Original Article

Comparison of the Effect of Maximal and Submaximal Aerobic Physical Activity on Saliva Enzymatic and Non-Enzymatic Antioxidant Indices in Middle-Age Women

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Abstract

Background & Objective: Some studies have demonstrated that aerobic exercise can reinforce antioxidant defensive system. To this end, the present study aimed to compare the effect of maximal and submaximal aerobic physical activity on saliva enzymatic and non-enzymatic antioxidant indices in middle-aged women.
Materials & Methods: In this quasi-experimental study, 24 women (35-45 years, Weight: 67.41±5.03, Height: 161.83±2.98 and BMI: 25.74±2.01) were selected purposefully and voluntarily who were randomly categorized into two groups, Maximal Aerobic Physical Activity (MAPA) and Submaximal Physical Activity (SAPA) (n=12/group). SAPA were done with 50 to 70 percentage of heart rate reserve for 3 sessions/week for 6 weeks. To do MAPA, Bruce Protocol (seven stages for 3 minutes) was applied. It started with 10% incline and 1.7 miles/hour speed and continued with 2% increase for every 3 minutes until exhaustion, 3 sessions/week for 6 weeks. Required saliva samples were gathered before the first session and 24 hours after the last session of exercise to be applied to evaluate Catalase, Superoxide dismutase, Malondialdehyde enzyme, and total antioxidant capacity. Data were analyzed using an independent T-test ($P \leq 0.05$).
Results: Research results represented a significant difference in saliva enzyme indices for MAPA and SAPA groups: Catalase (P_{CAT}= 0.003), Malondialdehyde (P_{MDA}=0.001), and total antioxidant capacity (P_{TAC}= 0.04). MAPA group showed higher average. In addition, the results indicated no significant difference in dismutase superoxide enzyme levels (P_{SOD}=0.88) in MAPA and SAPA groups.
Conclusion: According to the reported results, it may be concluded that due to its ability to increase antioxidant enzyme secretion, MAPA is applied to cope with oxidative stress.

Keywords: Maximal and Submaximal Aerobic Physical Activity, Malondialdehyde, Catalase, Superoxide dismutase, Total Antioxidant Capacity

Introduction

Aging is a progressive and dynamic process accompanied by numerous morphological, functional, hemodynamic and psychological changes which reduce abilities to adapt to the environment (1). The average age in developing countries is 24.3 while it is estimated 37.4 in developed ones. It is predicted that it will increase to 35 and 46.4 in developing and developed countries in 2050, respectively (2).

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Regular physical activities (PA) along with controlling the growth of chronic diseases improve general and physical health. Many studies have emphasized an essential relationship between PA and decreasing the risk of chronic diseases such as heart attack, brain stroke, diabetes, osteoporosis, and cardiovascular diseases (3-5). World health organization published a warning letter in 2003 in which physical inactivity was announced as one of the most severe health problems. It reported about 2 million annual deaths because of physical inactivity (6). Physical weakness and sarcopenia (loss of skeletal muscle mass and strength correlated with aging) are threats that cause the risk factors. The most important internal inducing factor of sarcopenia is increasing the production of free radicals and therefore, oxidative stress. In addition, the reduction of PA, as an external factor affecting sarcopenia, is significant (7). Older adults are the largest population in the upcoming decades. During middle-age which ends in old age, Middle-age people need to adapt and continue some PA. Doing exercise prevents an unusual increase in free radicals in resting and exercising status (1). According to the various studies on the advantages of physical activity, it is evident that doing exercise leads to an increase in the number of reactive oxygen species(ROS) (8) which is correlated with increasing oxygen consumption in the mitochondrial respiratory tract, in a way that total oxygen consumption elevates up to 15-20 times more than the regular condition during an exhausting PA, (9). Antioxidants are molecules capable of reducing oxidative stress effects (10). When low concentration antioxidants confront oxidative materials, they induce the reduction of their reactivity by attracting or releasing electrons of free radicals (11), and prevent or postpone the oxidation significantly. Antioxidants are divided into two groups of industrial chemicals which are added to products to prevent oxidation and are transferred to our body by foods and naturally existing compounds produced by the body. Externally supplied antioxidants are no enzymatic (12). Naturally existing antioxidants in the body are the main components of the defensive system, regulate the formation of cellular transferring molecules, and thereby balance the oxidative stress (13). Salivary glands contain important ionic and protein transferring mechanisms from blood to saliva which are transferred through blood vessels (to nourish salivary glands). Therefore, this mechanism will clarify the relationship between vessel system and mouth environment (14). Human saliva is composed of many molecules and enzymes such as Superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) (9). SODs are a ubiquitous family of enzymes that catalyze the dismutation of superoxide anions ($O_2^{\cdot -}$) as the first line of defense against ROS.SODs reduce $O_2^{\cdot -}$ to oxygen and hydrogen peroxide (H2O2), and H2O2 is further neutralized to water through enzymatic reactions by CAT (15). CAT is one of the most important antioxidant enzymes. As it decomposes hydrogen peroxide to innocuous products such as water and oxygen, CAT is used against numerous oxidative stress-related diseases as a therapeutic agent (16). MDA is the main form of aldehyde resulting from tissue lipid peroxidation and widely used as a biomarker of oxidative stress and clinically serious metabolic impairments (17). Total antioxidant capacity (TAC) is the antioxidant capacity of all antioxidants in a biological sample, not just the antioxidant capacity of a single compound (18). In many studies, the effect of exercise on the saliva secretion stream has been investigated. Some researchers have shown that the amount of saliva secretion in short-term activities with maximal and sub-maximal intensities increase significantly (19-21) while some others emphasized that saliva stream levels decrease during PA (22-24). Some researchers reported significant changes in these salivary enzymes following the close study of SOD and MDA in a stressful condition (25, 26). Some researchers reported no significant changes in related activities to these antioxidants (27). It should be noted that saliva is one of the most critical defensive mechanisms against ROS. Sampling saliva has advantages over blood sampling such as ease of sample, non-invasiveness, lack of sampling stress and there is less risk of exposure to blood diseases and contamination in saliva sampling than in blood sampling (28). Among numerous antioxidants in saliva, they have the greatest and the most critical contributions against produced free radicals after MAPA and SAPA and are considered as good indices of antioxidant conditions that are selected to measure. However, limited literature and contradictory results are available about doing MAPA and SAPA and related changes to antioxidant and non-antioxidant enzymes, and their significant role in health and conquering sequenced free radicals. Therefore, this study aimed to compare the effect of MAPA and SAPA on salivary enzymatic and non-enzymatic antioxidant indices in middle-aged women.

Materials & Methods

The present study is a quasi-experimental study with a pre-test and post-test design. It was approved with IR.USB.REC.1399.003 ID in ethics committee of University of Sistan and Baluchestan. In this research, volunteer participants, based on inclusion criteria such as age (35-45 years), no heart disease, asthma, diabetes, joint problems, and physical fitness participated in a 6-week period of MAPA and SAPA, 24 women were selected.

They were informed about the purpose and the method. In addition, they filled out the consent form for participation in the research procedure and were randomly divided into two research groups.

In order to evaluate saliva samples, after personal measurements like height and age and before applying the protocol, participants were asked to wash their mouth with distilled water and gather their saliva samples into their sterile tubes (29). Samples of saliva were taken from the participants during a rest day in the morning (9:00–10:00 AM), following overnight fasting (30). Participants were asked to sit on a chair, place their heads ahead and a little downward and then release the predefined amount of saliva every 1 minute for two to five minutes into the CBC tube. Gathered samples were immediately put into the ice and sent to the laboratory. After centrifuging in 2400 round/min (rpm), they had been placed in a fridge with -20°C until enzyme activity was measured. MAD was exploited through measurement and performed by N-Butane based on spectrophotometry method (11). To measure CAT activity, 50 Mmol Phosphate buffer (PH=7) was combined with 10 Mmol Hydrogen peroxide, then two quartz cuvettes were selected. 500 µl of buffer phosphate solution and hydrogen peroxide were added to the blank cuvette and 250 µl of buffer phosphate solution, hydrogen peroxidase, and 55 µl of saliva was added to the sample cuvette. They were measured using a spectrophotometer in a kinetic method with 240 nm wavelength for 1 minute in 5-second intervals. Finally, to calculate catalase enzyme activity, numbers were divided into 39.4 (31). In order to measure Peroxidase enzyme activity, a 4-Aminoantipyrine substrate in spectrophotometry, was applied. Its activity in 25°C was measured using a 0.3 mol buffer phosphate solution, 0.001 mol hydrogen Peroxidase, 0.002 mol 4-Aminoantipyrine, 0.15 mol phenol in 7.4 pH, and 510 nm wavelength spectrophotometry (9). TAC was studied based on the FRAP method. The method evaluates saliva potential in the oxidation of Fe^{2+} to Fe^{3+} in the presence of TPTZ. Its result is TPTZ-Fe blue complex with 800 nm maximum absorption. Saliva oxidation potential was measured through increasing the concentration of the so-called complex by the spectrophotometer, and then a standard curved was depicted based on Iron-Sulfate solution. The concentration of the mentioned solution was measured based on µmol/ml (32).

SAPA protocol

It includes 30 to 50 minutes of progressive aerobic exercise on a treadmill (mark-Italy) for 3 sessions/week, for 6 weeks at the 9:00–10:00 AM (alternative and discontinuous sessions) with 50% to 70% heart rate reserve intensity based on the Karvonen method (the speed and the length of time increased periodically during the exercise period) which was performed after 10 min warm up and stretches. Every session ended with 5 min cooling down (walking on treadmill and stretches) (11).

MAPA protocol

Bruce protocol (seven 3-minute stages) was used to do the maximal exercise until exhaustion. The starting point (stage 1) was 1.7 mph at 10% grade. It continued with 2% increase every three minutes until exhaustion (11) for 3 sessions/week, in 6 weeks at 9:00–10:00 AM. In the end, an independent-samples t-test was applied to study the effects of exercise on the studied variables.

Results

Table 1 presents personal and physiological specifications (average and standard deviation) of each group, including age, height, weight, and body mass index (BMI). Changes in indices during two steps of saliva sampling are presented in Table 2.

The levels of MAD, CAT and TAC in MAPA were significantly higher than SAPA after intervention. However, the change in SOD levels between the research groups was not significant (Table 2).

Discussion

The results showed a significant difference in MDA levels in MAPA and SAPA groups. They are compatible with Abdi Nejad et al.'s (2017) findings that assessed changes in this hormone after exhausting aerobic exercise. Hashemi et al. (2014) studied a group of children suffering from asthma. They reported a significant reduction in MDA levels.

To clarify the results obtained on increasing MDA levels in MAPA group compared to the

Table 1. Anthropometric characteristics of the participants

variables	groups	N	M±SD	*P*-Values
weight(kg)	MAPA	12	69.83±5.07	0.808
	SAPA	12	65.00±5	
height(cm)	MAPA	12	162.67±2.27	0.401
	SAPA	12	161±3.69	
BMI(kg/m2)	MAPA	12	26.40±2.03	0.985
	SAPA	12	25.09±1.99	
age(year)	MAPA	12	39.83±2.69	0.189
	SAPA	12	40.50±1.97	

Table 2. Changes in research indicators before and after the intervention

Variables	Group	Sampling time	M± SD	*P*-Value
MDA (n mol/ml)	MAPA	pre-test	0.49±0.25	0.001*
		post test	0.89±0.02	
	SAPA	pre-test	0.48±0.02	
		post test	0.58±0.03	
CAT(u/ml)	MAPA	pre-test	0.57±0.003	0.003*
		post test	0.93±0.01	
	SAPA	pre-test	0.57±0.005	
		post test	0.80±0.009	
SOD(u/mgP)	MAPA	pre-test	0.83±0.61	0.88
		post test	1.15±0.65	
	SAPA	pre-test	0.85±0.009	
		post test	1.13±0.24	
TAC(µm/ml)	MAPA	pre-test	1.87±0.37	0.04*
		post test	1.77±0.37	
	SAPA	pre-test	1.89±0.42	
		post test	1.62±0.29	

Independent-samples t-test results in the studied variables (P≤0.05).

SAPA group, it would be claimed that intense and/or heavy exercise stimulates lipid peroxidation (17) and following the increase in lipid peroxidation, MDA levels increase as a marker of increased oxidative stress. On the other hand, lower levels of MDA following submaximal aerobic exercise can be attributed to the antioxidant adaptations created as a result of aerobic exercise (33). It should also be noted that increasing MDA concentration in saliva is correlated with exercise intensity. Whatever the intensity level is higher, the MDA release is more significant (34).

The second studied variable is SOD. The results showed no significant difference in its levels for the MAPA group when compared to the SAPA group. Abdi Nejad et al. (2014) obtained the same results as the present study. Hashemi et al. (2014) reported different results in a study on children who have asthma. They showed no significant decrease in MDA levels. Deminise et al. (2010) studied the effects of maximal activities on SOD and reported similar findings. Gonzalez et al. (2008) reported inconsistent findings in which 24 healthy men and women were studied in a 10000 km race. In explaining the results, it can be said that after anaerobic exercise, under the influence of increasing the production of ROS, the gene rearrangement of antioxidant enzymes increases antioxidant capacity (35).

The results on CAT showed a significant difference between MAPA and SAPA groups. MAPA group demonstrated higher levels of CAT. Some previous research has reported conflicting results with the results of the present study (9, 28, 36). This increase is claimed to be due to increased oxygen consumption of the mitochondrial respiration so that during exhausting activity, the amount of oxygen consumption in the whole body increases from 15 to 20 times (37). Increased mitochondrial respiration increases H_2O_2 levels, Parker et al. (2018) reported that more H_2O_2 is produced during exhausting activity than gentle exercise (38). In fact, increasing CAT levels in strenuous exercise is an antioxidant defense process that occurs following an increase in H_2O_2.

Total antioxidant capacity demonstrated a significant difference in MAPA and SAPA groups. MAPA group demonstrated higher levels. The results in some studies are incompatible with our obtained conclusion (11, 28, 39). Increased production of active oxygen as a result of glycosylation, peroxidation, and autooxidation of glucose and conversion of glucose to acidic glucose leads to an increase in free radicals and subsequently leads to an increase in total antioxidant capacity. Saliva antioxidants play a significant role in saliva defense mechanism against free radicals. Any defects in this defensive system directly interfere with the pathogenesis of many diseases. In this study, saliva antioxidant capacity in the MAPA group is significantly more than what observed in SAPA group which will be suggested for reducing induced damages of free radicals. When these people are imposed to maximal activities, they use more natural antioxidants to deal with the reduction of body antioxidants. As these women are experiencing menopause, they mostly apply MAPA because they increase their antioxidant stores and makes some compatibility. To the best of our knowledge, our work is the first report about comparing the effects of maximal and submaximal physical activity on salivary antioxidant activity, but our findings are limited to inactive middle-aged women with a specific exercise protocol over a period of 6 weeks. Further research can be done on more diverse populations, other training protocols, and larger sample sizes.

Conclusion

As the results demonstrated, it is deducted that MAPA causes the optimized compatibility and increases the capability of a defensive system against oxidative stress. Elevating anti-oxidative defense levels causes enforcing-related factors to the immune system. Therefore, exceeding levels of these factors may lessen the growth of induced damage by aging.

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Conflicts of Interest

No potential conflict of interest relevant to this study was reported.

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