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The Effect of Aerobic Exercises in Hypoxic Conditions on the Levels of Saliva Testosterone, Cortisol and Testosterone to Cortisol Ratio in Active Young Men

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Abstract: The aim of the present study was to determine the effect of aerobic exercises in the hypoxic conditions on the levels of saliva cortisol, testosterone and testosterone to cortisol ratio in young active men. Eight active young men with the average age of 23.33±1.56 years, weight of 67.16±3.14 and height of 176±1.76 cm participated in four sessions of aerobic exercises including running for 30 min with the intensity of 70% of maximal heart rate in four normoxic and hypoxic conditions (altitudes of 2750, 3250 and 3750 m). Before, immediately after and one hour after the exercise, the saliva samples were collected. The analysis of the data was done using mixed-models statistical methods which showed that, in both sampling times after exercises, the concentration of saliva cortisol in the hypoxic condition was significantly lower than its amount in the normoxic condition ($P<0.05$); however, it was not significantly different between three altitudes ($P>0.05$). No significant difference was observed for saliva testosterone ($P>0.05$) but the saliva testosterone to cortisol ratio significantly increased in the hypoxic condition ($P>0.05$). The findings of the present study are in contrast to the previous findings in most cases, which could be due to the applied natural or simulated altitudes in different studies, exercising in hypoxic conditions, the period of staying in the hypoxic condition, etc. The best result which could be taken from this study is that there should be more studies on this issue in order to answer the problems resulted from the present work.

Key words: Hypoxic · Cortisol · Testosterone · Aerobic Exercise · Altitude

INTRODUCTION

High altitude has not been defined precisely. Most individuals develop clinical, physiological and biochemical changes above 3000 m. However, there are some variations and a number of people develop signs and symptoms of high altitude sickness at the altitudes as low as 2000 m [1]. Others have defined high altitude arbitrarily as the elevation of above 2500 m [2].

Hypoxia which exists in the altitudes higher than sea level can influence hormonal responses; although it has attracted the attention of some researchers in some cases, its effect on the hormonal response has not been found yet. It is assumed that more advances can be achieved in the aerobic capacity of people by simulations at high altitudes. Therefore, athletes try to use commercial systems which provide hypoxic environments in order to achieve their training goals. Nevertheless, the body's ability for maintaining hormonal balance may change due to doubled physiological pressure [3]. In addition to physiological conditions, physical exercises are stressful conditions which challenge body homeostasis [4].

Many changes have been observed in the levels of cortisol and testosterone under the conditions of physical pressure [5-7]. For the first time, Adlercreutz *et al.* [8] talked about the testosterone to cortisol ratio as a diagnostic tool which is the pressure indicator for training and exercises. The exact pattern of the response of this ratio to physical exercises is not known and there are different reports for its decrease [8], increase [9] and lack of change [10] after exercises and training. It has been said that hormonal responses in hypoxic conditions are

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larger than those in the normoxic conditions [11]. In the hypoxic conditions, the maximum oxygen consumption decreases and, as a result, the relative intensity of work pressure increases, too [12]. Exercises in the lack of oxygen can create changes in the hormonal response, even in the opposite direction of normal conditions [13].

No studies can be found in the related literature on the effect of hypoxic conditions on the saliva levels of cortisol and testosterone hormones; previous studies have examined the serum levels of these hormones in response to hypoxic conditions. A small number of studies have worked on the serum responses of these hormones to the hypoxic conditions and have presented different interpretations. For instance, Moncloa *et al.* [14] observed the significant increase of cortisol after being faced with the altitude of 4300 m. Marcello *et al.* [15] demonstrated the increase of cortisol and decrease of testosterone after climbing to the altitude and endurance exercises. However, Blegen *et al.* [3] did not observe any significant differences in the plasma cortisol considering the hypoxic and normoxic conditions. Moreover, Vasankari *et al.* [16] reported that the concentration of serum testosterone in the hypoxic conditions is higher than that in the normoxic conditions. On the other hand, Benso *et al.* [17] reported the significant decrease of testosterone with no change of the level of cortisol at the altitude of 5200 m above sea level. Bouissou *et al.* [18] did not observe any changes between cortisol levels in the normoxic and hypoxic conditions at the altitude equal to 3000 m in exercises with the intensity of 65% maximum oxygen consumption. Ermolao *et al.* [19] demonstrated the significant increase of cortisol on the first day of life in the altitude of 5050 m.

Considering the contradiction and lack of available information, it is essential to undertake more studies on the responses of cortisol and testosterone hormones to the exercises in the hypoxic conditions.

The aim of the present research was to determine the effect of aerobic exercises in four normoxic and hypoxic conditions equivalent to the altitudes of 2750, 3250 and 3750 m above sea level on the levels of saliva cortisol, testosterone and testosterone to cortisol ratio in active young men.

MATERIELS AND METHODS

Participants: From among 23 volunteers, 10 young male students in the universities of Tehran were purposefully selected. During the research, two participants asked for their removal; the 8 remaining participants who continued till the end of the research had the mean age of 23.33±1.56 years, height of 176±1.76 cm, weight of 67.16±3.14 kg, maximal oxygen consumption of 48.6±3.96 ml per kg of body weight per min, body mass index of 21.6±0.91 kg by the square of height and resting heart rate of 68.55±3.74 beat per min. During the last two years, they had regular physical training at least two days per week and had no disease background. Also, they did not take any medications during the study. It should be mentioned that, after selecting the participants, the required information about the research was given to them and they signed the written testimonial.

Exercise Program: On the first day, the aerobic power of the participants was measured using the Bruce treadmill test [20]. This session was performed in the presence of a physician. After 5 days, the participants attended the first day of four exercise sessions, between which there were 72 hours of resting. In order to avoid confusing results caused by the detrimental impact of exercise sessions on each other, the sequence of these exercise sessions was randomly determined for each person. Each participant performed the exercise of running on the treadmill for 30 min with the intensity of 70% of maximal heart rate once in the normoxic and three times in the hypoxic conditions. The hypoxic conditions were provided with the oxygen percentage of 15, 14 and 13% equivalent to the altitudes of 2750, 3250 and 3750m, respectively, above sea level using the Go2 altitude system made in Australia [21]. The altitude of normoxic conditions was equivalent to 1200 m above sea level in the city of Tehran. To calculate maximal heart rate, the following equation was used [22]: 208 - (0.7×age).

It should be mentioned that the participants were asked to avoid caffeine and alcohol the night before sampling and during the research in general. All the stages of sampling were done in equal conditions for them in order to naturalize the effect of circadian rhythm. In fact, each person started and finished all his exercise sessions at the time which was specific to him and equal for all his exercise sessions.

Saliva Sample Collection and Hormonal Analysis: Saliva samples were taken at three stages of before exercises, immediately after exercises and one hour after exercises into special containers. The collected samples were kept frozen at -20 degrees Centigrade until reaching the laboratory; there, laboratory measurement was immediately started. For each sample, cortisol, testosterone and testosterone to cortisol ratio were measured and calculated. Cortisol and testosterone were measured using the ELISA method by de Medi Tec kit

(Medikit Co.) with the sensitivity of 0.014 ng/ml and 2.2 pg/ml, respectively. Testosterone to cortisol ratio was calculated after converting hormonal values to nmol/l. To convert the units of cortisol and testosterone, cortisol×0.2759 and testosterone×3.48 formula were applied, respectively [http:// www.unc.edu/ rowlett/ units/ scales/clinical_data.html].

Statistical Analysis: All analyses were performed using SPSS 15 statistical software (SPSS Inc., Chicago, IL). P-values of less than 0.05 (P<0.05) were considered as significant.

RESULTS

For saliva cortisol, there was a significant difference within measures totally evaluated in the sessions ($F_{(8,69)}$ = 10.645, P < 0.001).

Also, in the separate evaluation of the measurements, significant differences were observed within measurements in the normoxic (1200 m), hypoxic (2750 m) and hypoxic (3250 m) sessions (All P<0.05) (Table 1). In addition, the results of the post hoc test for normoxic sessions (1200 m) showed significant differences between before intervention with immediately after intervention and one hour after intervention (Both P<0.001) situations. Also the results showed significant differences between before intervention and immediately after intervention situations for hypoxic (2750 m) (P=0.034) and hypoxic (3250 m) (P=0.027) sessions.

Also, there were significant differences among sessions for both immediately after intervention and one hour after intervention measurements (Both P<0.05) (Table 1). In addition, the results of the post hoc test for immediately after intervention measurements showed significant differences between normoxic (1200 m) and hypoxic (2750 m), hypoxic (3250 m) and hypoxic (3750 m) sessions (P=0.009, P= 0.035 and P=0.048, respectively). For one hour after intervention measurements, significant differences were observed between normoxic (1200 m) and hypoxic (2750 m) and hypoxic (3250 m) sessions (Both P=0.003).

For saliva testosterone, there was a significant difference within the measures totally evaluated in the sessions ($F_{(8,61)}$ = 7.695, P < 0.001).

Also, in the separate evaluation of the measurements, significant differences were observed within the measurements in all 3 hypoxic sessions (All P<0.05) (Table 2). In addition, the results of the post hoc test for all three hypoxic sessions revealed significant differences between before intervention and one hour after intervention situations (P=.003, P<0.001 and P=0.001 for 2750, 3250 and 3750, respectively, in hypoxic sessions). Besides, the difference between immediately after intervention and one hour after intervention situations was significant in the hypoxic (3250 m) session (P<0.001).

However, there were no significant differences among the sessions of before, immediately after and one hour after intervention measurements (P>0.05) (Table 2).

Table 1: Statistics results of saliva cortisol (ng/ml) tests within sessions

Session	Measures	Mean	Std. Deviation	Skewness	Kurtosis
Normoxic (1200 m)	Before	2.92	0.75	-1.52	4.25
	Immediately After	5.05	0.96	0.14	-1.77
	1 Hour After	5.22	0.97	0.83	-0.57
	Test results ¥	($F_{(2,14)}$ = 23.355, P <0 .001)			
Hypoxic (2750 m)	Before	2.98	0.52	-0.20	2.72
	Immediately After	3.67	1.10	0.29	2.10
	1 Hour After	3.37	0.97	0.45	1.19
	Test results ¥	($F_{(2,15)}$ = 4.504, P = 0.030)			
Hypoxic (3250 m)	Before	2.94	0.75	-1.61	4.24
	Immediately After	3.72	0.92	0.94	-0.23
	1 Hour After	3.06	1.21	0.54	-0.15
	Test results ¥	($F_{(2,14)}$ = 7.042, P = 0.008)			
Hypoxic (3750 m)	Before	2.92	0.74	-1.55	4.28
	Immediately After	3.67	1.04	-0.72	-0.75
	1 Hour After	3.74	1.59	0.17	-1.83
	Test results ¥	($F_{(2,14)}$ = 3.312, P = 0.068)			
Test results §	Before	($F_{(3,21)}$ =.256, P = 0.856)			
	Immediately After	($F_{(3,20)}$ =5.133, P = 0.009)			
	1 Hour After	($F_{(3,19)}$ =6.915, P = 0.003)			

¥Based on mixed-models analysis for comparing 3 measurements within each session

§Based on mixed-models analysis for comparing 4 separate sessions for each measurement

Table 2: Statistic results of saliva testosterone (pg/ml) tests within sessions

Session	Measures	Mean	Std. Deviation	Skewness	Kurtosis
Normoxic (1200 m)	Before	84.88	5.96	-0.32	-0.53
	Immediately After	92.50	3.30	-0.02	0.67
	1 Hour After	87.50	13.48	-0.79	1.86
	Test results ¥	($F_{(2,12)}$ = 1.593, P = 0.244)			
Hypoxic (2750 m)	Before	84.25	5.60	-0.35	-0.67
	Immediately After	92.13	5.77	-0.07	-0.63
	1 Hour After	101.50	16.64	-0.24	0.55
	Test results ¥	($F_{(2,12)}$ = 7.649, P = 0.007)			
Hypoxic (3250 m)	Before	84.50	4.50	-0.38	-0.39
	Immediately After	88.25	4.95	-0.76	0.80
	1 Hour After	97.13	7.08	0.73	0.01
	Test results ¥	($F_{(2,14)}$ = 21.910, P < 0.001)			
Hypoxic (3750 m)	Before	84.38	5.42	-0.32	0.01
	Immediately After	94.00	4.31	-0.21	-0.73
	1 Hour After	102.88	12.22	-0.18	-0.01
	Test results ¥	($F_{(2,13)}$ = 10.416, P = 0.002)			
Test results §	Before	($F_{(3,21)}$ =0.646, P = 0.594)			
	Immediately After	($F_{(3,22)}$ =1.909, P = 0.158)			
	1 Hour After	($F_{(3,21)}$ =2.146, P = 0.126)			

¥Based on mixed-models analysis for comparing 3 measurements within each session

§Based on mixed-models analysis for comparing 4 sessions separately for each measurement

Table 3: Statistic results of saliva testosterone to cortisol ratio (nmol/l) tests within sessions

Session	Measures	Mean	Std. Deviation	Skewness	Kurtosis
Normoxic (1200 m)	Before	0.040	0.019	2.503	6.825
	Immediately After	0.023	0.005	-0.011	-1.578
	1 Hour After	0.021	0.005	0.930	0.437
	Test results ¥	($F_{(2,13)}$ = 11.593, P = 0.001)			
Hypoxic (2750 m)	Before	0.037	0.008	1.070	3.190
	Immediately After	0.034	0.014	2.014	5.255
	1 Hour After	0.041	0.015	0.123	0.428
	Test results ¥	($F_{(2,14)}$ = 2.318, P = 0.135)			
Hypoxic (3250 m)	Before	0.039	0.018	2.528	6.900
	Immediately After	0.031	0.008	-0.523	-1.679
	1 Hour After	0.046	0.020	0.571	-1.285
	Test results ¥	($F_{(2,13)}$ = 6.746, P = 0.010)			
Hypoxic (3750 m)	Before	0.040	0.018	2.474	6.730
	Immediately After	0.035	0.013	1.202	0.433
	1 Hour After	0.041	0.019	0.187	-1.711
	Test results ¥	($F_{(2,13)}$ = 1.199, P = 0.332)			
Test results §	Before	($F_{(3,21)}$ =.572, P = 0.640)			
	Immediately After	($F_{(3,20)}$ =4.249, P = 0.018)			
	1 Hour After	($F_{(3,20)}$ =5.667, P = 0.006)			

¥Based on mixed-models analysis for comparing 3 measurements within each session (after logarithmic transformation on saliva testosterone to cortisol ratio)

§Based on mixed-models analysis for comparing 4 separate sessions for each measurement (after logarithmic transformation on saliva total testosterone to cortisol ratio)

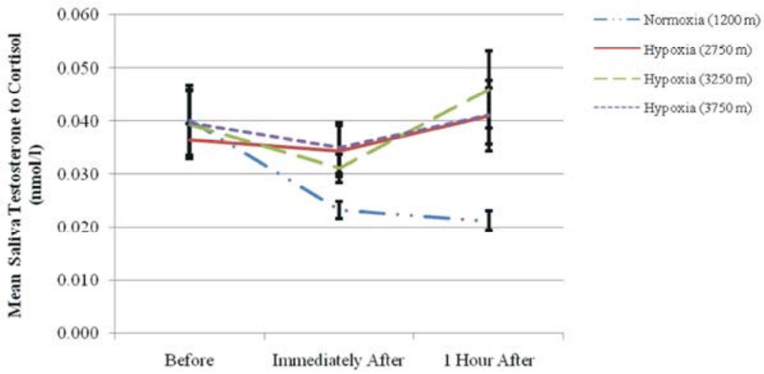

Fig. 1: Mean saliva cortisol (ng/ml) within 4 sessions

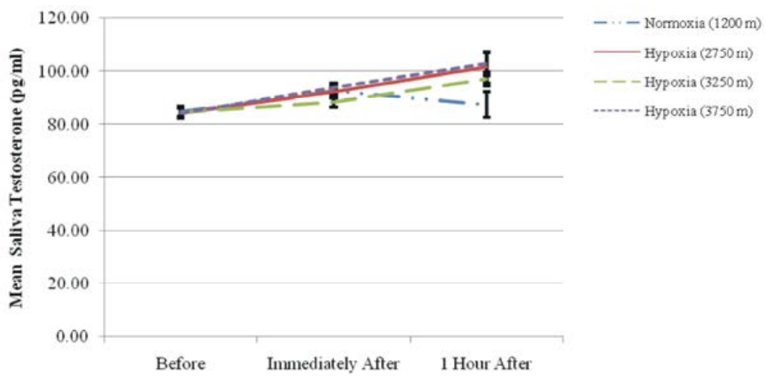

Fig. 2: Mean saliva testosterone (pg/ml) within 4 sessions

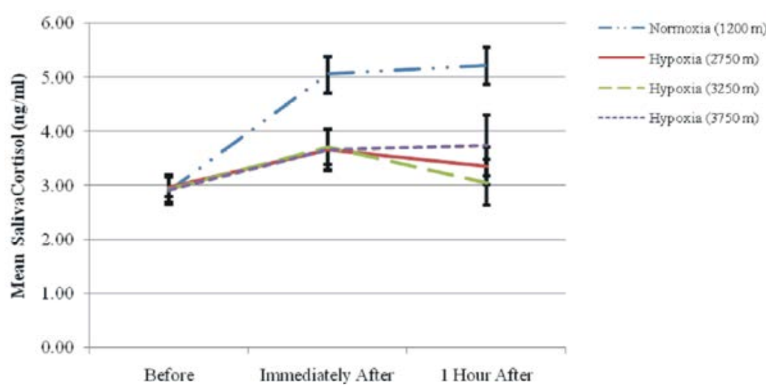

Fig. 3: Mean saliva testosterone to cortisol ratio (nm/l) within 4 sessions

Values higher than 2 and 3 for skewness and kurtosis measures, respectively and large values of SD for saliva testosterone to cortisol ratio along with the results of the K-S tests ($P<0.05$) rejected the normality of the data (Tables 3). Therefore, logarithmic transformations were followed for the variable.

For saliva testosterone to cortisol ratio, there was a significant difference within measures totally evaluated in the sessions ($F_{(8,69)} = 4.991$, $P < 0.001$).

However, in the separate evaluation of the measurements, significant differences were observed within the measurements in normoxic (1200 m) and hypoxic (3250 m) sessions (Both $P<0.05$) (Table 3). In addition, the results of the post hoc test for normoxic (1200 m) showed significant differences between before intervention, immediately after intervention and one hour after intervention situations ($P=0.006$ and $P=0.001$, respectively). However, for the hypoxic (3250 m) session, a significant difference was observed between immediately after intervention and one hour after intervention situations (P=0.016).

Also, there were significant differences among the sessions for both immediately after intervention and one hour after intervention measurements (Both P<0.05) (Table 3). In addition, the results of the post hoc test for immediately after intervention measurements demonstrated significant differences between normoxic (1200 m) and hypoxic (2750 m) sessions (P=0.022). For one hour after intervention measurements, significant differences were observed between normoxic (1200 m) and hypoxic (2750 m) and hypoxic (3250 m) and hypoxic (3750 m) sessions (P=0.011, P= 0.006 and P=0.045, respectively).

DISCUSSION

According to the findings of the present study, the concentration of saliva cortisol in the normoxic condition was significantly higher than that in the hypoxic condition. Immediately after exercises, saliva cortisol was significantly less in all three altitudes in the hypoxic conditions than that in the normoxic conditions.

In contrast to the findings of the present study, Blegen *et al.* [3], Benso *et al.* [17] and Bouissou *et al.* [18] did not observe significant differences in the concentration of serum cortisol between hypoxic and normoxic conditions. Moreover, Moncloa *et al.* [14], Marcello *et al.* [15] and Ermolao *et al.* [19] reported the significant increase of cortisol in hypoxic conditions compared with the normoxic conditions. The exact reason for this evident difference is not known. Most of previous studies have observed increase of cortisol in hypoxic conditions; however, in the present study, the opposite results were observed; although previous studies have investigated serum levels of cortisol, there has been a positive relationship between serum and saliva levels of cortisol [23, 24]. Blegen *et al.* [3] conducted their research in low-pressure chambers with oxygen concentration of 14.65%. The present study examined the altitude of 3250 m (14% oxygen).

In Benso *et al.* [17] study which was done in Himalayas, the participants went to the altitude of 5200 m; five of them reached the altitude of 8852 m, three to 8600 m and one to 7500 m. In their study also, cortisol did not make any significant changes although the participants went to high altitudes. Bouissou *et al.* [18] studied the altitude of 3000 m using low-pressure chambers and observed findings similar to those in the present study. In contrast, Moncloa *et al.* [14] who reported the altitude of 4300 m reported significant increase in the concentration of cortisol in the altitude.

Normally, if exercise intensity exceeds a threshold level, cortisol increases, too. It is believed that cortisol decreases in lower intensities and it is said that the required intensity for cortisol stimulation is 60% of maximum oxygen consumption [25].

If exercise intensity is more than the threshold level, muscle glycogen decreases; as a result, the body is forced to call the free fatty acids for supplying its energy requirements; therefore, cortisol concentration increases in order to analyze triacylglycerol and produce fatty acids. Thus, exercise duration is also a determining factor for cortisol secretion [26].

According to the findings of the present study, it seems that aerobic exercises for 30 min with the intensity of 70% of maximal heart rate can be a stimulator for the cortisol increase; it was interestingly in contrast with the previous findings and this increase was not significant in the altitude of 3750 m [the highest altitude in the present research] since it has been stated that exercises less than the threshold in the normoxic conditions can increase the level of blood cortisol [27].

These findings are in contrast with the statement that hypoxia increases sympathetic exercises [28]. It has been demonstrated that hypoxia leads to the increased adrenal sensitivity to ACTH [18]. The created changes in the serum cortisol from immediately after exercising to one hour after exercising remained unchanged for all four conditions.

Marcello *et al.* [15] reported testosterone decrease in the marathon race in the altitudes of 3400, 3860 and 5100 m. Benso *et al.* [17] observed testosterone decrease in Himalayan Mountains. Hypoxic conditions probably impose doubled pressure, which decreases testosterone in hypoxic conditions relative to normoxic conditions; however, the findings of the present study did not support this hypothesis. It was shown that, even after 8 weeks of mountain climbing exercises in mountains, the testosterone levels of participants were remained at a low level relative to sea level and this decrease was maintained until reaching the sea level [29].

In contrast, Vasankari *et al.* [16] demonstrated that levels of serum testosterone were higher before ski racing in the altitude of 1650 m relative to the sea level. It is possible that this contradiction is somehow related to hypoxic degree.

Previous studies have shown the relationship between serum testosterone and hypoxic degree [30]. Furthermore, intensity and duration of exercises should be considered while evaluating different findings. According to the findings of the present study, although exercising in normoxic conditions leads to saliva testosterone increase immediately after exercising and its decrease up to one hour after exercising, this change was not statistically significant. Both decrease [31, 32] and increase [33] of testosterone have been reported following persistent exercises.

In most of the cases, differences of the results can be due to the difference in the volume of exercises or training situation of the participants [34]. Most probably, intensity of exercises is the most influential variable. Wilkerson *et al.* [35] did not report any changes in the concentration of testosterone after 20 min of running with different low exercise intensities. In the present study, although exercising in normoxic conditions led to the increase of saliva testosterone immediately after exercising and its decrease up to one hour after it, which were non-significant, exercising in the altitudes of 2750, 3250 and 3750 m increased saliva testosterone immediately after exercising and this increase continued up to one hour after exercising, which were significant.

These findings can be controversial as well since testosterone decrease in altitudes has been usually reported [15, 17] although, in some cases, its increase has been also observed. Increased nitric oxide generated in the body followed by being placed at high altitudes can lead to damage to the synthesis of steroid hormones [36]. It has been shown that after entering NO into mice, their testosterone levels decreased [37]. Decrease in sex hormones has been determined in high altitudes [38].

It should be reminded that previous studies have investigated levels of serum testosterone in hypoxic conditions and, probably, the present study was the first one which measured saliva levels of testosterone followed by exercising in hypoxic conditions. The balance between anabolic and catabolic processes is stated via cortisol to testosterone ratio and this ratio has been recommended as an important indicator of exercise and pressure [8].

The findings of the present research revealed a significant difference between saliva testosterone to cortisol ratio of the four sessions of exercises in four different conditions both immediately after and one hour after exercises. In fact, saliva testosterone to cortisol ratio in normoxic conditions was less than its level in hypoxic conditions immediately after and one hour after exercises. In normoxic conditions, saliva testosterone to cortisol ratio considerably decreased immediately after exercises and showed a small amount of decrease after that up to one hour. In the three altitudes of hypoxic conditions, saliva testosterone to cortisol ratio decreased immediately after exercising; however, it increased up to one hour after exercises and, even in some levels, was more than the before exercise level. These changes were significant in normoxic conditions and the location of difference was between before exercising and immediately after exercising and also between before exercising and one hour after exercising. Moreover, these changes were not significant in two altitudes of 2750 and 3750 m; however, it was significant in the altitude of 3250 m. Nevertheless, significant difference was observed in the altitude of 3250 m between immediately after exercising and one hour after exercising. The response of this ratio was more catabolic in normoxic conditions than in three different hypoxic conditions. However, in contrast with the findings of this study, it has been said that hormonal changes in the altitude include significant decrease in testosterone levels and significant increase in cortisol levels [39].

A large amount of individual variability has been observed in testosterone and cortisol changes [6, 40]. The effect of exercising in hypoxic conditions on the testosterone to cortisol ratio has been considered to less extent; the cases which have investigated this issue only have measured the ratio between serum levels of hormones. Marcello *et al.* [15] reported findings which are in contrast with those of the present study.

Increase in testosterone to cortisol ratio in the recovery period after exercising indicates enough resting after that exercise and this increase was observed up to one hour after exercising in hypoxic conditions in the present study [41]. Future studies should consider frequent measurements. Finally, considering the contradiction of previous findings which are mainly in contrast to the findings of the present study, more investigations are required for making a precise conclusion and presenting a transparent result.

It can be concluded that the response of saliva hormones to exercising in hypoxic conditions was more anabolic that the response to the same exercise in normoxic conditions. Anyway, considering that most of previous studies have stated more physical pressure after exercising in hypoxic conditions compared with normoxic conditions, the best results from this study is that more investigations should be done in this regard in order to solve confusions resulted from these findings.

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