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Cortisol, testosterone and mood state variation during an official female football competition

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ABSTRACT

BACKGROUND: Endogenous hormones are essential on the control of physiological reactions and adaptations during sport performance. This study aims to compare the mood state and the salivary levels of cortisol and testosterone during an official female association football tournament.

METHODS: Twenty female football players (22.85±4.2 years) from the Portuguese women's national team were included in the study. Mood, salivary cortisol and testosterone levels were examined in five moments over the championship (M1, neutral measures; M2-M5, on every match day). Saliva samples were collected before breakfast and immediately after each match. Mood was measured by the profile of mood states questionnaire (POMS); hormone levels were measure by immunoassay methods.

RESULTS: Iceberg Profiles of POMS were observed during all the moments of evaluation (M2-M5), showing a decrease in vigor and an increase in tension and depression in both team defeats (M2 and M5). There is no relationship between the hormones levels and the outcome of the competition, once cortisol and testosterone decrease from pre-match to post-match in both wins (M2 and M5) and defeats (M3 and M4). For testosterone the observed decrease is significantly different ($P<0.05$) before and after all matches.

CONCLUSIONS: Our results show a pattern in mood states behavior. Cortisol and testosterone decrease after match and throughout the tournament, independently of the match outcome. The absence of hormone fluctuations related to competition performance points out that top-level professional football players training systematically and regularly seem to be very well adapted to competition stress effect.

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In top-level sports, to achieve peak performance, it is required that an athlete keeps optimal capabilities during competition. Through training, the best load is selected to provide a stimulus for sport-specific adaptation that will result in improved athletic performance.¹ However, competition encounters usually causes stressful situations that may alter, for example, the physiological and psychological athlete's state in different

ways.² Competition stress can be regarded as a complex psychophysiological process,³ attributing a significant degree of indeterminacy to the final outcome, particularly in team sports.

Over the years, some biomarkers have been used to measure and understand changes in physiological and psychological athlete's state. For instance, cortisol (C) is considered a stress response biomarker⁴ due to its

catabolic, anti-inflammatory and homeostatic function that influences the electrochemical and metabolic balance.⁵ This steroid hormone has a deep physiological and psychological effect during active stress responses, motivating human behavior toward or away from desirable and undesirable outcomes, respectively.⁶ While C is the main glucocorticoid hormone, testosterone (T) is considered the primary androgen, both with different but complementary biological functions. The main role of T is the development and regulation of male secondary sex characteristics and reproductive function, to stimulate nitrogen fixation and to increase protein synthesis in a wide variety of target tissues.⁷ Despite the gender difference in circulating levels,⁸ several studies^{9, 10} have been showing that T seem to exert similar influences on social behaviors across sexes, particularly on behavioral aspects of dominance.¹¹

The association between physiological and neuroendocrine stress during sports competition has been studied based on numerous variables namely the anticipatory effect of competition,¹² the effect of winning and losing experiences,^{13, 14} the effect of different pre-game motivational interventions¹⁵ and even the opponent's psychological state.¹⁶ When the individual appraises the competition as important, controllable and depending on their effort, an active coping response pattern is more likely to develop. This pattern is characterized by T increase and sympathetic nervous system (SNS) activation, accompanied by positive mood changes. On the other hand, when the competition is considered threatening or out of control, a passive coping response pattern characterized by insufficient T, SNS activation and increase in serum C, affecting player's mood and satisfaction will probably occur.² The importance of the competition (*e.g.* regional *versus* national level) seems also to be a differentiating factor in this relationship between hormonal and psychological responses to competitions in each sport.^{1, 14, 17} However, as mentioned by Mazur and Booth,¹⁸ it seems relevant to assume that hormones can influence behavior but are also affected by behavior itself as well as several other environmental and biological parameters. It has been recently shown that C has a suppressing effect on T,¹⁹ due to the indirect regulation of the expression of androgen receptors.¹¹ Indeed, the effects of T on dominance behavior will only be expressed when basal C levels are low.¹⁹ Based on this evidence, Liening and Josephs¹¹ suggest-

ed that an approach-motivated behavior (toward desirable outcomes) is a necessary condition to allow a T effect dominance type behavior, like the desire for status. This seems to be one pertinent reason for the absence, in some studies, of any T effect on human competitive behaviors.^{20, 21} In fact, the literature seem quite inconsistent, showing different hormonal patterns in relation to sport competition depending on gender,^{22, 23} age^{24, 25} or prior competitive experience.²² Actually, high-level athletes seem to show some neuroendocrine habituation in the response to potentially stressful competitions, showing non-significant increments of salivary cortisol awakening response.¹² The discrepancy of results may also arise from increased mental control of the athletes in some sport fields.¹⁴ Indeed, judo players have increased cortisol levels over the competition with no changes in testosterone levels.¹

The published data on this thematic is far from conclusive, as it is mostly conducted on males subjects. In addition, scarce data are available in the literature concerning the correspondence between psychological and neuroendocrine stress during competition among elite female athletes. It is also unclear what hormonal drift is expected over the course of one important championship, which generally consists of a number of stages over several days. Thus, the purpose of this study was to analyze the correspondence between psychological and neuroendocrine stress of elite female soccer players during a concentrated period of competition. For that, we monitor the mood state and the salivary-free T and C concentrations in women's national soccer team, over the first course (group stage) of a Women's World Cup Soccer Championship.

Materials and methods

Subjects

Twenty female elite soccer players of the Portuguese national soccer team were recruited for this study. Athletes exhibited a mean age, body mass and height of 22.85 ± 4.2 years, 59.25 ± 4.58 kg, 170.2 ± 5.7 cm, respectively; a mean for the percentage of fat mass of $18.73 \pm 3.07\%$, and a mean value of $48 \pm 3.27\%$ for the percentage of lean mass. All athletes had been competing regularly in different international teams, exhibiting, at the time of this study, a good overall performance.

Each subject had a full explanation about the proto-

cols and signed an informed consent before starting the study. The experimental procedures were performed with ethics commission approval and in compliance with national legislation and the Code of Ethical Principles for Medical Research involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Experimental procedures

This study was conducted during the group stage of the Women's World Cup Soccer Championship (Algarve Cup, 2012). At this stage of the competition (from February 26th until March 7th, 2012), the Portuguese national team participated in four consecutive competitive matches involving the national teams of Hungary (February 29th, A₂: lost match, 0-1), Wales (March 2nd, A₃: won match, 4-0), Ireland (March 5th, A₄: won match, 2-1) and China (March 7th, A₅: lost match, 0-1). Between competitive matches there were one or two interval days in which all the players participated in team training sessions of low volume and low intensity for recovering purposes and correction of strategic and tactical details. During the championship, the players were not subjected to any type of mental training or strategies to self-regulate mood dimensions before and after each match.

The main variables measured in this study were free T and C responsiveness, performed in two or three saliva samples per match day (A₂-A₅) and the mood state assessment questionnaire that was filled after each game. To control circadian fluctuations in hormone levels, three samples were collected during a neutral day (three days before the first match) to be used as baseline values (A₁): when getting up 30 minutes before breakfast (8:00), before lunch (11:00) and before dinner (18:00). In A₂, A₃ and A₄ it was only possible to collect two samples of saliva: before lunch (11:00) and after each match (around 18:00). In A₅ the match occurred in the morning, thus the saliva samples were collected at getting up, before breakfast (8:00) and immediately after the game (around 13:00 for A₅).

The morning samples were collected when the athletes were in a period of rest/recovery, at the hotel. The post-match samples were collected after each match, moving the athletes to a quiet room at the playing venue. Excepted for the baseline measures (A₁), only the

data from athletes that participated in the game (A₂-A₅) were considered for analysis (POMS scores and hormonal measures concentrations).

Free hormone assessment

Saliva was used to measure T and C responses due to its ease of compliance, low invasiveness, and ability to track the biologically active "free" hormones. Using established procedures,²⁶ saliva was collected by passive drool into sterile containers, approximately 2 mL over a timed collection period of 2 minutes, and these were stored at -30 °C until assay. After thawing and centrifugation (2000 rpm for 10 minutes), samples were analyzed in duplicate for T and C using immunoassay methods, specifically the Salimetrics® for T (Salimetrics Europe, Newmarket, UK) and the Elecsys Cobas® test for C (Roche Diagnostics International AG, Rotkreuz, Switzerland), following the manufacturers' guidelines. The minimum detection limit for the T assay was <1 pg/mL with intra-assay coefficients of variation (CV) of <6.4%. Results were calculated using a 4-parameter sigmoid minus curve fit ($r^2=0.9983$) as proposed by the manufacturer, including assay controls. The C assay had a detection limit of 0.308 µg/dL with inter-assay CV of <20%.

Psychometric assessment

Mood was measured using the POMS questionnaire.²⁷ We use the reduced version with 42 adjectives that was translated and validated for Portuguese the population.²⁸ This Portuguese version of the POMS provide valid and reliable measures of mood states in athletes and non-athletes.²⁸ Subjects were asked to reflect on their emotion over the day, using a Likert-type scale with 5 points (from zero to four). Scores were then obtained according to six factors analytically derived mood states:²⁶ tension (Ten), depression (Dep), anger (Ang), vigor (Vig), fatigue (Fat), and confusion (Conf). POMS total mood disturbance (TMD) was also calculated using the following formula:²⁷ $TMD = (Ten + Dep + Ang + Fat + Con + 100 - Vig)$.

These outcomes were assessed in a neutral day (A₁) and after every match day (A₂-A₅). The questionnaire was administered in A₁ to A₅ to all players around 8 p.m. by the same trained interviewer. It took athletes between 5 and 10 minutes to complete it.

Statistical analysis

Standard statistical methods were used for the calculation of means and standard deviations. The Kolmogorov-Smirnov test of normality and Levine's test of homogeneity of variance were performed to assess the normality of the distribution. Non parametric statistical tests were used to analyze data when one or more of the underlying parametric test assumptions were violated. Friedman followed by Wilcoxon's signed-rank test were used as appropriate to determine the location of the significant differences. The Spearman rank correlation was used to test the relationship between the POMS scores and hormones measures concentrations after each match. Statistical significance for all analyses was as accepted at $P \leq 0.05$. Data were analyzed using SPSS Statistics v.22.0 for Windows.

Results

Profile of mood state

The results of the profile of mood states obtained in all assessment moments (A_1 - A_5) are presented in Table I. One can note a typical iceberg profile, with a

higher score in the Vig (positive subscale) and a lower score in the negatives subscales in every single assessed moments. Significant differences ($P < 0.05$) were found over the tournament (A_2 - A_4) in TMD and in subscales Ten, Dep, Ang and Vig. It was also found significant differences in the some POMS scores between the following matches and baseline: TMD (A_2 , $P=0.011$), Ang (A_3 , $P=0.031$; A_4 , $P=0.041$) and Vig (A_2 , $P=0.006$; A_5 , $P=0.021$).

Cortisol and testosterone concentrations

The mean values for C and T concentrations at baseline and over the group stage of the championship are presented in Table II. At baseline one can note a significant decrease in the concentration of both hormones (C and T) throughout the day ($P=0.000$). On match days, C levels did not change significantly from pre-match to post-match moment ($P > 0.05$), excepted in the last match of the group stage (A_5) ($P=0.010$). Over the four matches, significant differences were found in C levels before matches ($P=0.003$) but not after matches ($P=0.131$) throughout the group stage. Comparison on the values before and after each game with baseline hor-

TABLE I.—Scores of profile of mood states (POMS) in all assessment moments through the competition.

POMS scores subscales	A ₁ (Baseline) (N.=20)	A ₂ (Lost match) (N.=16)	A ₃ (Won match) (N.=17)	A ₄ (Won match) (N.=16)	A ₅ (Lost match) (N.=16)	P value (over A ₂ -A ₅)
TMD	94.50±8.4	103.7±9.0*	92.6±6.6	94.6±7.9 +	94.1±6.6	0.002
Ten	3.6±2.9	4.7±2.1	3.4±2.4	3.6±3.4 +	3.0±1.7	0.029
Dep	0.4±1.3	1.3±1.4	0.1±0.3	0.06±0.2 +	0.1±0.4	0.003
Ang	0.8±1.5	2.0±2.4	0.1±0.3*	0.1±0.3* +	0.5±0.7	0.000
Vig	16.4±2.7	12.12±3.1*	16.6±2.9	15.3±2.4 +	14.7±2.9*	0.003
Fat	2.7±2.4	3.5±3.3	2.1±3.0	2.9±4.5	2.1±2.2	0.462
Conf	3.2±1.7	4.1±1.9	3.4±1.5	3.2±1.5	3.0±1.2	0.277

Data are expressed as the mean value ± SD. * $P < 0.05$ compared to POMS scores at baseline; † $P < 0.05$ compared POMS scores between moment A₂ with A₄.

TABLE II.—Free-salivary cortisol and testosterone levels at baseline and over the group stage of the championship.

	A ₁ (Baseline) (N.=20)			A ₂ (Lost match) (N.=16)		A ₃ (Won match) (N.=17)		A ₄ (Won match) (N.=16)		A ₅ (Lost match) (N.=16)	
	8:00	11:00	18:00	11:00	18:00	11:00	18:00	11:00	18:00	8:00	13:00
Cortisol (µg/dL)	0.73±0.23	0.39±0.09	0.35±0.27	0.48±0.15	0.46±0.12*	0.44±0.12	0.39±0.14	0.44±0.15	0.44±0.15	0.83±0.20*	0.61±0.22*
P value		0.000			0.495		0.074		0.670		0.010*
Testosterone (pg/mL)	172.4±63.0	108.0±33.0	78.25±40.8	63.0±29.4*	50.3±24.9*	78.1±29.1*	57.2±21.1	67.6±34.5*	48.8±22.1*	63.5±28.7*	45.6±19.58*
P value		0.000			0.038*		0.044*		0.011*		0.013*

Results are presented as the mean ± standard deviation (SD). * $P < 0.05$ compared to baseline at equivalent ordinary hours during the day.

hormone levels (between equivalent ordinary hours during the day), revealed significant differences in the concentration of C after the first match (A_2 , $P=0.004$) and before and after the last match (A_5 , $P=0.001$, $P=0.0018$, respectively).

Regarding T concentrations on match days, it was observed that T levels significantly decreased ($P<0.05$) from pre-match to post-match in all considered games. Over the four matches, no differences were found in the T levels before ($P=0.315$) or after the matches ($P=0.532$) throughout the group stage. Comparison on the values before and after each game with baseline hormone levels revealed significant differences in the concentration of T levels before and after each match ($P<0.05$), excepted after the second match (A_3 , $P=0.675$).

The Spearman correlation coefficient was computed to examine the relationship between the POMS subscales, salivary cortisol and testosterone concentrations reported after the match. No significant correlations were found between hormones and Mood, apart from a negative correlation ($P\leq 0.05$) between cortisol and Conf in the won matches (A_3 , $r=-0.567$, $P=0.023$; A_4 , $r=-0.564$, $P=0.023$) and between cortisol and Ten in the last match (A_5 , $r=-0.547$, $P=0.037$).

Discussion

The purpose of this study was to examine the psychological and neuroendocrine stress of women's soccer players over consecutive matches during an official international championship.

Regarding the psychological evaluation, that was assessed by the profile of mood states questionnaire (POMS), the results showed a normal non-inverted "iceberg profile", highlighting a significant decrease ($P<0.05$) in Vig scores after lost matches and a significant decrease ($P<0.05$) in Ang scores after won matches. In fact, the highest Vig score is coincident with the best team performance over the tournament (A_3 , won match, 4-0). It should also be noted that the loss in the first match (A_2) had a substantial impact on several negative subscales and therefore on total mood compared to baseline ($P<0.05$). This defeat in the first match of the tournament seems to have induced a much more profound effect on mood than the defeat in the last game, which led to the discontinuance of the team in the tournament. In general, these findings are consistent with

Morgan's mental health model of performance,^{29, 30} suggesting that the competition outcome significantly affect the mood states of elite female soccer players, being these results concordant with other female professional soccer teams^{1, 13} and also in other sports fields such as rugby.⁸ Studies conducted with male athletes showed the same psychological pattern, with negative mood in the losers, while winners showed a better appraisal of team performance.³¹

Concerning to the hormonal parameters, the baseline results showed a decrease in C and T levels from the morning to the evening, in accordance with the expected diurnal variation of these hormones as function of sleep-wake cycles.^{32, 33} A similar hormonal pattern for C was generally obtained over the tournament - on match days, C levels tended to decrease, particularly in the last match (A_5). These declines on salivary C levels from pre-match to post-match seem to be inconsistent with other research findings. Indeed, other studies have shown a significant C increase after female competitive matches in rugby,⁸ in basketball¹⁴ and also in soccer.^{13, 34} Our results also shown that pre-match and post-match C levels are independent of the game outcome. Different and contradictory results were found in tennis players as after losing matches players revealed a C level peak²³ and in basketball, where the C levels increased after the game in both winners and losers.¹⁴

Mazur's biosocial theory^{18, 35} refers that T levels tend to rise after a successful dominance confrontation and decrease after losing dominance contest. This relationship between the T level and the players' competing for status and victory has been reported by some authors.^{13, 23, 34} Our findings, as those of other studies,^{1, 14} do not support such association.

Finally, our study also reveals a weak correlation between hormone levels and mood states in elite female soccer players during this type of competitive stress. In fact, T and C responses did not show a significant relationship with mood changes, in accordance with others studies.^{1, 31}

There are many possible explanations for some of the literature inconsistencies and the divergence of part of our results with other studies. One particular cause for such conflicting results could be related to methodological differences between studies. The time lag between the end of the game and the saliva sampling would have been sufficient to allow for steroid changes in the blood

to be reflected in saliva. However, in a study with rower players, which provided saliva samples 20 min and 40 min post-competition, the C and T levels began to decline in males by 40 min post-competition, but not in females.²² Even so, this time lag is not always properly clarified among studies.

Another reason for some of the inconsistencies between our results and the literature could be related both to the importance of the competition and different levels of athlete's experience. None of the aforementioned studies included elite players or the assessment of psychological and hormonal levels over a competitive period with the characteristics of the world cup. Female elite soccer players considered on this study are subject to systematic training on a high-performance level, in addition to the competing demands of home clubs and national team they represent. Thus, our results may be explained, at least in part, by the increased ability of these athlete's to cope with competitive stress,³⁶ having a better control over mental challenges and stress during competition. The decrease in C levels towards the competition may arise from the fact that these elite female players are managing the challenge of competition. Effectively, C levels do not interfere with conciliatory behavior between individuals who were opponents a few moments earlier and teammates who were challenged in the heat of the competition.³⁴

The different time course of T response from males to females also contributes to explain different research finding between genders across the literature.²² Other reasons for different biobehavioral stress responses between genders may be related to evolutionary and social factors. According to Taylor *et al.*³⁷ (also enunciated by Sedghrooni *et al.*,¹⁴ women were mostly protective during our evolution as species, so they tend to form female-female social bonds in a tend-to-be-friend response to stress. On the other hand, men were responsible for the protection, and thus they tend to react to stress as "fight-or-flight". According to this theory, the friendlier the match is, the weaker T response on women is.

Notwithstanding the relevance of the present results, this study has some limitations. Firstly, it was not possible to measure salivary C and T levels when getting up (8:00), during the first three matches (A_2 - A_4) and before lunch (11:00), during the last match (A_5). Looking at the waking C response in all matches may have shown differences in relation to anticipatory stress. Secondly, no

information about the phase of menstrual cycle of each athlete was available which could have been helpful in explaining the impact on mood and hormone measures. Thirdly, the interpretation of our results is based on a single baseline measure, taken days before competition. It would have been valuable to have other measures before the competition preparation phase.

Despite these limitations, this study also has significant merits. Accessing elite athletes over consecutive matches during the most relevant international competition (World Cup) is quite rare. Thus, as far as we know this is the first study that reports the monitoring of the mood state and the salivary-free T and C concentrations in such conditions. To assure comparable results to other studies we used consistent standard methodology to measure mood and hormonal kinetics, prior and immediately following the game.

Future studies should measure the POMS scores before and after each game in order to assess the expected mood changes during the course of a competitive event, known as mood unbalancing effects model.³⁸ Furthermore, future research should link the C and T measures to match time and running distance during the game to better understand the psychological and neuroendocrine stress response according to different competition demands.

Conclusions

In summary, this study has shown a typical iceberg profile of mood states over four consecutive matches during the group stage of the Women's World Cup Soccer Championship: Vig subscale tend to decrease after lost matches and Ang scores decrease after won matches. The C levels did not change significantly over the first three matches, regardless of the competition outcome. However, the higher level of importance of the last match, beyond the defeat itself, seems to have determined a significant decrease in C from pre to post-competition. On the other hand, T levels decreased significantly ($P < 0.05$) after all considered competitions encounters. The relationship between hormone levels and mood states was inconsistent during the tournament. Thus, it may be reasonable to assume that in female elite players the competition stress despite being able to affect mood depending on the match outcome, only causes significant hormonal effects in competitions of manifest importance.

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