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**COMPARING THE EFFECTS OF ONE-SESSION AEROBIC EXERCISE IN THE
MORNING AND EVENING ON THE IMMUNE SYSTEM AND URINARY
COMPOUNDS AMONG BODYBUILDERS**

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ABSTRACT

This study aimed to investigate the effect of aerobic exercise twice (morning and evening) on the immune system and is a component of urine bodybuilders. The sample consisted of 20 athletes who voluntarily participated in this study. Factors measured included blood factors (white blood cells, neutrophils, lymphocytes, monocytes) and urine (total protein and creatinine) before and after aerobic activity (morning and evening) was measured. After determining the normal distribution by Kolmogorov-Smirnov test of paired t test with a confidence level of $P < 0.05$ were used. The results of this study showed that aerobic exercise causes a significant increase in white blood cells, lymphocytes, neutrophils, monocytes, and total protein and creatinine participants in the morning and the evening. The results showed no significant difference between the morning and afternoon sessions of aerobic exercise on markers of white blood cell, monocyte, total urinary protein excretion and urinary creatinine $P < 0.05$. However, the results showed no significant differences between the morning and afternoon sessions of aerobic exercise on lymphocytes and neutrophils factors $P < 0.05$. Based on these findings, it is concluded that aerobic exercise aerobic exercise in the evening than in the morning because it reduces the amount of stress hormones (catecholamines and cortisol) in the evening and increased white blood cells of the immune system is more secure provides bodybuilders. Also significant increase in urinary

excretion of creatinin bodybuilders due to reduced renal flow and reduced glomerular filtration. In intense physical activity as factors contributing to changes in the immune system, blood and urinary system is considered to be a bodybuilder.

Keywords: Total Protein, Creatinine, White Blood Cells, Lymphocytes, Neutrophils, Blood Samples, Monocytes

INTRODUCTION

The method of regulating the inflammatory response in different sports protocols and among different people who have different levels of physical fitness has been remained unknown and depends on the intensity, duration, type of activity and one's endurance capacity. Also, the extent of the inflammatory response in people who exercise regularly are significantly different than those who are immobile. But one time severe or long-term aerobic exercise may cause damage to the immune system responses and finally, leads to increase one's vulnerability, acute and chronic inflammation. Extreme sports with cognitive safety changes includes the release of inflammatory mediators, activity of the types of sub-units of white blood cells, the activity of acute phase proteins, increased activity of pre-inflammatory and anti-inflammatory cytokines and associated changes in markers of muscle damage [1]. In a study, **Ghaemi (1973)** showed that a single session of physical activities increases the concentration and accumulation of leukocytes in peripheral blood. But on the other hand, expanding and

increasing these changes in the aforementioned factors depends on the duration and continuity of the external conditions and also the capabilities and skills of the individual athlete. Therefore it could be said that the more active, longer and more intense, there will be more changes.

One of the issues in question in the area of sport and physical education that created numerous ambiguities in renal function during exercise is the phenomenon of protein in the urine after sports activities. Due to the fact that protein excretion in the urine is followed by increased activity and one of the factors of kidneys' damages is excessive protein excretion by the urine. Of the important issues in physical education that attracted the attention of many experts around the world is the role of physical activities on renal function, particularly protein excretion in the urine. Exercise and physical activity lead to changes in renal hemodynamics and the electrolytes. Effective renal plasma flow decreases during exercise that this decrease is directly related to the exercise intensity and

sometimes reach 25% at the time of rest during strenuous activities. Subsequent reduction in renal blood flow will impress glomerular filtration. Probably the most important of this mechanism is afferent and efferent renal capillary contraction in response to increased sympathetic activity and an increase in adrenaline and nor adrenaline [2]. During exercise, protein breakdown and decomposition of by-products of metabolism, such as lactic acid will increase and therefore the amount of protein in the urine and the concentration of hydrogen ions in the urine will increase. Urinary protein excretion in young people who do not have any symptoms of kidney disease may be reached even a hundred times in resting level in a heavy and severe exercise. **Touchberry (2004)** reviewed increased protein in the urine due to physical activity in a series of subjects after the activities. Protein excretion among athletes was significantly intermittent and it seems that the maximum amount of protein excretion is among the Maarten runners ranging between 16 and 60 percent [3]. The research results indicate a significant increase in protein excretion following exercise. The results of the investigation relates to the overall mechanism of protein excretion in the kidney during sport. Previous studies have shown that single session of aerobic exercise

can lead to changes in the immune system, protein excretion and urine creatinine. In addition, studies show that daily changes or diurnal cycle of physiological responses in the body indicates the fact that physiological responses at rest and during exercise throughout the day will change and body capabilities are subject to diurnal influences. The biological clock of the body is the cycle of biological activity occurs in 24 hours a day in the body and is changed by the exposure to environmental stimuli such as night and day. This internal clock is located at hypothalamus of the brain and is responsible for the coordination and control the body's sleeping-waking hours due to the darkness or brightness of its environment and accordingly the regulation hormone excretion and also body temperature. In this study, it is sought to answer this question that whether an aerobic exercise session twice in the morning and evening can cause changes in the immune system response and urinary protein excretion and creatine?

Subjects

The subjects consisted of 20 men in the field of bodybuilding at Jahrom Pre-province who voluntarily participated in this study. It should be noted that all the participants of this study were active for at least 4 years of experience in sports activities before the tests were

conducted. The method used in this study was a field and quasi-experimental method. Also, the present study is group pretest-posttest study.

Data collection tools

In this study, the following instruments were used to measure research variables:

- Blood samples (white blood cells, neutrophils, lymphocytes, monocytes), which is studied by SYSMEY system made in Japan.
- Urine sample (total protein and urinary creatinin), which were examined by Cobas auto-analyzer device made in Germany and Hitachi model made in Japan.
- Treadmill made in Taiwan (Health stream).

Research Method

The present study was conducted on bodybuilders' body at Jahrom pre-province that is in laboratory method using a group of subjects in that 20 members of the group are chosen and the sampling of blood and urine are taken from the subjects in the morning and evening such that sampling of blood and urine of these people were collected in the morning before eating breakfast (To remove the matters and fats that affect the measurement device parameters). Then, the subjects ran on the treadmill for 12 minutes

(high and low speed depending on the aerobic capacity of subjects) and then in the blood and urine samples were again taken. After 4 days of rest given the participants, the practice was repeated in the afternoon on the mentioned 20 bodybuilders before the implementation of aerobic exercise and after the implementation of aerobic exercise. After fastening the brachial tourniquet, two ml of venous blood was obtained from the subjects and was poured in plastic vials containing 20 microliter of EDTA solution (This solution is blood not to clot; 2 to 3 drops of this solution was mixed with one ml of blood and are incorporated for 1 minute to remain in liquid form in the time of doing study) and was taken to the lab for less than 30 minutes and blood samples were analyzed. Measurement factors in this section include the measurement of lymphocytes, neutrophils, monocytes, etc. Also, the subjects' urine samples (before and after the test) that were collected in urine containers was taken to the laboratory to check the protein and total urinary creatinin and the mentioned factors were examined. Total protein in milligrams in 24 hours and creatinine per gram in 24 hours was measured.

Statistical Methods

Excel software for drawing tables, means, standard deviations and graphs were used in

the part of descriptive statistics. Data normality was specified by the Kolmogorov-Smirnov Tests and in the case of data normality, parametric statistics was used. Paired t-test was used to compare variables. Significant level was also considered $P \leq 0.05$. The data analysis was performed using SPSS19 statistical software.

RESULTS

Dependent t-test results in **Table 3** show that there is a significant difference between the amount of white blood cells after aerobic exercise in both the morning and evening ($p = 0/013$, $t(19) = -2/742$), aerobic exercise increases the white blood cells of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is a difference between the white blood cells of bodybuilders in the morning and evening after aerobic training (Cooper Test).

Dependent t-test results in **Table 4** show that there is a significant difference between bodybuilders' lymphocytes after aerobic exercise in both the morning and evening ($p = 0/010$, $t(19) = -2/882$), aerobic exercise causes a significant change of lymphocytes of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is a difference between bodybuilders' lymphocytes in the morning and evening after aerobic training (Cooper Test).

Dependent t-test results in **Table 4** show that there is a significant difference between bodybuilders' neutrophils after aerobic exercise in both the morning and evening ($p = 0/099$, $t(19) = -1/73$), aerobic exercise causes a significant change of neutrophils of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is no difference between bodybuilders' neutrophils in the morning and evening after aerobic training (Cooper Test).

Dependent t-test results in **Table 5** show that there is a significant difference between bodybuilders' total protein after aerobic exercise in both the morning and evening ($p = 0/001$, $t(19) = -3/915$), aerobic exercise causes a significant change of total protein of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is a difference between bodybuilders' total protein in the morning and evening after aerobic training (Cooper Test).

Dependent t-test results in **Table 7** show that there is a significant difference between bodybuilders' monocytes after aerobic exercise in both the morning and evening ($p = 0/005$, $t(19) = -3/188$), aerobic exercise causes a significant change of monocytes of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is a difference between bodybuilders'

monocytes in the morning and evening after aerobic training (Cooper Test).

Dependent t-test results in **Table 7** show that there is a significant difference between bodybuilders' creatinine after aerobic exercise in both the morning and evening ($p = 0/0001$, $t(19) = -4/799$), aerobic exercise causes a

significant change of creatinine of bodybuilders. According to the survey results, the null hypothesis is rejected. Therefore, there is no difference between bodybuilders' creatinine in the morning and evening after aerobic training (Cooper Test).

Table 1: descriptive statistics for the pre-test and post-test of the measured variables in the morning and evening

Statistics Test variable	Morning before exercise	Morning after exercise	Evening before exercise	Morning after exercise
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Leukocytes The number of cells per microliter	7378 \pm 515/87	8384/65 \pm 699/54	7407/2 \pm 521/7	8661/00 \pm 524/81
Neutrophils The number of cells per microliter	4498/15 \pm 37/4	5107/60 \pm 503/52	4470/7 \pm 380/9	5230/25 \pm 394/62
Lymphocytes The number of cells per microliter	2058/55 \pm 274/18	2335/35 \pm 314/36	2030/55 \pm 259/90	2378/80 \pm 318/38
Monocytes The number of cells per microliter	257/65 \pm 18/39	293/60 \pm 22/04	265/25 \pm 23/60	311/65 \pm 28/49
Total protein Milligrams per deciliter	2/722 \pm 0/461	3/34 \pm 0/66	0/675 \pm 0/398	3/83 \pm 0/57
Creatinine Milligrams per deciliter	92/57 \pm 13/39	115/38 \pm 30/69	92/02 \pm 13/33	150/37 \pm 36/92

Table 2: T-dependent leukocyte test results of bodybuilders in the morning and evening.

Statistics Variable	mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-247/8	483/96	-2/742	19	0/013

Table 3 results of dependent T test- Bodybuilders' lymphocytes in both the morning and evening

Statistics Variable	Mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-71/45	110/86	-2/882	19	0/010

Table 4: results of dependent t test of bodybuilders' neutrophils in both the morning and evening

Statistics Variable	Mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-122/65	316/70	-1/73	19	0/099

Table 5: results of dependent t test of bodybuilders' total protein in both the morning and evening

Statistics Variable	Mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-0/539	0/616	-3/915	19	0/001

Table 6: Results of dependent t test of bodybuilders' monocytes in both the morning and evening

Statistics Variable	Mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-10/45	14/65	-3/188	19	0/005

Table 7: results of dependent t test of bodybuilders' creatinine in both the morning and evening

Statistics Variable	Mean difference	SD	T statistics	Degree of freedom	Significance
Morning-evening	-35/53	33/11	-4/799	19	0/0001

DISCUSSION

The results showed that there is a significant difference between pretest and posttest changes in white blood cell of the research subjects in the morning and evening. And with respect to higher scores of afternoon, it is concluded that the increase in white blood cells of research subjects in the afternoon was significantly more than the morning that is compatible with the study conducted by **Faraee (1997)** but incompatible with the research results of **Gleason (2000)** [4, 5]. In this study, it was shown that aerobic exercise

in the morning and evening increase white blood cells which several hormonal factors such as catecholamine's, cortisol and growth hormone influence. Also, the increase in white blood cells in this study justify several mechanisms of leukocyte changes in the study such as the presence of surface receptors on lymphocytes (beta receptors of Adrenoceptors-Lymphocytes). To act more quickly and affect regulation of epinephrine, these receptors causes an increase in the number of Lymphocytes and changes in leukocytes in blood circulation) by changing

adrenergic-leukocytes receptor density. Cortisol is considered as the factors of above hormone that acts in the direction and redistribution of white blood cells, lymphocytes and neutrophils into tissues. Increased cortisol causes an increase in release of neutrophils from the bone marrow, inhibiting the entry of lymphocytes into circulation, delay return in the return period to the initial state and recycling so that changes of these hormones due to exercise causes possible changes in cellular processes such as protein synthesis or emergence and the expression of surface receptors. Also, it was shown in this study that the mean white blood cells in the evening was more than the one in the morning which the possible cause is that the human immune system response during the day has a different response. Previous studies also showed that the level of stress of hormones such as cortisol (the ability to suppress the immune system) is much more in the morning than in the evening. A study at the University of London on 14 young swimmers in the morning and evening took place and it was shown that higher levels of cortisol in the morning is much more than the one in the evening. So it was concluded that the exercise activity in the evening is more useful for immune system. Because levels of

stress hormones is lower and the risk of inflammation is lower.

The research results showed that there is a significant difference between changes in pre-test and post-test of subjects lymphocytes in the morning and in the afternoon that are compatible with the research results of **Mel'nikov et al (2007)**, **Gleason (2000)** and **Nieman (2001)** who indicated that the number of lymphocytes was significantly increased immediately after exercise as well as the studies conducted by **Green et al (2003)** who reported that the number of Lymphocyte from 1 to 3 hours after moderate and intense activity decreases and finally returns to the resting time. It was shown in this study that in both groups, the number of Lymphocytes immediately after the exercise significantly increases and more importantly, it will reach more below than before the exercise in an hour after the exercise. Reducing the number of blood Lymphocytes in the return period to the initial state and its lower level from the level before the exercise cause immunosuppression. **Gleason et al (2000)** argue that the responses associated with an intense activity is very similar to reactions that are stimulated by infection which relates to an increase in the number of blood leukocytes (especially neutrophils and lymphocytes), respectively. They also believe

that with severe neutrophil (especially neutrophils in the blood), that is along with ongoing training efforts, that cause the discharge of these important cells in the bone in the long run that the accumulation of neutrophils in the blood will surely lead to the reduction of their maturity in the athletes than those who had no activity and made less the neutrophil phagocytosis activity of the blood among the athletes who train hard with much intensity. However, it was shown in this study that average lymphocytes in the evening is higher than in the morning, which is probably due to the reduction of hormones stress (catecholamine and cortisol) in the evening than in the morning.

The results of research showed that there is no significant difference between the pre-test and post-test changes of neutrophil among subjects in the morning and evening that is consistent with the results achieved by **Zarr and colleagues (2010)**, but inconsistent with the research results of **Gleason (2004)**, **Neumayr et al (2005)**, **Amirsasan (1995)** and **Mel'nikov and colleagues (2007)**. It seems that inconsistency in the findings of the investigation may be due to the differences in sporting activities, sampling time, level of physical fitness of subjects and intensity of exercise and physical activities in the days before the test. Intensity and type of exercise

will change the function of neutrophils so that that neutrophils from the bone marrow is quickly evacuated and create the relative increase in circulating mature neutrophils. Immediately after the exercise, the number of white blood cells increases due to the increased number of neutrophils and monocytes and less due to increasing the number of lymphocytes. Therefore, possible increased lymphocytes in the first time of practice and then increases in the number of neutrophils following the increase and decrease of lymphocytes at the end of activity is considered possible mechanisms of the number and percentage of leukocytes in the study which the amount of neutrophils in later hours may be increased to 24 hours.

The research results showed that there is a significant difference between the pre-test and post-test changes of monocyte among research subjects in the morning and evening. And due to higher average values of the evening amount, it is concluded that increasing Monocytes among research subjects in the evening time is significantly more than that of the morning time that is consistent with the study conducted by **Starkie et al (2000)**, which shows that changing their number was significantly increased after the exercise. The results of this study was consistent with the results of

Amirsasan (1995) Mel'nikov et al (2007), but inconsistent with the results of **Nieman et al. (2000), Gleason (2000) and Neumayr (2005)**. Probably the inconsistency of the research results with the results of this study done by these researchers can be due to the factors such as factors such as the severity, duration, size, type of activity, level of physical fitness, the bleeding time, gender and also subjects' age.

It was shown in previous studies that the exercise causes changes in the immune system by increasing norepinephrine. In this study, possible increase in blood monocytes may be due to their fundamental role in the production of some regulating factors such as cytokines [15]. In addition, monocytes may have an important impact on the performance of various immune cells such as NK. Also, it was found in this study that average monocytes in the evening is more than in the morning, which one of the possible reasons is changing the immune system response (monocytes) during the day, because stress hormones have circadian rhythm and their amount in the afternoon than is less than the morning.

According to the results, it is shown that there is a significant difference between pretest and posttest changes in urinary protein excretion of total subjects in the morning and evening.

And with respect to higher scores of evening, it is concluded that total urinary protein excretion in the evening time was significantly more than that of the morning time that is compatible with the research results done by **Alijani et al (2011), Kohanpoor et al (2012), Touchberry et al (2004), Finkelstein (2006) and Lippi et al (2008)**.

The present study is not compatible with the research results done by **Babae et al (2013)**. These authors reported that different movement patterns at the maximum intensity has little effect on urinary protein. Probably the reason to the absence of significant proteinuria in the study of Babae et.al is the intensity of activities used. In the study of these researchers, two exercise intensity of 70 and 85 percent of maximum heart rate was used that the two intensities are corresponding to 50 to 70% of aerobic capacity. It seems that the intensities used can be considered under a maximum level that possibly does not lead to increased lactate from the threshold level due to the features of intensity control under the maximum level.

Urinary protein excretion during and after exercise is a temporary phenomenon and is returned to the initial state with a half-life of approximately one hour. The extent of exercise-induced urinary proteinuria on the

intensity of exercise depends on its duration. Urinary protein is a phenomenon that is common among athletes, so that the basic mechanism to account for urinary protein has been considered as increased glomerular permeability, maximal tubular reabsorption capacity saturation or a combination of the two [17]. The role of factors such as prostaglandins, renin-angio-tension system, sympathetic stimulation, and reducing the negative charge of the glomerular membrane etc. in justifying permeability change of glomerular membrane subsequent to reduction in renal blood flow and filtration fraction increases during exercise has been proven [18]. Many factors cause urinary protein by influencing on the aforementioned factors is led to their change. But the most important factor affecting the incidence of urinary protein that most previous studies have reported it is the intensity of the exercise. The intensity of exercise activity leads to changing the functional sections, increased permeability of the glomeruli and saturating the re-absorption of tubules by activating the various mechanisms and changing in renal hemodynamics and reducing negative charge of glomerular dams [3]. Part of the response to protein excretion due to exercise can be due to increased blood concentrations for plasma exit of the vessels

or the renal glomeruli permeability changes during exercise. During exercise, protein and decomposition breakdown caused by metabolism, such as lactic acid increases and for this reason, the amount of urine protein and the concentration of hydrogen ions in the urine also increase. Urinary protein excretion in young people who do not have any symptoms of kidney disease, may be reached even up to a hundred times greater than the rest at a heavy and severe exercise.

According to the above results, it is shown that there is a significant difference between pretest and posttest changes in urinary creatinine excretion among research subjects in the morning and evening times. With respect to higher scores of evening time, it is concluded that increased urinary excretion of creatinine among research subjects is significantly more than the morning time that is consistent with the research results of **Alijani et al (2011) and Babae et al (2013)**, but inconsistent with the research results of **Mantel Pierre et al (2002) and Lippi et al (2008)**.

Poortmans and colleagues (2006) observed that after maximal exercise in sedentary elderly men with active young men, there was no significant difference in plasma creatinine levels. **Gleason (2000)** demonstrated that after 12 weeks of exercise and physical

activity, there is no significant changes in serum creatinine and serum urea nitrogen among healthy elite cyclists while the research results done by **Lippi et al. (2008)** stated that the mean serum creatinine levels after the activity in healthy passive subjects has significantly increased compared with professional and amateur cyclists. Although researchers examined aerobic activity during the morning and evening time, based on these findings, it seems that time, intensity and volume of training can have an impact on renal function. Increased creatinine levels, with increased catabolic state (such as heavy exercise) represents the pressure of physical exercise on skeletal muscles and the muscles depreciation. Changes in serum creatinine during physical activity, depending on the type of exercise, intensity, duration and type of metabolism, is different. It seems that reduction in renal blood flow and glomerular filtration reduction in intense physical activity are the main reasons for the increase in serum creatinine in athletes.

CONCLUSION

The results showed that aerobic exercise in the morning and evening makes a significant increase in the amount of white blood cells in the evening time than doing aerobic exercise in the morning time. There are significant differences between the group, time and

group-time interaction of white blood cells. In other words, presenting the dependent variable of the aerobic exercise in the posttest of the first session between groups as well as morning and evening groups and the interaction between these two groups causes a significant increase in the number of white blood cells. Therefore, it can be found from these findings that in terms of the type of activity, duration and its intensity as well as other conditions such as environment (temperature and humidity), various phases of physical activity makes different changes in the amount of white blood cells. Research suggests that a single session physical activity can increase and accumulate the concentration of the amount of environmental leukocyte. But on the other hand, expanding and increasing these changes in the aforementioned factors depends on the duration and continuity of activities and external conditions and the degree of competence and skills of the athlete. So it can be said that the more and longer activity with more intensity, the more changes will be. In fact, the difference between the results of the first session of the morning and evening time can be attributed to lack of the impact of time on the amount of the impact of the mentioned test on the number of blood leucocytes and the origin of this difference could be

attributed to the lack of time effect on the test itself as a place of physical activity. It is also to be noted that the increase in the number of white blood cells and its subsets correlates with the intensity and duration of exercise, but it has a converse relationship with the level of one's physical fitness. Changes in Leukocytes and their subsets during severe and long-term exercise depends on several factors, including duration and intensity of exercise and diet, hormones and cytokines density, body temperature and blood flow and other factors that their clarity needs further more precise research. Also, aerobic exercise in the evening time represents a significant increase in the white blood cells of bodybuilders. In fact, the test was in a high level in terms of intensity. On the other hand, the higher the temperature of 4 degrees in the evening than in the morning could also be a reason to intensify the effect of aerobic exercise in the morning. It also seems that other factors also play a role in the mechanisms of these changes. Response of the human immune system during the day has a different response. Previous studies also showed that levels of hormones' stress such as cortisol (it has the ability to suppress the immune system) and catecholamines in the morning than in the evening is much higher. However, the number of changes by the

exercise in the number of leukocytes and their subsets in the majority of studies is temporary and unstable and it is not clear how these factors affect the immune system.

Overall, these findings suggest that changes in the cellular immune system and urinary system following an aerobic exercise session in the afternoon than in the morning between group, time (morning and evening) and time-group interaction showed a significant difference. It seems that silhouette of the human immune system and urinary system response to exercise needs more study in a longer time and studying the effective variables on it. However, it can be recommended to athletes and coaches to pay special attention to the kind of exercise given the factors of morning or evening time; for the amount of hormones' stress that cause changes in the immune system changes with changing the time of day and their lowest amount is in the evening time. It can be concluded that the exercise in the evening is more useful for the immune system. Because levels of hormones' stress is lower and the possibility of creating inflammation caused the exercise in the evening is less than in the morning. In the end, based on the findings of this study, aerobic exercise in the evening is an effective agent for the immune system of blood and urine composition of bodybuilders.

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