

Changes of Dehydroepiandrosterone (DHEA) and Cortisol in response to competition in female volleyball players

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Abstract

Aims: Hormones' response to competition and their relation to sport competitions have been extensively investigated in male and less widely in female athletes. Stress is an irresistible part of every sport competition which is mostly caused by competition. In the present study, female volleyball players were examined for changes in dehydroepiandrosterone (DHEA) and salivary cortisol in response to competition.

Methods: This cohort study was performed on 10 members of a female volleyball team attending a regional tournament held in 3rd region of Islamic Azad University in year 2010, who were selected by purposive available sampling method. Saliva samples were collected 5 and 30 minutes before the competition, between the second and third set, and immediately and 30 minutes after two different volleyball competitions. Using ELISA method, the concentrations of DHEA and cortisol were measured in a duplicate manner. Data was analyzed by one-way variance analysis for repeated measurements using SPSS 16 software.

Results: No significant difference was detected in concentrations of DHEA and salivary cortisol ($p>0.05$). Salivary cortisol concentration showed a slight raise in players only in the middle of the volleyball competition ($p=0.04$), but the increase was not statistically significant on the whole ($p>0.05$).

Conclusion: Participating in amateur volleyball competitions has no influence on salivary DHEA level. Amateur volleyball players experience the highest cortisol changes, during a volleyball competition which leads to a loss. It can probably be concluded that salivary cortisol concentration will increase more drastically in losers compared to winners in amateur players during a volleyball competition.

Keywords: Dehydroepiandrosterone, Cortisol, Saliva, Volleyball Competition

Introduction

Importance of winning in the sport competition increases the number and severity of stressful encounters in the competition that athletes have to cope with these events during the competition. In this stress-creating situation coping with physical or mental stress is often accompanied with secreting the corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) in anterior pituitary and adrenal Glucocorticoid (like cortisol) as a haemostatic response of organism [1, 2, 3]. Since attending competition is associated with disruption in homeostasis and stress is irresistible part of competitions, in this regard hormones are considered as one of the major factors in fixing influenced homeostasis by competition [4]. Studies have shown that androgens, cortisol and dehydroepiandrosterone (DHEA) are more sensitive to competition than other hormones and some changes in their concentrations is observed through activation of hypothalamus-pituitary

-adrenal axis (HPA) [5, 6, 7, 8].

Today, diagnostic methods using salivary compounds are considered as important techniques in physiology, psychology, immunology, and sports medicine. Researches show that sport activities influence the rate of saliva content secretion and salivary compounds can be affected by the automated nervous system and HPA axis [9]. In this regard it has been reported that salivary cortisol levels are more appropriate indicator for showing incurred stress on the organism than the serum levels. Also, salivary cortisol has been introduced as the free moving cortisol indicator [10, 11]. DHEA is also a special adrenal hormone, that through being changed to sexual steroids (testosterone and estrogen) in several tissues, remains the anabolic effects and it can show the response of salivary androgen to exercise in women better than testosterone androgen [12].

Many researchers have reported that participation in sport competition increases the levels of salivary cortisol that this increase is associated with adrenal

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cortical section response to exercise and increase in ACTH [13], blood glucose during exercise [14], psychological arousal associated with competition [12] and the type of stress involved in competition [11]. Besides cognitive mental stress of the competition, winning the competition is also effective in the hormone concentration. Also half hormone of the winners and losers is different with each other [3, 10]. Hormonal responses in winners and losers after the competition have contradictory results. Results of Aizawa et al. study in football competition [15] and Salvador et al. study in judo competition [16] showed that salivary cortisol levels are higher in winners than losers. Filaire et al. reported higher increase in Testosterone in losers compared with winners [7]. While Salvador et al. and Edwards et al. stated that levels of salivary cortisol and salivary DHEA at the end of competition are similar in winning or losing results [5, 17]. On the other hand, Moreira et al. reported the continuous increase of cortisol in professional soccer players [18]. Prapavessis et al. also expressed that the result of athletes' characteristics and mood state is better in successful athletes comparing with unsuccessful athletes [19]. It seems that any type of physical or mental stress causes the increase in cortisol secretion and ultimately changes the behavior [20]. With Searching in the prospect of these studies it seems that the cortisol and DHEA hormone responses to competition is still ambiguous because many factors are effective in hormone responses in winners and losers such as risk factors, the sensitivity of the competition, winning and losing feeling prior to the competition, player's perception about the difficulty of the tournament, acquiring social status, belief in luck and the referee role in creating competition results, level of player's effort throughout the competition, gender and elite in player [21] that requires more studies in this issue. Based on scientific evidence, the balance between catabolic hormones (such as cortisol) and anabolic hormones (such as testosterone and DHEA) play an important application in implementing and recycling periods. When the organism is in the acute practice situation, the balance between these hormones is disrupted and this is as the same as what happening in sectional stress. If return to the sufficient initial state is not reached such as in acute practice, while the organism continues to increase the release of cortisol in ascending trend, it also reduces the amount of DHEA [7, 12]. In sport event in which the interval between competitions is short and there is no enough time for making players ready for next competition, this issue can be effective on players' performance.

Understanding these physiological issues, have practical implications for players, coaches and athletic programs planners to become ready for next competition through stress reducing actions.

On the other hand investigation of conducted study in this domain indicate that the majority of studies on subjects has been done on elite competitor subjects and the effect of competition on hormonal responses in amateur female athletes in team fields such as volleyball has not been clearly specified.

This study has been designed in order to answer the question of what changes are happening in DHEA and salivary cortisol hormones during a volleyball competition in amateur female volleyball players and whether there is difference in hormone patterns following winning or losing at the end of the competition. The purpose of this study is investigation of the changes in DHEA and salivary cortisol concentration during the competition in female volleyball players.

Methods

This cohort study was conducted in 2010. Among 140 female volleyball competitors in the 3rd regional Islamic Azad University completions, 10 members of a team were selected through purposive available sampling. The conditions in selecting subjects included items such as full physical health, no history of mental diseases and hormonal disorders, no use of hormonal drugs, and having a normal menstrual 28 cycle days.

First, the demographic specifications of subjects including age, height, weight, BMI, subcutaneous fat percentage and history of membership in team were determined. Measuring of weight was done by digital scale weight measures (BEURER, Model ps06m42; Germany), and subcutaneous fat thickness was measured using a caliper (Lafayette, model 1127; making U.S.) through two-point method in two areas of triceps and leg. All measures were conducted in three turns from right part of body and within 20 seconds interval between turns and the average of three times was recorded. Subcutaneous fat was calculated 0.735 using formula +1 (total fat, two points).

Subjects participated in university volleyball competitions. Samples of salivary were collected in all competitions, but two semi-final competitions that result of one competition was winning and the result of other was losing, were chosen for measuring the variables. The reason for choosing the two semi-final competitions was the same situation of two

competitions, the same difficulty level and the same sensitivity of two competitions. Both competitions began at 4 pm. Subjects ate the similar food in camping the night before the competition. Also the condition of their sleep and rest was also identical. Also the food in competition day was similar. 3ml of non-stimulated saliva was collected through active discharge method to 5 and 30minutes before the competition between second and third sets and 5 and 30 minutes after racing from all ten subjects for investigation the effect of competition on the Cortisol and DHEA concentration. Subjects were asked to avoid eating food or chewing gum at least 2 hours before sample collection and warm themselves 20 minutes before the competition. At the time of sampling, subjects at first washed their mouth and after drinking 200ml water, were placed in sitting position for few minutes and then threw their saliva in to collecting tubes. Collected samples immediately placed in ice chamber beside the competition field and immediately were transferred to laboratory and froze in -20° C (sample transfer lasted for 10 minutes)

in the day of laboratory analysis, all samples were first placed in room temperature to be out of the freeze mode. Then samples were centrifuged with round 3000rpm and the mucosa in them was sediment. DHEA and cortisol concentration of liquid in the upper part of tube was determined by commercial kit (DEMEDITEC; Germany) according to manufacturer instructions and using the ELISA (Model Stat Fax 2100, Company Awareness; making the United States). Expected normal values in DHEA in women aged 21 to 30 was in the range of 83 to 469pg/ml, but its dynamic range was 0 to 144pg/ml. The mean level was 206pg/ml, analysis sensitivity of kit was 2.186pg/ml, its functional sensitivity was 5.6pg/ml and the coefficient of variation was 12.5%. Cortisol normal range was 50 to 230ng/ml at 8 to 10 am and 30 to 150ng/ml at 4 pm, but its dynamic range was 0 to 800ng/ml. Its Sensitivity was 0.012ng/ml and coefficient of variation was 6.5%. To prevent the effects of environmental factors, all samples were tested in equal environmental situation (in terms of time, place and examiner)

Descriptive statistical methods were used for calculation of mean, variance and percentage of changes. For investigating the normality of data Kolmogorov-Smirnov test and for evaluating the changes in hormone concentration in each competition one-way variance analysis in repeated measurements were used. If significant difference was observed in determining the location of difference, a sort of paired T-test (dependent T) was used according to Boone-

Ferroni amendment. In addition, to examine the relationship between salivary cortisol and DHEA Pearson correlation was used. All statistical analyses were done by SPSS 16 software. Significant level was considered as $p < 0.05$ in all cases.

Results

The mean of subjects' demographic data including age, height, weight, BMI, subcutaneous fat percentage and the record of team membership have been shown in table 1.

Table 1- Subjects demographic specifications mean

Subjects specifications	Mean \pm Standard deviation
Age (years)	21.44 \pm 1.13
Height (cm)	163.22 \pm 3.53
Weight (kg)	58.73 \pm 5.61
BMI (kg /m ²)	22.67 \pm 3.76
Percentage of subcutaneous fat	20.15 \pm 2.42
History of team membership (years)	6 \pm 3.73

Table 2 shows the levels of salivary cortisol and DHEA in five stages. The amount of salivary cortisol in competitions lead to winning had constant process from half an hour before the competition to half an hour after the competition. Only half an hour after racing the cortisol values were slightly higher than resting values that was not statistically significant.

Salivary cortisol levels in competition leads to loss had increasing trend from half an hour before the competition to the middle of the game.

Cortisol concentrations in middle of competition (between the second and third sets) increased significantly in comparison with 5 and half an hour before the competition. Values increased significantly half an hour and five minutes before the competition while its concentration was reduced 5 and 30 minutes after the game.

The amount of Salivary DHEA levels increases from half an hour before the competition led to winning to the middle of it and then it has slight decrease followed by increase, therefore the amount of DHEA half an hour after the competition was higher than the amount of it before the competition. Although there was difference in the amount of DHEA between half an hour before the competition and middle of the competition, this difference was not statistically significant.

Salivary DHEA levels in competitions which led to loss increased from half an hour before the competition to five minutes after the competition and

then it slightly decreased that its amount was higher after the competition than its amount before the competition, but this difference was not statistically significant.

Table 2- Levels of salivary cortisol and DHEA in five measuring stages

Sampling stages→ Index↓		Half an hour before competition	5 minutes before competition	Between second and third sets	5 minutes after competition	Half an hour after competition
Cortisol (ng/ml)	Win	3.1±2.28	3.04±2.11	2.86±1.91	2.88±2.03	3.89±2.00
	Lose	3.23±2.20	5.00±2.50	7.52±2.61	4.16±1.92	3.62±2.43
DHEA (pg/ml)	Win	507.40±335.96	622.54±401.52	753.99±467.94	637.57±333.75	720.18±533.44
	Lose	597.04±340.04	661.35±372.64	823.22±423.06	856.70±363.10	671.27±411.20

Discussion

The results of this study showed that participation in volleyball competition had no significant effect on salivary cortisol and DHEA concentration. The amount of Salivary DHEA levels in the competition led to win had increasing trend half an hour before the competition to middle of the competition and as games followed, it decreased slightly and then it increased so the amount of it was higher after the competition than the amount of it before the contest. The amount of Salivary DHEA levels in the competition led to loss had increasing trend half an hour before the competition to five minutes after the competition and then it had a slight reduction that these differences were not statistically significant. Hasegawa et al. [3] in chess players, Kivilghan et al. in rowing players [22], Edwards et al. and Haneshi et al. in football players [2, 5] and Farzanegi et al. [23] reported the increase of steroid hormones during the competition, while Wang et al. observed the reduction in the DHEA concentrations after the golf competition in women who had practiced [24]. Perhaps the levels of athletes' physical fitness, the type of activity or the time of being in competition are the reasons for the contrast between their findings and the result of this study. But the filler et al. [25] in their study on female basketball and handball players and Moreira et al. [18] in their study on female soccer players have not reported the significant difference in salivary cortisol and DHEA levels following participation in the competition. Background available literature associates the reasons of changes in DHEA and Salivary cortisol level during the competition and practice to their secretive mechanisms of them [2, 3, 15, 16]. Maybe this issue is due to extensive variety of competition with respect to their intensity and length. Also, the subsequent lack of ACTH secretion, which regulates cortisol secretion, the amount of cortisol is reduced [3, 23], but the secretion of adrenal androgens that slightly is controlled by ACTH is not affected so much

[15, 17].

Another finding of this study, showed the increase in amount of cortisol in both winnings and losing competition and that this difference was not totally significantly significant, but within the competition (between the second and third sets) there was significant increase compared with before the competition. May be the lack of significant increase can be described through situational factors and personal aspects in some athletes. In Competition, stress is existed inherently [26]. Theoretically, stress as an additional stimulus may be created through interaction with other players, the situation and the importance of the competition [27]. Filler et al. stated that the real competition causes more hormonal responses in female handball and volleyball players compared with training exercises (in vitro) [27]. This finding is consistent with the results of present study and Haneishi et al. [2] study in which they observed the similar effect of competition in United States female soccer players compared with a regular practice session. Ellomi et al. reported the increase in cortisol concentrations after the rugby competition, but by changing the situation of the competition, they did not observe the increase in cortisol concentrations after similar severe activity in vitro situation. It seems that obtained results in vitro situation (Even if the intensity of the activity is too exhaustive) cannot be compared with competition situation [28].

Regarding the studies which showed the increase in amount of salivary cortisol following high or non-competitive games, it may be possible that psychological components and not physiological components have caused this increase. For example, physiological need to play golf is in the range of 35 to 41% of the maximum oxygen consumption. Therefore any increase in cortisol levels during playing golf is due to psychological competitive stress [29, 30]. Also Carli et al. observed the increase in cortisol concentrations in 26 semi-professional United States football players during a soccer competition compared

with a team belonging to a lower league class team (amateur) [31]. In the present study also volleyball players were amateur and had lower physical fitness. Therefore, the cortisol concentration totally did not have significant increase.

Another possible disturbing factor in significant increase may be the high within and between individual differences in response to salivary cortisol. This large difference has been reported by Viru et al. In this study, at least four different reaction patterns of plasma hormone responses were observed in well-practiced good athletes during the exercise [32]. Rietjens et al. believe that changes in cortisol levels in the well-practiced subjects after a period of severe exercise are widely variable [33]. This finding is in accordance with the findings of the study of Moreira et al. on the professional basketball players. These researchers reported the large variability in mucosal immune parameters in basketball players during 17 days practice [34]. Research has also shown a few weeks of heavy training is accompanied with the high response of stress hormones (ACTH, cortisol and catecholamines), in the way that it forces the special hormone receptors in target tissue to react less to the effects of these hormones. It seems that the competition level has not been so high that affects endocrine parameters significantly in the players. Therefore, it is suggested that in future studies the effect of a sensitive competition in professional competitions on the stress related hormones be examined.

Conclusion

Participation in amateur volleyball competition has no effect on DHEA salivary levels. Amateur volleyball players at the same time, experience the most changes in saliva cortisol concentration during a competition resulting in loss. It can be probably said that the amateur athletes' saliva cortisol concentrations during the volleyball competition increases more in losers compared to winners.

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