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EFFECTS OF TRAINING TIME ON SERUM IMMUNOGLOBULIN ALTERATIONS AND CORTISOL TESTOSTERONE RESPONSES IN MALE ATHLETE STUDENTS

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ABSTRACT: The purpose of this study was to examine the effect of incremental continuous running as well as morning vs evening-time training on changes in serum immunoglobulins including IgA, IgG, IgM, testosterone, and cortisol hormones responses. For this reason, 28 male athletic students were purposefully selected and randomly divided into the two groups; morning-time training group (N=14, weight = 68.2 ± 9.8kg, age = 19.5 ± 1.6 years, training time = 7: 30 AM) and evening-time training group (N=14, weight = 63.8 ± 8.4 kg, age = 19.81 ± 1.24 years, training time =16:30 PM). The participants were trained according to an incremental continuous running program with a certain heart rate for two months (16 sessions). To determine the amount of serum immunoglobulins as well as cortisol and testosterone hormones, the participants' blood samples was taken twice, once 24 hours before the first training session and once 24 hours after training session. Then, a 12 – minute running-walking test was used to measure the maximal oxygen consumption. The results showed there were not any significant differences between the amounts of IgA, IgM, IgG serum of both groups in pre-test and post-test (i.e., respectively, p = 0.727, P = 0.068, P = 0.14). Also, there were not any significant differences between the amounts of testosterone and cortisol hormones secreted of both groups in pre-test and post-test. However, there were significant differences between the concentrations of cortisol hormone of the two groups in pre-test (P = 0.006) and post-test (P = 0.0001). More ever, the results also showed a significant difference between the pre-test of the morning-time training group and the post-test of the evening-time training group (P = 0.0001). The findings of this research indicate that cortisol hormone is influenced by the time of training, which is due to circadian rhythm.

KEY WORDS: circadian rhythm, immunoglobulin, cortisol, testosterone, continuous aerobic, running

INTRODUCTION

It is necessary to pay attention to the important internal factors, biological clock, and the effect of such factors on physiological as well physical functions especially in different times of day and night. Recent evidences (knowing the effects of time on physiological variables) show that the human body undergoes a lot of changes during the day and the night, and has a special ability in each hour [1]. Reilly et al. [11,12] showed that reaction time, muscular strength, anaerobic power, and the amount of flexibility are significantly different in the evening than the morning. Such variation is probably due to the higher temperature of body and hormone secretion in the afternoon. This research was designed to study the changes in the response of testosterone and cortisol hormones in different times of a day to a continuous aerobic activity. Immunoglobulins are parts of immune system components, which are produced by the lymphocyte B in blood serum and tissue liquids in primates [7]. Stress is one of the stimulating factors of immune system, and physical activity can result in stress, which leads to changes in the system [2].

On the other hand, cortisol secretion increase in stress producing conditions (environmental effects, tension pressure, aerobic activity, injury, infection and so on), so it is called an adaptation hormone [2]. The amount of releasing factors, secretion of corticotrophin (CRH) and cortisol diminishes at the evening and increases in the early morning. It arises from periodical and round the clock changes in messages sent by hypothalamus that causes the change of cortisol secretion [3]. On the other hand, testosterone is a distinguished neutralizer of cortisol reciprocal effect at the time of physical activity. Testosterone increases in the early and long-term training and decreases with continuing of the activity. This endocrine adaptation causes the adjustment of muscular activity and glycogenolysis stimulation and facilitating of glyconeogenesis process [4,5]. Numerous studies have demonstrated the effect of various activities on hormones secretion and immunoglobulins [2,3,6,9]. Klentrou [7] showed that the intense aerobic activity decreases the amount of immunoglobulins and sets the body exposed to injury, especially in the upper respiratory tract infection while the physical activity with average intensity causes the increasing of IgA amount and diminishes the danger of suffering from infection. In another investigation reported by Nieman et al. [10], the effect of 30 minutes of walking on cortisol concentration and salivary IgA was evaluated. Their results showed that although the oxygen consumption had an 11 percent increase, a significant change was not observed in IgA and cortisol amounts.

There are a few sporadic investigations in regard to the effect of aerobic activity during the day and night on physical variables such as hormones secretion and the amount of immunoglobulins, with contradictory results. For example, Deshenes [5] studied the effect of physical responses in ten healthy men. The results of this study showed that aerobic activity has some effects on steroid hormone stimulation, but by changing time, he did not observe significant changes in amounts of testosterone and cortisol hormones. (Although, the amounts of testosterone and cortisol hormone at 8 AM in relation with 8 PM were in the highest possible level). On the contrary, Bird [1] studied the effect of aerobic activity by weight training with 75% of one repetition maximum, in two different time (6 AM and 8 PM) and concluded that performing an aerobic activity in the evening in comparison with the morning cause significant decrease in cortisol concentration and increase of testosterone ratio to cortisol ratio. In another investigation, Dimitriou et al. [6] studied the effect of physical activity time in day on cortisol and IgA changes in 14 male swimmers. Their aerobic activity included swimming at their 85% of the best swimming record. The subjects in the first day of experiment participated at 6 AM and then at 6 PM in other days in the same activities. The result showed that the amount of cortisol secretion was different in various time of the day, but the different times of training during of the day have no effects on IgA amounts. On the other hand, in a study, Li Li [9] showed that the effect of training on IgA secretion amount was different. He studied the effect of 2 hours of pedaling activity with the intensity of 60% of VO_2max, performed at 9 AM and 2 PM, and concluded that there is circadian rhythm in IgA concentration and the increase in IgA concentration from training has diminished in the evening and has increased in the morning. He also pointed out that the training (in each hour of the day) causes the saliva decrease and salivary IgA concentration increase. In any case, three hours rest after training is a suitable time for returning to the first state. In spite of that, it is not clear what the daytime effect on cortisol and testosterone concentration is and whether their probable changes have any effects on hormonal immune indicators (IgA). Therefore, this investigation was planned to study the effect of 16 sessions of increasing continuous running in two different times (in the morning and in the evening) on variables such as serum amounts of IgA, IgG, IgM, immunoglobulin and cortisol and testosterone secretion to shed more light on the matter.

MATERIALS AND METHODS

In this semi-experimental research, the effect of 16 sessions of increasing continuous running in morning versus evening time on the amount of cortisol and testosterone response, VO_2max, and also amount of serum immunoglobulin IgA, IgG, IgM, was evaluated. The statistical population of the research included the university male students. Twenty eight students were selected purposefully and then were randomly assigned to 2 training groups (in the morning n=14, age 19.5±16.1, weight 68±9.8 kg and the training time 8:30 AM) and (in the evening n=14, age 19.8±1.2, weight 63.8±8.4 kg and the time 4:30 PM).

For the purpose of measuring the first phase of the resting amounts of testosterone, cortisol serum, antibodies responses, IgA, IgG, and IgM of the subjects, blood sampling was performed separately 24 hours before the start of the experiment. Blood samples were collected by drawing 10 cc of blood in the morning-training group at 8 AM, and immediately, following the 12 minutes running-walking exercise performed for determining VO_2max. Similar protocol was used in the evening-training group at 5 PM The following equation was used for calculating VO_2max of subjects:

VO_2max (ml•kg-1•min-1) = [0.0268 + (D)] ±11.3

„D" is the traveled distance in meter.

Following 24 hours of resting, the basic training protocol for morning groups performed on 7:30 AM, on Sundays and Tuesdays every week, for 16 sessions. For the evening groups, the training time was set at 4:30 PM, Sundays and Tuesdays, every week lasting for 16 sessions. A 15-minute warm-up was performed before starting each training session. Finally, the second phase of blood sampling collection was performed for each group after 24 hours of completion of the exercise protocol. The research protocol for these two training groups is presented in Table 1.

For determining the maximal heart rate of subjects the formula, 220 – age was used. For controlling intensity, a Polar Clock (made in Japan) was used. Also, for measuring the other related variables, the following method was used:

TABLE 1. THE PROTOCOL OF THESE TWO TRAINING GROUPS

The Training Session	The Traveled Distance in Every Session (meters)	Intensity of Training (Maximal Heart Rate Percent)	Heart Rate (beat/ minute)
The First to the Fourth Sessions	2000	70 - 75	140 - 150
The Fifth to the Eighth Sessions	2400	75 - 80	150 - 160
The Ninth to the Twelfth Sessions	2800	80 - 85	160 - 170
The Thirteen to the Sixteen Sessions	3200	85 - 90	170 - 180

TABLE 2. THE MEAN AND STANDARD DEVIATION OF MEASURED MARKERS OF TWO GROUPS

Group	Variables	VO_2max (ml/kg/min)	IgM (mg/dl)	IgA (mg/dl)	IgG (mg/dl)	Cortisol (nmol/L)	Testosterone (nmol/L)
Morning	Pre-test	48.92±3.96	90.35±31.16	175±69.5	1171.57±202.26	620.6±142.8	19.86±463
	Post-test	54.11±31.2	107.86±32.39	183.5±70.82	1099.86±185.4	567.29±173.71	19.22±3.79
Afternoon	Pre-test	53.42±6.98	112.42±41.75	169.57±71.71	1085.35±0.36	411.92±176.91	16.85±4.52
	Post-test	57.21±6.36	80.57±32.77	155.57±50.96	1009.5±163.3	307.45±67.62	17.24±5.36

For measuring testosterone serum, Radioimmunoasay and related kite was used.

1.For measuring cortisol serum, Radioimmunoasay and cortisol Im 1841 kite was used.

2.For measuring IgA, IgG, IgM, immunoglobulins, the Immunoturbimetric test and Pars Azmoon kites was used.

Analysis of data was performed by using one way analysis of variance followed by Scheffe post hoc test. The mean and standard deviation of measured markers are presented for both groups (Table 2).

RESULTS

1. There was no significant difference between the measure of testosterone concentration (P=0.24) and measures of IgA, (p=0.72), IgM, (p=0.068), and IgG, (P=0.24) of two groups in the morning pre- test phase and in the evening post- test phase.

2. The results of ANOVA showed that the amount of cortisol was significantly different between the groups. Post hoc Scheffe test indicated that the significant difference for cortisol was between the two training groups of morning and evening in the pre test (P=0.006) and in the post-test (P=0.0001). In addition, there was also a significant difference between the pre-test of the morning training group and the evening training group (P=0.0001). There was also significant improvement in the amount of VO_2 max of the two groups in post test comparing them to the post-test (P=0.0001).

DISCUSSION

The purpose of this investigation was to study the time effect of continuous aerobic running in the morning versus the evening time on, IgA, IgG, IgM, cortisol and testosterone serum values. Considering the results presented in the Table 3, it can be concluded that the observed responses to the morning and evening exercises for immunoglobulins was not significant. These findings are in agreement with the results reported by Dimitriou [6] who also studied the effects of the training time (at 6 in the morning in comparison with 6:00 PM) on the values of the swimmers' IgA and found no significant differences. While, Li Li [9] claimed that IgA concentration followed the circadian circle and the amount of IgA secretion aroused from aerobic activity was different in various times of the day. It seems the training period is a determining factor in relation with the serum` immunoglobulin response which was confirmed by Li Li study. The change of IgA concentration was obtained after 2 hours of pedaling with the training time mean of 18 minutes. Also, the 24 hours interval of the second blood sampling with the last training session can have some influence. In this regard, the researchers have concluded that it is sufficient to have 3 hours of rest after training for immunoglobulins values returning to the basic values. In this study, in spite of manipulating independent valuable, the measure of testosterone secretion did not show a significant change (p=0.24). These results are in agreement with those reported by Deschenes et al. [5]. They did not observe any significant difference in testosterone and cortisol values by changing the time. While, Bird [1], found that the aerobic activity in morning time versus evening caused decrease in cortisol concentration and the increase of testosterone ratio to cortisol. The differences reported in these studies may have been due to the time of blood sampling after the training. In confirmation of this hypothesis, Tremblays et al. [13] showed a significant increase of testosterone immediately after the training that returned to its base value after 3 hours.

TABLE 3. THE RESULTS OF ANOVA TEST IN RELATION WITH THE VARIABLES, IGA, IGG, IGM, CORTISOL AND TESTOSTERONE

Variables	F - Value	p - Value
Cortisol	13.3	0.0001
Testosterone	1.42	0.24
IgA	0.437	0.727
IgM	2.52	0.068
IgG	1.80	0.14

The results of this study in regard to cortisol level showed that the two groups in pre-test (p=0.006) and post-test (p=0.0001) had a significantly different values. Also, the pre-test of the morning training group had a significantly different level comparing to the evening training group (p=0.0001). These results are similar to those reported by Bird [1], Dimitriou [6], investigations.

CONCLUSIONS

In conclusion, it seems that the cortisol secretion has a strong circadian rhythm dependency, since the amount secreted in the morning is more than other times of the day and the night. There is not such certainty about immunoglobulins and testosterone. On the other hand, Volek et al. (1997) believe that testosterone serum secretion and ever saliva testosterone have a circadian rhythm. This rhythm includes the testosterone increase at night [8].

The restrictions attached to time researcher devoted to the protocol, limited blood sampling collection only in two phases (immediately after the last activity and 24 hours after the last session of training) in order to assess the changes of hormones and immunoglobulins secretion. It is suggested that further research be conducted including blood sampling in different times (immediately after the last activity, a few hours after that, 12 hours after that and so on). More investigations are needed to study the effect of time of training on various immunoglobulins amounts and hormones secretion to make firmer conclusions in this regard.

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