

# CONCENTRATION OF SALIVARY CORTISOL AND TESTOSTERONE IN ELITE WOMEN FOOTBALL PLAYERS: ANALYSIS OF PERFORMANCE IN OFFICIAL MATCHES

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## Abstract:

This study aims to evaluate the relation between salivary concentrations of cortisol (C), testosterone (T), the ratio T:C and the individual performance of top-level female soccer athletes during official matches. Eighteen female athletes from a national soccer team (age 23.06±4.33 years) participated in the study. Four official matches were analysed and the on-field time of each player as well as the index of individual effectiveness were calculated. Players were classified in two clusters according to their individual performance (cluster 1 – poor individual performance; cluster 2 – good individual performance) using K-means and their hormonal variables were compared. The players of cluster 2 generally revealed higher values ( $p < .05$ ) in both the positive actions and individual effectiveness when compared with the players of cluster 1. The players of both clusters presented identical values of C, T and T:C at the four evaluated matches. The athletes of cluster 2 showed a significant increase in C ( $p < .05$ ) and a significant decrease in T and T:C before the games lost (M2 and M5). However, there was no linear relation between the variation of both hormones during the matches and the individual performance of players. Performance in competition revealed significant differences between players, though with no apparent relation to the hormonal kinetics of C and T before and after the matches.

**Key words:** *cortisol, football, soccer, sport performance, testosterone*

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## Introduction

Soccer is considered a collective sport in which the unpredictability and variability of relations and behaviours are a constant. To better understand players and team's on-field behaviour, it is necessary to select, combine and test different variables that allow understanding of different performance factors (Jones, James, & Mellalieu, 2004; Katis, et al., 2013; Kempe, Vogelbein, Memmert, & Nopp, 2014; Lago & Martín, 2007). Recently, the analysis of a group of situational variables such as match location, quality of opposition, match status, defensive formation of the opponent team, revealed their impact on both collective and players' behaviour (Delgado-Bordonau, Domenech-Monforte, Guzmán, & Mendez-Villanueva, 2013; Jones, et

al., 2004; Lago-Ballesteros & Lago-Peñas, 2010; Lago-Peñas, 2012; Taylor, Mellalieu, James, & Shearer, 2008). Knowing that the performance of a player is highly influenced by the context, sports performance assessment in soccer (as well as in other types of team sports) should consider the analysis of the effects of the situational variables on the capacity of both players and teams (Reina Gómez & Hernández-Mendo, 2012). For instance, the study of the effects of the type of competition on psychophysiological stress seems to be an evident example of such an analysis. In fact, from a situational standpoint, certain variables (e.g. away game/home game, winning/losing, and importance of the game) seem to provoke alterations in athletes' levels of stress with implications for

their physiological and psychological state (Casanova, et al., 2016; Salvador & Costa, 2009) and, probably, for their predisposition to compete at the highest level (Michailidis, 2014; Moreira, Arsati, de Oliveira Lima Arsati, da Silva, & de Araújo, 2009). In general, competitive stress seems to determine characteristic hormonal responses (Salvador & Costa, 2009) that prepare the organism for action, where steroid hormones (specifically cortisol and testosterone) are considered valid biomarkers in the monitoring of the stress level imposed by training and/or competition (Gleeson, 2002; Hellhammer, Wüst, & Kudielka, 2009; Salvador, 2005; Salvador & Costa, 2009).

Cortisol response to stress involves increasing the hypothalamus release of corticotrophin (CRH) and an increased sympathetic stimulation of the adrenal medulla. CRH stimulates the secretion of the adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn stimulates the production of cortisol by the adrenal cortex. Testosterone is an anabolic-androgenic steroid dynamically regulated by both exercise and winning in competition (Wood & Stanton, 2012). In men, testosterone is produced in Leyding interstitial cells (testicles), and in women, in the ovaries and additionally a small amount is produced in the adrenal glands. Immunoassays have been widely used for analysing steroid hormones in saliva because they are considered effective, simple and analytically sensitive (Gröschl, 2008).

Several studies have been conducted on this subject. They have demonstrated that the day-time circadian rhythm of cortisol and salivary testosterone seem to reflect significant changes in the anabolic/catabolic balance of athletes (Teo, Newton, & McGuigan, 2011; Wood & Stanton, 2012) as well as that they are considered markers of stress, excessive training and overtraining (Bateup, Booth, Shirtcliff, & Granger, 2002; Crewther, Cook, Cardinale, Weatherby, & Lowe, 2011; Gleeson, 2002; Hellhammer, et al., 2009; Strahler, Ehrlenspiel, Heene, & Brand, 2010). In fact, Hough et al. (2013) have recently demonstrated that the hormonal response of cortisol and testosterone after an intense training period of 11 days is significantly reduced. However, changes in steroid hormone synthesis during successive matches/competitions remains poorly understood. Moreover, the evidence for most of the published findings on hormonal responses to competitive stress have been conducted in men (Kivlighan, et al., 2005); between- and within-sex variations in competitive stress affecting testosterone and cortisol secretion has not sufficiently been comprehended yet.

Having the above-mentioned as a base and as far as our knowledge goes, no investigation exists that assesses the psychophysiological effect of stress on the quality of player's performance. It is worth noting that research has shown that these

hormones regulate neuromuscular performance through various physiological mechanisms of acute action (Crewther, et al., 2011; van der Meij, Buunk, Almela, & Salvador, 2010) with implications for players' coordination and capability to perform. Besides, the on-field coordination among players in space and time as well as their expertise to use the most appropriate technical actions according to variations in game environment is a determinant factor in the success of a soccer player (Ali, 2011), namely players' tactical ability to perform well throughout the game. Thus, this research aimed to evaluate the relation between salivary concentrations of cortisol, testosterone, the ratio T:C and the individual performance of top-level female soccer athletes over a concentrated period of competition.

## Methods

### Subjects

Eighteen female elite soccer players ( $23.06 \pm 4.33$  years,  $58.22 \pm 4.34$  kg,  $169.39 \pm 5.39$  cm) from the Portuguese women's national soccer team participated in this study. In order to calculate an ideal sample size, we used a statistical procedure based on the mistake of estimation  $\alpha$  by 5%, estimative of the effect size of 1.8, the power  $\beta$  of 80% and level of reliability by 95% through the statistic software G\*power® (V. 3.1.9.2, Universität Kiel, Germany) (Faul, Erdfelder, Lang, & Buchner, 2007), which suggests a minimum sample size of 10 subjects. Considering this, and although the initial sample consisted of 18 players called for this national team, only the players who actually participated in the four analyzed competitions were considered in the study, assuming a minimum participation criterion of more than 10 minutes ( $n=13$ , game 1;  $n=14$ , game 2;  $n=12$ , game 3;  $n=14$ , game 4). All the athletes have been considered high-level players, having vast competitive national and international experience. Female goalkeepers were not considered in the study given the specificity of their involvement and performance during the game, clearly different from the field players. All participants were duly informed about the objectives of the research and they provided written informed consents (with an addendum describing all study procedures) before participating in the study. The experimental procedures were approved by the institutional ethical commission, in accordance with the requirements of the Declaration of Helsinki.

### Design

This study took place during the group stage of the Algarve Cup (an international tournament for women's national soccer teams). In this phase of the competition the Portuguese team participated in four games with the national teams of Hungary (M2 – game lost, 0-1), Wales (M3 – game won,

4-0), Ireland (M4 – game won, 2-1) and China (M5 – game lost, 0-1). The matches were spaced by intervals of one or two days, on which the athletes participated in training sessions of low volume and intensity to recover and align strategies and technical details for the next game.

## Procedures

### Assessment of individual performance

In order to assess the performance of individual female players in each game (M2, M3, M4, and M5), the video records of the games were considered. These records were made available by the Portuguese Football Federation. In each match, the performance of the athletes was registered through a notational analysis system (adapted from Hughes & Bartlett, 2002; Lago-Peñas, Lago-Ballesteros, & Rey, 2011), which allowed to quantify the frequency of both the positive and the negative players' actions with the ball: passes, shots, dribbles, fouls suffered, tackles (steals), interceptions, possession lost, and cards (Table 1). Actions starting with the ball stopped were not accounted for. According to the outcome of actions in regard to play unfolding, the variables were classified in two distinct groups: positive actions (+) and negative actions (-). Each group of actions (positive and negative) was normalized by the on-field time (minutes) of each athlete.

For establishing the individual performance of each athlete, a total effectiveness rate was calculated by calculating the difference between the sum of positive actions and negative actions and dividing that total by the total playing time: =  $[(\Sigma \text{positive actions} - \Sigma \text{negative actions}) / \text{total on-field time}]$ .

The notational analysis was executed by the first author after a process of training for one week.

During the training process, the first and the second authors analysed three games and discussed the obtained results to improve analysis reliability. After the initial process of training, the first author started the process of analysis of the games considered in the study.

### Hormonal evaluation

Three days before the first game, three saliva samples were collected in order to control circadian fluctuations of hormonal levels (Crewther, et al., 2011). These were used as baseline, the initial comparative value ( $M_1$ ): upon waking up, 30 minutes before breakfast (8 a.m.), before lunch (11 a.m.) and before dinner (6 p.m.). In the first three games ( $M_2$ ,  $M_3$ , and  $M_4$ ) saliva was collected before lunch (at approximately 11 a.m.) and immediately after each game (approximately at 6 p.m.). In the last match ( $M_5$ ), that took place in the morning, saliva collection took place upon waking up and before breakfast (approximately at 8 a.m.) and immediately after the match (approximately at 1 p.m.).

Only the biological samples from the athletes who took part in each game ( $M_2 - M_5$ ) for more than ten minutes were considered. Similarly, the value corresponding to the concentration differences in both hormones between the initial moment (before the tournament) and the moment of each competition only respected the athletes involved respectively in each game (e.g.  $\Delta C_{M1-M2}$  or  $\Delta T_{M1-M2}$ ). In addition to the fact that it is common to divide the game into 10-minute periods of analysis, players who were not considered in the analysis had only a negligible participation in the game (around 2-3 minutes and in most cases without any frequency of action with the ball)

A sufficient quantity of saliva was collected in sterile containers (approximately 2 millilitres),

Table 1. Actions carried out with the ball [adapted from Lago-Peñas et al. (2011) and Hughes & Bartlett (2002)]

Action	Designation	Description of the action
Passes	Successful (+)	Passes successfully received by teammates
	Intercepted/lost (-)	Passes that are unsuccessful and are lost or intercepted by the adversary
Shots	At the goal (-)	Shots defended by the goalkeeper, not leading to a goal
	Goal (+)	Shots that led to a goal
	Off target (-)	Shots outside the goal frame
Dribble (1on1)	Unsuccessful (-)	Actions of advancing the ball in opposition to one defender; possession lost
	Successful (+)	Actions of advancing the ball in opposition to one defender; possession kept.
Fouls	Committed (-)	Infractions committed that did not lead to warning/ expulsion (yellow/ red card)
	Suffered (+)	Infractions suffered that did not lead to warning/ expulsion (yellow/ red card)
Tackle (steals, possession regained) (+)		Actions in which the ball has been recovered from the adversaries
Interceptions (+)		Actions in which the ball is intercepted from the adversaries
Possession lost (-)		Actions in which a player loses the ball except unsuccessful dribbles and unsuccessful passes
Cards	Yellow	Yellow cards awarded
	Red	Red cards awarded

Note. (+) number of actions counted as positive; (-) number of actions counted as negative.

and it was frozen up to the moment of laboratorial analysis. After defrosting and centrifugation, the samples were analysed in duplicate for both hormones using methods of immune-analysis, specifically Salimetrics® for testosterone (Testosterone, Salimetrics Europa, UK), using a xMark™ Microplate Absorbance Spectrophotometer (Biorad, Hercules, CA, USA), and test Elecsys Cobas® for cortisol (Cortisol, Roche Diagnostic GmbH). Later, the ratio (T:C) for each assessment moment was calculated.

### Statistical analysis

Standard descriptive statistical methods were used for the calculation of means and standard deviations. We used the non-hierarchical K-means method applied directly on the original data in order to group the athletes into two clusters with a minimum of intra-cluster variance regarding individual performances during the game. Outliers were eliminated given their negative consequences for this method of grouping data. We proceeded with the partition of the elements in two K classes and to the subsequent calculus of the centroid for each class, having the following variables of classification as a basis: positive and negative individual actions (pondered in terms of on-field time) and individual effectiveness rate. The one-way analysis of variance (ANOVA) was carried out to identify the effect of each classification variable on each cluster. Discriminant analysis (stepwise method for  $p < .05$ ) was used to validate the clusters and to calculate the respective significance of the predictors. The non-parametric Mann-Whitney test was used to compare the differences between the clusters regarding the hormonal and notational parameters. Non-parametric test of Kruskal-Wallis was used to test the differences between the matches regarding the athlete's hormonal concentrations and performance. Linear regression was used to esti-

mate the relationship between the individual effectiveness rate (dependent variable) and the hormonal concentration variation during the game ( $\Delta C$  and  $\Delta T$ ). The data were analysed using the statistics program SPSS 23.0 for Windows.

## Results

### Individual performance variation

Table 2 presents the athletes' individual performance (positive actions, negative actions, and index of individual effectiveness) throughout the tournament: the individual effectiveness rate and the positive actions (per minute) were the variables with the highest effect ( $p < .05$ ) in all matches. Two clusters with a relative balanced number of members were generated (cluster 1 – lower individual performance; cluster 2 – higher individual performance). The discriminant function revealed a significant association between the clusters and all the predictors.

The results showed significant differences ( $p < .05$ ) between the clusters in all the matches in the parameters of “positive actions” and “individual effectiveness rate”. Throughout the tournament no significant differences were identified regarding the notationally evaluated parameters for the whole team. However, the female players classified under cluster 2 showed significant variations throughout the matches regarding “positive actions” ( $p = .001$ ), “negative actions” ( $p = .013$ ) and “individual effectiveness rate” ( $p = .013$ ). The players classified under cluster 1 revealed significant variations throughout the games regarding “individual effectiveness rate” ( $p = .011$ ).

No significant differences were identified ( $p > .05$ ) regarding the mean on-field time of the involved players over the four games under study ( $M_2 - 68.8 \pm 29.4$  minutes;  $M_3 - 70.0 \pm 30.6$  minutes;  $M_4 - 68.8 \pm 30.8$  minutes;  $M_5 - 64.4 \pm 29.2$  minutes).

Table 2. Average individual performance of the players throughout the competition (absolute value and for each of the generated clusters)

Parameter		Game 1 (M <sub>2</sub> , defeat)			Game 2 (M <sub>3</sub> , victory)			Game 3 (M <sub>4</sub> , victory)			Game 4 (M <sub>5</sub> , defeat)			
		N	M	(±SD)	N	M	(±SD)	N	M	(±SD)	N	M	(±SD)	
Positive actions (per minute)	Team	13	0.42	0.15	14	0.52	0.22	12	0.45	0.17	14	0.37	0.12	
	Cluster	1	6	0.29*	0.09	9	0.38*	0.10	9	0.36*	0.07	6	0.27*	0.07
		2 <sup>§</sup>	7	0.54*	0.08	5	0.79*	0.09	3	0.70*	0.12	8	0.45*	0.09
Negative actions (per minute)	Team	13	0.17	0.06	14	0.20	0.14	12	0.14	0.04	14	0.16	0.08	
	Cluster	1	6	0.17	0.08	9	0.14*	0.06	9	0.15	0.04	6	0.21*	0.06
		2 <sup>§</sup>	7	0.17	0.04	5	0.31* <sup>§</sup>	0.17	3	0.13 <sup>§</sup>	0.06	8	0.12*	0.07
Individual effectiveness	Team	13	0.25	0.15	14	0.33	0.17	12	0.30	0.18	14	0.22	0.15	
	Cluster	1 <sup>§</sup>	6	0.12*	0.09	9	0.24*	0.12	9	0.21*	0.06	6	0.07*	0.06
		2 <sup>§</sup>	7	0.37*	0.06	5	0.48*	0.12	3	0.57*	0.10	8	0.33*	0.10

Note. \*significant differences ( $p < .05$ ) between cluster 1 and cluster 2; § significant differences ( $p < .05$ ) throughout the four games.

### The hormonal concentration levels and the players' performance

According to Table 3, no significant differences were identified ( $p > .05$ ) between the two clusters regarding C, T and T:C in any of the four official matches analysed. However, we noticed that in both lost game ( $M_2$  and  $M_3$ ), the athletes integrated in cluster 2 (higher individual performance) presented significantly distinct values ( $p < .05$ ) before the match in relation to the hormonal concentration observed before the tournament regarding C [significant rise in match 1 and 4 ( $p = .046$  and  $p = .012$ , respectively)], T [significant fall in match 1 and 4 ( $p = .028$  and  $p = .012$ , respectively)] and T:C [significant fall in match 1 and 4 ( $p = .028$  and  $p = .012$ , respectively)].

The analysis of hormonal variation throughout the four matches allowed us to identify significant differences ( $p < .05$ ) in the mean value of C before the match in both clusters ( $p = .017$  and  $p = .003$  for cluster 1 and 2, respectively) and after the match for cluster 2 ( $p = .003$ ). Furthermore, the T:C ratio in both clusters varied significantly throughout the tournament, either before the matches ( $p = .028$  and  $p = .002$  for cluster 1 and 2, respectively) or at the end ( $p = .025$  and  $p = .002$  for cluster 1 and 2, respectively). No significant differences were identified ( $p > .05$ ) for the variation of T values before and after the four matches analysed.

Regarding hormonal variation during the match, there was a significant decrease of C in the players classified under cluster 1 in the first game that was won ( $M_3$ ,  $p = .021$ ). The variation of T throughout the tournament was always negative and it was significantly lower at the end of match 1 for the players classified under cluster 2 ( $p = .018$ ) and in match 3 for the players classified under cluster 1 ( $p = .012$ ).

The mean variation of the T:C ratio throughout the tournament was always negative, with a significant decrease in match 1 for the players under cluster 2 ( $p = .018$ ) and in match 3 for the players classified under cluster 1 ( $p = .012$ ). In spite of these results, as we can see in Figure 1, no significant linear relation was established between the variation of both hormones during the game ( $\Delta C$  and  $\Delta T$ ) and the individual effectiveness rate in any of the matches analysed.

### Discussion and conclusions

The purpose of this research was to evaluate the relationship between salivary concentrations of cortisol, testosterone, the ratio T:C and the individual performance of top-level female soccer athletes during four consecutive official matches. In general, the results show the non-existence of a significant relationship between the hormonal concentrations of C and T and the players' individual performance throughout the tournament.

Throughout the years, the emergence of different performance analysis measures has allowed a better understanding of the success/failure of players and teams in team sports (Ali, 2011; George, Ionel, & Cristian, 2014; Lago-Peñas, 2012; Rampinini, Impellizzeri, Castagna, Coutts, & Wisløff, 2009; Reina Gómez & Hernández-Mendo, 2012). The assessment of the actions carried out by the players during the competition, even though it does not allow to capture the dynamics of the game, has allowed to measure some of the characteristics of the successful teams (Delgado-Bordonau, et al., 2013; Lago & Martín, 2007; Lago-Balasteros & Lago-Peñas, 2010; Lago-Peñas, et al., 2011; Rampinini, et al., 2009; Taylor, et al., 2008). In this

Table 3. Hormonal concentration (cortisol and salivary testosterone) and ratio level T:C in each cluster along the tournament

Parameter	Register	Cluster	Game 1 ( $M_1$ )		Game 2 ( $M_2$ )		Game 3 ( $M_3$ )		Game 4 ( $M_4$ )	
			Mean	( $\pm$ SD)	Mean	( $\pm$ SD)	Mean	( $\pm$ SD)	Mean	( $\pm$ SD)
Cortisol ( $\mu\text{g}\cdot\text{dl}^{-1}$ )	Before the game	1 <sup>§</sup>	0.40	0.13	0.50	0.13	0.45	0.19	0.73	0.18
		2 <sup>§</sup>	0.55 <sup>¥</sup>	0.16	0.41	0.11	0.42	0.09	0.86 <sup>¥</sup>	0.20
	After the game	1	0.42	0.09	0.38 <sup>*</sup>	0.12	0.45	0.12	0.55	0.22
		2 <sup>§</sup>	0.64	0.48	0.49	0.17	0.33	0.01	0.60	0.22
Testosterone ( $\text{pg}\cdot\text{ml}^{-1}$ )	Before the game	1	51.17	30.52	83.63	38.78	68.78 <sup>¥</sup>	39.86	64.75	35.08
		2	74.71 <sup>¥</sup>	29.53	77.60	18.05	72.33	34.27	59.63 <sup>¥</sup>	23.63
	After the game	1	43.50	21.44	63.14	14.77	45.25 <sup>*</sup>	22.33	41.25	18.71
		2	54.71 <sup>*</sup>	31.23	61.40	18.60	46.00	14.80	47.14	22.79
T:C	Before the game	1 <sup>§</sup>	118.76	66.03	170.32 <sup>¥</sup>	69.79	164.43 <sup>¥</sup>	73.79	84.45	32.84
		2 <sup>§</sup>	136.86 <sup>¥</sup>	33.08	193.08	33.71	176.55	97.79	71.14 <sup>¥</sup>	26.04
	After the game	1 <sup>§</sup>	101.26	30.34	155.62	40.92	102.86 <sup>*</sup>	58.34	79.17	27.53
		2 <sup>§</sup>	93.42 <sup>*</sup>	23.39	133.22	48.77	138.32	45.95	71.28	28.27

Note. ¥ significant differences ( $p < .05$ ) between the value measured before the tournament and the value observed on the day of the competition; § significant differences ( $p < .05$ ) throughout the four games; \* significant differences ( $p < .05$ ) between the value assessed before the competition and the value registered after the competition.

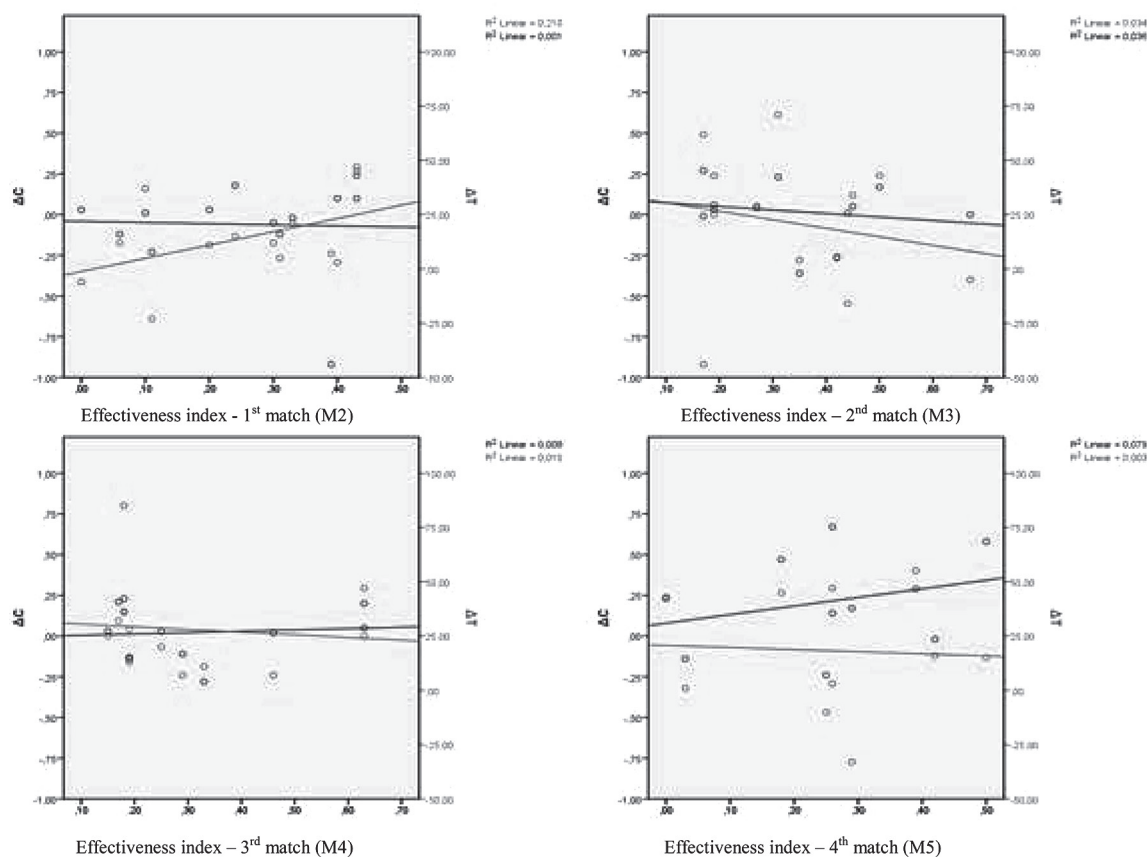


Figure 1. Simple linear regression between individual effectiveness in each game and the respective values of  $\Delta C$  and  $\Delta T$ .

study, after evaluating the players' performance over the tournament, one can notice the existence of significant differences between both clusters in the parameters of "positive actions" and "individual effectiveness rate". This labelling allows us to affirm that the performance of the players can be grouped at least into two clusters; in a regular form, the players belonging to cluster 2 show a better performance than the players belonging to cluster 1. However, the individual effectiveness rate varied significantly ( $p < .05$ ) throughout the four matches, presenting higher values in the won matches in both clusters, which seems to suggest the existence of a relationship between the individual effectiveness rate and the team success, as it has been verified in previous studies (Delgado-Bordonau, et al., 2013; Lago-Ballesteros & Lago-Peñas, 2010; Lago-Peñas, et al., 2011; Rampinini, et al., 2009).

When the differences in hormonal concentrations were analysed considering the quality of individual performance in the competition, the results did not follow a simple pattern of interpretation, at least having as a basis the notational and situational variables considered. In relation to cortisol, the results showed an increase in its concentration values when anticipating the competition, having as a basis the hormonal concentration observed before the tournament. In general, this result agrees with the consulted literature that reports rises in

cortisol regarding anticipation of the competition in both genders and in different sports (Bateup, et al., 2002; Carré, Muir, Belanger, & Putnam, 2006; Kivlighan, et al., 2005). However, from our point of view, there are no references in literature that support the fact that this rise in cortisol happens in a particularly significant form before the games that resulted in defeat (M<sub>2</sub> and M<sub>5</sub>) and in athletes classified as good performers in the competition. Some authors (Strahler, et al., 2010) refer to the possibility of a hormonal regulation effect in the anticipation of competition on athletes of a higher competitive level, because they are able to cope better with competitive stress. Curiously, the obtained results seem to suggest that a significant rise in cortisol in the anticipation of games of a higher importance (games 1 and 4; in particular, in players with a higher individual effectiveness) may be revealing of greater competitive stress leading to a negative match outcome. This is not a very solid result and it should be considered with some reserve, given that only four games, played in a very short period of time, were assessed.

The data also reveal the absence of significant differences in cortisol levels before and after the matches when linked to individual effectiveness rate. Besides, as Figure 1 reflects, there is no significant relation between  $\Delta C$  and individual effectiveness rate in the various matches that were analysed.

In some way, this result agrees with the study by Bateup et al. (2002) that does not report any relation between skill and sport experience and the levels of cortisol in the anticipation of the competition in female athletes; the authors suggest the existence of a not very individualistic behaviour in the expression of aggression during the game, which seems typical in female players (Taylor, et al., 2008). It is important to refer that the  $\Delta C$  during the matches does not follow a constant pattern over the tournament, and apparently it is not related with the result (Oliveira, Gouveia, & Oliveira, 2009).

Regarding testosterone, our results show a considerable decrease in the anticipation of the matches 1 and 4 for players classified in cluster 2. This result is coherent with the results proposed by Kivlighan et al. (2005) in rowing athletes, showing that in female athletes the testosterone response in the anticipation of competition tends to be negative. In any way, the absence of a relation between  $\Delta T$  throughout the four games and the individual effectiveness rate of the athletes (see Figure 1) seems to demonstrate the absence of dependence between these two variables. Also, it was verified that  $\Delta T$  during the games was not always positive in the four matches or coherent between the clusters. This lack of a constant pattern in  $\Delta T$  in competition is not convergent with the expected stimulating effect of the exercise in the production of testosterone (Mazur & Booth, 1998).

For ratio T:C, and according to the anabolic and catabolic effects described by the literature for each of the hormones, it is globally recognized that a higher ratio generally indicates a positive reaction of the athlete to the training, while the opposite indicates an intense stress effect upon the body (Gleeson, 2002). In fact, the alterations verified in the concentrations of C and T reflected themselves in a decrease of the ratio T:C on the competition days that assumed significant values in the athletes of cluster 2 before the competition with a result of defeat. According to some authors' opinion, this is due to the athletes' reaction to the competitive situation (Salvador, 2005; Salvador & Costa, 2009).

We also observed a T:C variation throughout the tournament and for both clusters. Even though there were no significant differences of pre- and post-game values linked to the individual effectiveness rate, we observed that in the last match (defeat) the T:C levels were the lowest ever. The highest levels appeared in the won matches and the lowest in the lost matches. These results seem to demonstrate, respectively, a more positive or a more negative state of the female athletes before a competition, which has an impact on the match outcome (Salvador, 2005; Salvador & Costa, 2009). Our results further show the significant differences in post-match results in relation to the basal values of reference. This reveals an acute post-effort variation

expected and coherent with the literature (Meeusen, et al., 2004).

In essence, our results seem to be in tune with that the literature has referred to about competitive stress in elite athletes, as hardly being perceptible (Moreira, et al., 2009) and as depending on the nature of the sports confrontation (Casanova, et al., 2016). From our point of view, the nature of confrontation is an intuitive and not very replicable parameter, resulting from the athletes' perception about the importance of the competition, the affinity they feel with the context where it occurs and still depending on the effectiveness of the adversaries (Lago-Peñas, et al., 2011). Given that the place where the competition takes place was different in matches with both defeat and victory, and there are no data (or known studies) about the importance that the athletes attribute to each game, we believe that the effectiveness of the adversaries will become a descriptive parameter of competitive stress. We actually observed that in the first and last match (both lost, but with the specificity of being the first match and the most decisive match for continuity in the competition, respectively) the players classified in cluster 2 presented significant alterations of C, T and T:C in the anticipation of the games. Even though this has not been the object of specific analysis in this study, we further noticed the existence of significant differences in the  $\Delta C$  among the games, with scope values clearly higher in match 1 and 4. Thus, we believe that the hormonal response can only be discriminatory of sports performance in unfavourable competitive contexts probably associated to adversaries with a higher effectiveness in individual performance (van der Meij, et al., 2010). Future studies on this topic should consider samples of different sports level, controlling parameters of individual (and/or collective) sports effectiveness of the adversaries.

Notwithstanding, for the relevance of the present results, it should be pointed out that the current study has some limitations. Firstly, there is no information about the hormonal response on rest days between competitions, whose kinetics could reveal a difference in hormonal concentrations in relation to the competition days, considering that there are studies that indicate hormonal alterations during rest time (Kivlighan, et al., 2005) and the need for a period of 48 hours to regain the normal values of both hormones – C and T (McLellan, Lovell, & Gass, 2010). Secondly, it is important to note that, despite the effectiveness of the algorithm used in this non-hierarchical K-means method, the number of volumetric clusters has previously been defined by the user, constituting a rigid partition without overlapping of items (Steinley, 2006). Future research may study the applicability of this method and its variants, using other performance parameters and larger samples. Thirdly, the opponents' sporting

performance was not analysed (and/or the international ranking of each selection inclusively), which information could have been useful for the understanding of the hormonal response of our players, especially in the anticipation of the game (Oliveira, et al., 2009).

The present study analysed the variation of the hormonal concentrations of cortisol, testosterone and of the ratio T:C throughout an intensive period of four high-level competitions, considering the individual performance of each athlete. It was possible to group the female athletes (according to

their actions of involvement with the ball) in two distinct levels of individual performance with the differences in the contribution that each athlete gave to the team's result. However, no significant differences between the two individual sports performance groups were identified regarding the hormonal concentration of C, T and T:C before and after the games. Thus, the data suggest that high-level female soccer players are prepared to deal with stress in each competitive situation, which, in turn, does not seem to determine significant variations in the individual effectiveness during the game.

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