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Emotions, immunity and sport: Winner and loser athlete's profile of fighting sport

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ABSTRACT

Several studies have focused on the relationship between hormonal changes and affective states in sporting contexts relating to an agonistic outcome. More recently, pro-inflammatory cytokines have also been successfully associated with affective state modulation.

The aim of this study was to investigate whether athletes who won or lost show different levels of steroid hormones (testosterone and cortisol), pro-inflammatory cytokine IL-1β, or expressions of anger and anxiety during six training fights in seasonal competitions down to the main seasonal competition.

In 25 male kick-boxing athletes (age ± SD, 28.68 ± 5.34), anger states (RS score) and anxiety states (AS score) were assessed by STAXI-2 and STAI-Y, respectively. Cortisol (C), testosterone (T) and IL-1β salivary levels were measured by the ELISA method. The saliva samples were taken in the afternoon, 30 min prior to the start and 30 min from the end of both simulated and official competitions.

The results showed that the RS score, T, T/C ratio salivary levels increased during the season, whereas the AS score, C and IL-1β suggested an opposite trend. Close to an official competition, the RS score, T, T/C ratio and IL-1β salivary concentrations were significantly higher, and then decreased during competition. By contrast, the AS score and C levels significantly increased throughout the official competition.

In addition, significant differences were found for hormones and IL-1β concentrations as well as psychometric assessment close to the outcome of an official match. Athletes who lost showed an higher AS score and C level, while those who won were characterized by an higher level during the pre-competition RS score, T, T/C ratio, and IL-1β. Note that these factors were positively and significantly correlated at the pre-official competition time, while in a linear regression analysis, IL-1β, T and T/C ratio concentrations explained 43% of the variance in the RS score observed at the same time (adjusted $R(2) = 0.43$, ANOVA $P < .05$).

Our data suggest that the beginning of an agonistic event could trigger emotional responses which correspond to different biological processes instead that of a simulated fight. In particular, IL-1β could be a potential new biological marker of anger and the combined measurement of these factors may be a useful way of understanding athletes' change in relation to their performance.

1. Introduction

During the past decades, sporting contests have been considered a reliable domain in which to study emotional states, mainly those relating to aggressive behavior. In particular, dyadic sports, such as martial arts (e.g. judo, karate and taekwondo) (Filaire et al., 2001), wrestling (Robazza et al., 2006), as well as team sports, such as basketball (He et al., 2010), football (Alix-Sy et al., 2008) and rugby (Pesce et al., 2013), have been used as a model of competitive aggression to study the neuro-endocrine response in human agonistic behavior. They appear to provide close parallels with the dyadic encounters usually studied in animals (Archer, 2006; Salvador, 2005; Salvador et al., 2003; Suay et al., 1999).

Steroid hormones such as testosterone (T) and cortisol (C) are strongly associated with the expression and regulation of competitive aggression (Archer, 1991, 2006). C plays a central role in the physiological and behavioral response to a physical challenge or to a psychological stressor, triggering the activation of the Hypothalamic Pituitary Adrenal axis (HPA) and hormone released from the adrenal cortex (Tsai et al., 2011). Studies have also inves-

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tigated the relationship of T with aggressive behavior. It is known that T is an index of dominance during competition for resources, as well as correlating with the activation of behavior to achieve and maintain a high social status (Archer, 2006). T and C levels are dependent on individual emotional responses and coping patterns, and *vice versa* (Mazur, 1976; Parmigiani et al., 2009; Salvador, 2005). Furthermore, the T/C ratio has been previously used to evaluate training responses and predict the performance capacity (Chennaoui et al., 2004; Kraemer and Ratamess, 2005); it is considered to reflect states of anabolism when it is high and states of catabolism or tiredness when it drops by 30% or more (Meusen et al., 2004).

In sports, the assessment of salivary composition can provide a feasible and reliable tool to monitor T and C. Taking a saliva sample is non-invasive and provides a plasma-comparable method (Papacosta and Nassis, 2011). Measuring C in saliva can successfully provide a reference for blood C levels. Significant correlations have indeed been reported between blood and salivary C concentrations at rest (Lippi et al., 2009; Rantonen et al., 2000). In addition, salivary measures of T can provide a reliable indicator of serum or plasma concentrations, as salivary T concentrations were found to be strongly correlated with measures of bioavailable T (Morley et al., 2006). In addition, a recent study by Riis et al. (2014) revealed that within several cytokines analyzed, only IL-1β showed an association between levels in plasma and saliva at rest.

As far as psychological factors are concerned, the examination of athletes' emotional responses to competition has developed into a focal area of sport psychology with a lot of research focusing on anger and anxiety responses by athletes in the run-up to competitive events (Woodman and Hardy, 2003). According to the "Interactional Model of Competitive Stress" (Cerin et al., 2000), the interpretation of cognitive and affective responses to a competitive event are influenced by personal factors and situational factors (approach to a competition) and interaction among them. Hence, different emotions may co-ordinate the response to competition in a different way and therefore influence neuro-endocrine processes in a distinct and specific manner (Moons et al., 2010). In particular, Strahler et al. (2010) showed a significant increase in the cognitive and somatic anxiety scores just before official competitions. Likewise, in the case of anger, many studies have suggested that this component is triggered before a competition (Filaire et al., 2001; Gould et al., 2002; Salvador et al., 2003).

It has been suggested that the expression of these emotions in the athletic environment is influenced by biological variables, which include neurological and endocrine mechanisms (Parmigiani et al., 2009). A role has been proposed for pro-inflammatory cytokines in regulating the bi-directional communication between the nervous and immune systems, clarifying how the network between these systems can affect behavior, mood and cognitive processes, not only in pathological conditions. Of note, the activation of immune system has been successfully related to anxiety and anger (Kiecolt-Glaser et al., 2005; Kraus et al., 2003; Suarez et al., 2004). The immune response could be activated by pathogens or tissue injuries, and also by environmental stimuli such as a competitive event. This last, stimulates the relative neural responses, and finally mediates the activation of the endocrine and immune system (Maier, 2003).

In a previous study, we pointed out by microarray analysis, that the gene expression profile of peripheral blood mononuclear cells (PBMCs) is significantly modulated by the nearing of an official sport event in rugby players. Interestingly, we observed a higher IL-1β mRNA expression by PBMCs and IL-1β plasma levels (Pesce et al., 2013). Again, other studies on animals suggested that this cytokine could act directly at a cerebral level, as well as modulating by peripheral level the activities of various neurotransmitters involved in triggering aggressive behavior (Anisman et al., 2008; Kiecolt-Glaser et al., 2005; Zalcman and Siegel, 2006).

In addition, O'Connor et al. (2009) showed that salivary IL-1β significantly correlated with specific blood oxygenation level-dependent (BOLD) activity of specific brain areas during verbal test. These findings reinforce the idea that peripheral mediators at a local site as mouth is communicated to the brain, and potentially influences the emotional processing.

The aim of this study was to investigate the individual hormonal (T and C) and IL-1β variation, and also the expression of anger and anxiety in kick boxing athletes pre- and post- simulated fights during the training season and towards the main seasonal competition, in order to analyze these variables in relation to the agonistic outcome. In end, psychological assessment was carried out employing psychometric tests to determine levels of anxiety and anger states, while biological variables were measured by taking saliva samples.

It was hypothesized that the real competition elicits several biological and psychological changes. In particular, anger may be an emotion which facilitates the performance in a competitive environment unlike anxiety. Moreover, given the previous results on the rugby players (Pesce et al., 2013), we hypothesize a possible role of IL-1β in the modulation of aggressive behavior.

2. Materials and methods

2.1. Participants

The participants were an opportunity sample of twenty-five male kick boxing athletes. Their ages ranged from 23 to 35 years ($M = 28.68$, $SD = 5.34$). Written informed consent was obtained from all participants before starting the experiment. The study was approved by the Ethical Committee of the "G. d'Annunzio" University of Chieti-Pescara, Italy, and it adhered to the APA ethical standards for the treatment of human participants.

Athletes were in good physical health, without medication, and had no history of psychiatric or somatic disorders. The average Body Mass Index (BMI) based on 25 participants was within the normal range ($M = 22.21$, $SD = 2.11$) and all participants identified themselves as non-smokers. In line with recent findings, the C-reactive protein (CRP) saliva level was measured as a nonspecific marker for inflammation and utilized as an exclusion criterion. The athletes recruited did not show salivary CRP levels > 1600 pg/mL (corresponding to serum CRP levels > 3 mg/L) at any experimental times (Byrne et al., 2013; Ouellet-Morin et al., 2011).

They did not show statistically significant differences in CRP levels between each simulated fight and official match ($P > .05$) and between pre- and post- simulated and official competition. Salivary concentrations ranged from 451.4 to 1518.9 pg/ml (mean ± SD, 969.9 ± 314.3).

2.2. Study design

The current study was framed within a wider project which involved following up the subjects throughout the sports training season in order to analyze their psychobiological adaptation during this period. Each training session consisted of a fight simulation.

To evaluate the influence of the variables on the competition and on its outcome, the following assessment was carried out: 30 min pre- and 30 min post- to the simulated fight (T0–T5), and 30 min pre- and post- (T6) official match, salivary samples were collected for hormonal and IL-1β determination. The warm-up was not considered as part of the match. Simultaneously with the salivary collection, subjects completed a psychological assessment for anger and anxiety.

During the official match, the athletes participated in the first match of The Italian Championship of Kick Boxing, Muay Thai, Savate Shoot Boxe Italian Federation (FIKBMS), in which the match foresaw three rounds lasting two minutes, with an interval of one minute between rounds.

Assessment during the training season (fight simulations) and on the day of the official competition was thus performed as follows:

- 5 months before the official match (T0).
- 4 months before the official match (T1).
- 3 months before the official match (T2).
- 2 months before the official match (T3).
- 1 month before the official match (T4).
- 3 days before the official match (T5).
- Official match (T6).
- 30 min before the beginning of the simulation/official match (PRE).
- 30 min after the end of the simulation/official match (POST).
- Of 25 athletes, 13 won and 12 lost after the official match.

2.3. Psychological assessment

The anger level of each subject was evaluated by the STAXI-2 questionnaire (State Trait Anger Expression Inventory – 2 – Spielberger, 1994). STAXI-2 is a questionnaire made up of 57 items that measure the experience of anger seen as an emotional state characterized by subjective feelings of different intensities (state of anger), the tendency to perceive a great number of situations as annoying or frustrating (anger trait), and finally as an expression of the same (anger/out, anger/in, control/out, control/in). STAXI-2 is composed of six scales: five subscales and an index of anger expressions that can be derived to provide a summarizing measure of the expression and the control of anger. The raw scores of every scale were transformed into standardized scores, using tables of conversion from the scores obtained from the Italian Normative Sample (Comunian, 2004). The questionnaire has also been used in other sports (Robazza et al., 2006).

The anxiety level of each subject was evaluated by the STAI-Y (State Trait Anxiety Inventory – Y – Spielberger et al., 1983). STAI is a self-assessment scale designed to measure both the state and the traits of anxiety. The questionnaire consists of 40 items to which the subject must respond to, in terms of intensity (from ‘hardly ever’ to ‘nearly always’). Items are grouped into two scales: Anxiety State, where anxiety is conceived as a particular experience, a feeling of insecurity, helplessness as perceived damage that can lead to worry or to escaping and avoidance; Anxiety Trait, i.e. the tendency to perceive stressful situations as dangerous and threatening and to respond to various situations with a different intensity.

The scores obtained from the two scales were interpreted using the reference tables in the Italian Normative Sample (Pedrabissi and Santinello, 1989). The reference ranges for the scores are as follows: STAI score >57 high; STAI score between 40 and 57 normal; STAI score <40 low.

2.4. Collection of samples

Saliva samples were obtained at the same time as psychological assessment was undertaken. Saliva was collected in a temperature-controlled room in which the participants washed their mouths with distilled water (30 s $\times$ 3 times). Thereafter, the production of saliva was stimulated by the use of a Chewable cotton swab (Salivette; Sarstedt, Nümbrecht, Germany) at a frequency of about 60 mastications per minute. All samples from each subject were run in duplicate in the same assay. Finally, saliva samples were frozen at -80 °C. The subjects were kept off alcohol consumption and intense physical activity for 24 h, in order to avoid any effects on T, C and IL-1β production before sample collection (Afshar et al., 2015; Bianco et al., 2014; Walsh et al., 2011). In order to determine blood contamination of saliva samples, a Salivary Blood Contamination Enzyme Immunoassay Kit (Salimetrics, State College, PA, USA) which measures the level of transferrin in saliva samples was used. According with the findings by Schwartz and Granger (2004), the athletes with salivary levels of transferrin $\geqslant$5 mg/L were excluded.

The salivary samples were all collected in the afternoon, between 15:30 and 16:00, for minimize the variations that might affect the hormone levels.

2.5. Enzyme linked immunosorbent assay (ELISA)

The salivary levels of IL-1β, T, C, and CRP were assessed by the commercial kit SearchLight Multiplex Assay ELISA (Endogen, Woburn, MA, USA) according to the instructions of the producer, on saliva samples collected within 30 min of the beginning of training, and of the match, and within 30 min towards the end thereof. Plates were scanned using a specialized Charge Coupled Device cooled tool. The integrated density values of the spots of known standards were used to generate a standard curve. Density values for unknown samples were determined using the standard curve for each analyte to calculate the real values in pg/mL. The reproducibility of intra-and inter-assay precision was >90%. Experiments with duplicate values >10% were repeated.

2.6. Statistical analysis

Data were checked for normality and homogeneity of variance and analyzed with parametric or non-parametric data respectively.

The state of anger, state of anxiety, hormonal and IL-1β differences in seasonal training, pre- and post-competition tests, as well as between those who won and those who lost were analyzed by two-way ANOVA for repeated measures (1 between the winner-loser factors; 1 within the pre–post factor) followed by the Bonferroni post hoc test.

Correlations between T, C, T/C ratio, IL-1β levels and anger state and anxiety scores were performed using nonparametric Spearman. We previously analyzed the linearity of correlation by examining scatterplots among T, C, T/C ratio, IL-1β, and psychometric scores. Again, RS scores were regressed on the T, T/C ratio and IL-1β to examine the co-existing effects of these biological variables on anger. Likewise, AS scores were regressed on C to examine the relative effect on anxiety.

Statistical analyses were performed using SPSS 19.0 statistic (SPSS Inc., Chicago, IL, USA) for Windows (IBM). Results are described as means ± SD for each assessment performed in triplicate. The level of a statistically significant difference was defined as $P < .05$.

3. Results

3.1. Psychometric scores during season

Anxiety was directly assessed using STAI-Y. In 25 athletes, the anxiety state (AS score) was measured 30 min pre- and post- the seasonal training competitions (T0-T5) and as well as the main seasonal competition (T6). At the same time, the anger state (RS score) was assessed using STAXI-2. The results reported in Fig. 1A show that the AS score followed a decreasing trend over time during simulated fights. The repeated measures ANOVA analyses showed that the effect within subjects was significantly decreased from T0 to T5 in all athletes ($F_{(11,\ 264)} = 17.06$, $P < .0001$). In particular, when we considered point by point contrast within subjects, the AS score

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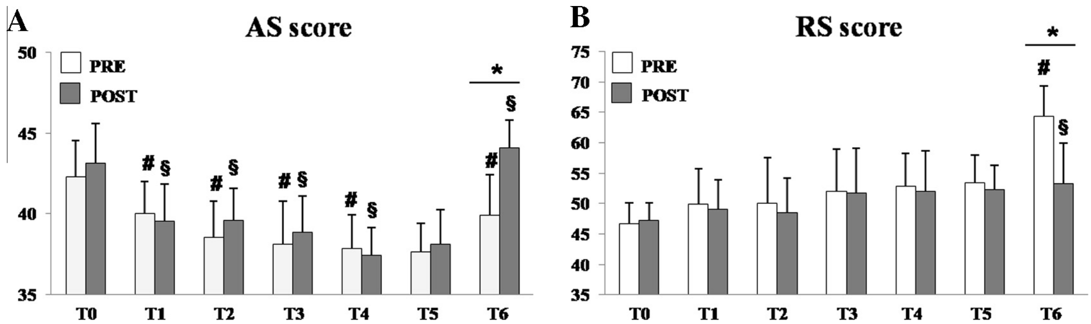

Fig. 1. Psychometric evaluations of anxiety and anger. Significance within RS score and AS score from T0–T5 and T0–T6 was obtained by repeated measures ANOVA, considering within subjects effect. (A) The anxiety state score was assessed by STAI-Y. AS score significantly decrease within simulated fights (T0-T5), $F_{(11,\ 264)} = 17.06$, $P < .0001$. B) The anger state score was assessed by STAXI-2. Data are expressed as means ± SD ($n = 25$). #Indicates a significant difference vs. previous pre-competition time points (repeated measures ANOVA, within subjects contrast); §indicates a significant difference vs. previous post-competition time points (repeated measures ANOVA, within subjects contrast); *$P < .05$ indicates a significant difference between 30 min pre- and post-competition values (repeated measures ANOVA, within subjects contrast).

showed a significant increase throughout the main competition (T6) ($F_{(1,\ 24)} = 37.48$, $P < .0001$), but that score did not increase across any seasonal training competitions. However, all AS scores were in the low or normal range.

We again found that, when we considered the RS score from T0 to T6, the Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi 2 = 49.73$, $P < .01$, and therefore, a Greenhouse-Geisser correction was used ($F_{(3.81,\ 91.44)} = 13.56$, $P < .0001$). The RS score significantly increased only 30 min pre- the official competition (T6). Nonetheless, during this moment we observed a peak value, after which the RS score reverted to the previous value ($F_{1,\ 24} = 35.79$, $P < .0001$). We did not observe any variation during season and between pre- and post- training competitions (Fig. 1B).

3.2. Biological variables during season

Comparisons of simulation fighting and the official competition values of salivary C, T, T/C ratio and IL-1β are reported in Fig. 2A–D. We found through analysis of variance with repeated measures ANOVA, that the salivary concentrations of C significantly decreased during the season starting from the beginning of training (T0–T5) ($F_{11,\ 264} = 27.07$, $P < .0001$). In considering within subjects contrast, the salivary C value was significantly higher pre-

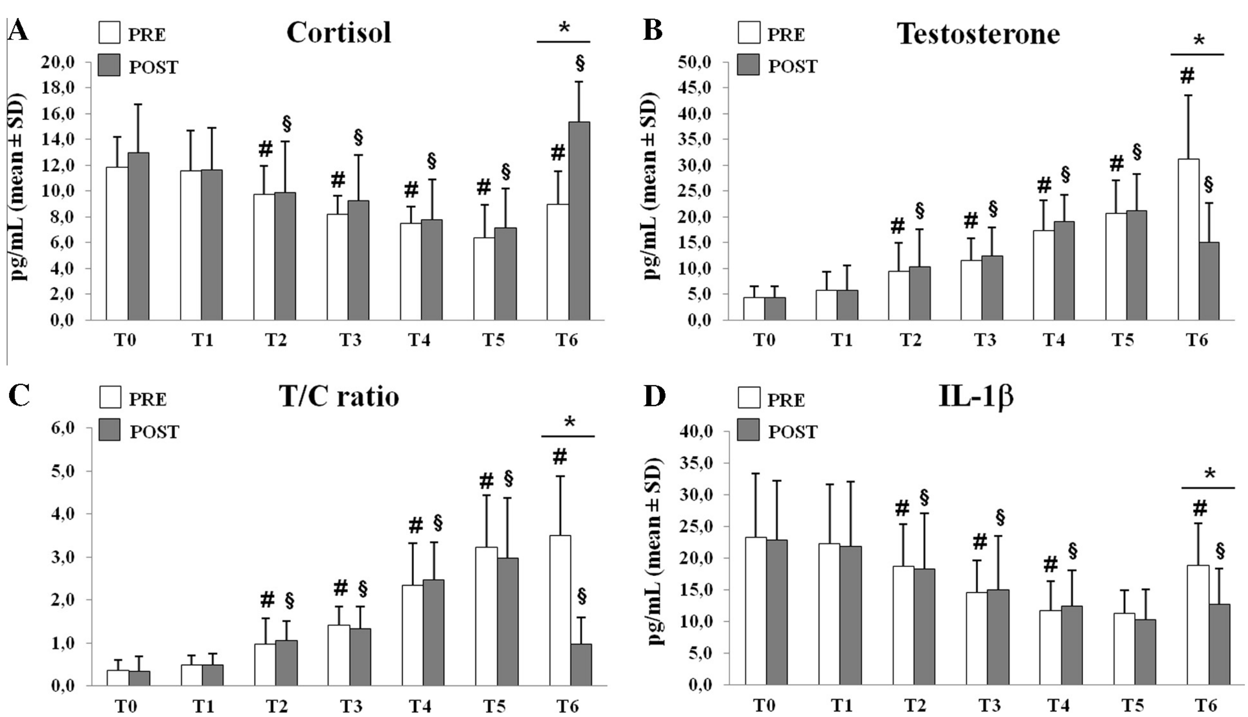

Fig. 2. The ELISA measurement of salivary biological markers in kickboxing athletes during training seasonal competitions performed at different times (T0–T5) until the official competitive event (T6). Significance within T0–T5 and T0–T6 was obtained by repeated measures ANOVA, considering within subjects effect. (A) Salivary cortisol concentration decreases across all simulated fights, from T0 until T5 ($F_{11,\ 264} = 27.07$, $P < .0001$). (B) Salivary testosterone concentration increases from T0 to T6 ($F_{13,\ 312} = 35.46$, $P < .0001$). Its level significantly changed during official competition (T6) ($F_{1,\ 24} = 39.22$, $P < .0001$), (C) T/C ratio shows similar trend to those observed for *T*. Its value significantly increase from T0–T6 ($F_{13,\ 312} = 13.80$, $P < .0001$). The value of T/C ratio significantly decreases across the official competition (T6, pre- vs. post-competition, $P < .005$). (D) Salivary IL-1β concentration significantly decrease within simulated fights (T0–T5) ($F_{11,\ 264} = 24{,}23$, $P < .0001$). Its value increases 30 min pre- the official match and decreased during the competition ($P < .01$). Data are expressed as means ± SD ($n = 25$). #Indicates a significant difference vs. the previous pre-competition time point (repeated measures ANOVA, within subjects contrast); §indicates a significant difference vs. the previous post-competition time point (repeated measures ANOVA, within subjects contrast); *indicates a significant difference between the 30 min pre- and post-competition values (repeated measures ANOVA, within subjects contrast).

official competition when compared to previous simulated pre-competition (T5) ($F_{1,\ 24}$ = 5.48, P < .05) and post-official competition when compared to previous simulated post-competition (T5) ($F_{1,\ 24}$ = 12.19, P < .005) (Fig. 2A). On the other hand, salivary T significantly increased during season (T0–T6) ($F_{13,\ 312}$ = 35.46, P < .0001). Its levels significantly reduced strictly after the main seasonal competition ($F_{1,\ 24}$ = 39.22, P < .0001), but we did not observe variations across the simulation fighting competitions (Fig. 2B). According to Fig. 2C, the T/C ratio increased during the season (T0–T6) ($F_{13,\ 312}$ = 13.80, P < .0001). The official competition induced a reduction in T/C values. Indeed, at T6 the post-match values were significantly lower than the pre-match ($F_{1,\ 24}$ = 14.98, P < .005).

Finally, salivary IL-1β showed a significant negative trend over time from T0 to T5 ($F_{11,\ 264}$ = 24,23, P < .0001). Close to the official competition, the salivary IL-1β concentrations significantly increased 30 min pre-, and then suddenly decreased 30 min post-competition ($F_{1,\ 24}$ = 10.78, P < .01) (Fig. 2D).

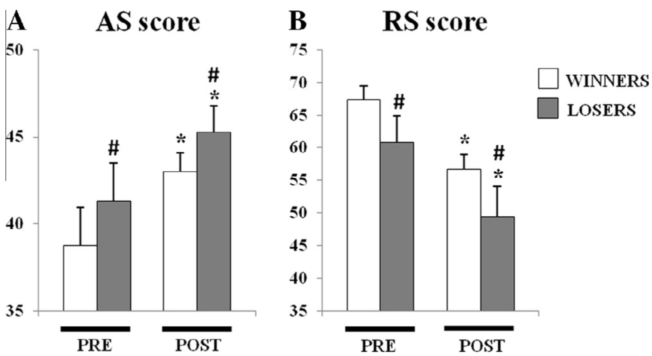

Fig. 3. Psychometric evaluations of anxiety and anger in those who won (n = 13) and those who lost (n = 12) 30 min pre- and 30 min post- the official competition. The anxiety state score (AS score) was assessed by STAI-Y. The anger state score (RS score) was assessed by STAXI-2. Those who lost showed higher anxiety scores pre- and post- the official match more than those who won. At the same time winner athletes show significantly higher RS score than losers. Significance was obtained with repeated measures ANOVA (1 between the winner-loser factors; 1 within the pre-post factor) followed by the Bonferroni post hoc test. Data are expressed as means ± SD. *P < .05 vs. pre-competition level; #P < .05 vs. winner athletes.

3.3. Psychometric scores and biological variables in winners and losers

When the focus was on those who won and those who lost, winners and losers, we did not observe any variation during seasonal friendly competition (data not shown). As shown in Fig. 3A, the analysis of our data with repeated measures ANOVA, suggest that athletes who lost, were characterized by significantly higher AS score 30 min pre- the official competition ($F_{1,\ 23}$ = 15.41, P < .005). Interestingly, winners showed significantly higher RS score than those who lost throughout the same competition ($F_{1,\ 23}$ = 19.04, P < .005) (Fig. 3B). In general, our analysis mainly highlighted that those who won and those who lost are characterized by differing levels of emotional states at the beginning of the official match.

Athletes that won showed significantly lower levels of C throughout the official competition than athletes that lost ($F_{1,\ 23}$ = 21.45, P < .0001) (Fig. 4A). Furthermore, one notes that the T, T/C ratio and IL-1β salivary levels were higher in athletes that won rather than those that lost (respectively, $F_{1,\ 23}$ = 7.5, P < .05; $F_{1,\ 23}$ = 7.9, P < .05; $F_{1,\ 23}$ = 12.03, P < .005) (Fig. 4B–D).

3.4. Associations between psychological and biological variables

Furthermore, we analyzed the possible correlations that occur between the biological variables, RS and AS scores. We considered

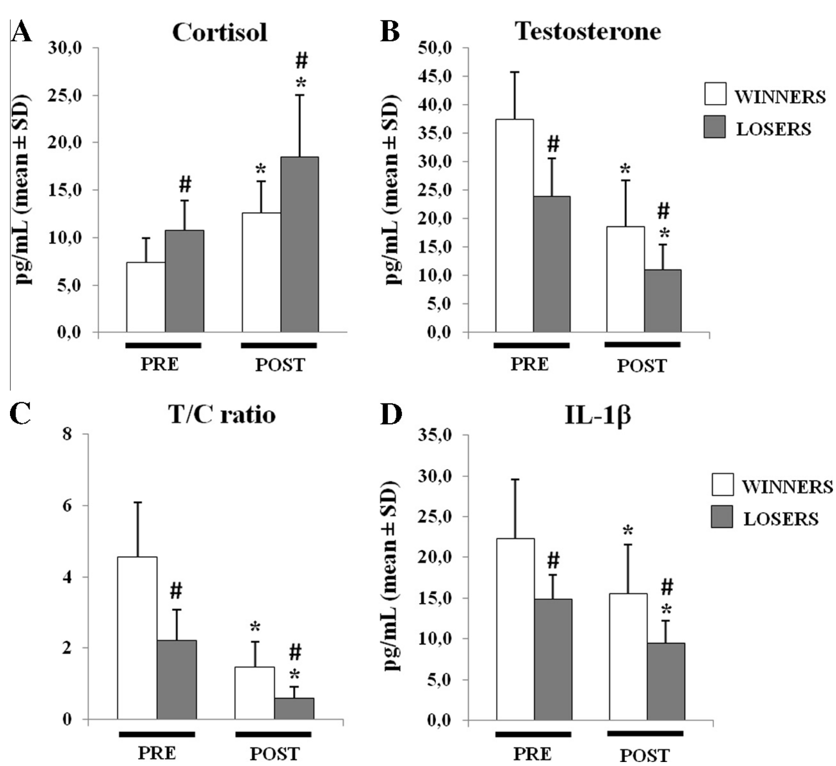

Fig. 4. The ELISA measurement of Testosterone (T), Cortisol (C), T/C ratio and IL-1β in winner (n = 13) and loser (n = 12) athletes 30 min pre- and 30 min post- the official competition. Salivary levels of T, C, T/C ratio and IL-1β were assessed by the ELISA method. Those who lost, showed higher C salivary levels pre- and post- the official match than winners. Remarkably, athletes who won, showed a significantly higher T, T/C ratio and IL-1β salivary levels than losers. Significance was obtained with repeated measures ANOVA (1 between the winner-loser factors; 1 within the pre-post factor) followed by the Bonferroni post hoc test. Data are expressed as means ± SD. *P < .05 vs. pre-competition level; #P < .05 vs. winner athletes.

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Table 1
Spearman rho (ρ) correlations between Age, AS score, RS score, Testosterone (T), Cortisol (C), T/C ratio and pro-inflammatory cytokine IL-1β salivary levels in male kick-boxing athletes (n = 25). The psychometric assessment and biological evaluations were performed 30 min before the main seasonal competition.

	Age	AS score	RS score	Cortisol	Testosterone	T/C ratio	IL-1β
Age	–	−.117	.303	.009	−.061	.014	.371
AS score		–	−.592*	.545*	−.251	−.432	−.192
RS score			–	−.428	.581*	.664*	.525*
Cortisol				–	−.549*	−.711*	−.331
Testosterone					–	.796*	.558*
T/C ratio						–	.586*
IL-1β							–

* $P < .05$

the values obtained 30 min pre- the official competition, because all variables were significantly modulated at this time. In particular, the RS score was significantly increased only at the pre-official competition. Table 1 shows the bivariate correlations obtained. The analysis showed that age was not related to participants' RS and AS scores as ascertained for the time point considered. Interestingly, the salivary level of IL-1β significantly and positively correlated with RS score, T and T/C ($\rho = .525$, $\rho = .558$ and $\rho = .586$, respectively; $P < .05$). In addition, RS scores were positively correlated with salivary concentrations of T and T/C ratio ($\rho = .581$ and $\rho = .664$, respectively; $P < .05$). AS scores were positively correlated with C salivary levels ($\rho = .545$, $P < .05$) and negatively related to scores assessing anger ($\rho = -.592$, $P < .05$). Furthermore, C proved negatively associated with T levels ($\rho = -.549$, $P < .05$).

Table 2 presents the multiple regression analysis results. RS score, showing significant zero-order correlations with the biological variables was regressed on T, T/C ratio, IL-1β salivary levels in a simultaneous regression analysis, using age as control variable. The overall model was significant, $F_{(4,\ 20)} = 3.51$, ANOVA $P < .05$, accounting for 43% of the RS score variance.

The AS score was regressed on C, using age as control variable. The analysis shows that C account for 26% of variance of cases for AS score, $F_{(2,\ 22)} = 3.98$, ANOVA $P < .05$.

4. Discussion

The aim of this study was to evaluate the psychobiological response to a competitive event and whether these factors may affect performance. We duly assessed the temporal trend state levels of anger and anxiety in 25 male kick-boxing athletes. At the same time, we monitored salivary levels of testosterone, cortisol, as well as their ratio and IL-1β as endocrine and immune activation markers. We focused on pre- and post- simulated and official competitions, to identify potential asymmetric factors that may be predictors of outcome.

As in our previous findings, we observed that an on-coming official competition, triggers feelings of transient anger (Pesce et al., 2013). Moreover, STAXI scores of RS were significantly higher in athletes that won as opposed to those that lost at the official pre-competition stage, compared to earlier simulate pre-competition stages, and then suddenly restored at the post-competition period (30 min after).

Table 2
Regression analysis summary for biological variables predicting RS score and AS score. RS score was regressed on Age, T, T/C ratio and IL-1β (n = 25; $F(4, 20) = 3.51$; $P = .049$). AS score was regressed on Age and C (n = 25; $F(2, 22) = 3.98$; $P = .047$). RS score = Rage State score, AS score = Anxiety State score, T = Testosterone, C = Cortisol.

Dependent variable	Independent variables	R^2	R^2 adjusted	F	ANOVA (p)
RS score	T, T/C ratio, IL-1β, Age	.584	.428	3.510	.049
AS score	C, Age	.399	.259	3.983	.047

Concerning salivary T levels, we observed an increasing seasonal trend over time in all athletes, and significantly increased pre-official match where we reported a peak in the value of this hormone. The increase was significantly higher in athletes that won. At the same time, the levels of T positively correlated with the anger state scores. These data are in accordance with several studies suggesting that increases in T levels are evident following resistance exercise, where acute increases in anabolic hormones are essential for muscular adaptation (Kraemer and Ratamess, 2005). It has also been shown that the maximal salivary T levels of each individual were linked to extra gains in strength, and several studies have indeed suggested that a relationship exists between measures of salivary T and strength-related performance capacity (Beaven et al., 2008; Crewther et al., 2009).

Again, aggression research in human studies have revealed an interesting property of T dynamics: it fluctuates rapidly causing reactive aggression in response to stimuli. Studies on a limited number of subjects have shown a positive relationship by T with aggressive phases of the game in judo contests and hockey players (Batrinos, 2012). Hence, the highest scores in RS and increases in the levels of T in winners might be useful by the way of physical and mental preparation for a competition. In agreement with several studies (Parmigiani et al., 2009; Salvador et al., 2003), these results seem to suggest a link between the pre-event anger in terms of T and performance outcome.

The anxiety state (AS score), on the other hand, follows an inverse sporting season temporal trend, decreasing from three months (T2) to three days (T5) to start of official competition (T6) and then increasing significantly during this match both in group that won and group that lost. C shows a similar temporal trend and positively correlates with the AS score. Closely to the official competition, these findings are in agreement with other work which suggests an acute increase in salivary C following a rugby match, and after restored within 4 h (Elloumi et al., 2003). Likewise, salivary C concentration displayed an acute increase following a competitive kickboxing match (Moreira et al., 2010), a wrestling competition (Coelho et al., 2010), and after a treadmill Bruce test in active male participants (Rahman et al., 2010).

Nonetheless, these results also suggest that C is not strictly linked to an agonistic activity but is affected by the cognitive and emotional perception of the event (Parmigiani et al., 2009). Thus, increases in C and allows the organism to adapt to environmental demands. Activation of HPA, with a consequent release of C by the adrenal cortex, thereby influences behavioral responses to physical and/or situational changes (Filaire et al., 2009). Furthermore, our data are in accordance with the finding that increased exercise-induced C's response is usually characterized by a hormonal adaptation to exercise (Viru and Viru, 2004).

Our data also shows how the saliva T/C ratio significantly increased during season. Close on competitions, we did not observe any variations across simulated fights. However this ratio significantly decreased across official competition and notably, athletes that won were characterized by higher values than athletes that lost during this match. We first showed that this ratio was positively associated with a score assessing anger strictly to an official competitive event.

A previous study Elloumi et al. (2008) described a decrease in the T/C ratio that was associated with a decrease in physical performance and increase of states of tiredness.

Concerning IL-1β, minimum salivary levels were recorded 3 days before the official match (T5). It is interesting to note that IL-1β values rose again close to this competition when future winners showed significant higher levels of cytokine than those who lost. At this time IL-1β was positively correlated with the T, T/C ratio and RS score.

Several studies on animals have previously emphasized a role for IL-1β in brain regions associated with the expression of aggressive behavior, clarifying its activity (i.e. endogenous neuromodulator) (Siegel et al., 2007). Our data suggested a new potential role for this cytokine also in the modulation by peripheral level the activities of neurotransmitters involved in aggressive behavior. We previously observed higher IL-1β plasma levels in correlation with state anger score (Pesce et al., 2013). However, it is plausible also suppose a role for salivary fraction of this cytokine in modulating aggressive behavior. Recent results, by Riis et al. (2014), indeed purposed a significant correlation between salivary and plasma level of IL-1β, and O'Connor et al. (2009) interesting showed the positive correlation between BOLD activity of specific brain areas and salivary IL-1β in a verbal test. To this end, our results newly suggested significant association between IL-1β and state anger score, supporting that mediators at a local site as mouth may indirectly influence emotional processing and changes in motivational state.

Drawing some early conclusions, our results suggest that anger and anxiety may be characterized as motivational behavior which triggers different endocrine and IL-1β salivary release (Moons et al., 2010). Our data are in accordance with findings that propose a role for C in anxiety responses; they also argue the implication of T, T/C ratio and IL-1β in the expression of anger in a sporting context. In particular, T, C, T/C ratio, IL-1β salivary concentrations and anxiety and anger responses are influenced by real sport competition rather than a simulated fight. Indeed, biological variable concentrations and emotional scores change significantly close to the official match. Previous studies showed anticipatory changes in hormone levels of T and C and in the responses of anxiety and anger before a sporting event, emphasizing how the competition demands several physical and psychological changes compared to other exercises of an equivalent duration (Elloumi et al., 2008; Filaire et al., 2001). Note that we first suggest that IL-1β may be a possible asymmetry factor in agonistic outcome.

Concerning trends observed during the training season, our data were in accordance with the substantial evidence that IL-1β is a powerful stimulus of cortisol and AS score, because these values change in parallel (Gądek-Michalska and Bugajski, 2010; Goshen and Yirmiya, 2009). However this finding thus not appears to be the case during the official competition. The positive correlation between the Anger score, the T, T/C ratio and IL-1β levels, and their higher levels close to the official competition in all athletes, suggest that these factors could play an important role in competitiveness, agonistic behavior and impulsiveness, as well as performing an important function in regulating body homeostasis in response to exercise. In conclusion, these findings may help to explain the already evident connection between anger expression and the activation of the immune system (Mommersteeg et al., 2008; Pesce et al., 2013; Ranjit et al., 2007; Suárez et al., 2002, 2003; Tsuboi et al., 2008). More general, aggressive behavior is important again to maintain the dominant status such as access to female and food, protecting young individuals fighting off predators and competing with con-species for resource and territory. For this reason high immune-competence could be a compensatory mechanism to protect aggressive individuals against their high risk of exposure to immune stimuli (Granger et al., 2000). In line with this, dominant status would be related to a low risk of disease and a rapid recovery from illness (Archie et al., 2012).

Aggressive behavior may occur even in non-pathological conditions, as in healthy subjects undergoing environmental stress. Thus, one can suppose that IL-1β may play a key role in the initial phase of the response, when the body is predisposed to reacting, consequently enhancing aggressive behavior (Pesce et al., 2011). If not, the presence of repeated stimuli (e.g. physical exercise during season training), could induce the development of an endogenous micro-environment, also due to the presence of other cytokines of muscle origin (i.e. IL-6 and TNF-α) (Pedersen and Fischer, 2007), in which IL-1β elicits anxiety rather than potentiating aggressive behavior (Bhatt et al., 2008; Siegel et al., 2007). This process appears to be less pronounced when athletes were adapted to a training workload at the end of season, and in which they were indeed characterized by a significantly higher T/C ratio. This could contribute in explaining the increased levels of IL-1β found in our sample close to the official match; the competition forms a challenging situation which usually stimulates higher psycho-physiological responses in the participant. This cytokine may function as an integration point for immune and neuroendocrine signals, through the stimulation of the sensory afferents of the vagus nerve as well (Goshen and Yirmiya, 2009).

We proposed a frame in which individual sport contact athletes experienced offensive anger characterized by approach motivation and associated with high levels of T and IL-1β and lower levels of C, instead that of defensive aggression that is fear motivated and related to a high C concentration (Kalin, 1999). In accordance with this, in those who won the process is amplified. Our data are in agreement with a previous study suggesting that in healthy non-violent men stronger immune responses were linked to high T levels and low C levels (Rantala et al., 2012). This may be reflected in the hormone profile as increases in T which, in turn, may produce falls in C reducing its immunosuppressive effects.

Finally, it is possible to regard an upcoming sporting event as a stimulus which induces anxiety and anger, and these conditions are associated with an increase in hormonal (T, C, and T/C ratio) and IL-1β salivary levels. Over and above the role of hormones T and C in relation to sports performance, which have been widely studied in international literature, one should emphasize the increased levels of salivary IL-1β before an official competition, and their significant and positive correlation with anger state scores. However, we were not able to extend our findings to other pro-inflammatory mediators and at this moment we could restrict our observations to salivary IL-1β, due to the poor correlation observed for other cytokines with plasma level (Riis et al., 2014).

Future studies are needed to better define the role of the IL-1β and other cytokines in relation to the expression of anger.

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