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Circadian Effects on the Humoral Immune System (IgG, IgA and IgM) and Serum Cortisol after a Strenuous Exercise until Exhaustion

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Abstract: The purpose of the present study is to examine the effect of a session of morning and evening strenuous exercise until exhaustion on the humoral immune system (IgG, IgA and IgM) and serum cortisol among the students of aviation admitted to the Air University in 2004. The project was conducted on 15 aviation students who had registered for the course of Physical Education 1 and were selected through random sampling. The research was semi-empirical which involved a single-group design specific to correlated groups carried out through four stages of blood sampling - before the exercise, after the morning exercise, after the evening exercise and a week after the exercise. Then, the change in data before and after the morning and evening exercises was analyzed using t-test for correlated samples and the comparison between the morning and evening data was done using two-way analysis of variance for correlated samples. Thus, the researcher used a cycle ergometer (JK-2040, Germany), Baharafshan kit, single radial diffusion-in-gel for measuring the immunoglobulins and Immunotech kit for measuring the level of cortisol. The results showed that a morning and evening session of strenuous exercise until exhaustion had a significant effect on the humoral immune system (IgG, IgA and IgM) and serum cortisol. The difference in the level of IgM and cortisol was significant in the morning exercise and the difference in the level of IgA, IgM and serum cortisol was significant in the evening exercise.

Key words: IgG, IgA, IgM, cortisol, diffusion gel.

INTRODUCTION

Physical education and sports are areas of human knowledge that have found a special place in the contemporary age and recent advancements in these areas have led to the increasing interest of enthusiasts in motor-sports activities (Rezayee, H.R., 1990).

One of the vital systems of the body is the immune system without which human life will be at stake. The function of many body systems depend upon the immune system. Sports immunology and hormonology have developed over the recent years and the information obtained has revealed more links between such sciences as sports sciences, medicine, immunology, pathology and behavioral sciences which will consequently contribute to the healthy life of humans and will prevent the hidden infectious diseases and their detrimental impacts (Mackinnon, L.T., Chick, T.W., Van As A. and Tomasi, T.B., 2004). Athletes who perform prolonged strenuous exercises are prone to diseases, in particular upper respiratory tract infections (Gleeson, M. 1995, Neary, J.P., Malbon, L. and McKenzie, D.C., 2002 and Tharp, G.D., 1991) and lack of physical activity is considered these days as one of the biggest risk factors for many diseases such as heart disease, obesity, insulin-dependent diabetes, high blood pressure and osteoporosis (Stites, D.P., 1990 and Delves, P., Martin, S., Burton, D. and Ivan Roitt, 1987).

Considering the critical job of pilots and that they must have a fit and healthy body, the present research was carried out on pilots to study the effect of strenuous exercise until exhaustion in the morning and evening on the humoral system and serum cortisol and it helped them in determining the type of exercise programs with respect to the condition of the immune system in the morning and evening.

Immunoglobulin:

Immunoglobulins are glycoproteins found in the blood and other bodily fluids which contain antibodies and are produced to protect the body against pathogens. All antibodies are immunoglobulin molecules but not all immunoglobulins are antibodies (Mackinnon, L.T., 1999).

Immunoglobulin G (IgG):

IgG is the most abundant immunoglobulin found in the body comprising up to 50-80 percent of all the immunoglobulins. The average IgG in the blood serum of an adult is about 11 mg/ml. In normal people, most of the antibodies such as those against bacteria, viruses, parasites, fungi, soluble toxins and Rh antigens belong to the IgG group (Pakzad, P., 2004 and Mackinnon, L.T., 1999).

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Immunoglobulin A (IgA):

This immunoglobulin can exist in monomeric, dimeric and secretory forms. About 15-20 percent of all the immunoglobulins in the body consist of IgA. IgA exists in the saliva, colostrum, mucosal secretions of the digestive system, urinary and genital tracts and in all the bodily secretions in general (Pakzad, P. 2004 and Mackinnon, L.T., 1999).

Immunoglobulin M (IgM):

IgM comprises 5-8 percent of all the serum immunoglobulins and the average amount of IgM in an adult is 1 mg/ml. IgM is the first antibody that is produced in response to antigens in organisms. Thus, it is particularly useful to measure the level of IgM in patients (Pakzad, P. 2004).

The structure of immunoglobulins was first introduced by British and American scientists in 1972. Immunoglobulins or antibodies refer to a group of soluble proteins that attach to antigens. These proteins which act as antibodies are called antigens regardless of the target antigen (Hosseini, F., 2000).

For a long time researchers have tried to carry out studies on the effect of exercise and physical education on the immune system and the results of these studies suggest that changes in the immune system depend on the intensity of exercise as well as its type and duration, physical fitness, age, the amount of time spent for exercise and the methods used for measuring the humoral immune system. Most of these studies are carried out by Mackinnon *et al*., (1989), Nieman *et al*., (1988-1992), Thomas *et al*., (1982-1990) and Simon *et al*., (1991). Roy G. Shepherd, the famous Canadian researcher, carried out many research studies in this regard and published many articles during 1991-1995.

Recent studies show that a session of exhausting exercise and excessive daily exercises are followed by an abnormal increase in lymph node infections and upper respiratory diseases (Mackinnon, L.T., 1999). Chen (1983) studied 111 athletes and came to the conclusion that under normal conditions, the level of IgM, IgG and IgA in serum are at a normal range. The level of serum immunoglobulins decreased significantly in endurance and semi-endurance runners at the early stages of strenuous exercise, i.e. before they were adapted to strenuous exercise. But the level of serum immunoglobulins increased significantly in the athletes who performed intense physical exercises and performed three 15-second running stages completely and with maximum speed or those who had performed the marathon (Chen, J., 1983).

Eliakim *et al*., (1998) studied the cellular and humoral response of the immune system following aerobic exercises. Seven elite female gymnasts and six untrained girls (10-12 years of age) participated in this study. Cellular indices such as lymphocytes, granulocytes and monocytes increased significantly after the exercise and 24 hours later, they returned to their initial levels. Humoral indices such as immunoglobulins A, M and G and IgG subclasses (IgG1, IgG2, IgG3 and IgG4) did not change significantly after exercise. Further, no significant difference was observed between the gymnasts and the control group in the concentration levels (Eliakim, A., Wolach, B., Kodesh, E., Gavrieli, R., Radnay, J., Ben-Tovim, T., Yarom, Y. and Falk, B., 1997).

Hanson and Flaherty (1981) carried out a research on the level of IgG, IgA and IgM in cyclists and came to the conclusion that these levels did not change after 2 hours of cycling (11). Dimitriou *et al*., (2002) studied the circadian effects on the level of IgA and salivary cortisol. The results showed that certain times of the day (morning and evening) significantly affect the level of immunoglobulin A and salivary cortisol before exercise (Dimitriou, L., Sharp, N.C.C. and Doherty, M., 2002).

Chatard *et al*., (2002) studied cortisol concentration, DHEA and performance and exercise in elite swimmers. This research was carried out on nine elite swimmers (4 female and 5 male athletes) during an exercise period of 37 weeks. The findings revealed that the level of cortisol concentration and DHEA has no significant relationship with performance.

Neary *et al*., (2002) studied the relationship between serum cortisol, salivary cortisol and urinary cortisol during resting. Salivary and urinary blood samples were taken from eight subjects (3 females and 5 males) after a day of recovery. The level of resting cortisol was at its highest between 7:30 and 8 A.M. A strong correlation was observed between serum cortisol (99%) and salivary cortisol (97%) and between serum cortisol and urinary cortisol (99%) during 24 hours (Muns, G., Liesen, H., Riedel, H. and Bergmann, K.C., 1989).

Methodology:

Population:

The population of the present research consists of the aviation students admitted to the Air University in 2004, who had registered in the course of Physical Education 1 during the period 2004-2005. The questionnaires and consent forms were distributed among the subjects.

Sample and Sampling Method:

15 aviation students who had registered in the course of Physical Education 1 voluntarily participated in the present research. In addition, the subjects were active from October 2004 until the day of blood sampling and they were engaging in a preparation course for entering the flight battalion and according to the health

questionnaire, they were not under any medication until blood sampling nor did they suffer from any physical illness and they were in perfect health.

MATERIAL AND METHOD

1. 60 Venoject tubes for blood sampling and sending the samples to the laboratory.
2. A Hermle centrifuge with a power of 14000 rpm for separating serum from blood.
3. 120 laboratory tubes for holding the serum of the subjects.
4. Immunotech kit made in Czech Republic for measuring the level of cortisol.
5. Accessories such as medical alcohol, cotton piece, adhesive plaster, etc.
6. Baharafshan kit, single-radial diffusion-in-gel (SRID) for measuring the level of immunoglobulins.
7. 3 magnetic stationary bikes (JK-2040, Germany).

The single radial immunodiffusion assay (SRID) was used for measuring immunoglobulins. This method was invented by Feinberg in 1957 and was later developed by Mancini, Carbonara and Heremans (Hosseini, F., 2000). The material and tools needed for measuring IgG, IgA and IgM were: 2- and 5-ml samplers, 10-ml Hamilton syringes, SRID triangle for measuring the diameters of the circles, glass plates, a scale, millimeter papers and physiological serum for diluting the concentrated samples.

Research Design:

To examine the effect of a session of strenuous exercise until exhaustion on the humoral immune system (IgG, IgA and IgM) and serum cortisol, the researcher studied 15 aviation students of the Air University both in the morning and the evening. One day, early in the morning at 6:30-8:00 A.M. and before the beginning of exercises, blood samples were taken from all the subjects and then the exercises were performed in groups of three in which each subject ran 800 meters for warm-up and continued the rest of their exercises on the cycle ergometer. This exercise was performed in three consecutive stages: first, they pedaled on the cycle ergometer for three minutes with 55% maximum heart rate; when this percentage increased, they pedaled for another three minutes with 70% maximum heart rate and finally, they continued with 85% maximum heart rate until exhaustion and when the subjects themselves found that they are exhausted and can no longer continue pedaling, they would dismount form the cycle ergometer and at that very point, blood sampling would be done.

After a week, at the same day of the week but from 18:30-20 in the afternoon, the experiment was again conducted and blood samples were taken from the subjects both before and after exercises. The collected samples were sent to Bahar Laboratory where blood serum was separated using a sampler; the serum of each subject was poured into two tubes one of which was used for measuring the level of immunoglobulins and the other was used for measuring the level of cortisol. Finally, the tubes were placed in a tube holder and were placed in a refrigerator for freezing.

Due to the lack of necessary facilities and equipment for an empirical research, a semi-empirical design was u. In the present research, blood samples were taken from 15 subjects at four stages.

Results:

1. There is a significant difference in the level of serum IgG of subjects before and after a morning session of strenuous exercise until exhaustion.

Table 1: The calculated statistical indices for morning serum IgG (mg/dl).

Indices / Serum IgG	Number of Subjects	Mean	Standard Deviation	Calculated T
Before the Exercise	15	1428.40	203.078	-1.740
After the Exercise	15	1472.13	210.234	
$p \leq 0.05$		df = 14		

2. There is a significant difference between the mean serum IgA of subjects before and after a morning session of strenuous exercise until exhaustion.

Table 2: The calculated statistical indices for morning serum IgA (mg/dl).

Indices / Serum IgA	Number of Subjects	Mean	Standard Deviation	Calculated T
Before the Exercise	15	327.47	147.004	-1.87
After the Exercise	15	324.33	138.056	
$p \leq 0.05$		df = 14		

3. There is a significant difference between the mean serum IgM of subjects before and after a morning session of strenuous exercise until exhaustion.

Table 3: The calculated statistical indices for morning serum IgM (mg/dl).

Indices / Serum IgM	Number of Subjects	Mean	Standard Deviation	Calculated T
Before the Exercise	15	165.67	24.029	-2.782
After the Exercise	15	171.33	22.493	

4. There is a significant difference between the mean serum IgG of subjects before and after an evening session of strenuous exercise until exhaustion.

Table 4: The calculated statistical indices for evening serum IgG (mg/dl).

Indices / Serum IgG	Number of Subjects	Mean	Standard Deviation	Calculated T
Before Exercise	15	1446.93	167.062	-5.846
After Exercise	15	1516.30	179.968	

5. There is a significant difference between the mean serum IgA of subjects before and after an evening session of strenuous exercise until exhaustion.

Table 5: The calculated statistical indices for morning serum IgA (mg/dl).

Indices / Sources of Variance	Mean Squares	Degree of Freedom	Calculated F	P Value
Circadian Effect (Morning and Evening) (A)	2394.017	1	4.907	-2.915
Exercise until Exhaustion (B)	294.817	1	2.698	0.123
Their Interaction (A × B)	22.817	1	0.320	0.581
$p \leq 0.05$			df = 59	

6. There is a significant difference between a morning and an evening session of strenuous exercise until exhaustion in the level of serum cortisol of subjects.

Table 7: The calculated statistical indices for evening serum cortisol (micGm/dl).

Indices / Sources of Variance	Mean Squares	Degree of Freedom	Calculated F	P Value
Circadian Effect (Morning and Evening) (A)	628.56	1	43.150	0.000
Exercise until Exhaustion (B)	98.517	1	38.282	0.000
Their Interaction (A × B)	14.406	1	2.272	0.154
$p \leq 0.05$		df = 59		

Discussion and Conclusion:

The mean level of serum IgG of the subjects before and after performing a morning session of strenuous exercise until exhaustion was in the normal range. Although the percentage of the changes in the level of serum IgG showed a difference of 628.56, considering the significance level of T, this change was not statistically significant; thus, performing a morning session of strenuous exercise until exhaustion does not affect the concentration of serum IgG of subjects.

However, the mean level of serum IgG of subjects before and after an evening session of strenuous exercise until exhaustion increased up to 98.517% and this increase is statistically significant considering the significance level of T. Thus, performing an evening session of strenuous exercise until exhaustion has a significant effect on the concentration of serum IgG.

This result is consistent with the results of Portman (1971), Chen (1983), suggesting the increase in the level of serum immunoglobulins after strenuous and maximal exercise, while it is inconsistent with the results of Hanson and Flaherty (1981), Nieman (1992) and Gleeson (1999).

The findings of this research showed that strenuous exercise until exhaustion with an intensity of 85% VO_2 max in the morning and the evening affected the humoral immune system of the subjects, that is, physical exercises until exhaustion had a significant effect on the humoral immune system of the subjects.

The factors of humoral immune system and serum cortisol showed an increase after a morning session of strenuous exercise until exhaustion, but the increase in IgG and IgA was not significant, while serum IgM had a significant effect. Further, in an evening session of strenuous exercise until exhaustion, all the said factors increased where the increase in IgG and IgA and serum cortisol was significant and only IgM did not show any significant effect.

What can be observed from the broad range of research studies carried out in this regard is that physical exercise and sport are factors that can directly or indirectly affect the function of the immune system of the body and make changes in the process of the body's protection system. Moreover, the results of the studies suggest that changes in the immune system depends on exercise intensity, exercise design, physical fitness, blood sampling method, measurement methods, exercise duration and even diet.

Despite the different results, most researchers unanimously believe that short-term exercise with moderate intensity will strengthen the innate and acquired immune system which will protect the body against diseases, especially upper respiratory tract infections (Hanson, P.G. and Flaherty, D.K., 1981 and Mackinnon, L.T., 1992). In contrast, prolonged and strenuous exercises as well as stresses due to high-level athletic competition decrease and suppress the proponents of body's immune system which will in turn expose the individual to diseases. The results of the study of Frank and colleagues too show that the immune system can adapt itself to physical activities to the same extent as other body systems such as blood circulation system, the heart and respiration system.

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