Effect of different intensity aerobic activity on total protein concentration in saliva

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Abstract
The biochemical composition of saliva in order to study effects of physical activity has been considered by many researchers. The aim of this study has been effect of different intensity aerobic activity on salivary total protein concentration. In this study, 10 male non-athletes academic (with an average height of 172/9 ± 4/25 cm, weight 67/8 ± 0/08 kg, body fat percentage, 15/98 ± 3/85, VO2max, 38/49 ± 6/43 ml/kg/min ,age 23/2 ± 2/35 years) randomly selected. For the measurement of maximal oxygen uptake, subjects performed an incremental continuous protocol on treadmill. In The main test, subjects did activity in three separate sessions for at least 7 days between them and at 2.30 pm. In order to compare the mean changes of total salivary protein concentrations between the three modes of activities used ANOVA test. The results indicated that the concentration of total protein in each exercise intensity decreased. That might parasympathetic nerve activity lead to increase in salivary fluid and ultimately a reduction in the total protein concentration. However, several factors moreover intensity of activity can also influence in the factor studied in this research.

Keywords: saliva, total protein, exercise intensity, aerobic activity

Introduction
Proteins are molecules that are responsible for what happens in the cells and organs. We
can consider them active molecules because it may contain enzymes that catalyze chemical reactions within the body or in the form of antibodies or receptors on human lymphocytes protect against diseases (Houston, et al., 2001). Saliva contains a continuum immunological and non-immunological protein with antimicrobial properties (Axelsson, et al., 2000; Carlsson, et al., 1983) which is vital to maintain and improve the health of the oral tissues and studding that as a good way to investigate in non-invasive biochemical metabolism has been used (Humphrey, et al., 2001). Exercise can alter the secretion of saliva and cause changes in various combinations, such as immunoglobulins, hormones, lactate, proteins and electrolytes (Chicharro, et al., 1998; Li TL Gleeson, et al, 2004). Oral cavity is a microbiological environment that includes different organisms that can impress unto sizes by the person's diet and oral health habits. However, saliva is the main factor that holds control of all the different groups of organisms using a chemical weapon. These ranges are from simple components such as urea or thiocyanate to enzymes and secretary immunoglobulin complex proteins. In normal conditions, in a healthy mouth, all these factors will help to maintain the ecological correct balance of mouth (Axelsson, et al., 2000); Fox ,et al., 2004); Levin, et al., 1989); Levin ,et al., 2004); Macquire, et al., 2003).Today the biochemical composition of saliva in order to study effects of physical activity has been considered by many researchers (Allgrove, et al., 2008); Li TL Gleeson, et al., 2004); Ljungberg, et al., 1997). Among the biochemical composition of saliva can note different electrolytes (sodium, potassium, calcium, chlorine, magnesium, bicarbonate, phosphate) and proteins in the form of enzymes, immunoglobulins, and other antimicrobial agents and some important mucous glycoprotein, polypeptides and oleic peptides, as well as glucose and nitrogenous products such as urea and ammonium(Edgar, et al., 1992); Humphrey , et al., 2001). These components affect on each other and are responsible for salivary various functions (Ferraris, et al., 2006). Salivary defense mechanisms are the first line of protection against foreign microorganisms that are harmful to the human body entered through the gastrointestinal tract. In addition to foreign pathogens, these factors prevent the uncontrolled growth of internal small molecules by different mechanism (Tenovuo, J., 1998). However, because human saliva has different defense mechanisms that act in a coordinated manner may indicate the effects of the agreement and it is difficult to describe the role of individual defense factor (Lenander-Lumikari, M., 1992).

Materials and Methods

Subjects
In this study, 10 male non-athletes academic (with an average height of 172/9 ± 4/25 cm, weight 67/8 ± 0/08 kg, body fat percentage, 15/98 ± 3/85, VO2max, 38/49 ± 6/43 ml/kg/min ,age 23/2 ± 2/35 years) randomly selected. Before participating in the study they became familiar with the concept of the test and after completing the questionnaire on health the consent form was received.

Determine the maximum oxygen consumption
For the measurement of maximal oxygen uptake, the subjects did a Continuous incremental treadmill protocol (Mc dowell, et al., 1991). Before the test, Subjects were asked to avoid of intense activity for 24 hours before performing of test, they took 5 minutes to warm up the body before the test. Test performed on a treadmill with no incline with an initial speed of 1/87 m/sec
every three minutes, 0/44 m/s, the speed was increased until the subject reached volitional exhaustion. Using heart rate belt (polo 033) and RPE using the Borg scale was recorded every minute during the test. During the whole test gas exchange parameters were determined using a gas analyzer (Quark b2 Cosmed Italian company part 2001 n. Coo827-d 2 - 91) was analyzed. Software Quark b2 record lot about respiratory gases during the test and also subjects VO2max was estimated in the test.

Design exercise
In The main test, subjects did activity in three separate sessions for at least 7 days between them and at 2.30 pm. The subjects were asked to eat lunch at 12 noon with 500 ml of water after lunch and, take a toothbrush. 10 participants did one of three intensity activity per each session randomly and an average of 18 minutes at a heart rate corresponding to 50% of maximal oxygen uptake, 75% and repeat increased continuity test to volitional fatigue was used to determine VO2max operated on a treadmill. Heart rate estimated for each subject using linear regression (based on information Swain, et al, 1994) and using equations heart rate reserve Karvonen to predict heart rate from VO2max test results.

Unstimulated saliva samples on three occasions before, immediately after and one hour after exercise from the subjects were collected. Recipes and dental care, the type of toothpaste, food habits and time to collect them after meals, carefully for all subjects was controlled. The time for collecting saliva in subjects related to salivary flow rate varied between 2 to 5 minutes. Approximately one milliliter of saliva samples in sterile tubes for doors (1.5 mL) were collected after centrifugation until fermentation experiments were kept in the freezer -18°C.

Determine the total protein concentration
For this purpose, we used the method of Bradford. After solving 100 mg Kumasi Brlyant Blue Powder 250 G at 96 ml 50% ethanol, the amount of 100 ml phosphoric acid (W / W) 85% was added to it. The volume of the resulting solution with distilled water reached to 1lit then with filter paper has been smooth. This solution can be used up to a month in 4C°. The contents of the tubes were mixed well, after 15 minutes the samples absorption was read at a wavelength of 595 nm. After recording the standard solutions absorption and samples of protein, standard curve plotted.

Statistical methods
In order to compare the mean changes of total salivary protein concentrations between the three modes of activities used ANOVA test. Data analysis by SPSS software, version 13 and drawing figures by Excel have been performed. Also, in this study, the level of significance P≤0/05 has been assumed.

Results
Referring to Table 1 the comparison between the two concentrations of total protein measurement time separately, It is clear that average total protein immediately after exercise (P=0/001) and one hour after exercise (P=0/001) is significantly reduced compared to the previous activity. But immediately after the activity than one hour after the activity (P=1/000) the difference was not significant (P≤0/05). Further analysis revealed that at 75% of VO2max in the comparison between the two concentrations of total protein measurement time separately, Average total
protein immediately after exercise were significantly decreased compared to the previous activity (P=0.016) The decline continued until one hour after the exercise (P=0.001). However, the time immediately after exercise (P=0.390) There was no significant difference (P≤0.05). And finally at exhaustive exercise in comparison between the two concentrations of total protein measurement time separately, it is appear the mean total protein immediately and an hour later, relatively with a significant level (P=0.027) and (P= 0.001) is significantly reduced compared to the previous activity. Despite a decrease in the concentration an hour after exercise than immediately after exercise (P=0.889) this decrease was not significant (P=0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>50% VO₂max</th>
<th>75% VO₂max</th>
<th>Exhaustion</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exercise</td>
<td>4.53±1.52</td>
<td>5.45±1.54</td>
<td>6.56±1.41</td>
<td>-</td>
</tr>
<tr>
<td>Immediately after exercise</td>
<td>1.90±1.07</td>
<td>3.61±0.87</td>
<td>5.06±1.19</td>
<td>0.001</td>
</tr>
<tr>
<td>1 h after exercise</td>
<td>1.84±0.87</td>
<td>2.78±1.04</td>
<td>4.51±1.25</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Significant level was considered P≤0.05.

**Discussion**

The results showed that the concentration of total protein in each exercise intensity decreased. So that the concentration of total protein immediately after exercise than before exercise decreased significantly and this decline continued until one hour after exercise but in this case to the time immediately after exercise, decrease was not significant. Results of this research have contradiction with a lot of research regarded in this area. Salminen et al., 1962; Steerenberg et al., 1997; Blannin et al., 1998; Reid et al., 2001, stated that aerobic exercise increases the concentration of salivary proteins(Blannin, et al., 1998); Reid, et al., 2001); Salminen, et al., 1963); Steerenberg, et al., 1997). The amount and composition of saliva secretion depend on collective actions of multiple transmitter and Biological messenger on secretary cells. Changes in protein profile and electrolyte composition may be the result of integrated and complex relationship between different controls systems arise. Intensity exercise may be necessary for the saliva. It can be expected that Short-term, low-intensity exercise causes only minor changes or no changes in the salivary electrolytes and other components, While intense and
prolonged exercise may be cause significant changes. Salivary gland metabolism after prolonged strenuous exercise can be affected. It is known that severe and prolonged Exercise has strong but diverse hormonal and physiological effects on various organs. Also, the synthesis of proteins by reducing storage of flow rate of salivary gland cannot be stopped after marathon race. It seems from among the various biological factors, secretor transmitters are important for the synthesis (Mandel, et al., 1989). Steerenberg and colleagues suggested that increase in salivary protein concentration may be due to dehydration (Steerenberg, et al., 1997). Increased sodium and proteins concentration of salivary may be due to the increased concentration of salivary components as a result of evaporation, which is caused by pulmonary ventilation during exercise. This mechanism seems to be quite true. Thus, salivary protein concentrations change during exercise in accordance with plasma levels. This is due to the increased permeability barrier of salivary - plasma that is due to lack of oxygen during exercise. Glomeruli membrane and plasmacerebrospinal fluid barrier permeability increase during oxygen deficiency. Also, transfer of some enzymes from the cell to the plasma due to exercise, causes increase of permeability of the cell membrane (Salminen, et al., 1963). A flow rate and composition of saliva during exercise is affected by the sympathetic nervous system activity and hypothalamic-pituitary-adrenal axis. Salivary glands are innervate by both sympathetic and parasympathetic nerves. In general, it is observed that sympathetic stimulation (via noradrenalin) leads to increased concentrations of salivary proteins (alpha amylase). While, in response to parasympt hetic stimulation, increase in salivary fluid output occurs (Fox, et al., 2004). Therefore, in the present study decrease in concentration of salivary total protein at different intensity aerobic activity it can be defined that parasympathetic nerve activity may lead to increase in salivary fluid and ultimately reduce the total protein concentration.

**Conclusion**

Although in this research the intensity of the exercise could not have a significant effect on salivary total protein concentration, but the effectiveness of exercise intensities was also different. However, moreover exercise intensity, several factors can also influence in the factors studied in this research that can be examined in future.

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