Measurement the Immunological and Hormonal Parameters in Intensive Excises in the Environment 33°C

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Abstract: Heat stress is one of the physical stresses which play a role in arising central body temperature and making changes in immune and hormone responses. The purpose of this study is to examine the effect of one stage severe aerobic exercise in warm environment on active women's immune and hormone factors. 16 physical education students with average age of 20.25 ±0.9 years, maximal oxygen uptake of 42.14±9.8 ml/kg/m and body mass index of 22.11±2.66 kg/mm have randomly divided in tow experimental and control groups. The experimental group has pedaled the ergometer wheels with VO2max 75% for 60 minutes and the control group has no activity during the test. The environmental temperature and humidity were maintained 33° centigrade and 40% respectively fixed during the test. Blood samples for measuring the levels of IL-10, TNFα, CRP, cortisol, epinephrine were taken in three periods pre, immediately and 2 hours after termination of the activity from participants. There wasn't any significant difference between control and experimental groups in density of IL-10, TNFα, when they were doing intensive activity in warm weather. There were only significant differences between groups on density of CPR and immediately and two hours after activity (p≤0.05). These data demonstrate that a 60 min exercise heavy in hot environment under these conditions can not affect on immunity and hormonic responses in activity women

Keywords: IL-10, TNFα, CRP, Cortisol, Epinephrine, Women’s Active

1. Introduction
Stress is the reaction of body to environmental devastating stimulants which derange the physiological natural balance of the body. Many hormones will be released while stress which some of them affect the physiological systems of body including immune system and its functions. Central nervous systems, in response to stress, respond in two ways; first it stimulates the sympathetic nervous system and second makes axis of hypothalamus-pituitary-adrenal active to stimulate secretion of cortisol which this axis play an important role in controlling the immune system and affect the immune system as antagonist of growth hormones and prolactin (vejgany, 2002). Cytokines are liquid glycoproteins which were produced by immune and unimmune cells of body (Moldoveanu et al 2001) and functionally divided into two groups of anti-inflammatory and pre-inflammatory (Pedersen et al 1998). Stress hormones which affects immune system regulate secretion of some cytokines by decreasing the production of cytokines pre-inflammatory including IL8, IL12 and TNF-α by auxiliary T-cells (Th1) as negative regulation and stimulate the production of cytokines anti-inflammatory including interleukine-10 and 13 (Phillips, 2001). Heat, against cold, increases the response of neutrophil, lymphocytes and natural killer cells and systemic releasing of cytokines (Mousavi & Abdullahi, 2003). Thus the athletes should be informed the signs of heat damages and coaches and authorities should arrange the exercises and matches in low environmental stress (Moran, 2001). However the effects of mediated immunity in physiological changes relative to temperature and internal connection between changes in central temperature, stress hormones and cytokines during external hyperthermia is not proven (Jimenez, 2007) but mechanism of immune system changes concerned with exercise is affected by various factors which among them changes in density of plasma cytokines and nervous hormones factors as cortisol, catecholamines and growth hormones have the greatest impact on it (Pedersen & Hoffman, 2000). The level of body fitness, intensity, duration and the kind of activity are the factors which affect the function of immune system (Mousavi & Abdullahi, 2003) but there is little information about other factors like temperature, unfavorable environmental conditions, nutrition, psychological stresses of exercise and match (Baj et al 1994).
Otherwise Bochuma and Knochel stated that human body work on a relatively restricted thermal domain and if temperature of body go higher than that the symptoms of thermal pressure like weakness, vertigo, cramps and finally reduction of performance occurs (Bouchama and Knochel, 2002). However this information about exercise in unfavorable environment brings about some research documents, presenting unit interpretation of the effect of body activity on immune function is difficult because of diversity of activities in intensity, duration, concerning other physiological factors like the role of hormones and also psychological factors and the results are contradictory (Gayyni, 2001).

On the other hand there is few studies examine the effect of exercise with various intensity, duration or verities on women immune factors which in few of them has examined ambient temperature (Bouchama and Knochel, 2002). The function of body systems especially immune system is different in men and women (Baj et al 1994). This fact that whether desired level of exercise makes the internal immune function work better without injuring it or stimulate it overly or not, are not clear yet and are more unclear especially in women who have the higher level of inflammation markers than men (Giraldo et al 2009). Despite knowing this fact, there are few studies examining cytokines responses to exercise (Ostrowski et al 1998) and it seems necessary to examine it with regarding to style of life, different research methods and statistical societies, cross-sectional and scattered researches, contradiction in results and lack of unit interpretation of findings especially limited information about women. Thus the purpose of this study is to investigate one stage intense exercise in warm environment on active woman immune and hormone factors.

2. Materials and methods

Subjects: Sixteen female physical education students healthy (table1) and voluntar participated in this study [Mean ± SD Age 20.25 ± 0.9 years, Body Weight (BW) 59.44 ± 7.6 kg, Percentage Body Fat (PBF) 26.19 ± 4.3%, Maximal Aerobic Capacity (VO2max) 42.14 ± 9.8 ml.kg.min and Body Mass Index (BMI) 22.11 ± 2.66 Kg/m2] consuming no drugs and narcotics during the study and performing regular aerobic activity three days in a week and were divided randomly into two treatment (E, 8 subject ) and control (C, 8 subject) groups.

Table 1. Participants’ demographic specifications for experimental and control groups

<table>
<thead>
<tr>
<th>characterstics</th>
<th>group</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vo2max(ml.kg.min)</td>
<td>E</td>
<td>47.01 ± 11.49</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>37.28 ± 4.49</td>
</tr>
<tr>
<td>Age (year)</td>
<td>E</td>
<td>20.25 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>20.25 ± 0.88</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>E</td>
<td>163 ± 5.75</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>165 ± 4.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>E</td>
<td>58.44 ± 7.55</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>60.44 ± 8.70</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>E</td>
<td>22.06 ± 2.98</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>22.16 ± 2.52</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>E</td>
<td>25.43 ± 4.60</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>26.95 ± 4.30</td>
</tr>
</tbody>
</table>

Study protocol: Participants’ height, body mass, body composition and maximal oxygen uptake were measured by strand test on ergometer. Blood cell count was done to be assured of participants’ health. The empirical group entered with light and uniform covers after primary reading and warming body up with doing stretching exercises and then closing rate meter to the environment with 33° centigrade air temperature and 40% humidity and pedaling about 1minute to warm up. Desired heart beat for keeping the intensity of desired activity was calculated formerly and was informed to all participants and wanted them to keep desired beating with regulating the speed of pedaling. Participants on ergometer pedals until reaching desired beating and keep this intensity for 60 minutes. They leave the desired environment after doing the activity and repose in normal environment with 21° centigrade and normal humidity. Participants were read immediately and 2 hours after activity.

Control group were read before entering desired place and then sat in a 33° centigrade environment for 60 minutes without doing any activity, and when the activity of empirical group finished, leave the controlled environment together and subsequent reading were done. Before doing the activity, body temperature of all participants were measured to be assured of participants’ health and non-existence of fever. It was also recommended to participants do not participate in match or heavy activity and use from advised diets (including proteins) 48 hours before carrying out the test , meanwhile it was recommended do not use vitamin C and carbohydrates because of their probable effect on immune system 12 hours before doing the test. Participants restrained from using any kind of
drinking and food except for mineral water which they have while doing the exercise and 2 hours after that. The tests were done between 9 am to 2 pm to control the effects of circadian rhythm on dependant variables.

**Biochemical analysis:** Blood was collected in SS-T Vacutainer and serum was separated by centrifugation at 2500 rpm for 10 min at 22-24˚C. The serum was divided into aliquots and stored at -80˚C until analysis of inflammation related proteins. TNFα, IL10 were measured in serum by ELISA method (Diaclone, Bensacon, France). cortisol, hs-CRP were determined in serum by ELISA method (Diagnostics Biochem Canada, Ontario, Canada). Sera epinephrine level measured in serum by ELISA method (IBL GmbH diagnostics, Hamburg, Germany).

The coefficients of variation, %CV (inter assay precision), were 5.8% for TNFα, 5 % for IL10, 6.4% for CRP, 5.4% for cortisol and 7.3% for epinephrine.

Detection limits (sensitivity) for the analyses were 8pg/ml for TNFα, 5pg/ml for IL10, 10ng/ml for CRP, 0.4ug/dl for cortisol and 10pg/ml for epinephrine. All samples were analyzed at the same time, at the end of the study, to minimize systematic variation.

**Statistical analysis:** To determine measured variable average descriptive statistics was used and to compare averages and their differences in two groups dependant T-test was used. To investigate changes within a group ANOVA with repeated measurement and Bonferroni paired test was used. Collected data were analyzed by SPSS software and average level of alpha error 0.05 was considered as a significant level (version 14).

### 3. Results

It wasn’t seen any significant difference between TNF-α, IL-10, cortisol and epinephrine in spite of changes in their serum three periods of pre, immediately and two hours after activity in two groups and the difference between groups wasn’t significant (figures 1-2-4-5). There weren’t any significant differences in density changes of CRP variable in three levels of activity between two control and experimental group and there was only one significant difference between two groups in immediately and two hours after activity (p ≤ 0.05) (figure 3).
Fig. 4. Serum cortisol concentrations prior to (pre), immediately following (post) and 120 min into recovery from (2h post) 60 min of ergometer exercise at 33°C (E) or sit down at 33°C (C)

Fig. 5. Serum epinephrine concentrations prior to (pre), immediately following (post) and 120 min into recovery from (2h post) 60 min of ergometer exercise at 33°C (E) or sit down at 33°C (C)

4. Discussion and conclusion

Immune system response during physical exercise in challenging environments is the subject that must be studied more. The environment condition may affect immune system performance thoroughly. The immune system responses may alleviate via intelligent evaluation of study participants in terms of environmental compatibility but instead of potential contribution of neurological-psychological effects, the psychological impact of heat, cold or altitude are among environmental challenges which are remained oblivious and there is limited information about immunity disorder intensity and duration due to exposure to stressful factors (Shephard, 1998).

The purpose of this study is to examine the effect of one stage severe aerobic exercise in warm environment on active women’s immune and hormone factors. The results of this study showed that TNF-α density decrease and then insignificantly increase after activity in empirical group, this finding is contradictory to Pedersen’s review study 2000 which has stated heavy exercise make TNF-α increase, however review study didn’t examine the effect of ambient temperature which is a main factor of this study but we can’t attribute these contradiction in results to this factor (Agha Alinejad et al 2009). Furthermore the result of this study is contradictory to Ostrowski and colleagues which reported tumor necrosis factor alpha increase after marathon match and one reason for this contrast can be intensity and duration of marathon activity (Ostrowski et al 1999). Rhind and colleagues examined the effect of 39° centigrade environmental temperature on men following pedaling ergometer while floating in water to thorax and reported that TNF-α increase after 40 minutes exercise (Rhind et al 2004), in Rhind’s study higher environmental temperature is an important factor because exercise accompanies increasing rectal temperature and increases the circulating stress hormones which increase cytokines. It is reported that changes in catecholamine interfere in interaction between lymphocytes and vascular endothelial cells which increase cytokines exist in circulation of blood (Moldoveanu et al 2000) that with considering this fact that epinephrine in this study didn’t change while doing the exercise, we can justify the decrement of TNF-α. Jimenez and colleagues stated that TNF-α didn’t change after one session exercise with moderate intensity in 35° centigrade temperature (Jimenez et al 2008a).

One of the reasons of contrast between this study and mentioned studies can be high level of resting serumal in athlete participants of study and permanent effects of severe inflammation, intensity and duration of activity, different ambient temperatures, difference in sexuality and diversity of activities and duration of heat exposure. The increase of TNF-α density until two hours after activity in this study is in one direction with Moldoveanu and colleagues’ study (Moldoveanu et al 2000) which reported that two cytokines IL-6 and TNF-α increase in 2 to 24 hours after doing 3 hours activity with moderate intensity, however intensity and duration of activity and ambient temperature varied with this exercise.

The results of this study, the decrement after activity and then insignificant increment until two hours after activity for IL-10 in empirical group, showed that this finding differ from Jimenez and colleagues’ (Jimenez et al 2008a) result that stated no change in density of this cytokine following doing activity in 35° centigrade temperature and one reason of contrast can be the differences in ambient temperature, sexuality, intensity and duration of activity. Some researchers know IL-10 as a cytokine anti-inflammatory which increase after doing heavy exercises (Phillips 2001) but Moller (Moller 2000) and Gannon & colleagues (Gannon et al 1997) didn’t observe any changes in IL-10 after exercise with stimulating lymphocytes or in its plasma density.
However the results of this study is concerned with normal environment, temperature probable effects, different intensities and duration of exercise, athletes’ fitness level, differences in age and sexuality and changing biological activity of some cytokines by internal inhibitors of protein carriers or liquid receiver makes it difficult to generalize and compare results and researcher didn’t find any study which follow similar scheme in women. Jimenez and colleagues investigate active and inactive hyperthermia effects on immune and hormones responses and stated that catecholamine plays an important role in stimulating components of immune cells and producing IL-10 while doing exercises and prolactin and catecholamine have an opposite role against immune responses (Jimenez et al 2007). Thus not measuring prolactine and probable effect of this factor in this study should be considered.

Study findings showed process of insignificant increase of CPR until two hours after doing the activity in empirical group which this finding is contrary to Jimenez and colleagues’ finding that examine the effect of 60 minutes exercise with moderate intensity in 35° centigrade temperature, They haven’t seen any changes in CPR (Jimenez et al 2008a) and one reason of this contrast can be exercise intensity, sexuality, and participants’ primary level of fitness. Our findings are in one direction with Albright study which reported significant increase in CPR of male athletes while doing severe and moderate aerobic exercises for 40 minutes on ergometer and after doing that (Albright 2006), besides our findings is compatible with Scharhag and colleagues’ results which examine the effect of exercise with moderate intensity on male athletes (Scharhag et al 2005), however in this study there wasn’t any thermal limit for doing exercise. It was said that body activity affect CPR changes and known factors which affect this response are body composition, body mass, the tense of body samples measurement, plasma volume changes, estrogen, nutrition and cigarette. Furthermore lower levels of CPR arising from exercise may be because of increases in IL-10 and IL-1ra; nevertheless there are few reports which examine one separate session of exercise on inflammatory markers (Plaisance & Grandjean 2006). The CPR increase is probably because of IL-10 decrement after activity, however the effect of other anti-inflammatory factors and other cases mentioned above but didn’t measure in this study should be considered. Existence of positive correlation between IL-6 and CPR have been mentioned in some reports and not measuring this cytokine in this study and its role as a liver hepatocytes stimulant in producing severe phase proteins while doing exercise and their other probable effects on each other should be considered (Petersen & Pedersen 2005).

No change in cortisol and then insignificant decrement of it until two hours after activity in empirical group is contradictory to Starkie and colleagues’ finding which examine the effect of 90 minute exercise on ergometer and heat stress on production of cytokines and stress hormones at both 15° and 35° centigrade temperature in men and reported that cortisol increase at both of two temperatures (Starkie et al 2003). One reason of present contrasts in findings can be high basic level of cortisol in this study and duration of activity. Peake and colleagues reported significant increase of cortisol in men following 60 minutes exercise on treadmill with different intensities which the mount of this increase was higher while doing intense exercise (Peake et al 2005). On the other hand, Giraldo and colleagues examine the effect of 60 minutes pedaling ergometer with moderate and severe intensities in women and reported that cortisol has decreased in both exercise protocols and stated that the possibility of catecholamines and cortisols and sex hormones participation in immune changes related to exercise intensity is debatable (Giraldo et al 2009).

 Whereas mentioned studies was done in normal environment, comparing findings with this study isn’t without difficulty, because unfavorable ambient temperature stresses body and activize axis of hypothalamus-pituitary-adrenal to stimulate secretion of cortisol. This factor is the element of controlling immune system and as a growth hormone’s antagonist and prolactin affects immune system. Thus not measuring these two hormones and their probable effects in this study should be considered. Chatard and Copeland in separate researches stated that cortisol response depends on numerous parameters including physical exercise intensity and duration, body fitness, nutrition, altitude, mentality, circadian rhythm and inappropriate environmental parameters (Chatard et al 2002, Copeland et al 2002). cortisol affect B and T lymphocytes and decrease the production of cytokines and antibodies and natural killer cells activity. On the other hand affect more B cells than T cells and cause mobilization of cells and immune responses acceleration (Vejgany, 2002). IL-1 and IL-6 can increase adrenocorticotropin secretion and consequently cortisol release which is a negative feedback among immunity-nervous-hormonal systems which is more likely to be a protecting mechanism against inflammation (Phillips 2001). However in this research these two factors haven’t been assessed, inflammatory response with production of acute phase proteins and secretion of cortisol are the samples of normal physiologic and
immune response which is yet ambiguous (Phillips 2001).

No changes in epinephrine and then its insignificant decrease of intensity until two hours after activity was one of these study findings in empirical group which is in contrary to Rhind and colleagues’ results in examining the effect of 18° and 39° centigrade environmental temperatures while doing exercises on ergometer with moderate intensity which reported the increase in epinephrine density (Rhind et al 2004). Furthermore this study finding is contradictory to Starkie and colleagues’ addition results (Starkie et al 2005). Jimenez and colleagues examine the effects of active and inactive hyperthermia on immune and hormone responses and reported that plasma’s catecholamine level increases only when doing active exercises (Jimenez et al 2007). Jimenez and colleagues also examine immune function after 60 minutes moderate exercise with or without clothes and stated that doing exercise with clothes causes higher increase in rectal temperature and increase epinephrine and norepinephrine more than usual, furthermore they said that auxiliary T lymphocytes significantly decrease at the end of recovery period while doing both exercises (Jimenez et al 2008b). One of contraries of this study can be high level of resting serum in participants and intensity and duration of exercise or duration of heat exposure. On the other hand Giraldo and colleagues examine the effect of 60 minutes pedaling on ergometer with moderate and severe intensities in women and reported that epinephrine increased in both of exercise protocols and stated that the possibility of catecholamines and epinephrine and sex hormones’ participation in immune changes related to exercise intensity is debatable (Giraldo et al 2009). Since this study was done in a normal environment, comparing results with this study is not without difficulty because unfavorable environment temperature stresses body and stimulates sympathetic nervous system to release catecholamine from adrenal.

Epinephrine may play a mechanical role in increasing production of cytokines while doing exercises, thus severe exercise cause epinephrine significantly increase and meanwhile IL-6 intensity increase to maintain glucose homeostasis (Plaisance & Grandjean 2006). Auxiliary T cells type 2 (Th2) control humoral immune and increase the activity of B cells and antibodies. Furthermore auxiliary T cells type 1 (Th1) which have β2 receivers regulate by epinephrine and norepinephrine released from central nervous system in response to stress (Phillips 2001). Exercise causes significant movement of leukocytes and its subsidiaries and it wasn’t recognized yet how much catecholamines have the responsibility of this effect (Jimenez et al 2007). Catecholamines may indirectly affect movement of white blood cells with releasing cytokines as IL-10 and releasing epinephrine while doing exercise is more related to entrance of lymphocytes to blood circulation and this thing maybe concerned with high ratio of adrenergic β in lymphocytes (Mousavi & Abdullahi 2003). Lymphocytes state high level of adrenergic β2 receivers and the density of this receivers increase with exercise and catecholamines. The connection between epinephrine and adrenergic β2 receivers brings about formation of CAMP intracellular messenger which can cause changes in cell and finally changes in its function (Agha Alinejad et al 2009). Stimulating β2 adrenergoreceptors while stress increase inordinate synthesis of cytokines pre-inflammatory and anti-inflammatory by increasing CAMP (Rhind et al 2001). It seems, with considering sated information, no change in epinephrine while doing exercise be in concerned with TNFα reduction.

Conclusion: however this study findings showed that changes in values of three period activity was insignificant in all dependant variables, but changes in density of these factors in three resting levels, after sitting 60 minutes in 33° centigrade and 40% humidity environment and after sitting 120 minutes in 21° centigrade and normal humidity environment probably show the effect of environment on these factors. There weren’t any significant differences between two control and empirical groups in TNF-α, IL-10, cortisol and epinephrine variables which can show in this study intensity and duration of activity was too low to make differences in tow groups. Thus we can say that environmental temperature presumably affects immune hormone responses in female athletes while they are doing severe aerobic exercises.

Acknowledgement:
We deem it necessary to give thanks to all people who assist us in concluding this study especially to Islamic Azad university physical education yadegar emam unit students and tarbit modarres university physical education office for their unstinting cooperation.

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