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Original Article

The Effect of Resistance Training on Plasma Hydrogen Peroxide Level in Older Women

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A B S T R A C T

Introduction: The prevalence of chronic diseases increases with age. Increased production of reactive oxygen species involves in the pathogenesis of cardiovascular diseases such as coronary atherosclerosis, hypertension, diabetic vascular complications, and heart failure. The present study aimed to explore the effects of resistance training on plasma hydrogen peroxide (H_2O_2) level of ageing women.

Methods: Twenty-four postmenopausal women (mean age = 67.37 ± 6.02, height = 153.02 ± 8.12, weight = 65.78 ± 12.03, body mass index = 26.87 ± 4.16, body fat percent = 18.61 ± 3.65, and waist-to-hip ratio (WHR) = 0.92 ± 0.4) were purposefully chosen and randomly divided into control and experimental groups each consisted of 12 subjects. Experimental group did resistance training for eight weeks as follows: three sessions per week with 40% to 65% intensity of a maximum repetition and 5% overload after each 6 sessions. Before and after 8 weeks of training, resting levels of H_2O_2 was measured and recorded. Data were analyzed by paired- samples *t*-test.

Results: A statistically significant decrease observed in plasma H_2O_2 level ($p = 0.041$) and also weight ($p = 0.048$), body fat percent ($p = 0.001$), WHR ($p = 0.037$), resting- heart- rate ($p = 0.021$), systolic blood pressure ($p = 0.006$) and diastolic blood pressure ($p = 0.002$) of participants in experimental group but there were not any statistically different in any of the variables, pre and post-test in control group.

Conclusion: Resistance training may be used as an intervention program for cardiovascular risk factors reduction.

Keywords: Hydrogen Peroxide, Women, Aging, Resistance Training

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Introduction

Chronic diseases are the leading causes of disability and death in aged people. In 2002, three main factors of death among persons 65 and older in USA have been cardiovascular diseases (31.8%), cancers (21.6%) and arrest (7.9%) (1). In Iran also coronary heart disease (38%), road accidents and cancers are main factors of death while chronic diseases are increasing (2). So precaution and postpone of chronic diseases in elderly people is considered an important issue in general health (3).

There is significant evidence that over production of reactive oxygen spices (ROS) plays a pivotal role

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in pathogenesis of cardiovascular diseases. The main sources of ROS generation are electron transfer chain of mitochondria, vascular NAD (P) H oxidase and Xanthine oxidase which produce superoxide anion O_2-. When some O_2- is dissociated fast by nitric oxide (NO) activity, signaling of O_2-, that does not convert to hydrogen peroxide (H_2O_2), is preserved and applies the extended signaling effects. The focus of this theoretical model is on patterns and mechanisms that by which H_2O_2 regulates various endothelial cell functions: Endothelial cell growth, cellular apoptosis, endothelium-dependent vasorelaxation, reorganization of cellular skeleton, endothelial inflammation reaction and endothelial regulated arterial regeneration. Perhaps this regulation of endothelial cell functions emphasizes on involvement of H_2O_2 in pathogenesis of arterial diseases. One electron reduction of molecular oxygen makes O_2- that acts as pre-formative for H_2O_2. Oxygen free radicals inactivate NO and decrease its bioavailability which contributes to vascular complications (4).

However H_2O_2 regulates endothelial cellular function through complicated mechanisms. Production of O_2- and so H_2O_2 in low level that have been preserved by essential activity of pre coupled endothelial NAD (P) H oxidase or potential leakage from Mitochondrion (5) breathing, are necessary for endothelial cellular growth.

Under pathological conditions, stimulated activity of arterial oxidase and so activated Xanthine oxidase or free eNOS produce high amount of H_2O_2 (6-8) that causes damage to biomolecules or that damages biomolecules of cells (9).

This duality function in H_2O_2 is like to character of NO. Arterial stretch and protective role in non-antibacterial pathological density are apoptotic in high amount of producing. Up to now from point of view of patho-physiologically unique molecule of ROS family has not been identified that have most correlation with arterial signaling. However experimental studies have revealed that the over production of H_2O_2 contributes to the pathogenesis of vascular disease (4).

Increases production of H_2O_2 from NAD (P) H oxidase is responsible for Angiotensin II-induced vascular smooth muscle cells hypertrophy (10). Although physical activity reduces the risk of many adverse health outcomes, prolonged and intense training can result in oxidative damage to tissues. It has reported that when VO_2 increases up to 10-15 times of resting level, free radicals generation increases dramatically (11). H_2O_2 in-vivo is the ultimate production of superoxide dismutase (SOD) and O_2-. H_2O_2 in many studies has been used as oxidizer condensation and shown that blocks arteries more than O_2-. Endothelial function is under interaction of oxidant and antioxidant mechanism (12).

It seems that regular physical activities stimulate antioxidant enzyme activities and glutathione levels in body fluids. As mentioned before, regulated aerobic training increases the production and activity of NO via up-regulation of endothelial NO synthase (eNOS) gene, in both elderly (13) and young people, but it reduces the lack of NO activity through strengthening of defensive capacity of antioxidant, increases SOD and glutathione peroxidase activity and reduces NADH/NADPH activity. Consequently ROS production is suppressed and NO bioavailability is augmented.

A molecular mechanism for explanation of circumstances of effect on NO inactive reduction suggests a role for ROS. NO via formation of Nitric peroxide reduced in presence of ROS. So induction of SOD activity through aerobic training is resulted in ROS elimination and finally increasingly reduction of NO level drop. Probably, this adaptive response is due to the cumulative effect of regular training on the expression of antioxidant enzyme-encoding genes (14). Despite the well-appreciated importance of exercise and oxidative stress relationship the effect of resistance training on H_2O_2 level is still unclear. Therefore, the aim of the present study was to investigate the effect of resistance training on H_2O_2 level in aging women.

Methods

*Procedure*s

This study was a quasi-experimental study with pretest – posttest design on 24 post-menopausal women from an Elderly House in Chaleshtar city, Shahrekord county, Iran (mean age 67.37 ± 6.02, height 153.02 ± 8.12 cm, weight 65.78 ± 12.03 kg, body mass index (BMI) 26.87 ± 4.16 kg/m^2, body fat percent 18.61 ± 3.65, and waist-to-hip ratio (WHR) 0.92 ± 0.4) with no history of disease, and resistance exercise.

Having given consent, volunteers were divided into control and resistance training groups, 12 subjects each. The test results of hormone exams were confidential and after the completion of the study were delivered to participants. At first a questionnaire physical activity level and medical background was completed. Height, weight, BMI, systolic and diastolic blood pressure and resting heart rate of all subjects were measured at 8-9 AM before each training session. These measurements were done by Beurer device (PM80, Germany) with the accuracy of 0.1 mm/hg. The skin fold test, used to calculate the body fat percent, which was measured in millimeters and taken from three sites on the right side of the body including front thigh, triceps and iliac crest using metallic caliper (SH 5020, South Korea).The measuring was repeated three times for every person and the average was recorded. Then using Jackson-Pollock method the body fat percent of participants were calculated (15).

H_2O_2 measuring: 48 hours before start of first training session and also after completing 8 weeks of resistance training, participants were drawn 5 ml blood from Antecubital vein. Participants were fast for 12 hours before blood collection. Blood samples were sent immediately to laboratory for plasma isolation by centrifuge and, frozen in the 70°C. samples were sent

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to medical diagnosis laboratory for analyzing and measuring of plasma H_2O_2 level with accuracy of 1 micro mole/litter (Eliza kit, Glory Company, USA).

Resistance training program

The experimental group participated in resistance training for 8 weeks, three 60-minute sessions in every week. Training program includes: 10 minutes warming up then 10 minutes circular stationary movement for overall 30-40 minutes, followed by 10 minutes cooling down at the end. Stations consisted of a package of 10 resistance trainings; foot press, chest press, shoulder press, front of arm , back of arm, let pool, knee extension (forceps and hamstring), knee bending, standing up on heel, (digastric) and up/down. Every training session consisted of three sets of twelve repetitions with the intensity of 40-65% of one maximum repetition. Resting time between stations and sets was 45-60 sec and 90 sec, respectively. Overload principal was designated in a way that after every 6 sessions, 5% weight of sinker was increased. To determine a maximum repetition formula this equation was used: 1 RM = displaced weight (kg)/ (1.0278-(number of repetition until tiredness) * 0.0278)) (16). The training protocol was designated According to Cadore et al. protocol which had been applied for aged people (17), also American college's special medical recommendations were considered (15, 16). All stages of exercising were under supervision of a body building trainer.

Ethical considerations

The study protocol was approved by Shahrekord university, Iran and also participation in the study was voluntarily and informed consents were obtained from the participants for participation in the study.

Data analysis

Data were analyzed using Statistical Package for the Social Sciences software using paired-sample *t*-test. $P < 0.05$ considered statistically significant.

Results

Mean and SD of participants' anthropometrics, before and after the intervention has been shown in table 1. A statistically significant decrease observed in weight, body fat percent, WHR, resting- heart- rate, systolic blood pressure and diastolic blood pressure of participants in experimental group but there were not any statistically different, pre and post-test in control group.

Table 2 shows the plasma H_2O_2 level of participant's pre and post training sessions in both groups. There was a significant difference between pre and posttest H_2O_2 concentration in the experimental group ($p = 0.041$) while the control group experienced no statistically difference ($p = 0.614$).

Table 1. Mean and standard deviation of participants' anthropometrics, before and after intervention

Variables	Experimental group			Control group		
	Pre test	Post test	p	Pre test	Post test	p
Age (year)	67.38 ± 6.82	-	-	67.63 ± 5.22	-	-
Height(cm)	154.15 ± 9.85	-	-	151.90 ± 6.39	-	-
Weight(kg)	64.53 ± 11.54	63.38 ± 10.90	0.048	67.03 ± 12.52	68.10 ± 13.01	0.13
BMI(kg/m^2)	27.08 ± 3.17	26.67 ± 2.98	0.051	29.33 ± 6.75	29.60 ± 6.63	0.24
Body fat %	18.38 ± 4.38%	17.01 ±4.02%	0.001	18.84 ± 2.93%	18.87 ± 2.90%	0.31
WHR	0.916 ± 0.044	0.861 ± 0.041	0.037	0.93 ± 0.037	0.94 ± 0.031	0.49
Resting- heart- rate	69.84 ± 2.07	66.76 ± 1.99	0.021	70.81 ± 2.31	71.01 ± 2.20	0.74
Systolic blood pressure	13.13 ± 1.85	12.36 ± 1.11	0.006	13.84 ± 0.98	13.88 ± 0.95	0.49
Diastolic blood pressure	8.64 ± 0.35	8.16 ± 0.18	0.002	8.76 ± 0.54	8.80 ± 0.50	0.31

Table 2. Comparison of mean and standard deviation of pre and posttest of H_2O_2

Group	Stage	Mean ±SD	df	t	p
Experimental	Pre-test	4.25 ± 0.42	11	2.31	0.041
	Post- test	4.04 ± 0.32			
Control	Pre-test	4.33 ± 1.20	11	-0.51	0.614
	Post- test	4.39 ± 0.92			

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Discussion

This study examined the effect of resistance training on plasma level of H_2O_2 in aged women. The results demonstrated the significant effect of resistance training on the reduction of plasma level of H_2O_2 in aging women. Rose et al. observed that H_2O_2 increases e NOS activity while deteriorates NO bioavailability (18). Hu et al. examined the bilateral actions of H_2O_2 on Phosphorylation and activity of eNOS (19). They found that in H_2O_2 treated cells AMP-activated protein kinase and Akt phosphorylate eNOS at serine residue results decrease of eNOS function in cardiovascular diseases.

Labinsky et al. compared endothelial function, O_2-, H_2O_2 production & oxidation pressure resistance of blood vessels between longest-living rodent, naked mole-rats and mice (20). Comparison of special clusters showed that there is a reverse correlation between ML and H_2O_2 in developing apoptotic cellular death. So endothelial connector function and reactive oxygen species don't correlated with maximum longevity, while increased potential longevity rises coincided by resistance of vessel, and is associated with pre-apoptosis stimulation.

Ochoa et al. examined the reciprocal effects of hypocloros acid (HOCL) and H_2O_2 on endothelial cell permeability. They found that low level of HOCL in in-vitro causes increasing endothelial permeability than H_2O_2, then intercellular level of cyclic adenosine monophosphate increases so inhibits the increased permeability resulted from other oxidants (21).

Lauer et al. investigated the role of H_2O_2 in training-induced eNOS up regulation. Recent researches showed that in cellular cultivation eNOS expression was increased both by layer pressure and H_2O_2, the phenomenon that was also associated with endothelial layer pressure and oxidative pressure. The results suggested that endogenic H_2O_2 plays an important role in endothelial Adaptation following training and this effect is accompanied with eNOS up regulation (22).

Young et al. examined the direct measure of H_2O_2 release or NO as a strong tool to determine real time of free radical release in biological model (23). Impaired function of endothelium is a common phenomenon that happens in many diseases such as local anemia damage, intravenous reinjection, diabetic vascular damages and clinical procedures that damage the blood vessels. Hallmark of endothelial dysfunction is decreased bioavailability of NO and so increasing of radical production, which stimulates inflammation reaction leading to tissue damage.

Measurement of free radical in biological models facilitates the determination of biochemical process that is responsible for regulation of these biomolecules production. This technic is not only a valuable tool for studying release of NO/ H_2O_2 but also a technique to identify pharmacologic factors that modify this release. Taylor et al. explored the effect of exercise on H_2O_2 endurance in field mouse heart in order to determine whether exercise can be a precondition of heart muscles facing of damages resulted from H_2O_2 or not. They concluded that exercise increases coronary blood flow and decreases H_2O_2 induced heart damage while it does not affect H_2O_2 induced cardiac dysfunction (24). Lee et al. demonstrated that H_2O_2 increased vascular permeability through vascular endothelial growth factor (VEGF) regulation and it is possible that ROS was involved in this response (25).

Cho et al. have shown that H_2O_2 stimulates VEGF production from activated neutrophils and macrophages. They found macrophages increase VEGF via induction of oxidation, which is mediated by neutrophils (26).

In the Cai study it was found that over production of reaction oxygen species in vascular system participates in developing heart disease (4). It seems that among dependent reactional oxygen, O_2- and H_2O_2 in redox signaling are in the most importance while O_2- causes endothelial dysfunction via inactivation of NO, and H_2O_2 effects on endothelial cell function via complicated mechanisms.

Conclusion

The results showed that resistance training with 40-65% of a maximum repetition causes reduction in amount of H_2O_2. So it is suggested to consider training in aged care daily programs.

Study limitations

The cultural contents of participants and also the female gender are the limitations of the study that should be considered in using the results.

Conflict of interest

The authors declare that they have no competing interests.

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