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Testosterone responsiveness to winning and losing experiences in female soccer players

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Summary In many animal species including humans circulating androgen levels in males respond to social challenges. This response has been interpreted as an adaptive mechanism that helps the individuals to adjust their behavior to changes in social context. According to this hypothesis socially modulated androgen levels (e.g. increased levels in dominants and decreased levels in subordinates) would influence the subsequent expression of social behavior in a status-dependent fashion. This rationale is partially based on male physiology and therefore has been rarely investigated in females. Here, we investigated if a hormonal response to a social challenge that produces changes in status is also present in human females. We have collected saliva from and administered questionnaires to female soccer players of both teams playing the final match of the Portuguese Female soccer league. Samples were collected on a neutral day and on the day of the game both before and after the match. The change in testosterone levels (i.e. post-game − pre-game values = Δ_T) was positive in the winners and negative in the losers and there was a significant difference in the testosterone change (i.e. Δ_T) over the game between winners and losers. Cortisol levels did not vary with contest outcome. An anticipatory rise in circulating levels of both hormones (testosterone and cortisol) was detected before the match. Paralleling the hormonal responses, changes in mood and anxiety state were also found between both teams, with more positive states being observed in winners and more negative states being observed in losers at the end of the match. These results suggest that testosterone also responds to social challenges in human females and that contest-induced mood changes may influence this response.

1. Introduction

In recent decades evidence has accumulated for a reciprocal relationship between androgens and behavior. The role of androgens on the regulation of aggressive behavior is well established and was the first behavioral endocrinology principle to be proposed as far back as the late 19th century (Soma, 2006). More recently, social challenges, such as fighting an opponent, have been shown to elicit an androgen response in a wide range of vertebrates including humans (Hirschenhauser and Oliveira, 2006). The accumulation of this type of data led to the proposal of two independent

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hypotheses that try to explain a reciprocal interaction between androgens and competitive behavior: the biosocial status hypothesis (Mazur, 1985; Mazur and Booth, 1998) and the challenge hypothesis (Wingfield et al., 1990). According to these two hypotheses changes in androgens levels driven by social competition would modify the behavior of the participants in subsequent interactions according to previous social information. Therefore, they provide a conceptual framework to explain the elevation of androgen levels in males during periods of increased social competition (Wingfield et al., 1990; Hirschenhauser et al., 2003; Archer, 2006). Several psychological variables have been proposed to mediate and moderate the hormonal response to social challenges and to competition. According to the cognitive activation theory of stress (CATS) previous social interactions produce stimulus or response outcome expectancies that would be instrumental in the subsequent responses to social stimuli (Ursin and Eriksen, 2004).

Indeed, the perception of contest outcome, winning or losing, seems to modulate the androgen responsiveness, since manipulated interactions where the animal is allowed to fight without perceiving a win or a loss of status (by fighting its own image on a mirror) fail to elicit an endocrine response (Oliveira et al., 2005). In general a victory increases (or arrests a decrease in) testosterone (T) post-competition levels whereas a defeat experience decreases circulating T, although some studies have failed to find an effect of the outcome on androgen response to competition (for reviews see Mazur and Booth, 1998; Salvador, 2005; Archer, 2006; van Anders and Watson, 2006; Salvador and Costa, 2009). This differential hormonal response to competition between winners and losers has been documented not only in contests that involve physical confrontation (e.g. tennis match, Mazur and Lamb, 1980; wrestling combat, Elias, 1981), but also in non-physical face-to-face competition such as chess and domino tournaments (Mazur et al., 1992; Wagner et al., 2002). Moreover, salivary T levels also respond to competition outcome in sports fans who are psychologically linked to the success of their teams but are not directly involved in the contest (Bernhardt et al., 1998). Together, these data suggest a cognitive activation of the endocrine response to social challenge. Concomitantly the tendency to engage in future social interactions is influenced by the outcome of a previous interaction (e.g. decreased in losers, Mehta and Josephs, 2006). These hormonal and behavioral responses to previous contest experience have been interpreted as an adaptation for individuals to express appropriate behavior to ever changing social environments, by adjusting their agonistic motivation accordingly (Mazur, 1985; Mazur and Booth, 1998; Oliveira, 2004).

The rationale for this hypothesis is based on male physiology and hence the evidence supporting it is mainly grounded on male studies. Recently there has been an emerging interest in understanding the validity of this hypothesis in females. T levels in women seem to be associated with social status and the expression of assertive behaviors (Grant and France, 2001; Cashdan, 2003; Edwards et al., 2006) and are predictors of reactions to wins and losses (Josephs et al., 2006; Mehta et al., 2008).

Moreover, dominance dependent T changes may modulate subsequent social interaction in females since T may modulate a set of psychological traits that are highly relevant for the dynamics and outcome of contest. T effects on competitive relevant traits include: increased selective attention to social threat (i.e. angry faces, van Honk et al., 1999), increased visuospatial ability (Aleman et al., 2004), fear reduction (van Honk et al., 2005; Hermans et al., 2006) and reduction of punishment sensitivity and enhancement of reward dependency (van Honk et al., 2004). T is also known to promote the activation to angry vs. happy faces in women brain areas involved in reactive aggression (e.g. amygdala and hypothalamus; Hermans et al., 2008).

On the other hand, competitive situations induced an increase in T and cortisol (C) levels in female rugby and soccer players (Bateup et al., 2002; Edwards et al., 2006) but not in women playing a video game, rowing in a ergometer sprint competition or competing on cognitive tasks (Mazur et al., 1997; Kivlighan et al., 2005; Schulteiss et al., 2005; van Anders and Watson, 2007). Interestingly, none of these studies on female competition reported differences in T levels between winners and losers (Bateup et al., 2002; Schulteiss et al., 2005; Edwards et al., 2006; van Anders and Watson, 2007). However, in both studies the researchers have followed a single team and so for the same event hormonal responsiveness data were only available for one of the two conditions (win/loss). Since the salience of different win/loss episodes may depend very markedly on its consequences for self-perceived status (e.g. winning/losing a final match that decides a championship vs. winning/losing a regular season game) data on hormonal responsiveness to the outcome of a competitive event for both parties are needed.

Here we report the hormonal responsiveness to competition both in winners and in losers of the final match of the Portuguese Female Soccer League. For this purpose we collected saliva samples from and administered questionnaires to female soccer players both before and after the final match and at the same time of the day in a neutral day (i.e. the same day of the week but without neither a game nor a training session). Since C can modulate the androgen responsiveness to competition we have also measured C levels in winners and losers.

2. Methods

2.1. Participants

Thirty-three female soccer players aged 24.24 ± 4.78 from two different teams that would play against each other the match that would decide the first place in the Feminine Portuguese Soccer League (FPSL) gave their informed consent to participate in the study. Players from both teams were sampled in a neutral day before the match and on the day of the match. Players from the winning team consequently became champions of that year's FPSL, and the players from the losing team classified in the second place of the FPSL. Since T levels vary during the menstrual cycle being higher in the follicular than in luteal phase (Goebelsmann et al., 1974; Rubinow et al., 1988; van Goozen et al., 1997), the menstrual cycle was monitored. Only 1 out of 33 players was in the luteal phase suggesting a synchronization of menstrual cycles of the women within each team. Therefore, it was decided not to enter the phase of the cycle as a covariate. Only 2 out of 33 players reported using oral contraceptives and they did not display abnormal T or C levels. Thus, data from these 2 individuals were also included in the analysis. Saliva samples were obtained from 31 players in the neutral day (13 players from the winning team and 18 players from the losing team) and from 29 players in the match day (13 players from the winning team and 16 players from the losing team).

2.2. Data collection and psychological variables

Saliva samples were collected for the assessment of T and C levels 30 min before the start of the match and before the warm-up (pre-game sample) and 30 min after the end of the match (post-game sample). Saliva was provided by passive drool without using any method that would stimulate salivation (e.g. gum). The match was played at the home field of the winning team on April 18, 2004. The game lasted 90 min (two parts of 45 min each) with a break of 15 min and the final score was 4-0. To control for circadian fluctuations in hormone levels time matched baseline samples were collected in a neutral day (same week day of the match, i.e. Sunday) at the time the match would begin (16:00 h) and 120 min later, at the approximate time the match usually finishes (18:00 h). We have used the cortisol awakening response as a measure of hypothalamic—pituitary—adrenal (HPA) axis activity (Pruessner et al., 1997; Clow et al., 2004). For this purpose the participants were asked to collect two saliva samples at home on another neutral day: the first sample shortly after waking up, while still lying in bed; the second sample 30 min later. In order to standardize hormonal determination, general information about possible saliva contamination of each sampling point was assessed.

For the assessment of mood and anxiety states, players completed questionnaires both before and after the match on the day of the game at the same sampling points when saliva was collected. Time to complete questionnaires varied from 5 to 10 min. Mood state was assessed using the Profile of Mood States (POMS) inventory (McNair et al., 1971), which is composed of six scales: tension/anxiety, depression, anger, vigor, fatigue, and confusion. Anxiety state was evaluated by the MRF inventory (Krane, 1994), which is composed of three scales: worry, tension and confidence. A total score was used in both cases by adding all scales of each inventory (Viana et al., 2001). The scales used were analogue where the subjects had to indicate the way they were feeling at that moment by marking a cross on a 10 cm line between two words of opposite valence. The answer to each scale was operationalized as the distance from the beginning of the line to the marked cross using an electronic Calliper (Mitutoyo Digimatic), and converted into a percent value. Using this type of analogue scales match importance (I) and match difficulty (D) were also measured. A score of threat perception was obtained as $I \times D$ (Martens, 1977; Cruz, 1994).

At the post-game sampling point the following variables were also measured using questionnaires: causal attribution of the outcome ("luck/other's team fatigue/we played well/other team played better"), self-evaluated efficacy during the event, contribution to the match outcome and degree of satisfaction with the outcome. The last three variables were measured using the analogue scale (0—100%) described above. Changes (i.e. Δ_i = post-game − pre-game values of variable i) in psychological state variables were obtained for 28 players (12 from the winner team and 16 from the loser team).

Team-focal behavioral observations (*sensu* Martin and Bateson, 2007) were conducted during the game by two observers (one per team). For each player the following behaviors were registered: fouls done and received, successful and unsuccessful passes (successful when the ball reached the target team mate), tackles (successful when it interrupted the other team offensive) and shots on goal (successful when targeting the goal). Based on these observations and considering the time each player was on the pitch, four indexes were computed: observed effort (=total number of occurrences per player; it is a proxy for the involvement of each player on the game), observed performance (=ratio of successful occurrences per total number of occurrences for each player), observed dominance [=ratio of fouls committed per total number of fouls (committed + suffered)] and observed aggression (=frequency of committed fouls). Behavioral data were obtained for 28 players (14 from each team).

The psychological traits anxiety, mood and aggression were assessed in the neutral day using the MRF (Krane, 1994), the POMS (McNair et al., 1971) and the DIAS (Björkqvist et al., 1992) inventories (not the analogue scales mentioned above), respectively. These inventories used a 5-points Lickert scale. Psychological trait data were obtained for all the 33 participants in the study.

Each subject was allocated a random number code so that sample collection both of saliva samples and psychological questionnaires could be treated anonymously.

2.3. Hormone assays

All samples were collected on polypropylene vials and transported on ice to the laboratory, where they were centrifuged at 3600 rpm for 10 min. Approximately 2 ml of the fluid compound of the saliva was recovered from each tube and was stored frozen at −20 °C until further processing. For the analysis of T levels samples were extracted with diethylether and assayed by radioimmunoassay (RIA), using a monoclonal antibody for Human Testosterone (immunogen 3-CMO-BSA, Cymbus Biotechnology Ltd., ref. CBL 68) and a specific label ([1,2,6,7-3H] Testosterone, Amersham Biosciences, ref. TRK402-250μCi). C levels were assayed by luminescence immunoassay (LIA), using a commercial kit for C determination in human saliva (IBL-Hamburg, ref. RE62011), without prior extraction. Intra-assay coefficient of variation was 5.84% and 8.26% and inter-assay coefficient of variation was 7.25% and 8.52%, for T and C, respectively. Hormonal levels measured were within the expected range of values for salivary T and C, given the age of the subjects and the time of day of the sample collection (IBL, 2006, p. 22). Changes in hormone levels (Δ_T = change in T levels; Δ_C = change in C levels) across the match were calculated as post-game values − pre-game values.

2.4. Statistical analysis

Since some data did not match the assumptions of parametric statistics we have used non-parametric statistics. The comparisons between the two teams were performed using Mann—Whitney *U*-test. The within-subjects comparisons between pre-competition and post-competition values and before the competition and the neutral days were performed using Wilcoxon matched pairs tests. Spearman rank correlations were used when testing the association between T levels and psychological variables. All tests were two-tailed. Statistical significance for all tests was: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and n.s. ($p > 0.05$). Data were analysed using STATISTICA 7 for Windows (StatSoft, 2007). In cases where sample sizes are smaller than the ones indicated above that is due to missing data for that specific analysis.

3. Results

3.1. Psychological and hormonal trait variables

The psychological trait variables measured (i.e. anxiety, mood and aggression) did not differ between the players of the two teams (Mann—Whitney U-test — anxiety total score: $N_W = 15$, $N_L = 18$, $U = 125.00$, $Z = -0.36$, $p = 0.72$; mood total score: $N_W = 15$, $N_L = 17$, $U = 121.00$, $Z = 0.24$, $p = 0.81$; aggression total score: $N_W = 13$, $N_L = 18$, $U = 97.50$, $Z = 0.78$, $p = 0.781$).

The cortisol awakening response in a neutral day (i.e. without soccer game) did not differ between players of the two teams suggesting that there are no differences in the reactivity of the hypothalamic—pituitary—adrenal axis between subsequent winners and losers (Mann—Whitney U-test: $N_W = 13$, $N_L = 18$, $Z = 0.63$, $p = 0.53$).

3.2. Psychological and behavioral response to competition and to match outcome

Before the start of the match (pre-game sampling point) threat perception was significantly higher in the team that lost the game (winners: average $\pm$ SEM = 51.0 $\pm$ 8.2%, $N_W = 12$; losers: 76.7 $\pm$ 4.2%, $N_L = 16$; Mann—Whitney U-test: $U = 38.00$, $Z = 2.89$, $p < 0.01$). Mood and anxiety states changed significantly both in winners an in losers between pre-game and post-game. In winners competition enhanced a positive mood state (Wilcoxon matched pairs test, winners pre-game vs. post-game: $N = 12$, $Z = 2.29$, $p < 0.05$) compared to losers who experienced a decrease in mood (Wilcoxon matched pairs test, losers pre-game vs. post-game: $N = 16$, $Z = 3.51$, $p < 0.01$). Therefore, the mood change over the game (i.e. Δ_{mood} = post-game — pre-game levels) was different between the two teams (Mann—Whitney U-test: $N_W = 12$, $N_L = 15$, $U = 3.00$, $Z = 4.25$, $p < 0.001$) (Fig. 1A). The anxiety state decreased in winners and increased in losers from the pre-game to the post-game, also resulting in a significant difference in the change of state anxiety (i.e. $\Delta_{anxiety}$) over the game between the two teams (Mann—Whitney U-test: $N_W = 11$, $N_L = 16$, $U = 21.00$, $Z = -3.31$, $p < 0.001$) (Fig. 1B).

Among the post-competitive measures only satisfaction with outcome was higher in winners than in losers (winners = 71.42 $\pm$ 10.95%, $N = 11$; losers = 10.28 $\pm$ 4.07%, $N = 16$; Mann—Whitney U-test: $U = 29.0$, $Z = 2.91$, $p < 0.01$). Perceived efficacy (winners = 54.59 $\pm$ 11.39%, $N = 11$; losers = 38.89 $\pm$ 5.64%, $N = 16$) and perceived contribution to outcome (winners = 35.02 $\pm$ 11.32%, $N = 11$; losers = 42.80 $\pm$ 5.33%, $N = 16$) were not significantly different between players of the two teams (Mann—Whitney U-test — efficacy: $U = 65$, $Z = 1.13$, $p = 0.26$; contribution: $U = 70$, $Z = -0.88$, $p = 0.37$). In terms of attribution of the outcome we grouped the responses in attributions to external agents (e.g. "the other team played better") vs. attributions to internal causes (e.g. "we played well"). All players from the winning team attributed the positive outcome to internal causes, whereas 18.75% of the losers attributed the negative outcome to external agents. The difference between proportions of attribution to internal causes between winners and losers is not significant ($P_W = 100\%$, $N = 11$ vs. $P_L = 81.25\%$, $N = 16$, $p = 0.14$).

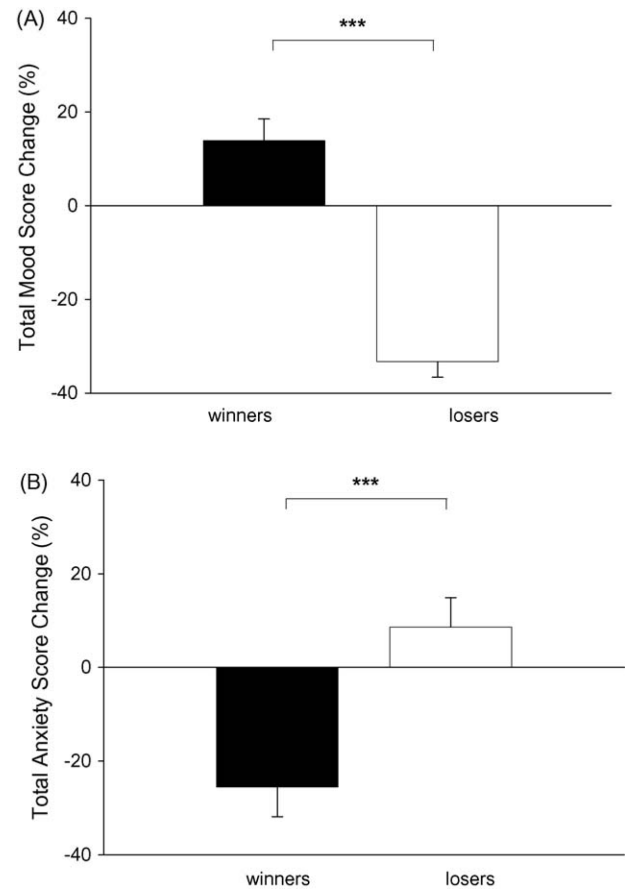

Figure 1 Change in mood (A) and anxiety (B) states during competition in winners and losers. *** indicates a p value <0.001.

Concerning the behavioral variables observed during the match the winning team revealed a better performance (Mann—Whitney U-test: $N_W = 14$, $N_L = 14$, $U = 19.00$, $Z = 3.75$, $p < 0.001$) and a non-significant trend to exhibit more effort (Mann—Whitney U-test: $N_W = 14$, $N_L = 14$, $U = 65.5$, $Z = 1.72$, $p = 0.09$) than the defeated team. There were no differences between the two teams neither in the observed dominance (Mann—Whitney U-test: $N_W = 14$, $N_L = 14$, $U = 90.50$, $Z = -0.63$, $p = 0.53$) nor in observed aggression (Mann—Whitney U-test: $N_W = 14$, $N_L = 14$, $U = 96.5$, $Z = -0.37$, $p = 0.81$).

3.3. Hormonal response to competition and to match outcome

To assess the anticipatory effect of competition on hormonal levels we have compared the T and C pre-game levels with

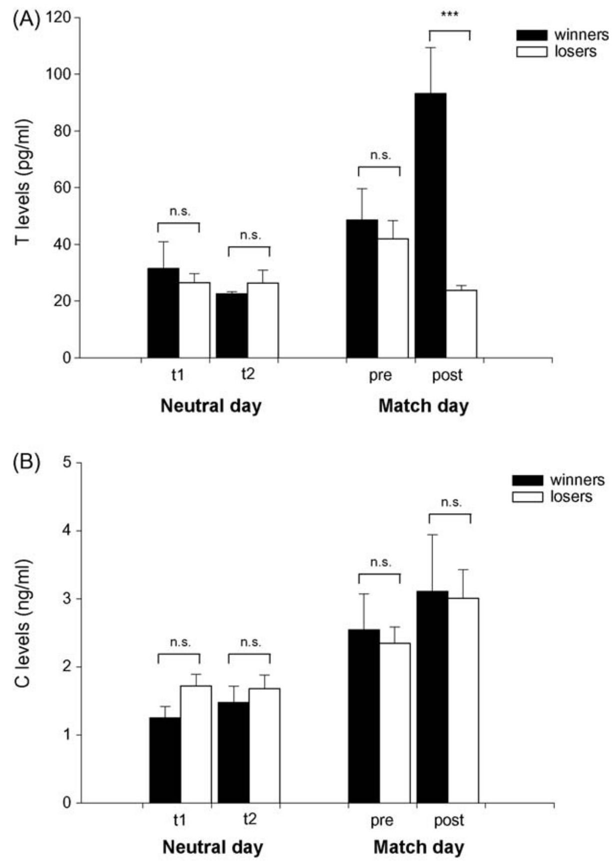

Figure 2 Pre-game and post-game testosterone (A) and cortisol (B) levels in winners and losers and their time matched baseline samples (t_1 and t_2) collected in a neutral day. *** indicates a p value $<$0.001.

levels of the same hormones measured at the same time in a neutral day. Pre-game values of T and C in players of the winning team did not significantly differ from values obtained for the neutral day (Wilcoxon matched pairs test: T, $N = 11$, $Z = 0.25$, $p = 0.80$; Fig. 2A; C, $N = 10$, $Z = 1.27$, $p = 0.20$; Fig. 2B). Players of the loser team exhibited a significant increase in T (Wilcoxon matched pairs test: $N = 16$, $Z = 3.46$, $p < 0.001$; Fig. 2A) and a non-significant change in C levels (Wilcoxon matched pairs test: $N = 13$, $Z = 1.64$, $p = 0.10$; Fig. 2B) in anticipation of the competition (i.e. pre-game values vs. neutral day values). However, when taken together the players of both teams, an overall anticipation effect is detected for both hormones (Wilcoxon matched pairs test: T, $N = 27$, $Z = 2.42$, $p < 0.05$; C, $N = 23$, $Z = 2.10$, $p < 0.05$).

On the day of the game there were no differences in T levels measured before the game between the two teams (Mann—Whitney U-test: $N_W = 13$, $N_L = 16$, $Z = 0.04$, $p = 0.98$), but the players of the winning team had significantly higher T levels at the end of the game (Mann—Whitney U-test: $N_W = 12$, $N_L = 16$, $Z = 3.67$, $p < 0.001$; Fig. 2A), with winners experiencing an increase and losers a decrease in T levels over the game (Wilcoxon matched pairs test: winners: $N = 12$, $Z = 2.70$, $p < 0.01$; losers: $N = 16$, $Z = 2.19$, $p < 0.05$; Fig. 2A). Changes in T levels measured at the same time of the day in a neutral day showed no significant differences between the teams (Mann—Whitney U-test — first sampling point corresponding to the start of the match: $N_W = 13$, $N_L = 18$, $Z = -0.12$, $p = 0.81$; second sampling point corresponding to the end of the match: $N_W = 13$, $N_L = 18$, $Z = -0.26$, $p = 0.39$), suggesting that the pre-game to post-game change in T levels (Δ_T) observed is not due to a differential daily temporal fluctuation between members of the two teams but it was linked to the competition event.

Both pre-game and post-game levels of C were not significantly different between winners and losers (Mann—Whitney U-test — pre-game: $N_W = 13$, $N_L = 16$, $Z = -0.48$, $p = 0.62$; post-game: $N_W = 13$, $N_L = 16$, $Z = -0.79$, $p = 0.43$; Fig. 2B). The pre-competition to post-competition change in C levels in winners was not significant (Wilcoxon matched pairs test: $N = 13$; $Z = 0.38$, $p = 0.70$; Fig. 2B) whereas there was a marginally non-significant increase in losers (Wilcoxon matched pairs test: $N = 16$, $Z = 1.86$, $p = 0.06$; Fig. 2B). Baseline values in the neutral day were not significantly different between players of the two teams (Mann—Whitney U-test — first sampling point corresponding to the start of the match: $N_W = 12$, $N_L = 15$, $Z = -1.56$, $p = 0.12$; second sampling point corresponding to the end of the match: $N_W = 12$, $N_L = 15$, $Z = -0.78$, $p = 0.45$; Fig. 2B).

3.4. Relationship between psychological variables, hormone levels and competitive behavior

Anticipatory (i.e. pre-game) T and C levels were not correlated with any of the measured behavioral (observed effort, performance, aggression and dominance) and state (mood and anxiety changes) and trait (aggression, mood, anxiety) psychological variables.

Since it has been argued that hormonal changes in response to competition are driven by changes in mood state and/or by other psychological states elicited by the competitive event (e.g. McCaul et al., 1992), we decided to investigate the relationship between mood and anxiety state changes over the game with hormonal responses. Change in mood state over the game was correlated with T change ($N = 27$, $R_s = 0.48$, $p < 0.05$), but not with C change over the game ($N = 29$, $R_s = -0.25$, $p = 0.20$). Similarly, there was a non-significant negative correlation between the fluctuation in anxiety state and the change in T over the game ($N = 27$, $R_s = -0.36$, $p = 0.06$), whereas the pre-game to post-game fluctuation in anxiety state was not correlated with the change in C ($N = 27$, $R_s = 0.04$, $p = 0.83$). To assess the direction of the putative causal relationship between the change in mood and the change in T levels before and after the match we have computed temporal cross-correlations. T levels prior to the match were not good predictors of mood changes over the match ($N = 27$, $R_s = -0.13$, $p = 0.50$). On the other hand, mood change over the match showed a negative correlation with T levels after the match ($N = 27$, $R_s = -0.57$, $p < 0.01$), suggesting the involvement of mood changes on T responsiveness to competition.

From the post-competitive measures taken only satisfaction with the outcome shows an association with the change in T over the game (Δ_T) ($N = 28$, $R_s = 0.42$, $p < 0.05$). Satisfaction was not correlated with C change (Δ_C) ($N = 28$, $R_s = 0.27$, $p = 0.16$). Both perceived efficacy and perceived contribution were not correlated with either T or C changes over the game (R_ss < 0.23, p values $>$0.27). This is probably

Table 1 Association between pre-competition and post-competition T and C levels and the observed competitive behaviors during the game. Sample sizes are: $N_{\text{pre-game}} = 23$; $N_{\text{post-game}} = 22$.

	T levels		C levels	
	Pre-game	Post-game	Pre-game	Post-game
Observed effort	−0.24	0.11	0.18	0.24
Observed performance	0.17	0.64***	0.20	0.56**
Observed dominance	−0.21	−0.30	−0.23	−0.01
Observed aggression	−0.29	−0.34	−0.15	0.11

** Indicates $p < 0.01$.
*** Indicates $p < 0.001$.

due to the fact that satisfaction with outcome is well correlated with post-competitive mood ($N = 28$, $R_s = 0.67$, $p < 0.0001$) and with mood change over the game ($N = 28$, $R_s = 0.63$, $p < 0.001$).

In order to disentangle the causal links between steroid hormones and competitive behavior, that is if pre-contest hormones are good predictors of observed behavior or if on the other hand observed behavior during competition predicts hormone levels at the end of the game, we have also computed temporal cross-correlations between T and C levels (pre-game vs. post-game) and observed behavior during the game. Pre-game neither T nor C levels predict any of the competitive behaviors measured (i.e. observed effort, performance, dominance or aggression; see Table 1). On the contrary, observed performance is positively associated both with post-game T and C levels (Table 1) and with the change of T over the game (Δ_T) ($N = 22$, $R_s = 0.54$, $p < 0.05$), suggesting an effect of performance on androgen and cortisol responsiveness to the game situation.

4. Discussion

The main goal of the present study was to evaluate the hormonal responses in winners and losers of a single competitive event of high valence in terms of social status. For this purpose we have sampled players of both teams during the final match of the Portuguese Female Soccer League. Overall players of the two teams exhibited an anticipatory rise both in T and C before the game. Over the game players of the winning team experienced an increase in T levels whereas T levels decreased in losers resulting in significantly higher T levels in winners than in losers at the end of the match. The change in T levels over the match was correlated both with observed performance and with an increase in positive mood.

The anticipatory rise in T has been detected in the majority of studies that investigated so far the hormonal responses to sports competition in men (Booth et al., 1989; Mazur et al., 1992; Suay et al., 1999; Salvador et al., 2003). Like the well-established rise in C levels in anticipation to a stressful event, also the pre-competition rise in T has been interpreted as an organismic adjustment to anticipated demands within the framework of allostasis (Salvador et al., 2003). This anticipatory effect seems to be relevant for the mental and physical preparation for the confrontation (e.g. psychomotor function, coordination, risk-taking strategies) and some studies suggested a link between the pre-event rise in T and performance (Salvador et al., 2003). For females the few data available are far from conclusive: one study has found a competition anticipatory effect on T for rugby players (Bateup et al., 2002), and two studies have detected decreased pre-competition T levels both in a rowing competition (Kivlighan et al., 2005) and in females playing a video game (Mazur et al., 1997). In our study the anticipatory rise in T levels was only present in players of the team that subsequently lost the game, and it was not correlated either with observed performance or with any of the psychological and behavioral variables that we have measured. These results contradict the postulated function of performance enhancement of pre-game increased T levels previously reported in the literature (Salvador et al., 2003). On the other hand, an anticipatory effect of competition on C levels was detected on both teams. Since a mild increase in C levels is known to prepare individuals for action it could be argued that the anticipatory effect of C played a causal role on performance and concomitantly on the match outcome. However, no correlations were found between pre-game C and observed performance. Again the available data for competition anticipatory C rise in women are conflicting: two studies have reported pre-competition increases in C irrespective of subsequent outcome (Bateup et al., 2002; Kivlighan et al., 2005) and one study did not find the effect (Mazur et al., 1997). More studies specifically designed to address the hormonal anticipation effect in female competitors are needed to clarify the relationship between pre-competition psychological and endocrine variables and their effects on subsequent performance and contest outcome.

This study is the first to find a differential T response to competition between female winners and losers. Players of both teams had similar pre-game T levels, but at the end of the game winners had significantly increased and losers decreased their T levels. These results contrast with the lack of effect of contest outcome on T levels reported in previous female studies (Bateup et al., 2002; Schulteiss et al., 2005; Edwards et al., 2006; van Anders and Watson, 2007). In previous studies that examined the effects of competition outcome in women two found an increase in T levels in both winners and losers of rugby and soccer games (Bateup et al., 2002; Edwards et al., 2006) and in the other two no significant differences in T changes were reported (Schulteiss et al., 2005; van Anders and Watson, 2007). At least three different hypotheses can be advanced to account for these contrasting results.

One possible explanation for the differences in testosterone responses between winners and losers found in the present study is that winners had higher physical exertion than did losers. Unfortunately in the present study we have not taken any measures on physical exertion that would allow us to rule out this possibility. Since C responds to physical exercise (Davies and Few, 1973; Bloom et al., 1976) and increased circulating C levels lead to an acute suppression of plasma T levels (Doerr and Pirke, 1976; Cumming et al., 1983), it has been suggested that a reduction in T following exercise may be caused by cortisol elevations in response to physical exertion (Nindl et al., 2001; Brownlee et al., 2005). The fact that in our study change in C levels did not respond to the competition outcome, rules out the possibility that the post-game difference in T is a byproduct of an exercise-induced differential activation of the HPA between winners and losers. Nevertheless, physical exertion must be examined in future research on the effects of competition on T levels.

An alternative explanation has to do with post-game sampling interval. In the present study post-game saliva samples were collected 30 min after the end of the match whereas in the other two field studies with negative results saliva was collected immediately after the end of the games (Bateup et al., 2002; Edwards et al., 2006). Therefore, it could be argued that the time lag between the end of the game and saliva sampling would have been sufficient to allow for steroid changes in blood to be reflected in saliva in our study, but not in the other two cases. However, Walker et al. (1984) observed a transfer of cortisol from blood to saliva within 1—2 min of the injection of the steroid in dexamethasone-suppressed individuals. Moreover, the negative valence of the victory/defeat probably builds up during the game as the end of the game approaches and depending on the dynamics of the event (e.g. sequence of scored goals). Thus, although one cannot rule out this hypothesis it is the least parsimonious of the three.

Finally, a third possible explanation for this disagreement in the data maybe related to the way the different studies were designed. In our study we have sampled the players of both competing teams in a single event. In the two previous studies that found an increase in both winners and losers (i.e. Bateup et al., 2002; Edwards et al., 2006) the authors sampled the same players when they won and when they lost different (sequential) events. Therefore, there is the hard to meet implicit assumption that the competitive episodes with opposite valences sampled (i.e. positive vs. negative outcome) had a similar salience. However, the "cognitive salience" of each competitive episode is most probably dependent on its consequences for the social status of the individuals: winning a game that determines the champion of that season should be more salient than winning a regular season game. According to this rationale competitive events whose outcomes imply changes in status (ascending vs. descending or missing the opportunity to gain status), such as the final match that we sampled in the present study, are more likely to generate differential hormonal responses to competition than an average game. This salience activation hypothesis is further supported by data on hormonal responses to a real game and a practice session in female soccer players that show a significant increase in post-game but not in post-practice C levels (Haneishi et al., 2007). This same explanation may also account for the mixing results published so far on the relationship between contest outcome and T change in men (Salvador, 2005). Since match cognitive salience seems to play a major role in the moderation of win/loss effects on T changes in women, we strongly suggest that this variable should be considered and measured in future studies (e.g. by comparing finals with mid-season matches, assessing self-reports of match importance, or by manipulating the importance of competition in lab settings).

It should also be noted that the differential T responses to competition between winners and losers addressed in this study are average responses between teams and that individual variation (not addressed here) in psychological traits and/or states may play a role in moderating endocrine responses to wins and losses. For example, it has been shown that power motive predicts T and C changes in response to competition outcome in laboratory settings (Schulteiss et al., 2005; Wirth and Schultheiss, 2006) and that pre-competition T levels modulate C changes after wins and losses (Mehta et al., 2008). Although this type of analysis is difficult to implement in field studies with real life competitive events where samples sizes are usually limited and there are serious constraints on the time available to collect data, variables that monitor individual differences should be included in future studies as much as possible.

The current study detected an association between performance, mood changes and T responses to competition. Pre-game T levels were not good predictors, neither of performance, nor of mood changes during the game. Conversely, observed performance was correlated with mood changes and with subsequent post-game T levels. Together, these data suggest that the observed performance that lead to a positive outcome (winning), with a concomitant change in status (champions of the National league), is associated with a change towards positive mood and an increase in T levels in winners. In fact higher T has been associated with winning and elation after competitive events (e.g. Mazur and Lamb, 1980; Booth et al., 1989), and a lack of T response to winning has been detected in cases where competitors won by luck rather than by personal effort (e.g. McCaul et al., 1992) or when they did not take the event seriously (e.g. Mazur et al., 1997). Even in a study where an association between androgen responsiveness and mood changes was not detected, T levels were positively associated with an indicator of performance in the game and negatively with external attribution to the outcome in winners (Gonzalez-Bono et al., 1999), suggesting that the individual's perception of his contribution to a positive outcome is mediating the T response in this case. Thus, mood changes seem to be consistently associated with T responses to competition. However, the causal link between mood and T changes is not clear and needs further research.

In brief, our results support the challenge hypothesis, that is a reciprocal interaction between androgens and competitive behavior in human females, and suggest that mood changes may play a role as psychological modulators of this response. The function of post-competition T changes in the modulation of subsequent behavior is a promising avenue for future research, as suggested by recent studies that indicate that T changes after a status loss predict the decision to compete again in losers in men (Mehta and Josephs, 2006), and that winning-induced increases in T levels in California mice enhances ability to win subsequent fights (Oyegbile and Marler, 2005).

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Conflict of interest statement

Hereby we declare that none of us (authors) have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence, or be perceived to influence, the work submitted here.

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