ARCHIVES of Exercise in Health and Disease

DOI: 10.5628/aehd.v3i1-2.116

Original Research Manuscript

The effect of one bout of incremental exercise on salivary immunoglobulin A (IgA) of high school students

Alireza Ramezani[1], Amir hossein Barati[1], Mohammad ali Azarbaijani[2], Mohammad Tohidi[1], Mehdi Abbaszadegan[1]

[1]College of Physical Education and Sport Sciences, Shahid Rajaee Teacher Training University, Tehran, Iran
[2]Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Objective: The aim of present study is study the effect of one bout incremental exercise on salivary IgA changes in boy athlete and no athlete high school students. ***Design:*** 15 no athlete and 30 athlete (15 wrestlers, 15 endurance runners) students were selected as subject randomly among students of Neishabur city in Iran. Subject ran on the treadmill to exhaustion range using Bruce protocol. Four milliliter unstimated saliva was collected pre, immediately and 2 hour after exercise. ***Results:*** The result showed that one bout incremental exercise caused significant increase in S-IgA concentration in non-athlete and endurance runners groups, and this increase continued to 2 hours after exercise. There was significant increase in S-IgA concentration in wrestlers group after activity. S-IgA concentration decreased after 2 hours of activity, but it did not reach to initial levels of pre-activity, this different was statically significant. ***Conclusion:*** Based on these findings, it is inferred that one bout incremental exercise in young students had no suppressor effect on the mucosal immune system and there is no probability of existence of the upper respiratory tract infection following this activities.

Arch Exerc Health Dis 3 (1-2):168-172, 2012

Key Words: An excessive activity session; IgA; young students

INTRODUCTION

It has been well-documented that performing physical activity has a positive effect on the body various systems. However, there are some differences regarding the effect of physical activity on the immune system (25). Although training of moderate intensity causes an improvement in immunologic capacities (22), intense and difficult training can have a negative effect on some immune system functions (9) and can result in immune system suppression (8). Training of high intensity can decrease lymphocytes, the number of NK cells, and antibody production (31). In some cases, immune weakness is so severe that the body status defensively simulates an open window; under this condition clinical infection can often be expected (23).

Although immunoglobulin A (IgA) constitutes only 10-15 percent of the total serum immunoglobulin, it is the dominant and main immunoglobulin in mucous secretion and its level in the mucous fluids compared to serum antibodies has a high coefficient with resistance to upper respiratory tract infections (URTI) (17). Salivary immunoglobulin A (S-IgA) is the first barrier against pathogenic factors in the mouth cavity and the upper respiratory tract infections and can cause an inhibition of the bacteria cohesion, antigen absorption in the overall mucous levels, and toxic and bacteria neutralization (21). The external body level is a wide area that can be a suitable environment for replacing pathogenic bacteria. The secretive immune system provides an impressive mechanism in host defense against nestling pathogenic microbes in the eyes, nose, upper and lower respiratory tract, gastrointestinal tract and urinary-reproductive levels (25).

The first study on the effect of physical training on mucous immunity was conducted in 1982. The results

Corresponding author:
Mohammad ali Azarbaijani: College of Physical Education and Sport Sciences, Shahid Rajaee Teacher Training University, Tehran, Iran • Email: m_azarbayjani@iauctb.ac.ir

of this study showed that an S-IgA concentration in the endurance of skiers was lower than recreational athletes, and this decrease became higher after the national ski competition (30). Many studies have been conducted on the effect of various physical activities on chronic (rest level) and acute responses of the mucous immune system specially S-IgA. Some studies indicated decreased S-IgA concentration in S-IgA response to an activity session (27, 24, 16, 12). On the other hand, other studies indicated an increase in S-IgA (1, 11, 14, 27) or unchanged levels of S-IgA (2, 4). In addition, a study reported the effects of several days, weeks and months of training on the rest S-IgA concentration. Like an acute response of the S-IgA, the results of studies were paradoxical and reported decreased (3, 5, 15), increased (7, 29) or unchanged levels in S-IgA (17). Researchers attribute these differences to the methodological factors used such as subjects, intensity, duration and type of training programs. An investigation of conducted studies in this area show a plethora of studies on the effect of exercise training on S-IgA response have been on adults and elite athletes subjects, so the effect of an intense exercise session to exhaustion on S-IgA response is not known in active young subjects. The present study investigated the effect of one bout of incremental exercise on S-IgA changes in male high school students, some of which were athletes.

MATERIAL AND METHODS

Subjects

In a quasi-experiment design, 45 male students ranging in age from 15 to 18 years were selected as subjects (15 non-athletes, 15 wrestlers and 15 endurance runners). The wrestlers and endurance runners had been involved in regular training for two years prior to the fulfillment of this study and competed actively in school province competitions. They were informed of the purposes and methods of the study before providing written consent. None of the participants were taking any form of medication. General characteristics of the subjects are presented in Table 1.

One bout of incremental exercise

To determine acute response of the mucosal immune system to one bout of incremental exercise to exhaustion, each subject ran on the electronic treadmill (Techno Jim HL 4200) to exhaustion using adjusted Bruce protocol (20). At the beginning of the test, treadmill speed and slope were 1.6 km/h and 10% respectively, so that speed and slope increased every three minutes.

Saliva sampling

Five milliliters of un-stimulated total saliva was collected via passive drooling before, immediately following and two hours after one bout of incremental exercise. In each stage of sampling, subjects washed their mouth using water and drank 200 milliliters of water in order to avoid dehydration. Then they collected their salivary samples into special saliva tubes. To reduce the effect of diurnal variations on S-IgA concentrations, saliva samples were obtained from individual subjects at the same time of day—between 8 a.m. and 11 a.m. After collecting, the samples were put into an ice closet and were frozen in -20° centigrade for examining at the appropriate time. Subjects were asked to avoid drinking caffeine 24 hours before the testing, and to avoid eating two hours prior to participating in the study (sampling). They were banned from undertaking any physical activity for 48 hours before the first sampling.

S-IgA assay

S-IgA concentration was measured by single radial immunodiffusion (SRID). This method includes forming a visible residual line of reaction between immunoglobulin and anti-immunoglobulin in suitable concentration. In this method, after providing an S-IgA plate with low concentration from Biogen factory, a five microliter sample was added into wells and from each plate, nine wells for each sample and three wells for designing a standard curve was used. Standards added in 1, 2, 3 wells with determined concentration and after absorbing solvent by gel after five minutes, the plate was adversely kept in a flat lot with 23 º centigrade for 48 hours. Then, through using a special ruler with diameters of residuals measured and using a standard curve design, the S-IgA concentration in each sample was reported by ml/dL.

Statistical analysis

Descriptive analyses were conducted for the characteristics of participants. We used the Kolmogorov-Smirnov test for normalization of data distribution. Homogeneous variances were done by a Levin test. One-way analysis of variance with repeated measures was used to evaluate the S-IgA changes in each group. One-way analysis of variance was used for comparing groups. The Tukey post-hoc test was used to identify significant differences. Except when otherwise indicated, an alpha level of $P \leq 0.05$ was accepted as indicating significance. All statistical analyses were performed using the SPSS program for Windows, version 16.

Table 1. Descriptive characteristics of experimental subjects

Group*	Age (year)	Height (cm)	Weight (kg)	BMI	Body fat%	VO_{2max} (ml.kg.min)
Non-athlete	16.01 ± 0.77	169.87± 4.74	60 ± 9.26	20.80±2.7	20.74± 2.2	38 ± 2.2‡
Wrestler	15.97±1.02	173.87±5.5	68.87±4.92	22.76±1.12	23.33±2.41	43.7±1
Runner	16.29± 0.89	173 ± 5.47	63.27± 5.76	21.01± 1.32	2.47± 2.23	47 ± 1.9

*Results are expressed as mean± standard deviation and ‡ denotes statistical differences between groups. See text for explanation of significant effect.

RESULTS

The first results showed that there are no significant differences among the groups concerning age and height, but there are significant differences between the weight, BMI, percentage fat and VO_{2max}; these differences themselves are due to differences between athletic and non-athletic subjects.

A comparison of subjects VO_{2max} showed significant differences among the three groups ($F_{2,24}$=95.73, P=0.001). The highest and lowest VO_{2max} levels were observed in runners and non-athletes, respectively. Runners' VO_{2max} had a significant increase as compared to wrestlers (P=0.001) and non-athletes (P=0.001). The VO_{2max} level of wrestlers was also significantly higher than non-athletes (P=0.001). A significant difference was observed between groups in Bruce test of performance ($F_{2,24}$=89.69, P=0.001). Running time was significantly higher in the runners group than in the wrestlers (P=0.001) and non-athletes (P=0/001) in the Bruce test. The running time in wrestlers was significantly higher than in the non-athletes (P=0.001).

The results of the responses of S-IgA show that one bout of incremental exercise had a significant effect on S-IgA concentration in the non-athlete students ($p\leq 0.05$). One bout of incremental exercise caused a significant increase in S-IgA concentration immediately after exercise ($p\leq 0.05$) and this increase continued two hours after exercise (Table 2).

Furthermore, one bout of incremental exercise had a significant effect on S-IgA concentration in wrestlers ($p\leq 0.05$). S-IgA concentration of wrestlers increased significantly immediately after exercise ($p\leq 0.05$). After this time, S-IgA concentration continued to decrease so that two hours after exercise, it had decreased significantly more than the immediately following level ($p\leq 0.05$), but there still was a significant increase compared to the resting level (Table 2). The final result showed a significant increase in S-IgA concentration in response to one bout of incremental exercise in endurance runners ($p\leq 0.05$). S-IgA concentration in endurance runners increased significantly immediately after exercise ($p\leq 0.05$). This increase was observed two hours after exercise. There was no significant difference between S-IgA rates immediately and two hours after exercise ($p\leq 0.05$) (Table 2).

DISCUSSION

The first study showed that one bout of incremental exercise to exhaustion level caused a significant increase in S-IgA concentration immediately to two hours after exercise. Various studies reported S-IgA changes following an exercise session to exhaustion (12, 16, 24, 28), but these changes were related to intensity of exercise (6). Some studies reported that moderate intensity training has not had an influence on S-IgA concentration (10). The physiological mechanism in S-IgA changes following physical activity is not known precisely, but a mechanism has been reported for this decrease. One of these factors is a change in S-IgA molecule transfer across mucosal epithelial. In this case, the sympathetic nervous system is activated during physical activity and causes vasoconstriction in the sub mucosal area of the mouth cavity; this phenomenon likely decreases the migration of produced S-IgA into the mouth cavity (18).

The regulation of saliva flow is a complex trend. The sympathetic and parasympathetic nervous system controls saliva output. Exercise training activates the sympathetic nervous system and, through the constriction of arteries that deliver blood to the salivary glands, causes decreased salivary output. Also, the sympathetic nervous system decreases the migration of the IgA from its producing lymphocyte B cells to mucosa in the mouth via arterial constriction of salivary glands (19).

In our study, it appears that due to the absence of psychological stress and a low duration of reaching the point of exhaustion, subjects have not experienced intense sympathetic stimulation and this is likely to cause S-IgA increase after activity in this case. Another mechanism is to the ventilation increase because of an increased need for oxygen during exercise; increased pulmonary ventilation causes changes in the level of mouth mucosa, so that this phenomenon suppresses S-IgA secretion or secretive segment from across the mucosal epithelial (19). In this case, investigation of the other immunoglobulin changes can prove the disorder in S-IgA transition to

Table 2. S-IgA changes pre, immediately and two hours after an incremental exercise session in non-athletes, wrestlers and endurance runner's students.

Group*	Pre	Immediately Post	2 hours Post
Non-athlete	3.1±0.9	3.85±1.86*	3.97±1.81*
Wrestlers	2.48±0.44	3.3±0.78*	2.9±0.67*†
Runners	2.59±0.75	3.48±1.34*	3.4±1.23*

Data has been reported on base of mean and standard deviation. *Denotes significant increase than rates of pre-activity and †denotes significant decrease than rates of immediately after activity.

mouth. The reason for this issue is the selectivity of the physical activity effect on salivary immunoglobulin. The transition of S-IgA and S-IgM from the epithelial barrier into the mouth requires the presence of the secretive segment, whereas IgG secretion from serum into saliva goes directly through inactive transition (31). Although the effect of physical activity on secretive segments has been studied, a prominent decrease in S-IgA and S-IgM and IgG are unchanged in response to physical activity. This represents the hypothesis that changes in the S-IgA transition trend into saliva content is one of the most effective factors in S-IgA decrease. The activity duration in this study was less than 16 minutes; it appears that the outstanding changes in the level of mouth mucosa did not occur and therefore, suppression of the S-IgA secretion or secretive segment across the mucosal epithelial is not probable.

It has been well-documented that increased saliva viscosity after activity is probably because of decreased water in saliva due to increased pulmonary ventilation and water evaporation. This phenomenon can cause increased S-IgA concentration during post activity measurements. The saliva water loss and its concentration were clear during sampling after activity and in our present study. Research findings also showed that the trend of an increase in S-IgA concentration continued for two hours after the activity. This increase can probably be attributed to changes in saliva viscosity in this study.

CONCLUSION

The results of this study showed that one bout of incremental exercise to exhaustion caused increased S-IgA concentration in young, athletic and non-athletic students and this increase is relatively stable for hours after activity. Based on these findings, it is inferred that one bout of incremental exercise in young students has no suppressor effect on the mucosal immune system and there is no probability of having upper respiratory tract infection following this kind of activity.

ACKNOWLEDGMENTS

The authors of this article acknowledge the head of physical education of Neishabur education department and all of the friends and students who participated in this study.

REFERENCES

1. Allgrove JE, Gomes E, Hough J, Gleeson M. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci* 2008; 6:653-661.
2. Bratthall D, Widerström L. Ups and downs of salivary IgA. *Scand J Dent Res* 1985; 93: 128–134.
3. Fahlman MM, Engels HJ. Mucosal IgA and URTI in American college football players: a year longitudinal study. *Med Sci Sports Exerc* 2005; 3:374-380.
4. Farzanaki P, Azarbayjani MA, Rasaee MJ, Jourkesh M, Ostojic SM, Stannard S. Salivary immunoglobulin A and cortisol response to training in young elite female gymnasts. *Braz J Biom* 2008;2:252-258.
5. Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL, Fricker PA. The effect on immunity of long-term intensive training in elite swimmers. *Clin Exp Immunol* 1995; 102:210–216.
6. Gleeson M and Pyne, DB. Exercise effects on mucosal immunity. *Immunol Cell Biol* 2000; 78:536–544.
7. Gleeson M, McDonald WA, Pyne DB et al. Immune status and respiratory illness for elite swimmers over a 12-week training cycle. *Int J Sports Med* 2000; 21: 1–6.
8. Gleeson M. Immune functions in sport and exercise. *J Appl Physiol* 2007;103: 693–699.
9. Hoffman-Goetz L, Pedersen BK. Exercise and the immune system: A model of the stress responses. *Immunol Today* 1994; 15:382-387.
10. Housh TJ, Johnson GO Housh DL, Evans SL, Tharp, GD. The effect of exercise at various temperatures on salivary levels of immunoglobulin A. *Int J Sports Med* 1991; 12:498-500.
11. Li TL, Gleeson M. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and alpha-amylase responses. *J Sports Sci*. 2004 ; 11-12:1015-1024.
12. Libicz S, Mercier B, Bigou N, Le Gallais D, Castex F. Salivary IgA response of triathletes participating in the French Iron Tour. *Int J Sports Med*. 2006; 5:389-394.
13. Liwe FY, Russell SM, Appleyard G, Brand CM, Beale J. Cross-protection in mice infected with influenza A virus by the respiratory route is correlated with local IgA antibody rather than serum antibody or cytotoxic T cell reactivity. *Eur L immunol* 1984;14:350-356.
14. Ljungberg G, Ericson T, Ekblom B, Birkhed D. Saliva and marathon running. *Scand J Med Sci Sports* 1997; 7: 214–219.
15. Mackinnon LT, Ginn E, Seymour G. *Effects of exercise during sports training and competition on salivary IgA levels*. In: Husband AJ (ed.). Behavior and Immunity. Boca Raton (FL): CRC Press, 1992.
16. Mackinnon LT, Ginn E, Seymour GJ. Decreased salivary immunoglobulin A secretion rate after intense interval exercise in elite kayakers. *Eur J Appl Physiol* 1993; 67: 180–184.
17. Mackinnon LT, Hooper S. Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining. *Int J Sports Med* 1994; 15: S179–S183.
18. Mackinnon LT. Immunoglobulin, antibody and exercise. *Exerc immunol Rev* 1996;2:1-35.
19. Mackinnon LT. *Advances in exercise immunology*.1st ed. Human Kinetics, 1999.
20. McInnis K, and Balady G. Comparison of submaximal exercise responses using the Bruce VS modified Bruce protocol. *Med Sci Sports Exerc* 1994; 26:103-107.

21. Mazanec MB, Nedrud JG, Kaetzel CS, Lamm ME. A three-tiered view of the role of IgA in mucosal defense. *Immunol Today* 1993;14: 430–435.
22. Nieman DC, Henson DA.Role of endurance exercise in immune senescence. *Med Sci sport Exerc*.1994;26:172-181.
23. Nieman DC. Exercise effects on systemic immunity. *Immunol Cell Biol* 2000; 78: 496–501.
24. Nehlsen-Cannarella SL, Nieman DC, Fagoaga OR et al. Saliva immunoglobulins in elite women rowers. *Eur J Appl Physiol* 2000; 82: 222–228.
25. Pedersen BK, Rohde T, Ostrowski K. Recovery of the immune system after exercise. *Acta Physical Scand* 1998;162:325-332.
26. Pedersoen BK, Rohde T, Ostrowski K. Recovery of the immune system after exercise. *Acta Physiol Scand* 1998;162:325-332.
27. Schouten WJ, Vershuur R, Kemper HCG. Habitual physical activity, strenuous exercise, and salivary immunoglobulin A levels in young adults: The Amsterdam growth and health study. *Int J Sports Med* 1988; 9: 289–293.
28. Steerenberg PA, van Asperen IA, Van Nieuw Amerongen A, Biewenga J, Mol D, Medema G. Salivary levels of immunoglobulin A in triathletes. *Eur J Oral Sci* 1997; 105: 305–309.
29. Tharp GD. Basketball exercise and secretory immunoglobulin A. *Eur J Appl Physiol* 1991; 63: 312–314.
30. Tomasi TB, Trudeau FB, Czerwinske D, Erredge S. Immune parameters in athletes before and after strenuous exercise. *J Cli Immunol* 1982; 2: 173-178.
31. Tomasi TB, and Plaut AG. *Humoral aspects of mucosal immunity*. In advances in Host defense mechanisms, ed. Gallin JI and Fauci AS.31-61.New York :Raven Press, 1985.
32. Walsh NP, Bishop NC, Blackwell J, Wierzbicki SG, Montague JC.Salivary IgA response to prolonged exercise in cold environment in trained cyclists. *Med Sci Sports Exerc* 2002; 10:1632-1637.