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Official Research Journal of
the American Society of
Exercise Physiologists

ISSN 1097-9751

Response of Salivary Antioxidants to Intense Exercise in Non-Athlete Men

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ABSTRACT

Nazari Y, Damirchi A, Sariri R, Taheri M. Response of Salivary Antioxidants to Intense Exercise in Non-Athletic Men. **JEPonline** 2012;15(3):1-9. Both enzymatic and non-enzymatic antioxidants in human saliva were subjected to alteration due to different factors. The purpose of this study was to determine the effects of a short period intense exercise on salivary flow rate, superoxide dismutase (SOD) activity, and uric acid concentration. Using a double-blind randomized design, 25 healthy non-athlete men (mean \pm SD = 21.34 \pm 3.11 yrs) volunteered to participate in the study. The purpose of the study was explained and, then, an informed consent was completed prior to treadmill runs at exhaustion. Their unstimulated whole saliva samples were collected before, immediately after, and 1 hr after exercise. Activity of superoxide dismutase was determined nitro-blue tetrazolium (NBT) as its specific substrate. The enzymatic assay was based on the ability of superoxide dismutase to inhibit the reduction of NBT by superoxide. The results showed a significant increase in uric acid and SOD activity immediately after exercise, which almost returned to the original level in 1 hr. It was concluded that an exercise of short-duration, high-intensity increases the antioxidant power of saliva, while only small changes are observed in saliva flow rate.

Key Words: Superoxide Dismutase, Short Period Tense Exercise, Flow Rate, Salivary Antioxidants.

INTRODUCTION

Anecdotal evidence suggests that various physical conditions are associated with an increase in resistance to infection (27). Athletes undergoing chronic high intensity training exhibit a propensity for upper respiratory tract infection (25). It is suggested that the increase in infection among athletes may be related to low resting levels of salivary immunoglobulin-A (31). This may be the reason that many health organizations have recommended aerobic type exercise performed for 20 to 60 min, 3 to 5 times per week at intensities of 50 to 80% VO_2 max in order to maintain cardiorespiratory fitness in healthy adults (2). The variations of many infection-related factors due to different exercises have become a new research line for both physical education and biochemistry scientists.

Human saliva is the first body fluid to encounter the exogenous materials, gases, and fluids that penetrate the body. Aside from lubrication and digestion, saliva plays an important role in the immunological and antioxidant defense systems (7). A significant increase in secretion rates and absolute concentrations of salivary antimicrobial proteins and markers has been reported by Algrove and colleagues (1) during intense training and competition as well as the first week of recovery. They also reported an inverse correlation between secretion rates of salivary immunoglobulin A (sIgA) and cortisol. This finding appears to indicate a possible role of cortisol in the strenuous exercise-induced immunosuppression. Alteration of salivary immunoglobulin has also been found in children infected with *Streptococcus mutans* (23) and in swimmers who are in training (31). The importance of salivary antioxidant enzymes is due to their power for decomposing various free radicals entering the oral cavity as well as the H_2O_2 produced by oral bacteria.

We have previously studied the response of salivary peroxidase to exercise intensity (10). The aim of this research was to further examine changes of enzymatic and non-enzymatic antioxidants in saliva of healthy volunteers. Thus, we studied alterations in oral superoxide dismutase (OSOD) and uric acid before and after a short period of intense exercise in a group of non-athlete men.

METHODS

Subjects

The subjects were 25 healthy non-athlete male university students (mean \pm SD; age = 21 ± 3 yrs; height = 172 ± 8 cm; mass = 66 ± 9 kg; VO_2 peak = 37.6 ± 7.4 mL \cdot kg $^{-1}$ \cdot min $^{-1}$; maximal heart rate = 180 ± 4 beats \cdot min $^{-1}$). They received local ethics community (Helsinki) approval before volunteered to participate in the research (Table I). All subjects understood the purpose of the study before providing written informed consent and then completed a comprehensive health questionnaire.

Table 1. Characteristic of the Non-Athlete Subjects who Ran for 20 min at a Heart Rate that Corresponded to 80% VO_2 max.

	Age (yrs)	Mass (kg)	VO_2 max (mL \cdot kg $^{-1}$ \cdot min $^{-1}$)	Height (cm)	Body fat (%)
Mean	21.18	67.08	37.6	172	17.55
SD	3.11	2.26	7.4	8	4.42
Range	20-26	50.6-70.3	29.5-42.1	167-180	10.2-21.1

Materials

The chemical reagents were of analytical grade and used as supplied by manufacturers without further purification. Enzyme activity assessment kit (Cayman chemical, Cat No.706002, USA) and uric acid kit were purchased from local importer representatives in Iran. All buffers were prepared freshly in Biochemistry laboratory, University of Guilan and their pH was double checked using pH meter. All general chemicals and reagents were of the highest purity available.

Design

The study was based on a randomized, double-blind, cross-over design. All subjects performed three trials: 1) before; 2) immediately; and 3) 1 hr after experimental trials. The first saliva sample was donated 5 min before the treadmill run and the next two 5 min after trial. The subjects were asked to brush their teeth and rinse their mouth with distilled water 3 hrs after lunch. After arrival to the laboratory, they rested for 15 min before starting the experimental trial. Then, they performed the treadmill run using the Astrand test at $8.01 \text{ km}\cdot\text{h}^{-1}$.

Determination of $\text{VO}_2 \text{ max}$

To determine $\text{VO}_2 \text{ max}$, all subjects performed a continuous incremental treadmill run to exhaustion. The test began at a velocity of $8.01 \text{ km}\cdot\text{h}^{-1}$, with an increase of $2.5\% \text{ km}\cdot\text{h}^{-1}$ every 2 min until exhaustion. Gas change parameters were analyzed during the run using a calibrated Sormedics Horizon Metabolic Measurement Cart (Sormedics, Anaheim, Calif). The values for heart rate (HR) were recorded every minute throughout the test.

Saliva Sampling and Measurement of Flow Rate

After the subjects' rinsed their mouth with distilled water, they were instructed to collect 3 ml of their unstimulated whole saliva samples in clean, dry, and sterile tubes. The flow rate was calculated by measuring the time required to collect 1 ml of saliva in minute. The saliva samples were immediately centrifuged at $800 \times g$ for 10 min at 4°C to remove squamous cells and cell debris. The resulting supernatant was stored at -18°C until used for assessment of antioxidants.

Determination of Salivary Uric Acid

The concentration of uric acid in saliva was determined using an enzymatic method described for assay of uric acid in serum (32). The 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.

Measurement of Superoxide Dismutase Activity

Superoxide dismutase activity in saliva samples was evaluated using an enzyme activity assessment kit (Cayman chemical, Cat No.706002, USA). This assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radical. The experimental section was performed by an experienced technician blind about cases. Each sample was assessed two times.

Statistical Analysis

Each assay was repeated at least duplicate and the results were presented as mean \pm SD values. Statistical difference between individuals was compared by unpaired t-test. Significant differences

between means were determined by Duncan's multiple range tests. *P* values less than 0.05 were considered statistically significant.

RESULTS and DISCUSSION

Salivary Flow Rate

Table 2 presents the results of flow rates before and after exercise. Salivary flow rates were expressed as volume of unstimulated saliva (ml) collected per minute. Although slight alterations were observed between saliva flow rate before and after exercise, the values were not significantly different. It was found that the saliva flow rate ranged from 0.7 to 1.2 mL·min⁻¹ before exercise, which was reduced to (0.6-1.0 mL·min⁻¹) after intense training. However, the value returned to almost the normal amount after 1 hr. This is an interesting finding since it indicates that dehydration during exercise does not seriously influence the normal flow rate of saliva.

Similar to other biological fluid such as tears (24,30), the flow rate of saliva depends on various external and internal factors (8,12,16,18). In contrast to the present results, during a concurrent piece of research (29), we found a significant decrease in salivary volume and rate of flow in smokers (which we felt was due to the presence of various toxic chemicals in cigarette smoke). This result can explain why heavy smokers complain of dry mouth even at young ages. Salivary volume and flow rate are of primary importance in maintaining a healthy environment for oral cavity (and especially the prevention of dry mouth syndrome). It is reasonable to conclude that regular exercise of almost any type can be beneficial to oral health.

Table 2. Changes in Salivary Flow Rate after a Short Intense Exercise. Each Value is the Mean Obtained from at Least 50 Measurements (25 cases each duplicate).

Flow Rate (mL·nm)	Before Exercise (n=25)	Immediately After Exercise (n=25)	1 Hr After Exercise (n=25)
Mean	0.41	0.39	0.42
Range	0.33-0.45	0.28-0.42	0.32-0.44
Standard Deviation	0.09	0.12	0.13

In agreement with the present research, it has been reported that the salivary flow rate is not significantly altered in response to short period of medium to high intensity exercise (1,10,11,26). However, some significant decrease in flow rate has been reported (4,13,19). Disagreement between these results may be related to lower activity of the parasympathic nervous system during the short period, low-intensity exercise (19). On the other hand, dehydration up to about 2% of whole body weight during high intensity exercise may also lead to lower flow rate of saliva (33). Therefore, in the

present study, neither dehydration nor stimulation of the nervous system has been high enough to reduce the salivary flow rate.

Salivary Uric Acid and SOD

Figure 1 compares the antioxidants before and after a short exercise period. It should be noted that in order to compare the responses of a non-enzymatic with an enzymatic salivary antioxidant, the concentration of uric acid is expressed as mg/L (instead of mg%). As seen in the figure, while uric acid concentration was decreased, the activity of SOD increased immediately after exercise. However, the level of both antioxidants returned to their normal value 1 hr after exercise. Uric acid is the most important non-enzymatic antioxidant in human saliva. It is entirely water soluble and induces its antioxidant action much more quickly than fat soluble antioxidants (14,28). Its reduction immediately after intense exercise may be related to evaporation, therefore, lowering the volume of saliva. The flow of saliva after a short period returns the uric acid concentration to its base line level. On the other hand, the increase in activity of SOD was expected, as stress appears to induce the enzymatic activity. This result is in agreement with our previous findings regarding the activity of salivary peroxidase in response to exercise intensity (10) and cigarette smoking (29).

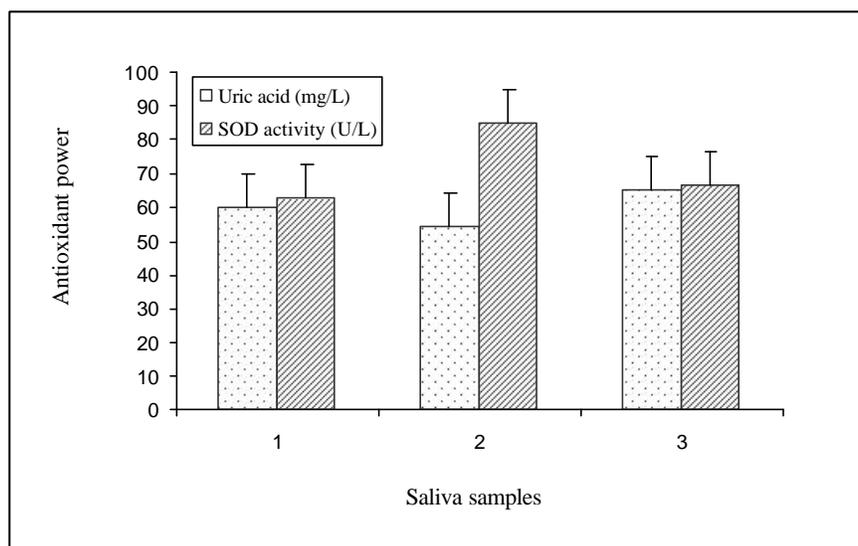


Figure 1. Variations of Salivary Antioxidants Due to Treadmill Run. Sample 1 Before, Sample 2 Immediately After, and Sample 3 1 hr after Treadmill Run.

The salivary antioxidant system is composed of various molecules and enzymes. The most important antioxidants in human saliva are water soluble uric acid, peroxidase, superoxide dismutase, and ascorbic acid (14). The concentration of lipid-soluble antioxidants is very low, contributing about 10% of the total salivary antioxidant capacity (21,22). It has been reported that the uric acid is responsible for about 70% of the total salivary antioxidant capacity (21). The present study showed that salivary uric acid was significantly decreased immediately after exercise, 6.01 compared to 5.21 mg%. The concentration of uric acid in stimulated saliva has been reported to be lower than this value (17). The results of this study are similar to reported values for uric acid in unstimulated saliva (34). Uric acid acts as a chelating agent and prevents the attack of free radicals as well as being a scavenger of the

generated free radicals. Figure 1 also indicates that the activity of SOD is increased almost significant immediately after intense exercise, which then returned very close to the original value 1 hr after exercise. Given that it is the second enzymatic antioxidant in saliva, getting back to normal activity is vital for providing total protection of the oral cavity.

While the beneficial effects of aerobic exercise on human health (5,9,11) are well-known, more recently, it has been reported that long periods of exhausting exercise increases cellular ability to prevent the attack of free radicals (3). On the other hand, Mastaloudis and colleagues (20) reported that the antioxidant activity of human body is not significantly altered even after long periods of high intensity exercise (20). This discrepancy may be related to the individual responses to external stimuli and, therefore, more research is needed. It is known that aerobic exercise leads to the increased production of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals. Hence, a higher production of antioxidants during this type of activity is a type of biological response for deactivation. Similar to the present study, an increase in the activity of SOD has also been reported in saliva of elite judoist after exercise (6). It is certainly possible that the type of stress may induce different alterations in antioxidant status of saliva. An increase of SOD activity has been reported in saliva samples obtained immediately after a 3-hr computer game compared to the samples before the game. This is a result that is entirely different from the present study (15), which is an especially interesting result that shows the refreshing stress of exercise compared to the stress caused by a static exhausting game.

CONCLUSIONS

Based on the results obtained in this study, it was found that aerobic exercise until exhaustion increases the activity of superoxide dismutase in saliva of non-athlete men. However, the most important water soluble salivary antioxidant (i.e., uric acid) was not significantly affected by aerobic exercise. It can be concluded that exercise induces production of free radicals and various reactive oxygen species. The increase of ROS in human saliva causes an increase in the activity of antioxidant enzymes including SOD. The antioxidant activity of SOD is of prime importance in protecting the oral cavity against the attack of free radicals, which may lead to dangerous disorders. Therefore, it is reasonable to conclude that exercise of moderate intensity is beneficial for oral health in terms of antioxidant capacity.

ACKNOWLEDGMENTS

The financial support by University of Guilan is highly appreciated.

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