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ORIGINAL ARTICLE

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Behaviour of saliva cortisol [C], testosterone [T] and the T/C ratio during a rugby match and during the post-competition recovery days

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Abstract Competition is a more demanding situation than other strenuous exercise of equivalent duration; it results in stronger physiological changes. The object of this study was to get information on the duration of the recovery period by measuring changes of saliva cortisol [C], testosterone [T] and their ratio T/C in a group of international rugby players ($n = 20$) during the week following a rugby match (6 days). Using non-invasive saliva assays, we were able to take samples during the day of competition and the post-competitive days. Hormone levels were assayed with a routine in-house radioimmunoassay (RIA) method. Throughout the competition, C levels increased sharply (about 2.5-fold compared resting values) and returned to basal values within 4 h. Conversely, the T level decreased slightly. During the recovery period, C levels were lower and T levels were higher than basal values, resulting in a very high T/C ratio until the 5th day. This high post-competitive T/C ratio phase is probably required to restore the break-down of homeostasis induced by the very hard mental and physical strain associated with a rugby match. Thus, a period of 1 week recovery appears to be the minimal duration between two competitions.

Keywords Competition · Cortisol · Recovery
Rugby · Saliva · T/C ratio · Testosterone

Introduction

The effects of physical exercise on cortisol [C] and testosterone [T] levels have been widely documented, with particular reference to the fact that [C] varies in the opposite direction to [T], thus, showing that physical exercise produces an imbalance between the anabolic hormone of testicular origin and the catabolic hormone of adrenal origin (Adlercreutz et al. 1986). Consequently, many studies have used the T/C ratio to emphasize in a clearer way the variations in these two hormones during the training season. This ratio diminishes when the training load and the performances capacities increase (Hoogeveen and Zonderland 1996; Mujika et al. 1996), conversely, below a certain threshold, it may indicate a state of overtraining (Adlercreutz et al. 1986; Häkkinen and Pakarinen 1991; Vervoorn et al. 1991). This imbalance may also be caused by a situation of stress, resulting from the mental strain (Bolm-Audorff et al. 1986), and, especially in sports competitions, from the coupling of mental and physical strain (Aubets and Segura 1995; Banfi et al. 1993; Filaire et al. 1997; Guezennec et al. 1992; Passelergue et al. 1995; Viru et al. 1992).

Another field which has been poorly investigated in sport concerns post-competition recuperation, although this domain is largely concerned in managing sport training. Some studies have shown that, despite a state of staleness during the days following an endurance competition, C levels were below resting values (Lac and Berthon 2000; Maron et al. 1977). In a recent study by our laboratory on wrestlers, a sport characterized by explosivity (Passelergue and Lac 1999), we found elevated T levels during the post-competitive period. In all cases, T/C ratios were elevated (Lac and Berthon 2000; Passelergue and Lac 1999). Thus, the aim of this study was to follow the changes in the salivary levels of C and T in rugby players during and after an international match, in order to see if some changes occur in the balance of these two hormones, and, if there are any, to control the duration of this imbalance. Rugby is very well adapted for this purpose since the duration of a rugby match (90 min including a 10-min half-time) pushes players to exhaustion, and the situation of an international contest leads to a maximal stake, thus to the highest mental constraint possible in the players.

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Methods

Subjects

Twenty well-informed consenting rugby players of the Tunisian team entered the study. All of them participated in the match either in the starting line-up or as a substitute. They were between 20 and 34 years old. They trained in their clubs three to four times per week, that is to say 8–10 h/week plus a 1.5-h match. During the week preceding the match, they took part in two daily training sessions from Monday to Saturday, that is to say 3–4 h per day. The competition took place on the Sunday between 2 pm and 3.45 pm. The main characteristics of the subjects are reported in Table 1.

Hormone measurements

For hormone measurements, samples of saliva were collected in three different situations as represented in Fig. 1:

- Three saliva samples during a rest day (without training) which took place 2 months before the competition, in order to get reference values: at 8 am, 4 pm and 8 pm.
- Four saliva samples during the day of competition at 8 am, just after the match at 4 pm, 2 h later and at 8 pm.
- Twelve saliva samples during the post-competitive period which lasted 6 days, (from Monday to Saturday because another match took place on the Sunday). Samples of saliva were collected every day at 8 am and 8 pm (Fig. 1).

Hormone levels were assayed in our laboratory in accordance with the previously described method (Lac et al. 1993). Briefly, after thawing, saliva samples were centrifuged to make them clear, and steroids were extracted in aliquots with 10 vol of diethyl-ether; dry extracts were solved in a phosphate buffer and the immunoassay done. The performances were – sensitivity: 15 pg, precision: 10.5%, intra-assay reproducibility: 6.1% for C and 20 pg; 9.3%, 10.9% for T. All the samples were done in the same series in order to avoid possible inter-assay variability.

Statistical analysis

Mean hormone levels are presented with the standard error. The analysis of variance (ANOVA) was used for comparisons of repeated measurements. Post-hoc *t* test for matched series was used to assess the statistical significance whose threshold was chosen at $p < 0.05$.

Results

Table 1 represents some anthropometrics characteristics of the subjects: mass (kg), height (cm), percentage of fat mass 6%).

Table 1 The main biometric characteristics of the participants ($n = 20$)

Age (years)	Height (cm)	Weight (kg)	Body fat (%)
25.2 (4.2)	180.0 (5.4)	88.0 (2.9)	13.8 (5.4)

Saliva C and T levels and T/C ratio measured in three different situations (rest, competition and post-competitive recovery period) are presented in Figs. 2, 3 and 4. C and T levels measured during the rest day served as references.

During the rest day, the C values ranged from 16.99 ± 1.37 at 8 am to 5.91 ± 0.55 nmol.l^{-1} at 8 pm in agreement with the circadian rhythm of cortisol.

During the day of competition, C levels recorded at 8 am and at 8 pm were comparable to the reference levels measured during the rest day at the corresponding time. The levels recorded at the end of the match (4 pm) were two-fold higher (148%, $p < 0.001$) than during the rest day at the corresponding time. There were no differences 4 h later at 8 pm. The C values recorded at 8 am during the post-competitive recovery period were statistically lower during the first 4 days (from Monday to Thursday; $p < 0.05$) than during the rest day and those recorded on the 5th and 6th days of the same period. In the same way, C levels measured in the evening of the post-competitive recovery period (8 pm) followed the same profile; they were statistically lower ($p < 0.05$) than the 8 pm value of the rest day on the 3rd recovery day only (Fig. 2).

T levels recorded at 8 pm during the resting day were lower than the values measured the same day at 8 am ($p < 0.05$) and at 4 pm ($p < 0.001$). During the day of competition, T levels recorded at 8 am and at 8 pm were comparable to the reference levels measured during the rest day at the corresponding time. The competition induced a statistical decrease of T values at 4 pm (−16%, $p < 0.05$) against the corresponding reference value. There were no differences between the 8 pm post-match value and the reference value. During the post-competitive recovery period, 8 am T levels were statistically higher on Monday, Wednesday ($p < 0.05$) and Saturday ($p < 0.01$) than the 8 am value measured on the rest day. T levels measured at 8 pm followed the same profile. They were higher ($p < 0.05$) on Monday and Tuesday than rest day value at 8 pm. They rose again on the 6th day and were statistically ($p < 0.01$) higher than the 8 pm rest value.

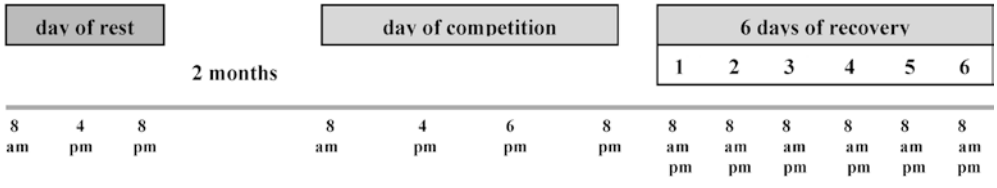

Fig. 1 Schedule of the saliva sampling (samples were collected at each hour mentioned)

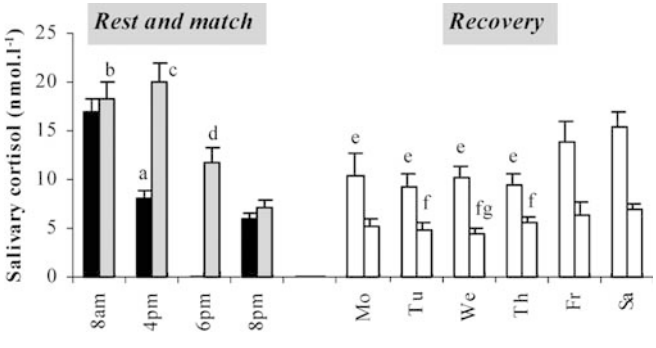

Fig. 2 Concentration of salivary cortisol during the rest day (*black bars*), during the competition day (*grey*), during the post-competitive recovery days at 8 am and 8 pm (*white*). Rest 8 am vs. rest 4 pm $p < 0.001$ and vs. rest 8 pm $p < 0.001$. Rest 4 pm vs. rest 8 pm $p < 0.05$. [a]Rest vs. match 4 pm $p < 0.0001$. [b]Match 8 am vs. 6 pm $p < 0.01$ and vs. 8 pm $p < 0.001$. [c]Match 4 pm vs. 6 pm $p < 0.001$ and vs. 8 pm $p < 0.0001$. [d]Match 6 pm vs. 8 pm $p < 0.05$. [e]Recovery 8 am vs. rest 8 am and vs. last day of recovery 8 am $p < 0.05$. [f]Recovery 8 pm vs. last day of recovery 8pm. [g]Recovery 8 pm vs. rest 8 pm $p < 0.05$

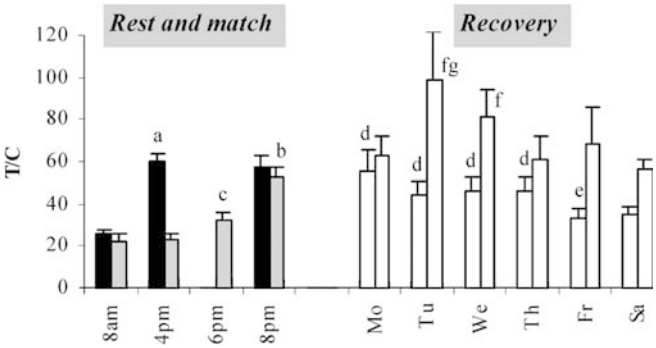

Fig. 4 Changes in T/C values during the rest day (*black bars*), during the competition day (*grey bars*) and during the post-competitive recovery period at 8 am and 8 pm (*white bars*). Rest 8 am vs. rest 4 pm and 8 pm $p < 0.001$. [a]Rest 4 pm vs. match 4 pm $p < 0.001$. [b]Match 8 pm vs. match 6 pm $p < 0.05$, and vs. match 8 am and 4 pm $p < 0.001$. [c]Match 6 pm vs. match 8 am and 4 pm $p < 0.05$. [d]Recovery 8 am vs. rest 8 am $p < 0.05$. [e]Recovery 8 am vs. first day of recovery $p < 0.05$. [f]Recovery 8 pm vs. rest 8 pm $p < 0.05$. [g]Recovery 8 pm vs. first and last day of recovery 8 pm $p < 0.05$

The 8 am and the 8 pm T/C ratios measured during the day of competition were similar to those recorded during the rest day at the corresponding times. The post-match level (4 pm) was significantly lower than the 4 pm rest day value (−62%, $p < 0.001$).

The T/C ratio increased between 8 am and 8 pm: (+43%, $p < 0.05$) at 6 pm and (+133%, $p < 0.001$) at 8 pm.

During the four first days of the recovery period, the 8 am T/C ratios were significantly higher than the 8 am value of the rest day. The 8 pm values were significantly higher than 8 pm reference values on Tuesday and Wednesday.

Discussion

The present study was conducted on a group of specifically rugby-trained males. All of them were submitted to the same training regime over the sporting season, that is to say 8–10 h weekly intensive training plus one match per week, which occurred generally on a Sunday. All saliva samples were collected at the same time during each sampling session in order to avoid the circadian rhythm effect.

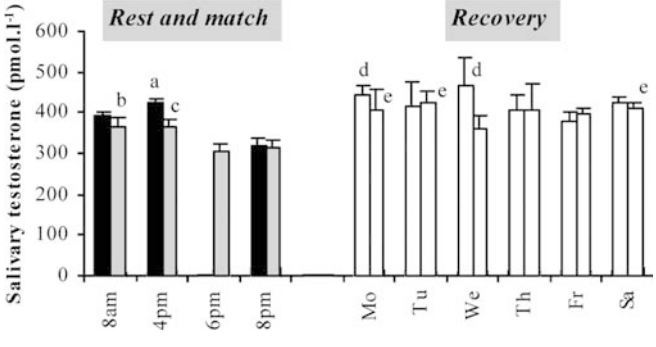

Fig. 3 Concentration of salivary testosterone during the rest day (*black bars*), during the competition day (*grey bars*) and during the post-competitive recovery period at 8 am and 8 pm (*white bars*). Rest 8 am vs. rest 8 pm $p < 0.05$; rest 4 pm vs. rest 8 pm $p < 0.001$. [a]Rest 4 pm vs. match 4 pm $p < 0.05$. [b]Match 8 am vs. match 6 pm $p < 0.01$. [c]Match 4 pm vs. match 6 pm and 8 pm $p < 0.05$. [d]Recovery 8 am vs. rest 8 am $p < 0.05$. [e]Recovery 8 pm vs. rest 8 pm $p < 0.05$

The match was played outdoor at a temperature of 19°C. It lasted 90 min including a 10-min half-time break. Rugby is a collective sport (15 players) where short sequences of intensive effort of 5–15 s duration alternate with 20–40 s periods of rest (Nicholas 1997). This characteristic of intermittence is reproduced during the 80 min of effective played time. As in soccer, a good aerobic background conditions the total distance run during the match. A rugby match, especially at a high sporting level as was the case here, corresponds to very intensive physical strain, with maximal energy expenditure within the time played; it also includes frequent impact against opponents and thus, it requires a constant self-controlled aggressiveness. Moreover, in this situation of international contest, it corresponded to a maximal stake, a fact which is known to act positively on the C level (Passelergue et al 1995).

The conditions of this competition, placing high physical and mental strain on a large number (20) of high level sportsmen, complied well with the aim of this study, which was to analyse the C and T response to a major competition and during the post-competitive recovery period. This hormone follow-up with repeated sampling including the competition phase was made possible using saliva samples, a non-invasive method for the assays of cortisol and testosterone (Kirschbaum and Hellhammer 1989; Lac et al. 1993; Vining et al. 1983).

Cortisol

Saliva C levels on the rest day ranged from approximately 16 nmol.l^{-1} for the morning value (8 am) to

6 nmol.l^{-1} for the evening value according to the circadian rhythm (Fiet et al. 1981; Kirschbaum and Hellhammer 1989). The 8 am level recorded on the competition day was slightly higher although not significantly different from the 8 am resting day level. Pre-competition cortisol levels are often higher, due to cognitive anticipation and anxiety (Aubets and Segura 1995; Filaire et al. 1997; Guezennec et al. 1992; Passelergue et al. 1995), but in the present case as the match began at 2 pm, that is to say 6 h later, this effect had probably not yet occurred.

The C values recorded just at the end of the competition at 4 pm were 2.5 times higher than those recorded at the same time on the rest day. C increases in response to all kinds of exercise higher than 60% maximal power (*P*max) in intensity and longer than 30 min (Kirschbaum and Hellhammer 1989; Lutoslawaska et al. 1991; Snegovskaya and Viru 1993). The higher the intensity and the longer the duration, the greater the response (Lac and Berthon 2000; Port 1991; Urhausen et al. 1987). The adrenal response is also stronger for intermittent anaerobic versus continuous aerobic exercise (Jensen et al. 1991; Vanhelder et al. 1985), which is the case in rugby. The psychological constraint associated with the situation of competition reinforces this cortisol response (Filaire et al. 1997; Passelergue et al. 1995). During this international rugby match, all these components were gathered together, explaining this sharp increase in saliva cortisol.

Cortisol levels then decreased progressively (6 pm was still higher than the 4 pm rest level) returning to the resting level at 8 pm, as is generally the case after strenuous exercise (Lac et al. 1997; Passelergue and Lac 1999).

During the post-competitive phase of recovery, we measured the hormone levels at 8 am and 8 pm during the week following the competition. A match of less importance occurred on the following Sunday. During this period of recovery, the C levels recorded in the morning (8 am) significantly reduced from the first to the fourth day compared to the 8 am values recorded on the rest day. The evening values followed the same tendency, although less pronounced, only the third day value being significantly lower than the 8 pm rest day value. Cortisol levels began to rise on the fifth day and were equivalent to rest levels on the sixth day. These results are in agreement with previous reports obtained after a competition (Maron et al. 1977; Passelergue and Lac 1999; Urhausen and Kindermann 1987). Conversely, reported results obtained after strenuous exercise monitored under laboratory conditions do not reveal any changes during the following days in cortisol levels (Hooper et al. 1995; Snegovskaya and Viru 1993; Vuorimaa et al. 1999). This observation highlights the fact that the results obtained under laboratory conditions, even if exhausting, are not comparable to competitive situations which create a higher physiological and psychological demand.

Testosterone

During the rest day, T levels decreased in the evening (8 pm) according to the circadian rhythm (Guignard et al. 1980). During the day of competition, morning (8 am) and evening (8 pm) values were comparable to rest day values. The match itself induced a slight drop (about 20%) in testosterone level. During exercise, testosterone has been reported to present an initial rise, followed by a decrease approximately 3 h later (Guglielmi et al. 1984). In this study, this fall is more precocious (a rugby match lasts 1.5 h) as we reported in a previous study on runners (Lac and Berthon 2000). At the cellular level, testosterone is known to exert a protective effect against proteolytic pathways (opposed to cortisol's effects) and a sparing effect on glycogen stores (Guezennec et al. 1986). The fall of testosterone limits these protective effects and allows the energy supply to be enhanced from these cellular substrates.

During the six post-competitive days, T levels were globally higher than rest day values, in the evening as well as in the morning. Such results were reported following a 100-km run (Morville et al. 1979); in contrast, low levels were reported after a triathlon (Urhausen et al. 1987). In two previous studies by our laboratory we found T levels during the post-competitive days to be equivalent in runners (Lac and Berthon 2000) and elevated in wrestlers (Passelergue and Lac 1999).

The corticotropic axis inhibits the gonadotropic axis at hypothalamic level by a direct effect (or via an increase of beta endorphin) of corticotrophin-releasing hormone (CRH) on gonadotrophin-releasing hormone (GnRH) secretion (Barbarino et al. 1989; Cumming et al. 1983). This may explain the decrease in T level just after the match when C levels are high. Reciprocally, this is in agreement with (but does not explain) the rise of testosterone during the recovery phase when cortisol is low. It would be of interest to measure CRH and adrenocorticotrophin (ACTH) on one hand and GnRH and luteinizing hormone (LH) on the other hand (which was not possible in saliva) because, today, we have no explanation for these post-competitive C and T changes and no proof that they are linked. However, as propounded by Aldercreutz et al. (1986) and used by many authors (Banfi et al. 1993; Häkkinen and Pakarinen 1991; Lac and Berthon 2000; Passelergue and Lac 1999; Urhausen et al. 1987; Vervoorn et al. 1991), we expressed these changes as the T/C ratio, which permits us to highlight the changes noted for each hormone.

From our results, it appears clearly that the match induced a marked drop in the T/C ratio (which was 2.5-fold lower at the end of the match compared to the 4 pm value on the rest day); conversely, the post-competition days correspond to a high T/C ratio from the first to the fourth day for the morning values and from the second to the third for the evening ones.

As already mentioned in a previous study (Passelergue and Lac 1999), these high post-competition T/C ratios are associated with a state of tiredness, allowing only a light training regime during this phase. Usually, it is admitted that, under a certain threshold, a drop in the T/C ratio is associated with tiredness, and its ultimate state overtraining (Adlercreutz et al. 1986; Banfi et al. 1993; Gastmann et al. 1998; Häkkinen and Pakarinen 1991; Hooper et al. 1995; Urhausen et al. 1987; Vervoorn et al. 1991). In this last case, the recovery capacity of the organism is overtaken when, conversely in the present study, the high T/C ratio recorded during the post-competitive phase fits with the normal physiological regulation necessary to restore the organism.

Conclusion

Whatever the biological determinants of the elevated T/C ratio recorded during the post-competitive period, it constitutes a biological marker of a breakdown in homeostasis following competition. Such hormonal changes were not observed following standardized laboratory exercises (Hooper et al. 1995; Snegovskaya and Viru 1993; Vuorimaa et al. 1999), which are probably unable to lead to complete exhaustion as can do a competition. In concrete terms, at least 5 days of resting (or with a light training regime) must be respected between two matches to obtain a correct recovery, which is generally but not always the case.

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