

## Alternations in salivary $\alpha$ -amylase due to exercise intensity

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### Summary

Oral  $\alpha$ -amylase, one of the most important salivary digestive enzymes, is subjected to alternation in response to various stress conditions. The aim of this study was to assess the effect of exercise intensity on salivary  $\alpha$ -amylase activity. Using a randomized design, 12 healthy male students (mean age, 23.22, sx = 2.34 years) completed treadmill runs with initial velocity of 6.73 km/h, and 1.58 km/h increase every 3 minutes until exhaustion. Un-stimulated whole saliva samples were collected over a 5-min period into pre-weighed sterile tubes before and after exercise and analyzed for  $\alpha$ -amylase activity. The saliva flow rate ranged from 0.08 to 1.40 ml.min<sup>-1</sup> at rest and was not significantly affected by the exercise intensity. Salivary  $\alpha$ -amylase activity was measured in the supernatant of centrifuged saliva before and after exercise. Treadmill runs at 50%, 75%  $VO_{2max}$  and to exhaustion caused an increase in the activity of salivary amylase immediately after exercise, which were returned to almost the original value one hour after exercise in the case of 50%  $VO_{2max}$  and 75%  $VO_{2max}$ . On the other hand, at treadmill runs to exhaustion the 150% increase in salivary  $\alpha$ -amylase activity was returned to about 120% its baseline value. It was concluded that short-duration, high-intensity exercise increase the activity rate of salivary  $\alpha$ -amylase, especially at higher intensities. However, the salivary flow rate was not changed noticeably at treadmill runs at 50%, 75%  $VO_{2max}$  and to exhaustion.

**Key words:** Exercise intensity, treadmill, salivary  $\alpha$ -amylase, saliva flow rate.

### Introduction

Saliva is a glandular secretion that is in constant contact with the hard and soft tissues of oral cavity. Many functions have been ascribed to saliva, including its role as a lubricant that coats the mucosa and helps protect the oral tissues against mechanical, thermal and chemical irritants [8]. Saliva is also involved in initial enzymatic digestion through one of its major constituents, amylase [9]. Other functions of human saliva include initiation of digestion through  $\alpha$ -amylase; buffering capacity; acting as an ion reservoir that facilitates the remineralization of teeth; antimicrobial activity involving secretory immunoglobulin A, lysozyme, lactoferrin and myeloperoxidase; agglutination, resulting in the clearance of bacterial cells; pellicle formation; providing a solvent and acting as a medium where tastants derived from foods are presented to taste buds; and acting as a medium for moistening dry foods to aid swallowing [8]. Biochemical composition of saliva may show various alternations due to physiological conditions [5], psychological stress [17] metabolic diseases [1] and physical activities [3]. Salivary  $\alpha$ -amylase, the principal salivary protein, is a calcium-containing metalloenzyme that hydrolyzes the  $\alpha$ 1,4

linkages of starch to glucose and maltose. Salivary  $\alpha$ -amylase is produced by the serous acinar cells of the parotid and submandibular glands. It is one of the principal salivary proteins appearing as a number of isoenzymes. It accounts for 10–20% of the total salivary gland-produced protein content and is mostly synthesized by the parotid gland. The enzyme is not only responsible for an initiation of digestion in the oral cavity but it is also considered to play an important role in binding to oral bacteria [16]. Salivary  $\alpha$ -amylase has been proposed as a marker of sympathetic nervous system [17]. The enzyme is produced locally in the salivary glands, controlled by the autonomic nervous system. It has been suggested that salivary  $\alpha$ -amylase could reflect catecholaminergic changes due to increased activation of the sympathetic-adrenal-medullary system. Concentration of salivary  $\alpha$ -amylase is reduced due to some certain diseases such as lymphoma patients receiving chemotherapy [13]. However, lymphoma patients who were on cytostatic drugs had no change in total protein and  $\alpha$ -amylase concentrations by chemotherapy [12]. Significant correlations between salivary  $\alpha$ -amylase and plasma norepinephrine and epinephrine in the exercise conditions have been found [5]. We have previously reported alternations in salivary enzymes due to some external factors such as smoking [15] and exercise intensity [6]. On continuing research in this area, a limited literature was found about alternations in salivary  $\alpha$ -amylase due to various factors including exercise induced changes. Therefore, the present study reports influence of treadmill runs to exhaustion on salivary  $\alpha$ -amylase activity in a group of young healthy, non-athlete students. The hypothesis was that there must be changes in biological activity of salivary amylase due to exercise intensity.

### **Materials and methods**

#### ***Materials***

The chemical reagents and solvents were of analytical grade and used as supplied by manufacturers without further purification. A commercially available direct  $\alpha$ -amylase kit based on the hydrolysis of a substrate by  $\alpha$ -amylase in the presence of a chromogen was used (Chem Enzyme). The kit contained  $\alpha$ -Amylase substrate, maltotrioxide to which a chromogen (2-chloro-4-nitrophenyl) is attached. Enzyme activity was determined in supernatant of saliva samples collected from volunteers. All buffers were prepared freshly within our laboratory and their pH was double checked using pH meter.

#### ***Subjects***

The subjects were 10 healthy non-athlete male university students (mean age 23.22 years,  $s_x = 2.34$ ; height 1.72 m,  $s_x = 0.04$ ; body mass 67.08  $s_x = 8.42$ ;  $\dot{V}O_{2\max}$  38.49 ml  $\text{kg}^{-1} \text{min}^{-1}$ ,  $s_x = 6.43$ ; maximal heart rate 185  $\text{beats min}^{-1}$ ,  $s_x = 3$ ). They received local ethics community (Helsinki) approval before volunteered to participate in the research (Table I). All volunteers were informed of the aims and procedures for the study before providing written informed consent and completing a comprehensive health questionnaire.

Table 1. Characteristic of subjects run for 18 min at heart rate values which corresponded to 50, 75 and 80%  $\dot{V}O_{2max}$ .

Age (years)	Mass (kg)	$\dot{V}O_{2max}$ (ml/kg <sup>-1</sup> /min <sup>-1</sup> )	Height (m)	Body fat %
Mean: 23.22	67.08	38.49	1.72	15.98
SD: 2.34	8.42	6.43	0.04	3.84
Range: 20-26	52.9-72.8	31.12-49.29	1.69-1.81	10.2-21.1

**Experimental design**

Three samples of timed, un-stimulated saliva were collected from each subject, before, immediately after, and 1 h after exercise. Tooth caring instructions, type of tooth paste, eating habits and collection time after food were carefully controlled uniformly for all subjects.

**Determination of  $\dot{V}O_{2max}$** 

The Rating of Perceived Exertion (RPE) has long been encouraged for use in the group exercise setting in addition to heart rate checks. But, in recent years, it has increasingly become the primary means for determining the intensity of exercise. In this research RPE was also considered along with heart rate. The heart rate check provided the convenience of not having to use the gas mask and, therefore, provided a more reliable method for volunteers. Although this is a subjective measure, a person's exertion rating may provide a fairly good estimate of the actual heart rate during physical activity [4].

The exercise started at 2.30 pm and the experiment was repeated three times at 7 days intervals. To determine their  $\dot{V}O_{2max}$ , all volunteers performed a continuous incremental treadmill test to exhaustion. The test began at a velocity of 6.73 km/h, with an increase of 1.58 km/h every 3 minutes 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 were recorded every minute all throughout the experiment.

**Estimation of heart rates**

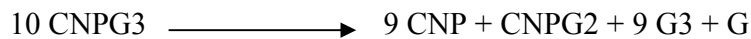
All 10 subjects performed randomly ordered 18 min treadmill runs at a heart rate corresponding to approximately 50, 65 and 80% of  $\dot{V}O_{2max}$ . To predict heart rate from the results of  $\dot{V}O_{2max}$ , the linear regression was used in each case.

**Saliva collection, handling and storage**

Timed un-stimulated whole saliva samples (3 ml) were collected in clean, dry in sterile pre-weighted tubes as discussed previously [6]. The duration of saliva sampling was altered among individuals depending on their flow rate. The collection time required to obtain 3 ml of saliva was 2.0-5.0 minutes for different subjects. The flow rate was calculated as the time required for collection of one ml saliva sample. All samples were immediately transported on ice to Research Laboratory of Biochemistry at University of Guilan. After arrival, they were 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 determination of  $\alpha$ -amylase activity. To minimize any error, all of the resulting supernatants were used for measuring  $\alpha$ -amylase activity on the same day.

**Assay of  $\alpha$ -amylase**

Salivary  $\alpha$ -amylase was measured using 2-chloro-4-nitrophenyl- $\alpha$ -D-maltotrioside (CNP3) as substrate, to which a chromogen 2-chloro-4-nitrophenyl was attached to a molecule of maltotrioside. This is a direct amylase assay without using enzymes such as  $\alpha$ -glucosidase/glucoamylase. CNP3 is hydrolysed by  $\alpha$ -amylase producing 2-chloro-4-nitrophenyl (CNP) directly and the concentration of CNP is measured at 405 nm [20].



In a typical reaction mixture at 37°C, 25  $\mu$ l of the saliva sample was added to 1 ml of the substrate reagent and mixed rapidly. Absorption was measured at 405 nm after exactly one minute followed by a second measurement after 5 minutes. The increase in absorption was related to the activity of  $\alpha$ -amylase.

$$\alpha\text{-amylase activity (U/ml)} = \frac{\text{Increase in absorption} \times 1025}{12.9 \times 25 \times 5}$$

The value of 12.9 is absorption coefficient for 1 mM of CNP at 405 nm. The terms 1025 and 25 are the total and sample volumes respectively.

The statistical differences are given in the result section. One unit of activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per min under standard conditions. To test the effect of freezing on the biological activity of salivary amylase, the enzyme activity was also measured on some fresh supernatant of saliva samples. No significant difference was observed between thawed and fresh samples. Therefore, only the frozen samples were used for continuing studies.

**Statistics**

Each assay was repeated triplicate and the results were presented as mean  $\pm$  SD values. Statistical difference between groups was compared by un-paired t-test, p values less than 0.05 were retained as significant.

**Results**

The range of saliva flow was 0.08-1.40 ml.min<sup>-1</sup> at rest and was not affected statistically significant by the exercise. The mean activity of  $\alpha$ -amylase (U/ml) is summarized in Table II. These are the mean value obtained from 2-3 repeats in each case. No significant (P>0.05) differences were observed within the repeated tests. The correlation coefficients for inter-assay repeats ranged from 0.66-0.96. The results of repeated tests ANOVA at all intensities showed a significant increase in salivary amylase activity especially at high intensity treadmill exercise as compared to pre-exercise level. On the other hand, treadmill exercise at low and moderate intensity, although caused a rapid increase in the activity of  $\alpha$ -amylase, but it was almost returned to the original value after about one hour. Table II shows that the highest activity of salivary  $\alpha$ -amylase was observed immediately after treadmill test to exhaustion. The rise in enzyme activity did not return 100% to the resting value. However, at 50% VO<sub>2max</sub>, almost 95% of the enzyme activity was observed after one hour.

Table II. Mean  $\pm$ SD activity (U/ml) of  $\alpha$ -amylase for subjects run for 18 min at heart rate values which corresponded to 50%, 75% and exhaustion.

Sample	50% $VO_{2max}$	75% $VO_{2max}$	Exhaustion	P values*
Pre-exercise	198 $\pm$ 3.0	199 $\pm$ 3.2	199 $\pm$ 3.0	0.05
Immediately after exercise	212 $\pm$ 3.5	223 $\pm$ 3.1	245 $\pm$ 3.0	0.02
One hour after exercise	201 $\pm$ 3.0	208 $\pm$ 3.3	220 $\pm$ 3.0	NS

Values presented as Mean  $\pm$  SD.

\* P values were compared by t-test; NS – not significant

### Discussion

In this research we found that activity of oral  $\alpha$ -amylase was increased by a factor of about 120-150% in response to various exercise intensity. The results presented in Table II show that the enzymatic activity increases with different degree due to various intensities of treadmill exercise. However, the intensity of exercise affects its remaining at high level of activity. It can be seen in Figure 1, when the intensity of exercise is lower, the increase in  $\alpha$ -amylase activity immediately after exercise is followed by its returning to almost original value after one hour. However, the higher rise in enzyme activity at exhaustion does not return to near original value, but it only decreases slowly.

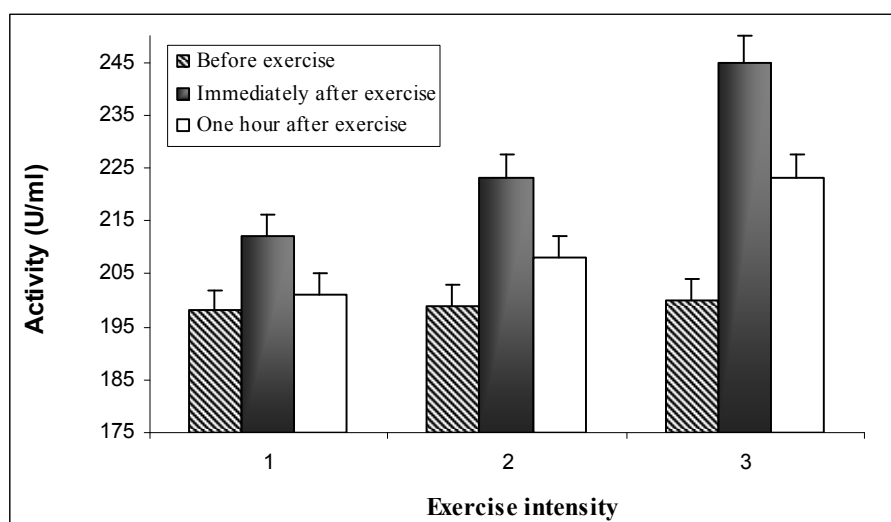


Figure 1. The effect of exercise intensity on biological activity of salivary amylase.  
1. 50%  $VO_{2max}$  2. 75%  $VO_{2max}$  and 3. Exhaustion.

The result of our study is consistent with findings from studies with younger research participants [7]. They reported increase in salivary  $\alpha$ -amylase due to moderate stress in infants. Specifically, the findings suggest that by 6 months of age levels of salivary  $\alpha$ -amylase are responsive to environmental stimuli. Marked increase of  $\alpha$ -amylase concentrations has also been reported in response different exercise such as running [18] and bicycle exercise [21].

### Conclusions

A treadmill exercise with low to moderate intensity (50-75%  $VO_{2max}$ ) causes a noticeable increase in the activity of salivary  $\alpha$ -amylase. The higher the intensity of exercise the longer would be the length of time that amylase remains at high activity level. On the other hand, the highest intensity exercise i.e., to exhaustion can not only increase the activity of  $\alpha$ -amylase to a higher level, it also keeps it elevated for at least one hour after exercise. Therefore, digestion of carbohydrates begins more easily and rapidly during the first one hour after high intensity exercise.

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