

The Effect of Aerobic and Anaerobic Exercise on Various Immunological Parameters of Athletes

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The aim of the research is to study the effect of aerobic exercise and anaerobic exercise on some immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) on athletes. The researcher used the experimental method by using (pre-post-60 minutes post measurements) on a double experimental group because it suits the nature of the research. The sample was composed of 10 runners with ages ranging between 20 and 21 chosen by the intentional method. They were divided into 2 groups, aerobic and an anaerobic group. Each group had 5 athletes. The aerobic group had to run a 10 km marathon while the anaerobic group had to run 4X600 m. The athletes approved that blood samples would be withdrawn in all measurements of the research before, immediately after and after 60 minutes. The results showed statistical differences between the pre, post and 60 minutes post measurements of the IL-6 and IL-10 in the aerobic group meanwhile there was no statistical difference between the measurements of the immunoglobulin's in the aerobic group. On the other hand there were statistical differences between the pre, post and 60 minutes post measurements for the anaerobic group in IL-6 and IL-10 and the immunoglobulins (IgG & IgM) meanwhile there was no statistical difference for IgA the pre, post and 60 minutes post measurements in the anaerobic group. The researcher recommends studying of the difference in the immunoglobulins' concentrations at different times of the day. As well as conducting further research to study the effect of variable intensity loads on the immune system especially the immunoglobulins -IgG, IgM and IgA.

Key words: Alpha heavy chain immunoglobulin, Interleukine 6, Interleukine 10.

Introduction

The continuous efforts to achieve new records in different sports led to the increase of the training intensities. This had a direct impact on the different body systems and their functions. Abdel-Alfattah and Layla (1999) stated that increasing the exercise intensity beyond the athletes' abilities lead to the weakening of the immune system. This increases the chances of being infected before competitions which becomes an obstacle to achieve desired levels. Glecson (2002) mentioned that athletes engaged in heavy training programs, particularly those involved in endurance events, appear to be more susceptible to infection. For example, sore throats and flu-like symptoms are more common in such athletes than the general population and once infected colds may last for longer in athletes who are training hard.

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Here is some evidence that this increased susceptibility to infection arises due to a depression of the immune system function. The causes of this and the reasons for the common association of recurrent infections with heavy training are the subject of current research worldwide.

Abdel- Alfattah (1989) stated that the exercise intensity is the main tool to improve the physical and physiological abilities of the athlete on scientific basis benefiting the athlete so reaching the ideal athletic state. There is an increased interest in knowing the changes that happen to the body systems as a result of high intensity training. He also stated that the training intensities increased so much especially in the 90's reaching 1700-2000 hours per year which is considered to be the maximum. Abdel-Alfattah (1989) highlighted that epidemiological evidence suggests a link between the intensity of the exercise and the occurrence of infections and diseases. The innate immune system appears to respond to chronic stress of intensive exercise by increased natural killer cell activity and suppressed neutrophil function.

Hilde (2012) concluded that the measured effects of exercise on the innate immune system are complex and depend on several factors: the type of exercise, intensity and duration of exercise, the timing of measurement in relation to the exercise session, the dose and the type of immune modulator used to stimulate the cell in vitro or in vivo, and the site of cellular origin .The effect of exercise on immunity is described in different relationships. Nieman (1994) mentioned that a J-shaped relationship best describes the relationship between infection sensitivity and exercise intensity. (Peters 1983; Ekblom 2006) explained the hypothesis is based on cross-section analysis of a mixed cohort of marathon runners, sedentary men and women as well as longitudinal studies on athletes and non-athletes that showed increased immunity with increased exercise training. Gleeson (1985) deduced from the J-shaped curve that moderate amounts of exercise may enhance immune function above sedentary levels, while excessive amounts of prolonged high intensity exercise may impair immune function.

Ekblom, Ekblom and Malm (2006) stated that individuals exercising moderately may lower their risk of upper respiratory tract infections (URTI) while those undergoing heavy exercise regimens may have higher than normal risk. When including elite athletes in the J-curve model, the curve is suggested to be S-shaped. This hypothesis states that low and very high exercise loads increases the infection odds ratio, while moderate and high exercise loads decreases the infection odds ratio. Nieman DC.(2000) mentioned that the immunological mechanism behind the proposed increased vulnerability to upper respiratory tract infections (URTI) after strenuous physical exercise is not yet described .Pedersen and Ullum (1994) stated that the phenomenon is commonly referred to as the "open window" for pathogen entrance .Nieman et al. 1998 ; Shephard RJ. 2001 explained that the "open window" theory means that there is an 'open window' of altered immunity (which may last between 3 and 72 hours, in which the risk of clinical infection after exercise is excessive. Fitch (2012) further explained that this means that running a marathon or simply engaging in a prolonged bout of running, increases your risk of contracting an upper-respiratory

system infection. Years of endurance training seems to incite airway injury and inflammation. Such inflammation varies across sports and the mechanical changes and dehydration within the airways, in combination with levels of noxious agents like airborne pollutions, irritants or allergens may all have an effect.

Williams, Strobel, Lexis and Coombes (2006) concluded that exhausting exercise can result in excessive inflammatory reactions and immune suppression, leading to clinical consequences that slow healing and recovery from injury and/or increase your risk of disease and/or infection. Also Ronsen (2003) agreed that both physical and psychological stress have been regarded as potent suppressors of the immune system. Pedersen, Hoffman (2000) questioned whether or not physical exercise is beneficial or harmful for the immune system.

These 3 relationships describe the mechanism by which the immune system responds to the different exercise intensities. The importance of this study is that with the increase in the exercise loads to the maximum levels of the athletes' physiological abilities, it is crucial to determine the consequences on the different body systems especially the immune and cardiovascular systems. The aim of this research is to determine the effect of aerobic and anaerobic exercise on some immunological parameters for athletics.

The purpose of the study was to investigate:

- a) The effect of aerobic exercise on some immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) on long distance runners (pre-post-60 minutes post measurements).
- b) Compare and find the difference between the immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) in an aerobic exercise for long distance runners (pre-post-60 minutes post measurements).
- c) The effect of anaerobic exercise on some immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) on middle distance runners (pre-post-60 minutes post measurements).
- d) Compare and find the difference between the immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) in an anaerobic exercise for middle distance runners (pre-post-60 minutes post measurements).

The following was hypothesized:

- a) Significant difference in the concentration level of the immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) in the aerobic group (pre-post-60 minutes post measurements).
- b) Significant difference in the concentration level of the immunological parameters (IgG, IgM and IgA) and the cytokines (IL-6 and IL-10) in the anaerobic group (pre-post-60 minutes post measurements).

Method

Participants

The sample was composed of 10 athletes with ages ranging between 20 and 21 chosen by the intentional method. They were divided into 2 groups, aerobic and an anaerobic group. Each group had 5 athletes and homogeneity was performed among

them. The criteria for choosing the participants were the players had the personal will and motive to participate in the research and the complete knowledge of the steps of the research. Also they approved that blood samples will be withdrawn in all measurements of the research before, immediately after and after 60 minutes. Finally the physical and medical state of the players were confirmed and not to get injured during the research Procedure.

Measures

The researcher used the following measures:

- Track used to conduct the research for both groups.
- Stop watches for timing the performance and resting time.
- Centrifuging device to separate blood components.
- Test tubes for collecting the blood samples and preserving them without coagulating by adding heparin so completing the separation process.

Procedure

Aerobic group

- The research for the aerobic group was conducted on Monday 6th July 2015 at 8 o'clock at the track of Physical Education College in Cairo –Helwan University.
- Warm up exercises were performed including light jogging and stretching exercises for 25 minutes so getting the muscles, joints and ligaments ready for the physical activity.
- The group did the pre-measurement of the immunity components through a blood sample withdrawn.
- The group performed an aerobic activity running 10 km.
- The group did the post-measurement of the immunity components through a blood sample withdrawn.
- The group took a passive rest.
- The group did the 60 minutes post-measurement of the immunity components through a blood sample withdrawn.

Anaerobic group

- The research for the anaerobic group was conducted on Sunday 5th July 2015 at 8 o'clock at the track of Physical Education College in Cairo – Helwan University.
- Warm up exercises were performed including light jogging and stretching exercises for 25 minutes so getting the muscles, joints and ligaments ready for the physical activity.
- The group did the pre-measurement of the immunity components through a blood sample withdrawn.
- The group performed a repeated performance of 6 x 400m running with an interval of 5 minutes between them.
- The group did the post-measurement of the immunity components through a blood sample withdrawn.
- The group took a passive rest between each performance and after the 6 performances.
- The group did the 60 minutes post-measurement of the immunity components through a blood sample withdrawn.

Results

In table 1, the descriptive statistics for the study are shown.

Table 1 the Differences between Pre and Post and 60 Minutes Post Measurements for the Variables in the Aerobic Group

Mean	Measurement	variable	Mean Rank	chi-Square	sig
	pre-test	1183.000	1.400		
IgG	post-test1	1406.000	2.400	2.800	0.247
	post-test2	1407.600	2.200		
	pre-test	307.000	2.600		
IgM	post-test1	269.000	1.200	5.200	0.074
	post-test2	287.800	2.200		
	pre-test	200.000	1.800		
IgA	post-test1	229.000	2.200	0.400	0.819
	post-test2	212.400	2.000		
	pre-test	1.512	1.000		
IL6	post-test1	6.920	2.900	9.579	0.008*
	post-test2	6.120	2.100		
	pre-test	5.980	3.000		
IL10	post-test1	3.246	1.200	8.400	0.015*
	post-test2	3.740	1.800		

Test for Several Related Samples- Friedman Test

It is clear from table 1 that there are statistical differences between the pre, post and 60 minutes post measurements of the IL-6 and IL-10. The values of Friedman Test are statistically significant at the level of 0.05. Meanwhile there is no statistical difference between the measurements of the immunoglobulin's measured in the research.

In table 2, measurements of the variables il-10 and il-6 were taken before and 60 minutes after the performance in the aerobic group

Table 2 Differences between Pre and Post and 60 Minutes post Measurements for the Variables IL10 - IL6 in the Aerobic Group

variable	Differences between	Ranks	N	Mean Rank	Sum of Ranks	z	Sig
IL6	pre-test and post-test1	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
	pre-test and post-test2	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
	post-test1 and post-test2	Negative	4	2,25	10	1.841	0.066
		Positive	0	0	0		
Ties		1	0	0			
IL10	pre-test and post-test1	Negative	5	3	15	2.023	0.043*
		Positive	0	0	0		
	pre-test and post-test2	Negative	5	3	15	2.023	0.041*
		Positive	0	0	0		
	post-test1 and post-test2	Negative	1	4	4	0.944	0.345
		Positive	4	2,75	11		

Tow-Related-Samples Tests - Wilcoxon Signed Ranks Test

Table 2 indicates that there is a significant difference between pre and post measurements of the variables in the aerobic group for the benefit of post measurement.

In table 3, Measurements of the Immunological Variables were taken before and 60 minutes after the performance in the anaerobic group.

Table 3 Differences between Pre and Post and 60 Minutes Post Measurements for the Immunological Variables in the Anaerobic Group

Mean Measurement	variable	Mean Rank	chi-Square	sig
	pre-test	1098.000	1.000	
IgG	post-test1	1302.000	2.600	7.600
	post-test2	1274.000	2.400	
	pre-test	260.600	3.000	
IgM	post-test1	203.400	1.400	7.600
	post-test2	208.800	1.600	
	pre-test	201.000	2.400	
IgA	post-test1	195.000	2.000	1.600
	post-test2	188.800	1.600	
	pre-test	1.458	1.000	
IL6	post-test1	6.260	3.000	10.000
	post-test2	4.380	2.000	
	pre-test	6.020	3.000	
IL10	post-test1	3.740	1.000	10.000
	post-test2	4.360	2.000	

Test for Several Related Samples- Friedman Test

It is evident in table 3 that there are statistical differences between the pre, post and 60 minutes post measurements for the second anaerobic group in the parameters being measured except IgA. The values of Friedman Test are statistically significant at the level of 0.05.

In table 4, measurements of the variables IgG and IgM were taken before and 60 minutes after the performance in the anaerobic group.

Table 4 Differences between pre and post and 60 Minutes Post Measurements for the Variables IgG - IgM in the Anaerobic Group

variable	Differences between	Ranks	N	Mean Rank	Sum of Ranks	z	sig
IgG	pre-test and post-test1	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
	pre-test and post-test2	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
IgM	post-test1 and post-test2	Negative	3	3.33	10	0.674	.500
		Positive	2	2.5	5		
	pre-test and post-test1	Negative	5	3	15	2.023	0.043*
		Positive	0	0	0		
IgM	pre-test and post-test2	Negative	5	3	15	2.023	0.041*
		Positive	0	0	0		
	post-test1 and post-test2	Negative	2	1.5	3	1.225	.221
	Positive	3	4	12			

Tow-Related-Samples Tests - Wilcoxon Signed Ranks Test

Table 4 indicates that there is a significant difference between the pre and post measurements of the variables in the anaerobic group for the benefit of post measurement.

In table 5, measurements of the variables IL-10 and IL-6 were taken before and 60 minutes after the performance in the anaerobic group.

Table 5 Differences between Pre and Post and 60 Minutes Post Measurements for the Variables IL10- IL6 in the Anaerobic Group

variable	Differences between	Ranks	N	Mean Rank	Sum of Ranks	z	sig
IL6	pre-test and post-test1	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
	pre-test and post-test2	Negative	0	0	0	2.023	0.043*
		Positive	5	3	15		
IL10	post-test1 and post-test2	Negative	5	3	15	2.023	0.043*
		Positive	0	0	0		
	pre-test and post-test1	Negative	5	3	15	2.023	0.043*
		Positive	0	0	0		
	pre-test and post-test2	Negative	5	3	15	2.023	0.041*
		Positive	0	0	0		
post-test1 and post-test2	Negative	0	0	0	2.023	0.043*	
	Positive	5	3	15			

Tow-Related-Samples Tests - Wilcoxon Signed Ranks Test

Table 5 indicates that there is a significant difference between the pre, post and 60 minutes post measurements of the variables in the anaerobic group for the benefit of the post measurement.

Discussion

Based on the theoretical concepts and previous studies as well as the statistical results of the research, the researcher discussed the results according to the purpose of the research as follows:

The first purpose is to study the effect of aerobic exercise on the immunoglobulins IgG, IgM and IgA and the cytokines (IL-6 and IL-10) on long distance runners. It is clear from table 1 that there are statistical differences between the pre, post and 60 minutes post measurements of the IL-6 and IL-10. The values of Friedman Test are statistically significant at the level of 0.05. Meanwhile there is no statistical difference between the measurements of the immunoglobulins measured in the research. Hanson and Flaherty (1991) have found no changes in serum immunoglobulins immediately and 24 hours after a 13 km sub maximal exercise but Putlur and Miskowski (2004) have seen suppression in the serum, salivary and basal immunoglobulins' secretion after a lengthy maximal exercise period.

Mackinnon (1999) mentioned that moderate exercise but of longer duration has led to a decrease in plasma IgA and IgM levels. Karacabey et.al (2005) have shown that acute moderate exercise of one hour duration has led to decrease in IgG, IgA and IgM. Karacabey et.al(2005) have investigated the effects of the aerobic exercise on humoral immune system parameters (IgG, IgA and IgM). Subjects performed 30

minutes of aerobic exercise on the treadmill after determination of the workload using the Karvonen protocol, IgG, IgA and IgM values immediately post exercise were not significant. Four hours post aerobic exercise all the value returned to baseline level. Two days post aerobic exercise IgG, IgA and IgM values were found to be significant.

Eliakim et al. (1997) 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. Hanson and Flaherty (1991) 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.

Aslan and Mosa (2011) contrasted that prolonged and strenuous exercises as well as stresses due to high-level athletic competition decreased and suppressed the components of the body's immune system which will in turn expose the individual to diseases. The results of the study of as referred from Frank and colleagues also showed 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. The second objective is to study the effect of anaerobic exercise on immunoglobulins IgG, IgM and IgA and the cytokines (IL-6 and IL-10) on short distance runners. It is evident in table 4 that there are statistical differences between the pre, post and 60 minutes post measurements for the second anaerobic group in the parameters being measured except IgA. The values of Friedman Test are statistically significant at the level of 0.05.

It is evident from table 4 that for the IgG measurements there is a statistical difference between the pre and post measurements in favor for the post measurement at the level of 0.05. Also there is a statistical difference between the pre and the 60 minutes post measurements in favor for the 60 minutes post measurement at the level of 0.05 as the values for IgG increased after the post and 60 minutes post measurements. There is no statistical difference between the post and 60 minutes post measurements. Moreover for the IgM measurements there is a statistical difference between the pre and post measurements in favor for the post measurement at the level of 0.05. Also there is a statistical difference between the pre and the 60 minutes post measurements in favor for the 60 minutes post measurement at the level of 0.05 as the values for the IgM decreased after the post and 60 minutes post measurements. There is no statistical difference between the post and 60 minutes post measurements.

Pederson, Rohde and Ostrowski (1998) concluded that the exercises used in the research motivated the production of the immunoglobulins. This agrees with Pederson et.al, where they stated that the immune system responds to the increase in physical activity whether moderate or high intensity. Also Keast and Morton (1983) stated that scientifically planned exercise that considers increasing and decreasing the intensity as

well as giving resting periods doesn't negatively affect the immune system, on the contrary sometimes strengthens the immune system.

Reda Rashed (1995) stated that performing exercises of varied intensities lead to an increase in the immune bodies. This also agrees with what Medhat Kasem (2000) stated from Prlepkina, that intensity exercises increase the immunoglobulins IgG and IgM.

The results of the research matches with Hadeer Sayed (2004) who set an exercise program of 50% aerobic exercise and 50 % anaerobic exercise for 400M swimmers with an average of 3 weekly exercise sets .The total were 40 exercise sets for 10-11 years swimmers. There was a statistical increase in the IgG and IgM immunoglobulins which was explained by Hadeer to be due to the performed exercise program.

Nelhsen et.al (2005) have shown that acute exercise up to 60 % of max VO2 leads to a temporary increase in IgA ,IgG and IgM values . Mackinnon (1999) have shown that IgG and IgM levels increase after a short but maximal exercise. These changes in the production of immunoglobulins have been related to the stimulation of the catecholamines. Kenney (1995) inferred that the differences among subjects can be attributed to their adaptation to exercise.

Table 3 states that there is no statistical difference for the IgA which means that high intensity training didn't affect the IgA which corresponds with Makinon (1997) that high training loads have a transient effect on some immunological parameters as decreasing the production of IgA ,white blood cells, the cytokine level in the blood and natural killer cells as well as decrease the production of the IgA .This agrees with Pederson, Rohde and Zacho (1996) that high intensity training leads to suppression of the immune functions and the release of IgG antibodies. The research results corresponds with the study of Ihab Sabry (2010) that aims of knowing the effect of high intensity training on the concentration of immunoglobulins IgG,IgM and IgA in the blood on wrestlers. The results showed that proper physical training with suitable variations in the intensity over various periods of time led to an increase in the IgG and IgM immunoglobulins meanwhile there was no change in the IgA immunoglobulin. Ldimitriou and Doherty (2002) have shown that a group of swimmers performed 5x400 m front crawl at 85 % of their seasonal best time with one minute rest between each 400m.This exercise did not have a significant effect on IgA concentration. Also no significant interaction was observed between time of day and exercise induced effects on IgA concentration.

Table 2 and 5 showed that aerobic and anaerobic exercise have a positive effect on the cytokines IL-6 and IL-10 for the 2 groups as there is a statistically significant increase in the post and 60 minutes post measurements in comparison to the pre-measurements.

Numerous studies (Tsigos et.al. 1997; Steensberg et.al.2003) have investigated the changes in the circulating concentrations of cytokines following exercise .Cytokines response are generally dependent on the combination of mode, intensity,

and duration of exercise. In the case of IL-6, prolonged running produces the greatest increase in plasma IL-6 concentration. (Gabay et.al. 1997; Bente and Pedersen 2001) stated that IL-6 is the first cytokine present in the circulation during exercise; the appearance of IL-6 in the circulation is by far the most marked, and its appearance precedes that of the other cytokines.

Steensberg et al. (2003) showed that evidence has accumulated over the past decade that cytokines play a much broader role during exercise. Cytokines act in a hormone-like manner during exercise, mediating metabolism in working skeletal muscle, liver and adipose tissue, angiogenesis and neurobiology. Peterson and Pedersen (2005) first proposed that exercise-induced increase in IL-6, and IL-10 exert beneficial anti-inflammatory effect to counteract obesity and insulin resistance. Bente et.al (2001) showed that the peak IL-6 level is reached at the end of the exercise or shortly thereafter, followed by a rapid decrease toward pre exercise levels. The basal plasma IL-6 concentration may increase up to 100-fold after exercise. Because IL-6 is a classical inflammatory cytokine, it was first thought that the IL-6 response was related to muscle damage. However, it has become evident that muscle damage is not required to increase plasma IL-6 during exercise. Rather, eccentric exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery. Several epidemiological studies (Cesari et.al 2004; Colbert et.al 2004 and Panagiotakos et.al 2005) have reported a negative association between the amount of regular physical activity and the basal plasma IL-6 levels: the more physical active, the lower basal plasma IL-6.

Christian and Fischer (2011) related that basal plasma IL-6 is closely associated with physical inactivity than other cytokines associated with the metabolic syndrome. Exercise is known to cause major physiological, hormonal, metabolic, and immunological effects. The question is whether exercise-induced IL-6 mediates some of these effects. Of note, IL-6 may act locally within the contracting muscle during exercise or within the adipose tissue during recovery, while most other cells and target organs are exposed only to IL-6 released into the systemic circulation. Regarding the systemic effects of IL-6, the dose-response relationship and timing has to be considered. First, it should be noted that marked increases of plasma IL-6 only occur if the exercise involves a considerable muscle mass working for a considerable amount of time at a considerable intensity. Otherwise, a systemic IL-6 increase may be small or absent. Regardless, the exercise-induced peak plasma IL-6 concentration will usually not exceed 100 pg/ml. Second, the peak plasma IL-6 concentration occurs at the cessation of the exercise (or shortly after), thus the systemic effects induced by IL-6 are for the most part expected to occur during recovery from exercise.

Santos (2012) explained that the increase in IL-6 after exhaustive exercise is that IL-6 is produced by the contracting muscle and is released in large quantities into the circulation. Studies have shown that prolonged exercise may increase circulating neutrophils' ability to produce reactive oxygen metabolites, but the release of IL-6 after exercise has been associated with neutrophil mobilization and priming of the oxidative activity. Ostrowski et.al (1999) stated that interleukin-10 (IL-10) is an important anti-inflammatory cytokine increasing with exercise.

(Zembron 2010 ; Pedersen 2011) mentioned that IL-10 is produced not only by other cells in the body but also by the cells belonging to the immune system, and the other cytokines affect the production. It is stated that the increase in IL-10 after exercise may be related to the muscle damage. Peake (2005) related the muscle damage and the intensity of the exercise causing IL-10 to increase which is an anti-inflammatory cytokine .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.

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