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Salıvary cortisol levels in elite male handball players during a match*

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ABSTRACT

Cortisol is a corticosteroid hormone produced by the adrenal cortex and it is associated with the stress response of the body. It is an important marker for determining the level of stress during or after exercise. The purpose of this study was to examine a sports team for the level of physiological and psychological stress generated by a handball competition via non-invasive saliva analysis. Fourteen athletes aged 20.7 ± 2.5 years who were members of the Ondokuz Mayıs University male handball team participated in the study. A total of three saliva samples were taken, one before, one during half-time and one immediately after an important match for the team in terms of the group standpoint. and were analyzed by ELISA. Data were analysed by repeated measures test and Mauchly's test of sphericity; also $p<0.05$ denoted statistical significance. As a result of the analysis, salivary cortisol levels were found to be significant in the three different samples taken from the athletes ($p = 0.018$) At the onset of the ıy defferent competition, cortisol levels were also seen to increase in parallel with the rise in the stress levels of the athletes. The highest cortisol levels of the athletes were found in the samples taken between halves during the match. It was found that submaximal exercise used in the team sport of handball had a significant effect on salivary cortisol levels. In samples taken during an event with a high level of importance for the team, significantly variable levels of cortisol as a stress hormone were expressed in the athletes. Therefore, saliva cortisol measurement appears to be an important parameter that can be used to develop stress-management and other necessary strategies in sport branches such as handball where the mental and physical stress is intense.

1. Introduction

Exercise brings about endocrinological changes to balance homeostasis during challenging coordinative movements, thus leading to physiological and psychological stress. It triggers a coordinated series of physiological responses which influence systems like the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system in particular, two of the basic stress activation systems (Soria et al., 2015; Furtado et al., 2016).

Cortisol is considered a stress hormone. It is released when the body is exposed to any threat or danger which stimulates the sympathetic nervous system by activating energy sources, thus enabling the body to cope with possible dangerous situations (Triviño-Paredes et al., 2016). Cortisol is a corticosteroid hormone produced by the adrenal cortex and it is associated with the stress response of the body. It regulates the level of energy use by activating amino acids in the skeletal muscles, thus promoting glycogenic activity (Öztürk, 2008; Wingfield et al., 2015). It is an important marker for determining the level of stress during or after exercise (Yazdanparast et al., 2009). Sufficiently intense exercise has an effect that can trigger cortisol release. Circadian and diurnal rhythm, nutrition, sleep, previous exercise and body composition are other important factors that can affect cortisol release (Kanaley et al. 2001).

When there is a high amount of cortisol in the body, the positive effect of the defense mechanism is reversed and the organism is driven to utilize its own defensive measures. Therefore, it is important to keep cortisol release constantly in balance. Athletes perform in a very stressful environment, especially when they need to make quick decisions during a competition. The organism can develop responses against this situation in different ways. In this sense, regular training and good preparation are positive ways that can help to avoid this stress (Dinçer, 2011).

Many studies have shown that in different species, exercise increases the level of cortisol (Kindermann et al., 1982; Farrell, 1989; Soria et al., 2015; Wingfield et al., 2015; Kayacan et al., 2016; Klentrou et al., 2016; Koutsandréou et al., 2016). However, data in the literature suggest that cortisol release varies with physiological activation parameters such as exercise intensity and duration and physical conditions (Filaire et al., 1996; Yazdanparast, 2009). One study reported that in mild-intensity exercises, no increase in cortisol release was observed, while in high-intensity exercise, there was a significant increase in cortisol levels as a response against stress (Günay, 2013).

There are several different methods for cortisol analysis. Saliva cortisol analysis has been widely used recently (Takagi et al., 2013; Cobb et al., 2016; Soroko et al., 2016), because it is non-invasive, does not require venipuncture and makes the sample collection phase easier and faster for subjects of different age groups.

Handball is a popular global sport played by more than 30 million athletes throughout the world. As an intermittently intense physical activity lasting over an extended time period, it requires both aerobic and anaerobic strength and durability. Despite this fact, not many studies have been done to date dealing with the influence of handball on the hormonal composition (Nedić et al., 2016).

The purpose of this study was to examine a sports team for the level of physiological and psychological stress generated by a handball competition via non-invasive saliva analysis.

2. Methods

Fourteen athletes aged 20.7 ± 2.5 years who were members of the Ondokuz Mayıs University male handball team participated in the study. A total of three saliva samples were taken, one before, one during half-time and one immediately after an important match for the team in terms of the group standpoint. Under the supervision of the team coach, saliva samples were taken by means of an Ependorf tube using the passive drool method. For the samples, the saliva spontaneously filling the mouth was collected in the saliva tube while the chin was tilted forward toward the chest. The collected saliva samples were then stored at -20 °C in the Inonu University Medical Faculty Physiology Laboratory and were later analyzed using the ELISA method.

ELISA cortisol procedure

The steps of the cortisol ELISA test are briefly described as follows. A 96-well ELISA plate was coated with Cortisol: BSA at the determined concentration. A 1% bovine serum albumin (BSA) solution was used to block the uncoated areas of the wells. Standard sample quantities of the cortisol antibody in the established concentration were then pipetted into the wells. The plate was incubated for 45 min at 37 °C. Following incubation, the plate was washed four times to remove unbound cortisol and antibodies and then dried by lightly wiping with a paper towel. Anti-Rabbit IgG antibodies labeled with the biotin concentration capable of binding to the cortisol antibody were pipetted into all the wells and the plate was incubated for 30 min at 37 °C. After incubation, the washing was repeated in the same manner. Streptavidin peroxidase at the determined concentration to bind biotinylated antibody was then pipetted into all the wells. The plate was incubated at + 4 °C for 15 min and following incubation, washing was repeated again in the same manner. Substrate solutions containing tetramethylbenzidine were then pipetted into all the wells and the plate was incubated at room temperature for 15 min to complete the color formation. Without delay, a stop solution containing H2SO4 was pipetted into all the wells and the resultant yellow color was read on a plate-reader spectrophotometer (Biotek, Synergy HT, USA) at 450 nm. Standard curves were generated with the Gen 5 computer program and the concentrations were determined according to these standard curves. The sensitivity of the test was 1-1000 ng/ml. The intra-assay coefficient of variation (CV) was 10.4% for the low-concentration samples and 8.1% for the high-concentration samples; the inter-assay CV was 13.3% for the low-concentration samples and 12.2% for the high-concentration samples.

Determination of match difficulty

In order to determine the difficulty level of the match, a 10-point Likert-type scale was used for the sportsmen. The rating ranged from 1 (very easy) to 10 (very difficult). The scale was also applied to other group matches and the difficulty levels of the matches were calculated. An easy match was rated as 1-4 points, a moderate one as 4.1-7 points and a difficult competition as 7.1-10 points. According to this classification, the difficulty level of the match for which the saliva samples were taken was found to be high (Nazem et al., 2011).

Statistical analysis

The IBM SPSS v.21 software package was used for statistical analysis. Mean and standard deviation values were given as descriptive statistics for data with normal distribution. In order to investigate the differences between the averages of pre-match, half-time and post-match replicated measurements, the ANOVA test was used to analyze the data for the replicated measurements from the parametric tests. The validity of the sphericity assumption was tested using Mauchly's test of sphericity. The sphericity assumption results were used in the comparison of the replicated measurements when the sphericity assumption of $p > 0.05$ was met, while the Greenhouse-Geisser result was used when the assumption was violated. Fisher's least significant difference (LSD) test was used to perform binary comparisons to determine which time interval caused the difference between the measurements that were found. Statistically, the alpha value was accepted as $p < 0.05$.

3. Results

First, to determine whether the assumption of sphericity had been achieved, the Mauchly sphericity test was applied to the normally distributed data. According to the findings of this test, the sphericity assumption values were examined to obtain the assumption of sphericity ($p = 0.478$) and the alpha value was found as $p = 0.042$. Moreover, the magnitude of the effect was determined to be 0.216. As a result of the analysis, salivary cortisol levels were found to be significant in the three different samples taken from the athletes ($p = 0.018$) (Table 2). At the onset of the competition, cortisol levels were also seen to increase in parallel with the rise in the stress levels of the athletes. Mean cortisol levels were 41.4 ng/ml before the match, 65.2 ng/ml during the match and 54.8 ng/ml after the match (Table 1).

Table 2. Comparison of samples according to the phases of the match

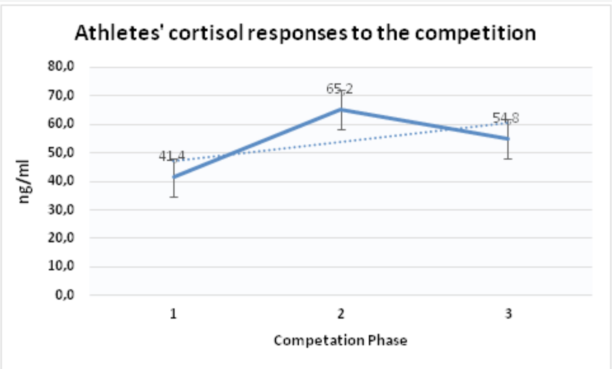
Phase*	Phase*	Mean Difference	SD	P
1	2	-23,821	7,245	,018
	3	-13,429	9,636	,560
2	1	23,821	7,245	,018
	3	10,393	9,660	,905
3	1	13,429	9,636	,560
	2	-10,393	9,660	,905

*** 1. Pre-game, 2. Half-time, 3. After game**

Table 1 shows the arithmetic means and standard deviations of the salivary cortisol levels of the 14 athletes participating in the study according to the phases of the match.

Table 1. Descriptive statistical data for the athletes according to the phases of the match

	n	$\overline{X}$	Sd
Pre-game	14	41,41	31,904
Half-time	14	65,23	37,909
After game	14	54,84	26,392



1. Pre-game, 2. Half-time, 3. After game

Figure 1. Athletes' cortisol responses to the competition

When the cortisol competition responses of the athletes are examined, it can be seen that the highest levels were reached in samples taken during the match between the halves (Fig. 1). Directly after the match, the level of cortisol in the athletes decreased.

4. Discussion

In the present research, the aim was to investigate the stress level of athletes via non-invasive methods during a handball game, where the aerobic energy structure is used more intensely, although it is recognized that this type of activity affects both aerobic and anaerobic metabolism (Ascensao et al., 2008). The stress response has complex molecular, cellular, physiological and behavioral effects on the organism. Physical stress can occur as a result of heat, cold, radiation, trauma and infective or toxic agents. Emotional causes can also be a factor. The stress response system is tonically active and is in fact necessary for the continuance of any organism. The HPA components CRH, ACTH and cortisol (the classic stress-related neurohormones of the stress system) have been reported in the literature as having a significant effect on stress response [Türsen, 2011]. However, this effect may vary according to the energy metabolism used during physical activity. For example, Filaire et al. (1996) conducted a study to investigate the effect of physical exercise and training on salivary cortisol concentration in women. They collected a total of six saliva samples throughout the day from a total of 31 adult women divided into three groups which included seven sedentary controls, 14 handball players and 10 swimmers (with both latter groups at the national level).

There was no significant difference between the swimming and sedentary groups for any of the samples collected during the day, whereas a significant increase was detected in the handball players after training (18.00-19.30, $p < 0.05$). In another study, cortisol concentrations were found to be significantly high in samples taken from 13 male athletes after inter-university competitions held in the Southern University Football Division, when aerobic energy was heavily used (Edwards et al., 2006). These results demonstrate that the type of sport is effective on the saliva cortisol concentration and stress level and support the results found in the present study.

It has been reported in the literature that stress due to high-intensity aerobic exercise causes significant increases in ACTH and cortisol levels (Raynaud et al., 1997; Skoluda et al., 2012; Moghadasi and Najafi, 2017). For example, Hill et al. (2008) found that for 30 min of intensive forms of exercise at 40%, 60%, and 80% maximal oxygen consumption (VO2max), there was a parallel between the increase in cortisol levels and the increase in oxygen consumption, i.e., the stress level increased as the exercise became more strenuous (Hill et al., 2008). Tsai et al. (2014) determined that the cortisol level of athletes increased during acute endurance exercises (Tsai et al., 2008). Minetto et al. (2008) put 15 footballers through intensive exercise programs and found a statistically significant increase in cortisol after the exercise program compared with before, in terms of the awakening response and daytime cortisol levels (Minetto et al., 2008). The release of cortisol is the result of activating the energy stores that help by intervening in emergency situations. Changes in the parameters mentioned above can be interpreted as signs of the activation of these stress pathways (Jayasinghe et al., 2014). It has been stated that stress symptoms emerge physiologically (physically), psychologically or behaviorally, and that the magnitude of these stress types influences the individual's physiological state (Tilbrook and Clarke, 2006). Before a match, athletes become anxious, fearful and excited, and they express these feelings physiologically, mentally and socially. This may cause the athlete to become nervous before a match. This state of anxiety can also manifest itself in a crucial situation during the competition. A critical game strategy applied by handball coaches is to attempt to break the player exchange or concentration of the opposing team, but such applied strategies and moves can also be a stress factor in their own players as well. This is because sports branches such as handball, in which the physiological burden constantly increases during the competition, are different from the steady-state branches of exercise. The sport of handball is a significant source of stress on the aerobic metabolism of an athlete (Buchheit et al., 2009). In addition, handball includes many anaerobic actions that require body contact, sepeated acceleration, sprinting, throwing, blocking, pushing, and quick about-turns. Among the most important factors that increase stress in sportsmen are the risk of muscle damage-based injury, performance decline and fatigue resulting from the necessity of participating twice a week during a handball season, where the competition is intense (Baker et al., 2004; Margonis et al., 2007).

In this context, given the physiological and physical requirements of the sport of handball, the hypothesis that athletes may be exposed to a stressful situation is logical. Filaire et al. (1999) collected samples from 20 top women athletes 5 min before and after a handball and a volleyball match and measured saliva cortisol, androstenedion and dehydropyandrosterone (DHEA) via radioimmunological assay. Three different psychometric scales (the Trait Anxiety Inventory, Bortner Scale, and Personality Questionnaire for Sport Participation) were used to measure the psychological levels of the participants. The results showed that although the cortisol levels after the competition had increased significantly from before in both groups, the degree of anxiety the handball players. These findings indicated that the adrenocortical changes during the handball and volleyball competitions had increased the level of anxiety related to losing or winning by affecting their personal characteristics.

As a consequence; It was found that submaximal exercise used in the team sport of handball had a significant effect on salivary cortisol levels. In samples taken during an event with a high level of importance for the team, significantly variable levels of cortisol as a stress hormone were expressed in the athletes. It is thought that cortisol may affect the performance either positively or negatively, depending on the orientation of the psychological stress of the athletes.

According to the findings, the salivary cortisol levels started to increase before the match, rose during the match and started to fall at the end of the match. Therefore, saliva cortisol measurement appears to be an important parameter that can be used to develop stress-management and other necessary strategies in sport branches such as handball where the mental and physical stress is intense.

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