Effect of Submaximal Aerobic Exercise at the Altitude of 3250 m on levels of Serum Cortisol, Testosterone and Testosterone to Cortisol Ratio in Active Young Men

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ABSTRACT

To date, relatively few studies have been conducted concerning the function of endocrine glands in hypoxic conditions. The purpose of this study was thus to determine the response of serum cortisol, testosterone and testosterone to cortisol ratio to aerobic exercises at the altitude of 3250 m. In this study, 9 young men with an average age of 23.33±1.56 years old performed two sessions of aerobic activities including 30-min running at an intensity level of 70% of maximum heart rate in both normoxic (1200 m) and hypoxic (simulated altitude of 3250 m) conditions. Blood samples were taken before, immediately after and one hour after physical activity and, for each sample, the levels of cortisol, testosterone and their ratios were determined. In order to compare the variables at the aforementioned altitudes, the T-student test for dependent groups was used and Analysis of Variance with repeated measurement was run for investigating the variation of each variable per session. The results of this study indicated that testosterone level decreased significantly at the altitude of 3250 m (P=0.000). No significant change was observed in either of the testosterone level at the altitude of 1200 m (P=0.212), cortisol levels at both altitudes (P=0.413 and P=0.057 for 1200 and 3250 m, respectively) and testosterone to cortisol ratios at both altitudes (P=0.262 and P=0.296 for 1200 and 3250 m, respectively). Immediately and 1 hour after physical activity, testosterone level at the altitude of 3250 m was observed to be significantly lower than its corresponding level at the altitude of 1200 m (P=0.032 and P=0.010, respectively). There was also no significant difference between the levels of cortisol (P=0.651 and P=0.514 for immediately and one hour after physical activity, respectively) and testosterone to cortisol ratio (P=0.054 and P=0.116 for immediately and one hour after physical activity, respectively) of the two altitudes. It seems that, for a given duration and intensity of aerobic exercise, testosterone response in hypoxic conditions differs from that in normoxic conditions.

Key words: Testosterone, Cortisol, Hypoxia, Aerobic exercise, Altitude

Introduction

Hypoxia which exists at altitudes above sea level can affect responses of hormones and, although it has received the attention of the researchers in some cases, its effect on hormone response is not yet completely clear. Altitude is defined as a place with more than 1500 m height from sea level [32]. Air composition remains unchanged at the altitude; however, its barometric pressure decreases. As a result, oxygen partial pressure decreases and hypoxia happens [18]. It is also assumed that, by simulating high elevation, more development can be generated in the aerobic capacity of individuals. Therefore, athletes have already started using commercial devices simulating hypoxic environment in order to fulfill their training goals. Nevertheless, due to doubled physiological pressure, the ability of body to maintain hormonal balance may make some changes [4]. In addition to hypoxic conditions, physical activity is a stressful situation which challenges body homeostasis [23].

Axes of Hypothalamus-hypophysis-adrenaline and Hypothalamus-hypophysis-testis are affected by physical activities. Studying these effects along with involving the effective factors like hypoxic environment is beneficial in maintaining athletes’ health [3]. Considerable changes at the levels of...
Testosterone and cortisol have been observed in situ of physical pressure [11, 26,33]. Testosterone is a steroidal hormone and has anabolic effects in muscle tissues [13]. Cortisol is the most important human glucocorticoid and which is mostly known hormone with catabolic effects [19]. For the first time, Adlercreutz et al. (1986) introduced testosterone to cortisol ratio as a diagnostic tool for depicting exercise and training pressure [1]. The exact response pattern of this ratio to physical activities is not clear and its decrease [31] and lack of change [12] after the activity have been reported. It has been stated that hormonal responses in hypoxic conditions are larger than those in normoxic conditions [25].

Maximum oxygen consumption decreases in hypoxic conditions and, consequently, relative intensity of work pressure increases [8]. Activity with oxygen shortage can make some changes in hormonal responses, even in reverse directions [15]. There have been not only few but also controversial studies on the operation of endocrine glands in hypoxic conditions. Moncloa et al. (1967) noticed significant increase of cortisol after the exposure to the altitude of more than 4300 m [10]. Marinelli et al. (1994) demonstrated cortisol increase and testosterone decrease after climbing the altitude and doing endurance activities [16]. However, Blegen et al. (2005) observed no significant difference in plasma cortisol considering hypoxic and normoxic conditions [4]. Also, Vasankari et al. (1992) reported higher concentration of serum testosterone in hypoxic conditions relative to normoxic conditions [30]. Benso et al. (2007) reported significant decrease of testosterone and lack of change of cortisol level at the altitude of 5200 m above sea level [2]. Bouissou et al. (1988) found no change between cortisol levels in normoxic conditions and its equivalent hypoxic conditions at the altitude of 3000 m in the activity with the intensity of 65% of maximum oxygen consumption [6]. Panjwani et al. (2006) declared the increase of plasma cortisol after one hour of exposure to the altitude of 3500 m compared with its level prior to the exposure [28]. Ermolao et al. (2009) showed that cortisol significantly increased on the first day of birth at the altitude of 5050 m [9].

Due to the lack of available and sometimes contradictory information, this research aimed at comparing the effect of submaximal aerobic activity in normoxic conditions and the same type of activity in hypoxic conditions at the altitude of 3250 m above sea level on acute responses of serum cortisol, testosterone and testosterone to cortisol ratio among active young men.

**Material And Methods**

**Participants:**

The participants in this research were 9 active young men, whose characteristics are summarized in Table 1.

**Table 1: Means and standard deviations of the participants’ characteristics**

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Height (cm)</th>
<th>Weight (Kg)</th>
<th>Maximum oxygen consumption (ml/kg/min)</th>
<th>Index of body mass (IBM) (kg/m²)</th>
<th>Resting heart rate (rate per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.33±1.56</td>
<td>176±1.76</td>
<td>67.16±3.14</td>
<td>4.86±48.6</td>
<td>21.6±0.91</td>
<td>68.55±3.74</td>
</tr>
</tbody>
</table>

They had regular physical activities at least two days per week within the last two years. After declaring the call in Tehran Azad universities and stating research purposes, 9 qualified male students were purposefully selected out of 23 volunteers. They filled in a testimonial and received medical experiments and examination.

**Exercise Programs:**

On the first day, aerobic power of the participants was measured through Bruce treadmill test in the presence of a physician [17]. After five days resting, the participants participated in the first exercise session. Within two aerobic exercise sessions, they ran with the intensity of 70% of maximum heart rate for 30 min in both hypoxic conditions equal to the altitude of 3250 m and normoxic conditions equal to the altitude of 1200 m in Tehran (Marzdaran Blvd). The two training sessions were held with 72 h resting in between and they were requested to avoid any physical activities between the sessions. In order to avoid any misleading results derived from the adverse effect of exercise sessions on each other, the participants were divided into two identical groups. On the first day, one group exercised in normoxic conditions and the other in hypoxic conditions on a random basis. On the second day, this condition was reversed. Hypoxic conditions with 14% oxygen equal to the altitude of 3250 m above sea level was provided using Go2 altitude device (made in Australia) in the laboratory of the faculty of Physical Education and Sport Sciences, Shahid Beheshti University, Tehran [5]. Maximum heart rate was also calculated by the equation 208 – (0.7 x Age) [27]. In addition, exercise sessions were held at the same time to avoid any detrimental effect of circadian rhythm on the results.

**Using the Altitude Simulation Device:**
Go2 altitude simulation device is used for hypoxic exercises and creates physiological conditions corresponding to the desired altitude in the body [5]. In this device, natural oxygen enters into the machine via a filter; then, the air with the desired percentage is stored in an air bag through an output filter. This bag is then connected through a tube to the mask on the face of the participant. Once the device is switched on, it takes one minute to be calibrated. Afterwards, the MENU button on the device should be pressed and the hypoxia option should be selected on the display screen. There is a screen on the device body which shows the oxygen ranging from 9% to 15%. Desired oxygen percentage is determined by turning the key on this screen. As specified by the device itself, 14% is related to the altitude of 3250 m. Before starting the test, the finger indicator which is placed on the machine by a connecting wire is joint to the index finger of the participant in order to be able the device to automatically provide natural oxygen in case saturated arterial oxygen (Sao2) goes lower than 85%. After cross-checking these steps, the participants starts to exercise on the treadmill.

Blood Sampling and Hormonal Analysis:

The participants’ blood samples were taken from the middle vein (Basilic) using a 5 cc syringe before, immediately after and one hour after the exercise in each session while they were seated in a stable position. The collected samples were poured in sterile tubes containing K3EDTR. Heparinised tubes and EDTR were placed inside ice and remained at room temperature for some minutes. Afterwards, serum was separated from the plasma using centrifuge for 10 min with 3500 RPM. All the blood samples were maintained frozen at -20 ℃ until reaching the laboratory. It should be mentioned that the participants were asked to avoid using alcohol and caffeine on sampling days, from one night before the first exercise session until the end of sampling. Cortisol hormone was measured using ELISA method and dbc kit (made in Canada) with the sensitivity of 0.2 nanogr/cc and accuracy of 0.99. Testosterone and cortisol ratio was calculated after converting the values of both hormones to nanomole/liter. The formula cortisol * 27.59 and testosterone * 3.47 were used for converting the units of cortisol and testosterone, respectively [34].

Statistical Methods:

In this study, first, research data were investigated by running Kolmogorov-Smirnov test for assuring the normal distribution of data and determining the application of parametric or non-parametric statistical tests. Normal distribution of data was confirmed; therefore, Dependant Groups Student T-test was conducted to compare variable changes between normoxic and hypoxic conditions and Anova with repeated measures was run to investigate changes of variables within each exercise session (before, immediately after and one hour after the exercise). In case of any significant difference, Dependant Groups Student T-test with Bonferroni Corrections was used to find the source of such differences and to decrease error percentage. In the Analysis of Variance test, data sphericity was also checked in order to use Greenhouse-Geisser correction on the degree of freedom if required. To ensure similarity of variables prior to both exercise sessions, Dependant Groups Student T-test was conducted and no significant difference was observed at the level P≤ 0.05. In addition, significance level of 0.05 was considered for all statistical tests. The SPSS statistical software, version 16, was used for performing the statistical tests.

Results:

Table 2 shows mean and standard deviation of variables before, immediately after and one hour after each of the two exercise sessions at the altitudes of 1200 and 3250 m.

Table 3 demonstrates the results of Dependant Groups Student T-test for comparing the altitudes of 1200 and 3250 m.

Table 4 shows the results of Analysis of Variance with repeated measures for changes of variables within each exercise session.

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Variable</th>
<th>Before exercises</th>
<th>Immediately after exercises</th>
<th>One hour after exercises</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200 m</td>
<td>Cortisol (µgr/deciliter)</td>
<td>10.04±4.114</td>
<td>7.70±3.272</td>
<td>7.76±5.482</td>
</tr>
<tr>
<td></td>
<td>Testosterone (nanogr/cc)</td>
<td>6.86±1.624</td>
<td>5.92±1.418</td>
<td>6.04±0.899</td>
</tr>
<tr>
<td></td>
<td>Ratio (nanomole/liter)</td>
<td>0.097±0.0429</td>
<td>0.116±0.063</td>
<td>0.143±0.083</td>
</tr>
<tr>
<td>3250 m</td>
<td>Cortisol (µgr/deciliter)</td>
<td>10.04±4.114</td>
<td>8.20±2.951</td>
<td>6.17±1.52</td>
</tr>
<tr>
<td></td>
<td>Testosterone (nanogr/cc)</td>
<td>6.86±1.626</td>
<td>4.22±1.276</td>
<td>3.91±1.448</td>
</tr>
<tr>
<td></td>
<td>Ratio (nanomole/liter)</td>
<td>0.097±0.0421</td>
<td>0.071±0.033</td>
<td>0.092±0.054</td>
</tr>
</tbody>
</table>
Significant difference between exercise sessions at altitudes of 1200 and 3250 m was only noticed in testosterone immediately after (P=0.032) and one hour after (P=0.010) the exercise in which testosterone level at the altitude of 3250 m was lower than its level at the altitude of 1200 m.

Besides that, regarding the changes during each session before, immediately after and one hour after the exercise, the only significant difference occurred for testosterone and at the altitude of 3250 m which included testosterone reduction in both stages immediately after and one hour after the exercise (P=0.000). Dependant Groups Student T-test with Bonferroni Corrections showed a significant difference between before and immediately after the exercise (P=0.001) and between before and one hour after the exercise (P=0.001).

### Table 4: Results of Analysis of Variance with repeated measures in each session of physical activity

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Variable</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude of 1200 M</td>
<td>Cortisol</td>
<td>32.204</td>
<td>2</td>
<td>16.102</td>
<td>0.934</td>
<td>0.413</td>
</tr>
<tr>
<td>Altitude of 1200 M</td>
<td>Testosterone</td>
<td>4.725</td>
<td>2</td>
<td>2.363</td>
<td>1.713</td>
<td>0.212</td>
</tr>
<tr>
<td>Altitude of 1200 M</td>
<td>Ratio</td>
<td>0.010</td>
<td>2</td>
<td>0.005</td>
<td>1.460</td>
<td>0.262</td>
</tr>
<tr>
<td>Altitude of 1200 M</td>
<td>Cortisol</td>
<td>67.485</td>
<td>2</td>
<td>33.742</td>
<td>3.446</td>
<td>0.057</td>
</tr>
<tr>
<td>Altitude of 1200 M</td>
<td>Testosterone</td>
<td>47.497</td>
<td>2</td>
<td>23.749</td>
<td>18.728</td>
<td>0.000</td>
</tr>
<tr>
<td>Altitude of 1200 M</td>
<td>Ratio</td>
<td>0.004</td>
<td>2</td>
<td>0.002</td>
<td>1.315</td>
<td>0.296</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.

**Discussion and Conclusion:**

Based on the findings of this study, no significant difference in serum cortisol concentration was noticed between exercises at the altitudes of 1200 and 3250 m. Belgen et al. (2005), Benso et al. (2007) and Bouissou et al. (1988) did not observe any significant differences in the concentration of serum cortisol between hypoxic and normoxic conditions [2, 4, 6]. Contrary to these results and the findings of this research, Moncloa et al. (1967), Marinelli et al. (1994), Panjwani et al. (2006) and Ermolao et al. (2009) reported significant increase of cortisol in hypoxic conditions compared with that in normoxic conditions [9,10, 16, 28]. Probably, these contradictory results are in part related to the hypoxic degree. Belgen et al. (2005) conducted the research in low-pressure chambers with the oxygen concentration of 14.65% [4].

This research examined the altitude of 3250 m (14% oxygen). 15% oxygen is equal to the altitude of 2750 m and 14.65% oxygen is approximately equal to the altitude 3000 of m. In Benso et al.’s (2007) research which was conducted in Himalayas, the participants were taken to the altitude of 5200 m; five of them reached the altitude of 8852 m, three the altitude of 8600 m and one the altitude of 7500 m. In their research, although the participants climbed to high altitudes, cortisol level had no significant changes [2]. Bouissou et al. (1988) also studied the altitude of 3000 m using low-pressure chambers and obtained similar results to those of the current research [6]. In contrast, Moncloa et al. (1967) who studied the altitude of 4300 m reported significant increase in the concentration of plasma cortisol at the altitude [10]. Additionally, the participants of the research by Marinelli et al. (1994) started their competition at the altitude 3860 of m and ended it at the altitude of 3400 m; of course, they reached the altitude of 5100 m in between, too [16]. They also reported significant cortisol increase at the altitude [16]. Panjwani et al. (2006) simulated the altitude of 3500 m with lowpressure chambers and observed significant increase of cortisol compared with the normoxic conditions [28]. Ermolao et al. (2009) also reported significant cortisol increase on the first day of ascending to the altitude of 5050 m [9].

The investigations which declared cortisol increase at the altitude have undertaken their studies at higher altitudes compared with that of this research (3400 m to over 5000 m). Belgen et al. (2005) and Bouissou et al. (1988) who reported similar results to those of this research conducted their studies at the simulated altitude of about 3000 m [4, 6]. Anyway, Benso et al. (2007) reported no cortisol difference between the altitude and sea level though they performed the tests at altitudes higher than 5200 m, even some participants climbed to the...
Altitudes of 7500, 8600 and 8852 m [6]. Their research altitudes were so high that one needs to be cautious about the hypoxic degree. Probably, factors other than hypoxic degree also affect cortisol changes at the altitude. Participants in the research of Benso et al. (2007) only climbed to the altitudes but had no other physical activities [6]. The participants of Belgen et al. (2005) exercised with 40% and 60% of maximum oxygen consumption for 60 min in both conditions [4]. Bouissou et al. (1988) enquired their participants to exercise for 60 min with the intensity of 65% maximum oxygen consumption [6]. The participants of the two recent studies were healthy males while those of Benso et al. (2007) were superior trained males. It seems that, in addition to hypoxic degree, physical activity and exercise schedule of the participants influences changes of serum cortisol at the altitude. It has been approved that hypoxia increases cortisol; yet, it has not been clear that which altitude has a cortisol response different from the sea level. However, some researchers have concluded that the increase can be due to the increase of ambient temperature and not resulted from the reduction of oxygen pressure [7]. In any case, to clarify the ambiguities, more controlled studies should be performed considering various aspects. Moreover, in this research, no significant change was observed in blood hemoglobin during and after each exercise session. Based on the findings of this research, the concentration of serum testosterone following the exercise at the altitude of 3250 m was significantly lower than that at the altitude of 1200 m. Additionally, although testosterone decreased after both exercise sessions, it was only significant at the altitude of 3250 m. Marinelli et al. (1994) reported testosterone reduction in a Marathon race at the altitudes of 3400, 3860 and 5100 m [16]. Benso et al. (2007) also observed testosterone reduction at the high altitudes of Himalaya Mountains [2]. Reduced levels of testosterone at the altitude of 3250 m in this research could be probably attributed to the hypoxic stress [30]. Perhaps, hypoxic conditions impose high pressure which results in significant decrease of testosterone in hypoxic conditions compared with the normoxic one in this research. It has been reported that, even after eight weeks of mountain climbing exercises in the mountains, testosterone levels remain at lower levels than sea level until participants return from the mountains [21]. In contrast, Vasankari et al. (1992) showed that serum testosterone levels were higher than sea level before ski competitions at the altitude [30]. Perhaps this controversy is partly related to the hypoxic degree. Vasankari et al. (1992) obtained these findings at the altitude of 1650 m compared with the sea level [30]. Studies have shown the relationship between serum testosterone reduction and hypoxic degree [24]. Besides, reduction of sex hormones has been determined at high altitudes [29].

Based on the findings of the current research, serum testosterone to cortisol ratio both immediately after and one hour after the exercise at the altitude of 3250 m was lower than that at the altitude of 1200 m. Nevertheless, these differences were not statistically significant. The balance between catabolic and anabolic processes is defined by testosterone to cortisol ratio [1]. This ratio is an important indicator of exercise and training pressure [1].

The findings of this study showed that serum testosterone to cortisol ratio increased after exercising at the altitude of 1200 m while it decreased after exercising at the altitude of 3250 m. This increase continued for one hour after the exercise at the altitude of 1200 m; however, it moved toward resting amounts at the altitude of 3250 m although it was still slightly lower than the amounts before the exercise. Obviously, changes within each session were not significant as well but, when intensity and duration of exercises were kept constant, increase of testosterone to cortisol ratio at the altitude of 1250 m was converted to the ratio decrease at the altitude of 3250 m immediately after exercising. Hypoxia acts as an external stressful stimuli and increases sympathetic activity [22]. It has been recommended that hypoxia causes a rise or intercurrent sympatho-vagal shift toward sympathetic penetration at the resting state [22]. This sympathetic activity results in ACTH increase which stimulates cortisol secretion from adrenal glands [22]. However, in this research, due to the significant reduction of testosterone at the altitude of 3250 m, the ratio of testosterone to cortisol insignificantly decreased. Nitric oxide which increases at the altitude damages the synthesis of steroidic hormones; it has been considered that nitric oxide lowers outflow level of corticosteroids and testosterone [14,20]. It is possible that these differences between hypoxic and normoxic conditions are basically related to relative work pressure rather than direct effect of hypoxia per se because maximum oxygen consumption increases in hypoxic conditions [8].

In any case, exercising in hypoxic conditions may result in hormonal and metabolic changes which are in line with catabolic changes. This has been supported by some previous studies [9,10, 16, 28] and was in correspondence with the findings of this research. Nevertheless, further researches are required to study various hypoxic degrees and consider other effective variables in order to reach more precise conclusions.

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