THE EFFECT OF INTENSIVE EXERCISE ON SALIVARY IMMUNOGLOBULIN A, CORTISOL AND PH CONCENTRATIONS IN TEENAGE-GIRL SWIMMERS

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ABSTRACT: Salivary Immunoglobulin A has a leading role in the immune system of the upper respiratory tract. The aim of this research was to compare the effect of intensive morning and evening exercises on the concentration of immunoglobulin A (IgA), cortisol, and pH in the saliva of teenage swimmers. This research was quasi-experimental in nature. The sample consisted of 120 teenage-girl swimmers from high-schools of Gonabad city. Out of them, 20 girls were chosen through random convenient sampling method. The subjects did their allocated swimming exercise and their saliva samples were collected before the intensive exercise and then immediately after. The data was analyzed through SPSS software V.14 employing paired T-test. Maximum decrease in IgA and cortisol levels was observed in the morning and evening bouts, respectively, whereas the largest increase in pH level was found in the evening set. The concentration of salivary IgA and cortisol of the swimmers reduced significantly after exercise compared to the levels noted before exercise, on the other hand, there was a meaningful rise in pH after exercise compared to the one before (p=0.0001). Moreover, while salivary IgA and cortisol decreased after the evening set of exercises compared to the morning set, pH levels were meaningfully higher in the evening set compared to the morning one (p=0.0001). The results of this research showed that intensive morning and evening practices caused salivary IgA and cortisol to reduce and salivary pH to increase which could influence the onset of infection in the upper respiratory tract.

Keywords: Immunoglobulin, Saliva, Practice, Cortisol.

INTRODUCTION
Light and regular sport exercise plays a role in raising the standard of an individual’s health through a reduction of blood pressure and body weight, elevated glucose tolerance, and suppression of upper respiratory tract infection [1], etc. Exercise is a variable that brings about physiological and hormonal compatibility and positively enhances the immunological system from various aspects. Athletes and coaches believe that intense exercise and heavy training routines reduces the activity of the immunological system which becomes a factor in making the individual susceptible to upper respiratory tract infections [2]. In other words, intensive physical activity has a suppressing effect on the immune system [3]. Indicative of the same are a reduction in
lymphocytes, in count and activity of natural killer cells and in antibodies [4]. In certain cases immunity is so low that it can be compared to an open window through which the possibility of clinical infection is very high [5].

Immunoglobulin A however only makes up 10 to 15% of all the serum immunoglobulin in the body yet it is the main and dominant immunoglobulin in mucosal secretion and its level in the mucosal environment is closely correlated to the upper respiratory tract immunity in comparison to serum antibodies [6]. On the basis of the same, salivary IgA is the first barrier that disease-causing factors of the oral cavity encounter as it inhibits the adherence of bacteria, causes the absorption of antigens throughout the mucosal surface and neutralizes toxins and bacteria.

For the first time the effects of physical exercise on mucosal immunity were studied in 1982. The results of this research showed that salivary IgA concentration in long-range elite skiers was lower than that of leisure athletes – and reduced even further after contest [7].

There has been an assortment of studies since then on the effects of different physical activities on the immediate- and long-term responses (resting level) of the mucosal immune system. A review of these works shows disparity in their conclusions. Some of them concluded that one bout of activity caused a drop in concentration of salivary IgA [8 – 11] whereas others showed an increase of the same after exercise [12 – 15]. Further, there were others that cited no change in concentration levels [16, 17]. Researchers throw the blame of these apparent contradictions on methodical factors in use such as the samples, intensity, duration and type of exercise. In a six-month-long research on swimming practice, Gleeson has reported a reduction in concentration of salivary IgA [18].

Cortisol is the most important catabolic hormone in the body. It is a steroid hormone produced by the zona fasciculate of the adrenal cortex and has an overall catabolic effect [19]. Stress hormones, such as cortisol, inhibit immunity and reduce resistance to infections in elite trainers or athletes. A high density of cortisol under specific conditions prevents the production of antibodies and reduces the rate of salivary IgA [20, 21]. Moreover, mental and physiological stress influences the secretion of cortisol hormones in the adrenal glands [22]. Many researchers have emphasized the important role cortisol plays as a hormone modulating immunological responses and responsible for changes in the immunological system of the body after physical activity. There is proof that during exercise cortisol behaves as a mediator in inducing changes leucocytes counts and their redistribution [1].

To answer this question that whether muscular activity affects the immunological system and or which form of exercise weakens the immune system, various studies with different protocols have been carried out by researchers on the relationship of the activity of the immune system and physical exercise. Johansson et al. (2003) and Pederson (2007) have reported that cortisol rates have risen after long and rigorous physical exercise resulting in an increased entry of leukocytes into surrounding tissues consequently reducing their production and inhibiting their count in circulation. A reduction of lymphocytes (immunoglobulin generators) causes a reduction in activity of the body’s immune system [23, 24].

The results of research done by Mackinnon et al (2003) demonstrated that after three times a week exercise, the rate of salivary IgA reduced by 27% - 38%; the maximum 38% occurred after a bout of strenuous exercise. They conclude that intense interval exercise in elite athletes causes a reduction of salivary IgA and pH level thus playing an important role in the manifestation of upper respiratory tract infections [25].

Niemen et al did a research in 2006 to study the effect of 30-minute walks on the rate of salivary cortisol and IgA and found that although Oxyhemoglobin increased by 11%, there were meaningful differences in IgA and cortisol rates [5]. In a 2004 study, Farzanegi et al focused on the profile of salivary IgA and cortisol. They planned an increase in exercise routines of girl gymnasts and concluded that exercise did not have a meaningful effect on salivary IgA, but it increased the rate of salivary cortisol [17].

In the same year, Rajabi et al compared and studied the effect of single and double rigorous exercising bouts per day on salivary IgA and cortisol in female swimmers. Their analysis showed that single or double bouts of exercise caused no significant difference in the IgA and cortisol rates of female swimmers. [19].

As previous studies on the subject were contradictory and since a deeper study regarding the effects of physical activity on the functioning of the immune system in teenagers was needed, the present researchers designed a suitable exercise routine (specific to age, physique and physical fitness) in order to study changes in salivary cortisol and IgA level in teenagers. This research was undergone not only to compare previous results on the matter but also to give suitable recommendations and guide lines to coaches and athletes on the subject, so that they could turn to sports activities for the sake of keeping physically fit with more certainty.
MATERIALS AND METHODS
The present study is a quasi-experimental research embarked upon in 2011. The statistical population consisted of 112 teenage-girl swimmers from high-school level in the city of Gonabad; 20 of them were chosen through simple random sampling. The sample volume was calculated on the basis of a pilot study, mean comparison formula, and by considering beta and alpha coefficients at 0.2 and 0.5, respectively. Inclusion criteria included: a willingness to participate in the research, all sample units under study had to be athletes with at least a two-year-long background of routine exercise and a certificate from the doctor showing ample physical health.

In order to weigh the samples, a German weighing machine (Beuver) with an accuracy of 0.1Kg was used. To calculate the time for the execution of each session of the exercise routines and to count pulse rate, a Japanese chronometer (Q&Q) was used. Laboratory Kit DEXK276 with an accuracy of 1mcg/ml (by DEMED ITEC, Germany) was used to measure salivary IgA. And lastly for measuring salivary cortisol, the kit DE-SLV2930 (RAIDM, Italy) was utilized. The accuracy of the scales was checked with a 1Kg weight every morning and the accuracy of the chronometer was compared to a wristwatch. To confirm the accuracy of salivary IgA and cortisol readings, one out of ten samples was sent to two different laboratories, simultaneously.

Initially the characteristics of the samples were noted on individual specification sheets and each one underwent their exercises once at 11am and then again at 4pm on the following day (in order to neutralize any effects from over-exercising during the morning sets on the evening ones). The training program for the samples that were all swimmers and had fulfilled the inclusion criteria consisted of a 200-meter swim in crawl-style with a one minute rest after every 100 meters. Prior to taking saliva samples, the swimmers had to gargle for one minute using distilled water. This was done to eliminate any traces of substances similar to chlorine that could affect the salivary IgA and cortisol readings. Saliva collection was done using standard procedure: the samples sat erect on a chair while their head was bent forward and in order to prevent artificial secretion, they were asked not to make any attempt to produce saliva but to allow their saliva to drip into a 20ml plastic test tube for 4 minutes. The collected saliva was immediately put in dry ice and the suspended fluid was kept at -20°C until testing.

The first saliva collection was done in a composed state before exercise and the second was collected immediately after the intensive exercise. Further, a physician was also always present at the pool in case of any emergencies.

To identify normality of data in each coefficient, the Kolomogrov-Smirinoff test was used. The data was analyzed via spss software and the paired T-test was used to compare the salivary IgA and cortisol rates in the morning and evening bouts, before and after rigorous training.

RESULTS
The mean age of the samples was 14+/-5 years with an average height of 146+/-2.5cm. The mean weight of the individuals in the morning group was 46.63+/-2.3Kg whereas in the evening group it was 47.41+/-1.73Kg. The greatest drop in IgA was seen in the morning set while the largest decrease in cortisol was seen in the evening set.

| Table No. 1: Results of salivary IgA before and after exercise in morning and evening bouts |
|-------------------------------|----------------|----------------|
| **exercise** | **morning Mean(SD)** | **evening Mean(SD)** | **Statistics** |
| Before | 4.91(0.76) | 2.54(0.39) | t=14.47 p=0.0001* |
| After | 1.60(0.83) | 0.63(0.48) | t=4.43 p=0.0001* |
| Difference | 3.30(0.73) | 1.90(0.25) | t=5.31 p=0.0001* |
| statistics | T=12.21 | T=13.35 |
| p=0.0001** | p=0.0001** |

*Independent samples t-test was used. ** paired sample t-test was used
The IgA rate of the swimmers reduced meaningfully after exercise compared to before (p=0.0001), moreover it showed a significant reduction in the morning bout (p=0.0001).

Table 2: Results of salivary cortisol rates before and after exercise in the morning and evening sets

<table>
<thead>
<tr>
<th>Exercise</th>
<th>morning Mean(SD)</th>
<th>evening Mean(SD)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>830.76(80.31)</td>
<td>743.32(40.80)</td>
<td>t=5.34 p=0.0001*</td>
</tr>
<tr>
<td>After</td>
<td>488.72(38.34)</td>
<td>331.04(63.73)</td>
<td>t=9.22 p=0.0001*</td>
</tr>
<tr>
<td>Difference</td>
<td>342.04(84.24)</td>
<td>412.28(73.36)</td>
<td>t=2.83 p=0.0009*</td>
</tr>
<tr>
<td>Statistics</td>
<td>T=28.10</td>
<td>T=20.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.0001**</td>
<td>p=0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

*Independent samples t-test was used. ** paired sample t-test was used

Salivary cortisol of the swimmers reduced meaningfully after exercise compared to before it (p=0.0001). Plus, the evening readings showed a meaningful drop compared to that of the morning (p=0.0001) (Table 3). Salivary pH levels of the swimmers rose significantly after exercise in comparison with those before (p=0.0001). Further the amount was less in the evening bout compared to the morning one (p=0.0001) (Table 3).

Table 3: Results of salivary pH levels in morning and evening before and after exercise

<table>
<thead>
<tr>
<th>Exercise</th>
<th>morning Mean(SD)</th>
<th>evening Mean(SD)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>7.10(0.66)</td>
<td>6.12(0.21)</td>
<td>t=6.66 p=0.0001*</td>
</tr>
<tr>
<td>After</td>
<td>7.74(0.21)</td>
<td>8.05(0.22)</td>
<td>t=4.43 p=0.0001*</td>
</tr>
<tr>
<td>Difference</td>
<td>0.64(0.74)</td>
<td>1.93(0.37)</td>
<td>t=5.17 p=0.0001*</td>
</tr>
<tr>
<td>statistics</td>
<td>T=25.85</td>
<td>T=4.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.0001**</td>
<td>p=0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

*Independent samples t-test was used. ** paired sample t-test was used

**DISCUSSION**

The aim of this research was to compare the effect of rigorous exercise or training in the morning and evening on IgA and Cortisol concentrations in teenage swimmers. The findings of this research show that the chosen exercise routine affects the concentration of salivary IgA and cortisol in the morning and evening and causes a reduction of the same. The results are in line with the conclusions of Azarbaijani [26], Moenia et al [27], Janson et al [23], McKinnon et al [25], Niman et al [1] and McDowell et al [28], who have all found that exercise causes reduction in serum IgA. Moreover, the present research is in contradiction to a study by Lisan et al [29] that reports an increase in salivary IgA levels and to research work done by Ashterani et al [30], Farzanegi et al [17] and Rajabi et al [19] who observed no difference in salivary IgA level after exercise. It can be said that the contradictions could be due to differences in exercise programs (in the intensity, duration, amount, interval and the number of exercise sets in a day plus in the the type of muscles involved) and/or in the characteristics of the samples (age, gender and level of physical fitness). On the other hand, our study shows that vigorous exercise is connected to a reduction of salivary IgA rates; a number of reasons can be cited for the same. Major changes that occur with muscular activity are hormonal changes. These changes differ in amount according to the duration and intensity of the activity and thus trigger changes in the internal environment of the body. One such change in hormone that is brought about by exercise is the change in catecholamines which normally increase with
physical activity [31, 32]. Research has illustrated that an increase in percentage of inhibitor hormones like epinephrine and encephaline can have destructive effects on the immune system – by suppressing immunizing factors, they can cause athletes to contract infections of the respiratory tract.

Moreover, the amount of salivary IgA secreted is connected to factors such as the activity of the sympathetic nervous system, physical and psychological stress and a reduction in saliva flow. A reduction in saliva flow caused by exercise can be due to the fact that physical activity produces an elevation in the sympathetic system which in turn triggers vasoconstriction and decreased volumes of saliva secretion. Another influential factor is the effect that alterations in the molecular transportation of salivary IgA through the mucosal epithelium can have on saliva flow [19].

The findings of our research showed that intensive training in morning and evening bouts influences salivary cortisol, causing a reduction in the same. This conclusion is in line with results from research by Rajabi et al [19], Rudolph and Mcauley [33] that have cited exercise as a factor for reduction of salivary cortisol. However, our results contradict those of Azerbaijani [21], Farzaneghi et al [17], Janson et al [23], Niman et al [1], and Pederson at al., [24], Nehlson and Canare Ua [8], who has all noted an increase in cortisol levels after exercise. Further, our research also opposes the results of Dimitriou et al [19] who have noted that there is no change in cortisol levels after exercise.

Cortisol secretion does not follow a continuous and permanent flow and has a day-night rhythm in such a way that secretion is at its highest in the beginning hours of the day and drops as the day commences. Any kind of stress disrupts the day-night secretion rhythm and the concentration of cortisol in the plasma goes up dramatically. Evidently, this rhythm differs among individuals and may be influenced by sleeping patterns, exposure to darkness and light or even eating times.

There is concrete proof showing hormones as having a role in modulating changes, resulting from exercise, in the count of circulating leukocyte subsets. It has been clearly seen that hormones such as cortisol and epinephrine could affect and increase or decrease the supply of the lymphocytes in blood circulation and in the different organs like the liver, pancreas and bone marrow [19].

Taking the literature on this subject into account, we can conclude that light to medium intensity exercise causes a decrease in the secretion of epinephrine and cortisol thus causing an increase in the reproduction of lymphocytes which strengthens the body’s immune system. Whereas, involvement in intense and prolonged exercise along with mental stress caused by competition triggers added secretion of the two hormones resulting in the entry of lymphocytes to surrounding tissues and in a drop in circulatory count of the same thus lowering the immune system [26].

Cortisol rates can also be affected by the intensity, duration, type and environment of the exercise on the one hand and the fitness level, age, gender, diet and mental stress of the samples on the other. The reasons that the present research regarding cortisol by and large contradicts other studies on the subject could be because of different reasons. Firstly, the age of the samples differed with that in the other studies and because of their young age and their being unaware of the conditions of the research, they felt no stress. Further, all the studies that showed increase in cortisol levels were done in conditions when the samples were preparing for or participating in competition which is a stress factor and by itself causes elevated cortisol levels. On the other hand, the researchers of the present study have tried their best to keep the exercising conditions as stress-free as possible and this might be the reason for lower cortisol levels before starting training. Last but not the least and what is confirmed by most researchers is the fact that physical activity in water can by and large help to reduce stress [5].

The results of the present research show that intensive training in the morning and evening affects salivary pH causing it to increase. This finding is in line with research by Tomas et al [7] and McKinnon et al [9] who have cited a significant rise in pH after exercise. However, it contradicts the works of both Gleeson et al [3] who reported drops in pH levels after exercise and Mc Dowell et al [28] who concluded that there is no change in pH levels after exercise.

The reason for the increase in pH after exercise in the present research could be because of the fact that saliva collection was done immediately after exercise, so there might have been inadequate time for acid to be released from the muscles into the blood and therefore its effects could be seen in the saliva. Another possibility is that the tamponers of the blood become activated and reduce the acid in the blood, or it is even possible that the level of secretion of base ions like sodium, potassium and calcium increases. Finally, the dryness of the mucous in the mouth could also be the reason for an increase of pH, as swimmers usually breathe through the mouth while doing their laps [35].
CONCLUSION

Taking into account the research done on the subject, it has become evident that physical activity is an influential factor in modifying the working of the immune system though it depends on the intensity, duration, exercise plan, physical status of the individuals, as well as the measurement methods. On the whole, the present research showed a significant decrease in salivary IgA and cortisol level after a session of intensive training in the morning and evening, whereas at the same time, salivary pH showed a meaningful increase. These could have implications on the development of respiratory tract infections.

REFERENCES