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Salivary antioxidants status following two resistance exercise systems in young women athletes

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Abstract. Physical exercise induces biochemical changes in human body that modify metabolites in blood, saliva and other body fluids. Saliva biochemical analysis is rapidly developing as a tool for the assessment of physiological biomarkers of sports training. The aim of this study was to investigate changes in antioxidant status after performing two loading patterns, i.e. pyramid (PLP) and inverse pyramid (RPLP). In practice, 36 young women athletes (height 162.7 ± 9.5 cm, weight 55.8 ±5 kg, fat 21.8 ± 5.5, BMI 21.1 ± 2.2) voluntarily participated in the study. On two consecutive days (one week apart) they performed 6 different movements in resistance exercise protocols with two loading patterns, i.e. pyramid (PLP) and inverse pyramid (RPLP). Their un-stimulated saliva was then donated before and 5 minutes after exercise. Alteration in activity of superoxide dismutase (SOD), radical scavenging activity on 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) and concentration of uric acid (UA) was then investigated.
The results showed that after exercise, SOD activity was significantly higher in both PLP and RPLP when compared to their values at rest ($p<0.05$). Uric acid values increased significantly in both resistance exercise systems ($p<0.05$). In the case of radical scavenging activity (%DPPH), both PLP and RPLP showed significant increases 5 minutes after exercise sessions compared to pre-exercise ($p<0.05$).
Key words: *resistance exercise systems, salivary uric acid, superoxide dismutase.*

Introduction

Physical exercise induces biochemical changes in human body that modify metabolites in blood, saliva and other body fluids. Analysis of biochemical compounds in saliva is rapidly developing as a tool for assessment of physiological biomarkers of sports training. Method of salivary fluid collection is non-invasive, requires less medical training and can be performed even in the sports field. Saliva is a useful and alternative fluid to serum and plasma as it can be obtained rapidly and without stress. One of the fastest growing and most popular types of exercise in recent years is resistance exercise that is used for the purpose of general fitness, rehabilitation, or athletic performance.
With the purpose of manipulating training stimuli, several set structures have been employed. The system of loading and the volume of prescription from one set to the next, needs to be determined for multiple-set programs. The intensity and volume of each set during an exercise can increase, decrease, or stay constant. Two of the most popular structures are light to heavy (LHS) and heavy to light systems (HLS). A “LHS” is one in which load is increased in each set while repetitions remain the same or decrease. A “HLS” is one in which load is decreased with each set and repetition number is either maintained or increased.
Although regular resistance exercise training is associated with numerous health benefits and has been recommended by many major health organizations, several acute studies have demonstrated that a single bout of resistance exercise can result in activation of several systems of radical generation (1). These include xanthine and NADPH oxidase production, ischemia reperfusion, prostanoid metabolism, phagocytic respiratory bursts, disruption of iron containing proteins, and excessive calcium accumulation, often resulting from the performance of muscle-damaging isotonic or eccentric biased muscle actions, which commonly produce muscle injury (2-4). Since no information is available on the effects of an acute session of loading systems on salivary antioxidant biomarkers in women, the purpose of this study was to compare salivary

antioxidants biomarker response to an acute session of resistance exercise set structures (LHS and HLS) on trained women.

Material and Method

Thirty six healthy and well-trained voluntary females (age 20.54 ± 5.38 years, mass 55.91 ± 3.94 kg, body mass index, BMI: 20.64 ± 1.04 kg/m², body fat 18.29 ± 3.58 %) were selected from the university's staff and randomly assigned to three groups: LHS (n= 12), HLS (n=12), and control (C, n= 12). All the subjects experienced resistance training, and trained regularly for at least 2 years. The inclusion criteria consisted of the following: a) the subjects did not have any medical conditions that might be aggravated by participation in resistance exercise; and b) subjects did not use any mineral or vitamin supplements and they were nonsmokers. All subjects read and signed an informed consent document and were asked to not participate in any resistance training during the period of study. The Institutional Review Board of the University of Guilan approved the research protocol.

Anthropometric measurements. Two familiarization sessions were designed to habituate subjects with the testing procedures and laboratory environment. Then, indices of anthropometric (weight, height, BMI, body fat) were measured. Their height was measured by a wall chart and weight was measured by a digital scale. Body mass index was calculated by dividing the weight (kg) by square height (m^2). Body fat percentage was assessed using body composition analyzer (In Body3.0, South Korea) according to the manufacturer's protocol. Table 1 shows demographic characteristics of the subjects.

Table 1. The demographic characteristics of the subjects (values are mean ± SD, n=36)

Characteristic	Body fat (%)	Height (Cm)	Body mass index (kg/m²)	Age (Years)
Mean ± SD	18.29 ± 3.58	166.2 ± 3.11	20.64 ± 1.04	20.54 ± 5.38
Range	15.25-21.12	161.54-170.23	18.21-21.12	19.32-22.14

One repetition maximum testing (1RM). For measurement of one repetition maximum (1RM), we used a method described by McGuigan to determine the hack squat (HS), leg press (LP) and leg curl (LC) bench press (BP), let pulls down (LPD), and biceps curl BC (5).

Exercise protocols. Both tests were performed at the same time in the afternoon (5 PM) in one day and subjects were instructed not to engage in any strenuous exercise for the 72 h period preceding the exercise tests. In the light to heavy, and heavy to light systems groups: At the beginning of exercise session subjects performed a warm-up which included 10 min of general warm-up and stretching exercises for the whole body muscles, followed by specific warm-up in which subjects performed five repetitions with 30%1RM. Then subjects performed resistance exercise programs with heavy to light, and light to heavy systems in the following order: HS, LP, LC, LPD, BP and BC. In the protocol of LHS subjects began with a set of 6 repetitions with 85% 1RM then the resistance increased over 3 sets until only a 1RM was performed. In the HLS protocol, the same sets and resistances repeated but in a reverse order with the last set consisting of 6 repetitions (Figure 1). The rest interval between sets and exercises was 2 minutes. The control group did not perform any type of physical activity in the training session.

Saliva collection and storage. The saliva samples were collected 5 minutes before and immediately after exercise session when the subjects were in a comfortable position (6). Before donating their un-stimulated saliva samples, the volunteers had brushed their teeth, rinsed their mouth once with distilled water and kept their saliva for exactly three minutes. It is worth indicating that the exact time was taken to later calculate the flow rate of saliva. The samples were collected in calibrated centrifuge tubes and immediately centrifuged at 900×g for 10 min at 4°C to remove squamous cells and cell debris. They were then marked, sealed in a container and stored at -80ºC until further examinations.

*Materials.*2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH), was purchased from Sigma representative in Iran. Kits for uric acid and superoxide dismutase assay (Cayman Chemical, Cat No.706002, USA) were purchased from a local representative. All solvents were reagent grade and obtained from Merck representative in Iran. A UV-visible spectrophotometer (Ultrospec 3000, Pharmacia Biotech™, Sweden) was used throughout the research.

Measurement of uric acid in saliva samples. The amount of uric acid was measured by an enzymatic method described for assay of uric acid in serum (7). The complete assay was based on enzymatic reaction of uricase on uric acid to form allantoin and hydrogen peroxide (H_2O_2).

Production of hydrogen peroxide was coupled with catalytic oxidation of p-hydroxybenzoate and 4-aminoantipyrine oxidation in the presence of peroxidase. The pink chromophore thus formed was then detected at 505 nm.

DPPH radical scavenging assay. In this chemical assay, an antioxidant reduces the stable DPPH$^{\bullet}$ by donating a hydrogen to it. In the present piece of research, a modification of the method described in was used (8). The radical scavenging activity of saliva samples against stable DPPH° was determined using a UV-visible spectrophotometer (Ultrospec 3000 from Pharmacia Biotech). The color of DPPH$^{\bullet}$ changed from deep-violet to light-yellow due to its reduction. Briefly, 1500 l of freshly prepared DPPH$^{\circ}$ solution in methanol (6×10^{-5} M) was added to 77µL of centrifuged saliva in 1cm path length disposable microcuvettes and mixed. Absorption was measured at 517 nm (A_c) after solution remained for 30 min at room temperature and in dark. Using methanol as blank, the absorbance of methanolic solution of DPPH$^{\circ}$ was measured as control (A_c). All experiments were carried out in duplicate and radical scavenging activity was calculated by following relationship:

$$\text{DPPH radical scavenging activity (\%)} = [(A_c - A_S)/A_c] \times 100$$

Measurement of superoxide dismutase activity. Salivary superoxide dismutase activity was measured by an enzyme assay kit (Cayman chemical, Cat No.706002, USA). According to procedure given in the kit, tetrazolium salt was used for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was the amount of enzyme needed to exhibit 50% dismutation of superoxide radicals. The whole experimental section was performed by an experienced technician blind about cases.

Statistical analysis. Data were analyzed using SPSS 19.0 J (SPSS Japan, Tokyo, Japan) with advanced modules and values were expressed as mean ± SD. Kolmogorov-Smirnov test was used to normalize the data. Homogeneity of the sample was tested using Levine's test. All variables presented normal distribution and homogeneity paired-sample T-test was used to compare antioxidant status in different times of examination. Between group differences were determined using one-way ANOVA followed by Tukey post hoc test and values with $P\leq0.05$ were considered as significant.

Results

Salivary flow rate. Salivary flow rate is among important factors for maintenance of safe and healthy environment in the oral cavity. The flow rates were calculated in terms of saliva volume (ml) per minute. Table 2 shows the flow rates of saliva before and after exercise. Based on the data in this table, alternations in the flow rate of saliva observed before and after exercise. The differences, were not significant and the rate between 0.48-0.86 ml/min before and reduced to 0.42-0.81 ml/min after resistance exercise. It was noticed that salivary flow rate was more decreased in the case of RPLP type of resistance exercise which is due to the more intense nature of the activity. However, the loading system had no significant effect on the flow rate and it was decreased in both systems. It has been reported that the flow rate of saliva depends on various external and internal factors (9-11). We have previously reported a significant decrease in salivary volume and rate of flow in smokers (12, 13) and passive smokers (14). The decrease in smokers could be related to the presence of various toxic chemicals in cigarette smoke.

Baseline values of salivary antioxidants are presented in Table 3. There were no significant differences between the groups for baseline levels of salivary, SOD, UA and DPPH ($P\geq0.05$)

Table 2. The flow rate of saliva in women athletes after resistance exercise

Flow rate (ml/min)	Before exercise (n=36)		After exercise (n=36)	
	PLP	RPLP	PLP	RPLP
Mean±SD	0.58±0.06	0.58±0.07	0.52±0.12	0.48±0.11
Range	0.48-0.82	0.48-0.88	0.46-0.88	0.42-0.76

Table 3. Baseline values of salivary antioxidants

Variables	Control Group	LHS Group	HLS Group	P Value
SOD (U/ml)	44.32 ± 9.85	44.64 ± 13.40	46.91 ± 14.36	P≥0.05
UA (mg/100ml)	2.07 ± 1.19	2.29 ± 0.9	2.07 ± 1.58	P≥0.05
DPPH %	12.23 ± 3.7	11.55 ± 2.11	15.33 ± 4/05	P≥0.05

The SOD and UA values increased significantly in the two resistance exercise groups when compared to the resting values (P≤0.05, Figures 1 and 2). On the other hand, No significant difference was found between the three groups (P≥0.05, Figures 1 and 2).
Differences in DPPH radical scavenging activity in control, LHS and HLS groups are presented in Figure 3. The DPPH values increased significantly in the two resistance exercise groups when compared to the resting values (P≤0.05). In addition, DPPH values were significantly increased in HLS groups compared to the control group (P≥0.05, Figure 3). However, there were no significant differences between LHS and HLS groups (P≤0.05).

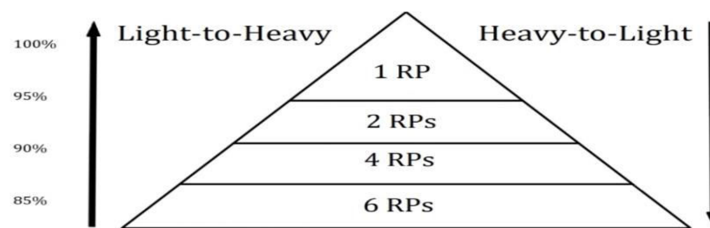

Figure 1. A schematic view of two resistance exercise systems

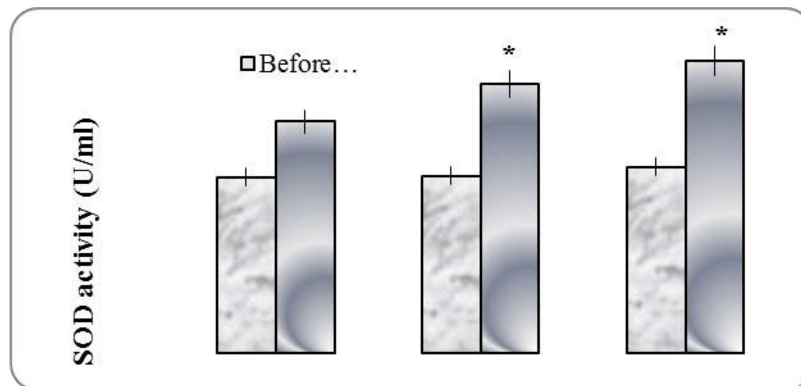

Figure 2. Activity of superoxide dismutase (U/ml) in saliva before and after exercise. Values are mean±SD. 1) Control group, 2) LHS grouop, 3) HLS group.*Significance difference compared to before exercise.

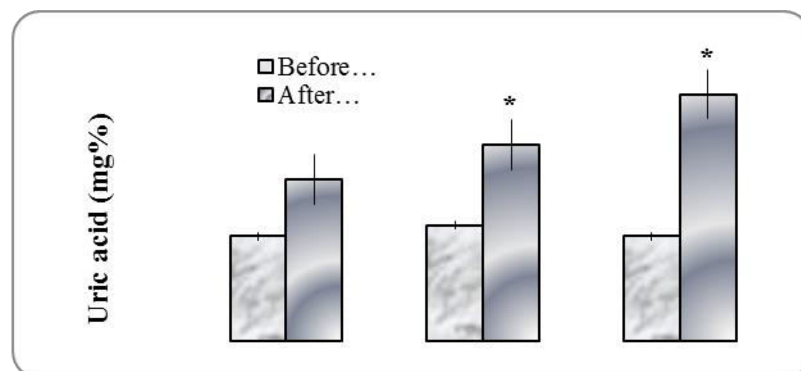

Figure 3. Concentrations of salivary uric acid (mg/100ml) before and after exercise. Values are mean±SD. 1) Control group, 2) LHS group, 3) HLS group.*Significance difference compared to before exercise.

Activity of superoxide dismutase. Resistance aerobic exercises could increase rate of production of reactive oxygen species (ROS). In a healthy person, the response to production of ROS is an increase in antioxidants including superoxide, and hydroxyl radicals. Figure 2 shows the biological activity of superoxide dismutase before and after various systems of exercise. It was observed that after exercise, SOD activity was significantly higher in RPLP compared to the values at rest. On the other hand, SOD values for the RPLP were higher when compared to the PLP (P>0.05). In support of these results, we have previously reported increase in activity of SOD in saliva of athletes men after intense exercise (15). It was found that the type of oxidative stress could affect the antioxidant status of saliva (15). The beneficial effect of exercise on SOD could positively be affected when combined with supplements such as garlic and green tea (16). In our previous studies we reported enhanced activity of other antioxidant enzymes such as peroxidase as a result of intense exercise (6). Other types of activity may alter the oxidative status leading to altered antioxidant activity. In agreement to our results, a significant increase in salivary SOD activity has been reported due to computer games (17). They followed the antioxidant activity of children up to three hours after the game and found that it was not returned to the original value (17).

Uric acid in saliva. Figure 3 shows concentration of uric acid is three groups before and after two exercise systems. No significant difference (P> 0.05) was observed between concentrations of uric acid in the two systems. However, based on the results of this study, concentration of uric acid s increased significantly in the two resistance exercise systems as compared to resting concentration (P<0.05). In support of our results, it has been reported that blood and saliva concentrations of uric acid after an acute session of resistance exercise were 23% and 38% respectively (18). They also found a significant correlation between uric acid concentration in saliva and plasma. On the other hand, uric acid in saliva has been examined after a race of 10km (19). Their results also support our findings in this study, as they reported a significant correlation between concentration of uric acid in saliva and aerobic exercise (19).

It is worth to notice that various types of exercise lead to higher accumulation of reactive oxygen species (ROS). However, a natural balance exists between oxidant concentration and the activity of antioxidant defense system to prevent oxidative stress (20). The unique antioxidant system of salivary fluid consists of enzymes and other secondary smaller metabolites. Most of salivary antioxidants are water soluble. It has been found that the powerful polar antioxidant, uric acid, possesses about 70% of the total antioxidant capacity of human saliva (21). Due to its special chemical structure, uric acid is able to scavenge various ROS and reactive nitrogen species (RNS) molecules and chelate the transition metals (7, 22). The activity of uric acid in saliva is, therefore, critical to keep the oxidant/antioxidant balance.

DPPH radical scavenging activity. Figure 4 shows the DPPH activity in salivary fluid due to performing PLP and RPLP systems. A significant increase was observed in DPPH activity between before and after exercise (P<0.05). On the other hand, looking at DPPH activity between PLP and RPLP, significant increase was observed at RPLP post exercise (P>0.05). Considering that resistance exercise can induce oxidative stress, the increase in antioxidant activity in terms of DPPH scavenging power is expected. In support of the present results, external and internal factors may lead to increase in antioxidant activity of saliva. We have previously found that salivary antioxidant level is increased in patients diagnosed with peptic ulcer (23), lupus erythematosus (24), as well as cigarette smokers (12, 13) and in healthy vegetarians (25).

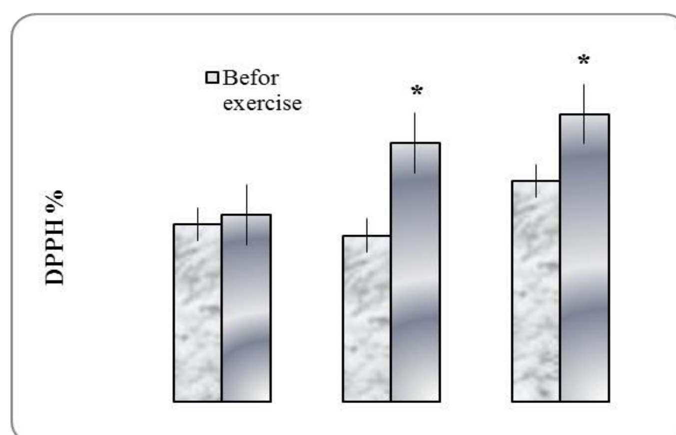

Figure 4. DPPH radical scavenging activity (%) before and after exercise. Values are mean±SD. 1) Control group, 2) LHS group, 3) HLS group.**Significance difference compared to before exercise*.

Conclusion

The normal response of human saliva to oxidative stress caused by resistance exercise is increase of its antioxidant power. Human saliva possesses advantage of easy and non-invasive sampling procedure. It contains many secondary metabolites including enzymatic and non-enzymatic antioxidant. Therefore, saliva could replace other body fluids such as blood for diagnostic and research purposes.

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