (16 weeks). NO production was determined based on NO_3 in the supernatant of cultured cells, an indirect measurement of NO synthesis.

It was collected 100µL of serum from participants and added 50 µL of Griess reagent (1,5% sulfanilamide in 5% of phosphoric acid (H_3PO_4), 0.1% N-(1-naphthyl) ethylenediamine in water)[13]. After subjects rested for 15 minutes at ambient temperature, reading was conducted in an ELISA reader (BIO-RAD, Model 680) with a 540 nm filter. Nitrate formed from NO oxidation must be quantitatively converted to nitrite for the analysis. Enzymatic reduction of nitrate to nitrite can be carried out using nitrate reductase. Methods for in-line reduction of nitrate to nitrite for automated nitrate analysis have been reported. Nitrite concentration was calculated using mean values of a standard sodium nitrite curve ($NaNO_2$), and data were expressed in µMol of nitrite/mL. The measurements and standard curve were performed in duplicate[14].

Cerebral artery assessment

Carotid and vertebral arteries were assessed by Doppler ultrasound, with GE Logiq 5 equipment and multi-frequency linear transducer (Milwaukee – UEA). Parameters used were evaluation of peak systolic velocity and end-diastolic velocity of the common carotid and left and right internal carotids, with the angle of insonation corrected to 60°. The resistivity index of arteries was calculated, resulting from the following mathematical formula, expressed in centimeters per second (cm/s): Resistivity index = Peak systolic velocity – diastolic velocity / Peak systolic velocity[15]. For the Doppler examination, the elderly were placed in the dorsal decubitus position. The measuring apparatus was positioned over the arteries to assess the neck region, and record degrees of arterial obstruction or hemodynamic compromise.

Intervention

The WG was submitted to a 16-week water exercise program, with combined aerobic and resistance exercises in three weekly sessions. Water temperature was approximately 30º C with an average ambient temperature of 34°C. The depth of the pool ranged between 1.20 and 1.40 meters.

The fifty-minute exercise program began with a warm of 5 minutes warm-up: stretching exercises with dynamic and static stretches while moving, small jumps with varied arm and leg movements; aerobic exercises (15 minutes) controlled by the Borg perceived exertion scale[16] between 4 and 7: different types of running, with or without arm movement; exercises using ski movements with anterior-posterior sliding, in adduction and abduction, jumping with legs extended and flexed; resistance exercises (25 minutes) were controlled by the Omni-Res scale[17] between 5 and 7: alternating body segments between upper and lower limbs and torso, with bending movements, extensions, abduction, adduction and rotations in the sagittal and frontal planes, executed on the spot or when moving. Each exercise, consisting of two series and alternating body segments, lasted 90 seconds, ending with stretching and relaxation (5 minutes): static stretching and muscle loosening; breathing and self-massage exercises.

During the first three weeks of the program, additional loads were not used and an adjustment was made to the subjective perceived exertion scales. The subjects' own body weight and that of the water, as well as the range and execution speed of exercises, were sufficient for successful adaptation to the water environment. From the third week onwards, low-density rubber materials were used (polyethylene co-vinyl acetate) in three different sizes, distributed according to loads tolerated by each participant. Exercise intensity was increased, with duration decreasing to one minute. Continuous encouragement was given to improve execution and strength applied to each activity in order to maintain moderate intensity in perceived exertion.

Statistical Analysis

Data were analyzed with the Predictive Analytics Software Program (PASW Statistics 18.0) and presented as mean and standard deviation. The sample exhibited normality and homogeneity of variance according to Shapiro-Wilk and Levene tests, respectively. Repeated measures analysis of variance (ANOVA) was applied to the factors group (WG and CG) and time (pre and post-test) for intra and intergroup comparisons of the variables cholesterol, nitric oxide level and arterial resistivity index. This was followed by Tukey's post-hoc test and Pearson's correlation was applied to analyze associations between study variables. A statistical significance value of $p<0.05$ was established for all tests.

Results

Sample characteristics, divided into an intervention group and control group, and are shown in Table 1.

Table 1. Sample characteristics divided into an intervention group and control group

Variables	CG (n=20)	WG (n=20)	t-test
Age (years)	69.53±5.33	68.90±5.22	0.707
Body mass (kg)	67.51±10.36	64.89±6.74	0.344
Height (m)	1.51±0.04	1.50±0.04	0.275
BMI (kg/m^2)	24.15±3.72	28.74±3.00	0.702
Waist-hip ratio	0.83 ± 0.03	0.81±0.04	0.131
Systolic blood pressure (mmHg)	137.83±10.33	137.95±15.30	0.977
Diastolic blood pressure (mmHg)	81.46±9.85	76.33±7.61	0.072
Resting heart rate (bpm)	74.07±9.35	76.61±8.57	0.375
Nitrite (µMol of nitrite/Ml)	10.55±3.15	8.97±2.61	0.542
RCCA (cm/s)	0.67±0.05	0.65±0.03	0.614
RVA (cm/s)	0.66±0.05	0.64±0.04	0.294
Total cholesterol (md/dL)	217.50±32.37	219.85±42.06	0.385
HDL-cholesterol (mg/dL)	43.30±14.79	45.05±12.08	0.461
LDL-cholesterol (mg/dL)	136.24±35.79	132.21±34.51	0.468

Note: BMI - body mass index; RCCA - Resistance of the right common carotid artery; RVA - right vertebral artery resistance; HDL-cholesterol - high-density lipoprotein; LDL - low-density lipoprotein. Independent Samples
Source: The authors

There was a significant increase in the level NO_2 after the intervention, with a 33% increase in the WG and a decrease of 7.2 µMol nitrite/mL in the CG, p= 0.023 and WG increased pre to post-test, p=0.003 (Figure 2).

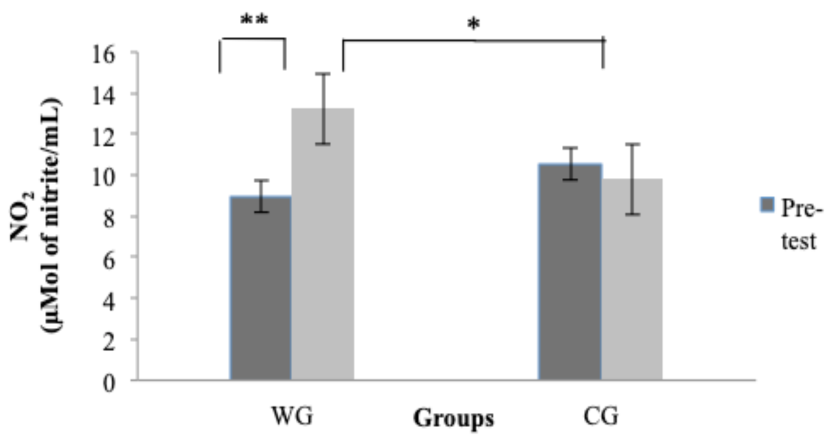

Figure 2. Nitrite level in plasma (NO_2) before and after 16 weeks of water exercise
Note: Data are expressed as mean and standard deviation. Water group (WG); Control group (CG). ** P < 0.01, WG comparison pre and post-test. * p<0.05, WG comparison with CG post-test
Source: The authors

A reduction of 6% was recorded for RCCA resistance in the WG after intervention, as well as a significant increase between WG pre and post-test, p=0.024 and between WG and CG post-test, p=0.000 (Figure 3).

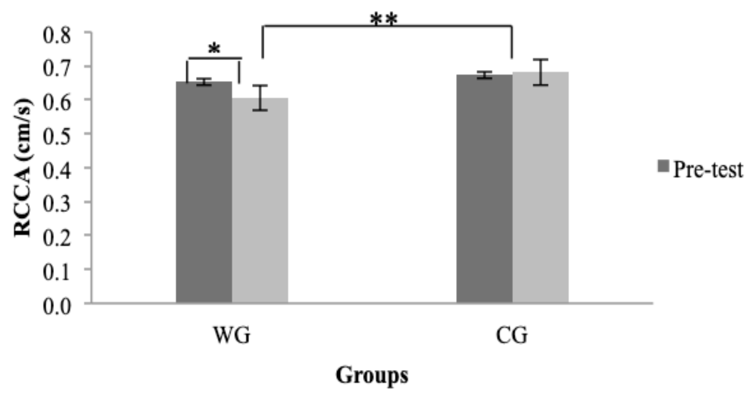

Figure 3. Resistance of the right common carotid artery (RCCA) before and after 16 weeks of aquatic exercise

Note: Data are expressed as mean and standard deviation. Water Group (WG); Control group (CG). *P < 0.05, WG comparison pre and post-test. ** p<0.01, comparison between WG and CG post-test

Source: Authors

The WG showed no significant reduction in RVA after intervention, p=0.093. However, a significant difference reduction was observed between the WG and CG after intervention (Figure 4).

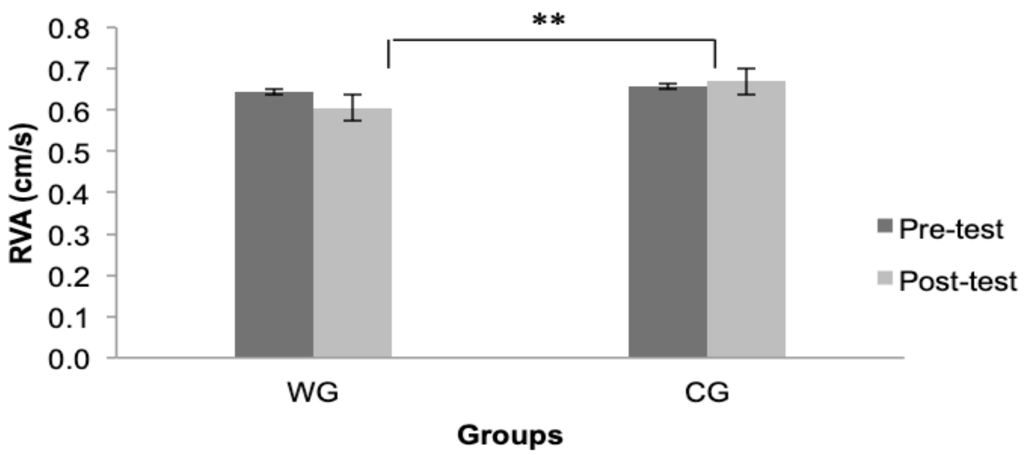

Figure 4. Resistance of the right vertebral artery (RVA) before and after 16 weeks of a water training program

Note: Data are expressed as mean and standard deviation. Water group (WG); Control group (CG). **P < 0.001, WG compared to CG post-test

Source: Authors

Atheromatous plaques compromising between 20 and 36% of vascular lumens were detected in carotid and vertebral arteries in 80% of the sample, all calcified with no risk of mobilization.

Negative correlation was found between RVA and NO_2 (r = -0.221 and p=0.049) and between NO_2 and CT (r = -0,269 and p=0,016) after intervention.

There was a significant reduction in cholesterol levels after intervention, where TC of the WG decreased from 180.35 ± 27.59 md/dL, p=0.003, HDL increased to 49.70 ± 10.64 md/dL, p=0.594 and LDL fell to 91.91± 23.06 md/dL, p=0.001.TC of the CG rose to 227.40 ± 30.89 md/dL, p=0.803.

Discussion

The main results of this study were: The effectiveness of aquatic exercise program proposed was evidenced by the significant increase in plasm NO_2, as well as the reduce in arterial resistance, total cholesterol and LDL levels in elderly women after 16 weeks of intervention.

The proposed exercise program may have influenced the increase in plasma nitrite level in elderly women significantly after 16 weeks of intervention. It has been a difficulty in comparing these results with the results of other studies, because it was found, in the majority of the literature studied, researches involving people with cardiovascular diseases[6,18], nitric oxide measured in the urine[8,19] and aerobic[6,18] and resistive exercises[5,8] applied separately, whereas our study population was considered healthy, the evaluation of NO was through the blood and that the proposed exercise involved in the same session aerobic and resistive exercises.

Few studies were found evaluating the plasma NO_2 alone[6,18], as in our study. In the two reported studies were analyzed NO_3 (nitrate) and NO_2 (nitrite) together (NOx^-) in hypertensive elderly, postmenopausal, after intervention with aerobic exercise on a cycle ergometer, not water. It was detected an of increase 15-35% after three months for high-intensity exercise[6], and an increase of 60% after six months at moderate intensity[18].

In a study of aquatic exercise intervention was observed significant excreted NO_2 increment, after three weeks of low intensity program for male population in the rehabilitation of coronary heart disease when compared to land drills[19] and in another study using population same characteristic applied resistance exercises of the lower limbs (leg extension) at moderate intensity for 12 weeks and also noted an increase of NO_2 and NO_3[20]. However, one should take into account that older men group mentioned in the above studies may not have been affected by hormonal deficits, giving them a physiological advantage over women in the maintenance of vascular tone especially when physically adhere to a lifestyle active.

Best hemodynamic changes were observed analyzing a plasma antioxidant enzyme in older women undergoing resistance training and moderate intensity for four weeks, and increased oxidative stress in a group that engaged in activities with varied exercises. According to the literature, the higher the oxidative stress, the lower the bioavailability of nitric oxide[5,9].

In the present study, there was an increase in the levels of NO_2, a reduction in oxidative stress suggested by arterial resistance and maintained the reduction of cholesterol levels assuming, these results observed in other studies. For this difference in the results, we added the fact that aquatic exercises were scheduled considered less stressful and moderate controlled by scales of perceived exertion[16,17] for all program sessions.

It was worked, in relation to resistance exercise, the method by alternating body segments to avoid an increase of the oxidative stress which may compromise the vascular resistance leading to endothelial dysfunction[21]. Although we found no intervention study with combined exercises in water that has assessed the same blood markers than ours, better hemodynamic adaptation was observed after resistance exercise in water applied for twelve weeks in healthy men[11]. Although there are controversies arguing that interventions with exercise in water and on land have shown similar hemodynamic responses[22,23].

The increased bioavailability of NO_2 reduction in plasma and in blood resistance observed in this study suggests relaxation of smooth muscle, maintained vasodilation and regulation of blood flow. Since these preventive factors that avoid the formation of atheromatous plaques in the vessel lumen, which was observed in RACCD and ADL after intervention[24]. This grouping plates so that atherosclerosis is the pathological degeneration of blood vessels in the middle layer consists of plasma lipids, smooth muscle cells, among other substances. The instability of atherosclerotic plaques leaves people susceptible to transient ischemic attacks and stroke[25].

In this study, small plates of calcified atheroma were found in the carotid and vertebral arteries of older and do not represent risk for cerebral arteries with obstruction of up to 70% of the vessel lumen are asymptomatic and other arteries assume their functions partially, maintaining blood flow2[26]. Therefore elderly were released to participate in the study without risk, as the participation in regular program of physical exercise reduces hemodynamic risk factors, increasing blood flow, favoring the shear stress on the circulatory system by stimulating the bioavailability of NO to the plates Existing were not increased. Some of these improvements have been suggested in our results, it had increased NO_2, maintenance of blood pressure and a reduction in cerebral vascular resistance. In addition, other results observed in our study refers to the negative correlations between NO_2, RVA and TC levels, which can be explained by the improvement in endothelial function of the elderly, for reduction in TC and RVA then correlated with the increase in NO_2 levels.

Similar correlations were found in the literature, but are supported by studies[10,23] that suggest that lowering cholesterol levels by promoting reduction of cardiovascular risk factors, preventing atherosclerosis, where large muscles are exercised as a way to combat aging and keep the arterial vascular function in healthy adults and elderly people[10] and in patients with coronary artery disease after practice exercises in the water and on land[24]. However, other authors did not observe a direct relationship between these variables[27], stating that the reduced arteriocoronarians risk factors are not measured only by adherence to exercise. Exercise is appointed as primary and secondary protective factor because of their possible beneficial effects on vascular function in the long term; But in the short term eight weeks, for example, reduction in risk factors were observed after resistance exercise program for individuals between 50 and 60 years of age[27].

In other studies with similar results to ours, older women were subjected to exercises in water, and observed positive hemodynamic adaptations to maintain desirable cholesterol levels and blood pressure regulation[28].

The results will allow better planning of water exercise programs for postmenopausal women including sessions of aerobic and resistance exercises together at moderate intensity which increase the bioavailability of NO, reduced cerebral blood resistivity and regulation of lipid profile.

The continuation of this study may overcome its limitations including avaliaton of other types of physical exercises, the elderlies depression level, education and marital status.

Conclusion

The aquatic exercise program proposed at 16 weeks, at moderate intensity, combining aerobic and muscular endurance exercise promoted positive effects on nitrite bioavailability in the blood and decrease in arterial resistance, cholesterol levels and blood pressure in older sedentary women. The correlation between Nitrite level in plasma, Right vertebral artery and total cholesterol leads us to conclude that endothelial function has improved.

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Received on May, 21, 2018.
Reviewed on Sep, 11, 2018.
Accepted on, Nov, 25, 2018.

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