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Salivary Testosterone and Cortisol Responses to Cycle Ergometry in Basketball Players with Different Training Volume

E. González-Bono, L. Moya-Albiol, S. Martínez-Sanchis, and A. Salvador

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Departament de Psicobiologia i Psicologia Social, Universitat de València, Spain

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Abstract This study analyzes the degree to which different amounts of training during a 4-month period affects salivary testosterone (Tsal), salivary cortisol (Csal) responses, and changes in the salivary testosterone/cortisol ratio (Tsal/Csal ratio) following acute physical effort. Two professional basketball teams with similar fitness levels carried out a maximal cycle ergometry at the beginning and in the middle of the sports season. In both sessions, saliva samples were collected to determine Tsal and Csal, and mood was assessed. Training was registered daily, total training volume being almost two-fold higher in Team 1 than in Team 2. No significant differences between the teams in anthropometric characteristics, mood, or cycle ergometer performance were found. The Tsal/Csal ratio response to ergometry decreased after training in Team 1 and increased in Team 2, whereas Tsal response did not change significantly and the Csal response to ergometry increased in Team 1 and decreased in Team 2. Results obtained are modulated by initial differences in the Csal values at baseline, with Team 1 showing higher levels than Team 2. In addition, the training volume applied was associated with the changes in the Csal and Tsal/Csal ratio responses to a maximal physical exercise in professional basketball players.

Introduction

Sports training can be defined as the exposition to repeated series of physical exercise programmed to produce an adaptive psychophysiological response with the aim of improving competitive performance. There has long been interest in finding ways to identify the point at which training ceases to be beneficial and the athlete begins to show signs of overtraining and staleness. This is important not only among elite athletes, but also among highly dedicated amateurs. In this context, testosterone and cortisol responses to exercise could be employed as endocrine indexes that reflect the impact of acute and chronic physical stress. More specifically, the testosterone/cortisol ratio has been proposed to indicate the adaptation to training, since it expresses the balance between anabolic and catabolic processes (Adlercreutz et al., 1986). Additionally, it could be useful in determining the intensity of an acute effort by studying the recovery of the subjects (Moya-Albiol et al., 2001; Salvador et al., 2001).

The effects of acute series of physical exercise and training (accumulative effects of repeated exercise of both competitive and non competitive type) have usually been analyzed separately. In general, increases in testosterone and cortisol levels after maximal and submaximal acute efforts have been reported, whereas changes in the ratio are not clearly known (Moya-Albiol et al., 2001). By comparison, testosterone decreases and cortisol increases have been described after training (Suay et al., 1997; Bosco et al., 2000), though others report no significant changes (López-Calbet et al., 1993). Recently, we found a decrease in serum testosterone/cortisol ratio after 5 months of training in professional athletes from several disciplines (Salvador et al., 2001).

Nevertheless, since athletes are continuously exposed to acute series of physical efforts during training periods, several studies have integrated both acute and chronic stressors. After training, the testosterone and cortisol responses to acute series of physical stress decrease (Tabata et al., 1990; Fry et al., 1993; Vasankari et al., 1993; Urhausen et al., 1998; Uusitalo et al., 1998; Filaire et al., 1998) or remain unchanged (López-Calbet et al., 1993; Hoogeveen et al., 1996; Urhausen et al., 1998; Uusitalo et al., 1998; Kiilavuori et al., 1999). These decreases and the consequent lack of variations in the testosterone/cortisol ratio (López-Calbet et al., 1993; Kiilavuori et al., 1999) after an acute series of exercise have been interpreted as adaptation of the adrenocortical system to aerobic training (Wittert et al., 1996). In the case of cortisol, a higher response after training has also been described (Häkkinen et al., 1989; Snegovskaya & Viru, 1993; Hoogeven & Zonderland, 1996). These contradictory results could be the result of several factors: the duration and intensity of acute stressors as well as the training periods, which differed among the studies. In addition, the evaluation criteria of the training periods were not consistent, and their quantification, when included, was poorly described. Moreover, the samples used differed in several variables such as gender, age, degree of fitness, and sports specialties, among others. All these aspects make it difficult to compare the outcomes from different studies to obtain a general perspective of steroid response to maximal acute stress after a period of training.

This study analyzes the effects of different volumes of training on salivary testosterone (Tsal), salivary cortisol (Csal), and salivary testosterone/cortisol ratio (Tsal/Csal ratio) responses in order to verify their value as biological markers of training. For this, two male professional basketball teams were examined at the beginning of the sports season and after 14 weeks of greatly differing amounts of training. Self-rated moods and fitness measures were also evaluated to monitor the adaptation to training. Taking into account the previously mentioned findings and the volume of training applied in the present study, significantly different responses of Tsal, Csal, and Tsal/Csal ratio were hypothesized. Nevertheless, the body of literature to support a determined hypothesis is different for each hormonal variable. Thus, a significant decrease in the androgenic response to acute exercise after the training was expected. With regard to cortisol, since drops and rises in the exercise-induced response have been reported after regular training, a more tentative training volume-related hypothesis was proposed, that is, the higher the volume, the lower the response. Finally, in accordance with other findings previously reported in our laboratory (Salvador et al., 2001), significant decreases in the Tsal/Csal ratio as a function of the training volume were hypothesized.

Table 1 General characteristics and motivation orientation (mean ± SEM).

	Team 1 (n = 10)	Team 2 (n = 8)
Age (years)	21.60 ± 1.07	21.50 ± 1.69
Height (m)	1.95 ± 0.02	1.95 ± 0.03
Weight (kg)	90.79 ± 3.73	93.56 ± 3.34
BMI (kg/m^2)	23.88 ± 0.75	24.55 ± 0.43
Fat (%)	9.65 ± 1.18	11.44 ± 0.76
Mastery	27.00 ± 7.41	23.50 ± 3.51
Competitiveness	26.25 ± 4.46	27.75 ± 11.79

Methods

Participants

The sample consisted of 18 male basketball players who belonged to two different teams (Team 1, n = 10; Team 2, n = 8). Both teams had a highly successful history in the National Spanish Basketball League, and in the sports season studied they reached the first and second positions of their group, composed of 14 teams. All subjects were healthy and free of drugs, and signed an informed consent approved by the local Committee of Medical Ethics. The anthropometric characteristics of both teams are presented in Table 1.

Experimental Design and Testing

Two periodical sessions (PSs), carried out before the "play-off" phase, concurrently at the beginning (PS_1) and in the middle (PS_2) of the sports season, were used to evaluate hormonal and emotional aspects. The first session (PS_1) was carried out at the beginning of the sports season, after 1½-month period without training sessions or competitions. The second session (PS_2) was performed 4 months later, during which time normal training and competitions had taken place. Subjects restrained from exercise 24 hours before either PS.

PSs were carried out in the Sports Medical Center in Cheste (Valencia, Spain) from 8:30 (one and a half hours after awakening) to 14:30 hours, where subjects arrived after overnight fasting. Firstly, between 8:30 and 9:00 hours, the first saliva sample was directly collected from mouth to tube (Unitek®) to determine hormonal levels and the Profile of Mood States (POMS) was administered. Afterwards, anthropometric measures were obtained according to the Pollock and Jackson method (Pollock & Jackson, 1984) by means of measurement of seven skinfold thicknesses obtained by a Holtain skinfold caliper at the subescapular, axilar, breast, triceps, abdominal, suprailiac, and anterior thigh. Thereafter, resting heart rate (Kenz-ECG 302) was measured prior to a maximal cycle ergometer test.

Before the first PS, subjects filled out a questionnaire in order to examine their attitudes regarding training and competition settings.

Cycle Ergometer Test

The test was performed as an incremental graded exercise (GXT) in a sitting position on a mechanically braked bike (Monark). It consisted of a 5-min warm-up period of unloaded cycling, the test phase, and a 5-min recovery phase of unloaded cycling. The test phase started at 30 watts and thereafter increased 30 watts every minute until voluntary exhaustion. Gas exchange was measured using a commercial breath-by-breath analysis system (Sensor Medics MMC 4400 tc), calibrated before each test with precision gases and a 3 l syringe, the maximum volume error accepted being below 1.5%. Heart rate (HR) was continuously registered using a three-lead ECG signal system (CM_5) and monitored in a Hellige Servomed SMS 182. Blood samples were collected from earlobe at rest, immediately after and at 1, 3, and 5 min of recovery to determinate lactate concentrations. Maximal power (W_{max}), oxygen volume uptake ($VO2_{max}$) and heart rate (HR_{max}) were registered and maximal lactate concentration (LAmax) was the maximum postexercise concentration of lactate recorded expressed in mmol/l. Twenty minutes after the test a second sample of saliva was collected.

Training Quantification

Training was registered daily for each subject between PS_1 and PS_2, attending to volume (min) and intensity developed. For this, coaches classified every exercise considering its intensity as a function of the HR as "high" when the exercise elicited a HR of 171–190 bpm, "medium" if the HR was 151–170 bpm, and "low" at 131–150 bpm. Since ambulatory monitoring of HR by pulsometer was not possible because of very frequent interferences because of proximity to each other, carotidean palpation of over 30 seconds was the procedure employed for HR measurement, a practice to which athletes are accustomed. In the first two training sessions, HR values reported by players were checked by pulsometers. Afterwards they were told to measure their own HR after every new exercise and to report the data to the training coach. Experimenters and coaches checked these criteria in the training field during previous sessions.

Total volume (in min) was obtained adding the volume performed in all the intensities. In their schedule, coaches take into account regular matches as part of the training, labelling time competing as "maximal" intensity (HR of 191–210 bpm) based on the pressure and anxiety experienced, in addition to physical effort. We maintained this category to control the time spent in the competitions by the subjects during the 4-month period.

Mood Self-Report

Mood was assessed by means of the POMS (McNair et al., 1971), which is composed of 58 items distributed into six subscales: Tension, Depression, Anger, Vigour, Fatigue, and Confusion. A total score (POMS-t) was calculated by adding all the scores of the scales, subtracting the vigor scale and adding 100. The higher the score, the worse the mood.

Competitiveness and Mastery Orientation

The attitudes regarding competitiveness and training were evaluated by "Perception of Success Questionnaire" (Roberts & Balague, 1989, 1991) composed of 26 items ranked by a 5-point Likert scale and distributed into two factors: Mastery-orientation (13 items), characterized by success being related to personal and individual effort; and competitiveness-orientation (13 items), characterized by success being related to showing higher capacity than others. Both factors are orthogonal, so that a score in one factor is independent of a score in the other (Roberts, Treasure & Kavussanu, 1996).

Hormonal Determination

All the saliva samples were centrifuged (5000 rpm, 15±2°C) and frozen at –20°C until determination by radioimmunoassay at the Central Research Unit laboratory (Faculty of Medicine, University of Valencia, Spain). Samples from each subject were run in duplicate in the same assay.

The Tsal assay required a previous extraction phase with ether. ^{125}I-testosterone tracer was added and decanted into a tube coated with a high specific antibody provided by a commercial kit (ICN Biomedicals, Costa Mesa, CA, USA). Duplicate internal and external control tubes were routinely included in every assay. Tsal levels were expressed in pmol/l, and sensitivity was below 6 pmol/l.

Csal was determined by a commercial kit adapted as was recommended in the protocol (Orion Diagnostica, Espoo, Finland). ^{125}I-cortisol tracer and a high specific antibody were used. Csal levels were expressed in nmol/l, the sensitivity was 1 nmol/l, and internal and external controls were included in the assays.

Intra- and interassay variation coefficients were below 5%. More details about hormonal determination have been previously described elsewhere (González-Bono et al., 1999).

The Tsal/Csal ratio was calculated by expressing Tsal in pmol/l and Csal in nmol/l, as initially suggested by Adlercreuz et al. (1986). This transformation has been followed in numerous studies (Tegelman et al., 1990; Vervoorn et al., 1991; Vervoorn et al., 1992; Banfi et al., 1993; Moya-Albiol et al., 2001; Salvador et al., 2001), which facilities comparative analysis.

Statistical Analyses

To measure the magnitude of hormonal responses, area under the curve (AUC) was calculated by means of the trapezoid formula (Kirschbaum et al., 1997). This method has the advantage of taking into account the baseline levels and the time in which hormonal response is prolonged. Although the period between salivary samples before and after the ergometric test was held constant among subjects, differences between individuals existed in cycling time. Thus, this parameter, directly related to the maximal power and physical fitness, has been considered. One-way ANOVAs were used to compare basal hormonal levels and anthropometric characteristics between groups.

For mood scores, ergometer performance and hormonal responses, repeated measures ANOVAs with SESSION as within-subjects factor and TEAM as between-subjects factor, were carried out, with Greenhouse-Geisser adjustments for degree of freedom where appropriate. As post-hoc tests, one-way ANOVA or repeated measures ANOVAs were used depending on the cases. Relationships between variables were carried out where appropriate by means of Pearson and Spearman correlations.

All statistical analyses were performed with SPSS 8.0 for Windows. Average values in the text are expressed as mean ± SEM: The α level was fixed at 0.05. η^2 statistic is offered where appropriate as estimation of the effect size (*f*).

Table 2 Training quantification (mean ± SEM).

Training volume (min)	Team 1 (*n* = 10)	Team 2 (*n* = 8)
Total volume*	14348.80 ± 266.91	8177.25 ± 214.55
Maximal intensity	196.00 ± 41.10	190.63 ± 40.25
High intensity*	7083.20 ± 103.44	5983.13 ± 161.89
Medium intensity*	3070.30 ± 80.65	1133.25 ± 32.91
Low intensity*	3999.50 ± 126.11	870.25 ± 61.53

*$P < .01$

Table 3 Measures in the ergometric test (mean ± SEM).

	PS_1		PS_2	
	Team 1	Team 2	Team 1	Team 2
Maximum HR (bpm)*	178.30 ± 3.03	173.50 ± 4.61	173.20 ± 2.44	170.37 ± 4.14
Maximal power (watts)	357.00 ± 8.31	341.25 ± 11.25	357.00 ± 8.31	352.50 ± 13.59
VO_2 max (ml/min)	44.29 ± 1.20	41.88 ± 1.22	44.60 ± 1.07	43.42 ± 1.36
LA max (nmol/l)	9.43 ± 0.49	9.52 ± 0.75	8.75 ± 0.68	9.01 ± 0.92

*$P < .05$

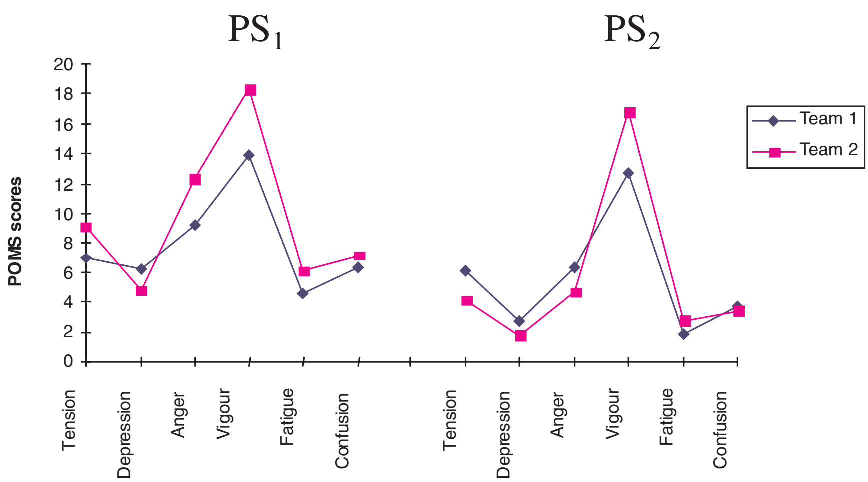

Figure 1 Mood profiles for both teams in PS_1 and in PS_2.

Results

No differences in the anthropometric characteristics and motivational orientation between either team were found (see Table 1).

Training Quantification

Total training volume was significantly different between teams, $F(1, 17) = 301.29$, $P < .001$. These differences were maintained for high, $F(1, 17) = 35.41$, $P < .001$, medium, $F(1, 17) = 413.05$, $P < .001$, and low intensities, $F(1, 17) = 423.71$, $P < .001$. Team 1 showed higher values in all cases (see Table 2).

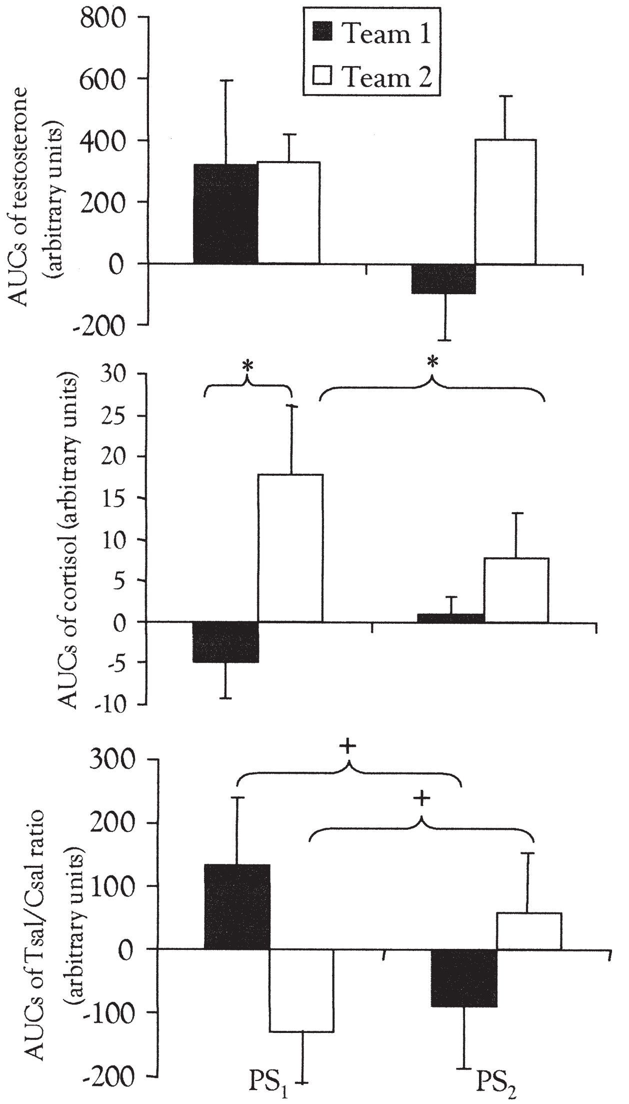

Figure 2 Hormonal responses (AUCs) of testosterone, cortisol, and Tsal/Csal ratio in both teams.

Table 4 Hormonal levels (mean ± SEM).

	PS1				PS2			
	Team 1		Team 2		Team 1		Team 2	
	Basal	Post-ergometry	Basal	Post-ergometry	Basal	Post-ergometry	Basal	Post-ergometry
Tsal (pmol/l)	200.15 ± 31.87	252.50 ± 56.04	179.12 ± 34.36	235.51 ± 41.79	151.38 ± 25.04	135.88 ± 19.69	96.41 ± 19.92	164.28 ± 23.86
Csal (nmol/l)*	5.64 ± 0.95	4.74 ± 0.92	3.10 ± 0.54	6.11 ± 1.23	3.08 ± 0.96	3.24 ± 0.78	2.69 ± 0.42	4.02 ± 0.79
Tsal/Csal ratio*	0.042 ± 0.009	0.065 ± 0.016	0.066 ± 0.015	0.046 ± 0.011	0.074 ± 0.016	0.060 ± 0.013	0.045 ± 0.013	0.055 ± 0.013

$*P < .05$ for the comparisons considering the hormonal responses, $+P < .05$ for the comparisons between teams considering the hormonal levels.

Mood

Team 1 and Team 2 did not show significantly different total mood scores in PS (119.40 ± 8.06 and 121.12 ± 6.99, respectively) and in PS (107.90 ± 5.05 and 99.75 ± 2.23, respectively). There was a significant effect for the factor SESSION, $F(1, 16) = 11.34$, $P < .004$; $P = .88$; $f = 0.42$, with all subjects improving their total mood scores after the training period (from 120.17 ± 5.30 to 104.28 ± 3.06). With regard to mood subscales, only vigor showed a significant main effect of TEAM, $F(1, 16) = 4.31$, $P < .05$, with Team 2 presenting higher scores than Team 1. When contrasts between teams were carried out with vigor scores in each session, differences were nonsignificant. Mood profiles for both teams with all the scores of each subscale of the POMS are presented in Figure 1.

Cycle Ergometer Performance

Neither of the teams differed in their performance during the maximal cycle ergometry in PS_1 or in PS_2 (Table 3). Only for the maximum HR was there a significant effect for SESSION, $F(1, 16) = 6.40$, $P < .02$; $P = .66$; $f = 0.29$, the values of the total sample changing from 176.17 ± 2.63 in PS_1 to 171.94 ± 2.24 in PS_2.

Hormonal Responses

Table 4 presents for both teams the Tsal, Csal, and Tsal/Csal ratio values before and after the maximal cycle ergometry in PS_1 and in PS_2, and Figure 2 shows the magnitude of the response. In PS_1, both teams differed in Csal levels at baseline, $F(1, 17) = 4.66$; $P < .05$), being higher in Team 1. The pre-ergometry Csal levels in PS_1 were positively related to vigor changes in the sports season, $r = 0.63$, $P < .005$. Because of the initial differences in Csal levels, analyses were carried out on the hormonal responses (AUCs) to the ergometry in both sessions, in an attempt to minimize these differences.

No significant effects were found in the case of Tsal response. For Csal response, the factor TEAM and the interaction TEAM × SESSION were significant, $F(1, 16) = 5.08$, $P < .04$; $P = .56$; $f = 0.24$ and $F(1, 16) = 7.34$, $P < .02$; $P = .74$, $f = 0.31$, respectively. Post-hoc analyses showed a significant effect for TEAM in PS_1, $F(1, 17) = 6.91$, $P < .02$, with higher Csal response in Team 2, and for SESSION only in this team, $F(1, 7) = 5.71$, $P < .05$; $P = .54$; $f = 0.45$, showing a decrease in Csal response after training.

For Tsal/Csal ratio response, the interaction TEAM × SESSION was significant, $F(1, 16) = 8.39$, $P < .01$; $P = .78$; $f = 0.34$, although post-hoc analyses only showed a tendency toward significance for the factor SESSION, with a diminution for the response in Team 1, $F(1, 9) = 4.44$, $P < .06$; $P = .47$; $f = 0.33$, and an increase in Team 2, $F(1, 7) = 4.71$, $P < .07$; $P = .47$; $f = 0.40$).

In addition, Csal levels prior to the first ergometry were negatively related to both Csal and Tsal/Csal responses in PS_1 (for both, $r = -0.52$, $p < .03$).

When differences between AUCs (PS_2 minus PS_1) for each hormonal variable were calculated in order to determine the net change after the period studied, a significant effect of the factor TEAM was found in Csal and in Tsal/Csal ratio, $F(1, 17) = 7.34$, $P < .02$ and $F(1, 17) = 8.39$, $P < .01$, respectively. Furthermore, these changes were significantly correlated to total volume of training, positively in the case of Csal ($r = 0.51$, $P < .03$) and negatively in the case of Tsal/Csal ratio ($r = -0.58$, $P < .01$). Finally, this all Csal changes were positively associated with Csal levels prior to the first ergometry.

Discussion

The results of this study showed that the differences between two teams of basketball players in the Csal responses to a maximal cycle ergometry found before the beginning of the sport season disappeared after a 4-month training period, whereas Tsal did not change significantly. Since neither team differed in overall characteristics, ergometric performance, scores of mood or motivation toward competition, the differences between the teams in PS_2 might be related – apart from other nonassessed variables – to training and to baseline differences in hormonal levels. In the team with the highest volume of training (Team 1) Csal did not change significantly. In Team 2, Csal levels were lower at base in both periodic sessions, and the response to ergometry was higher in both periodic sessions. The Tsal/Csal ratio response, a measure that relates both hormones, decreased after training in Team 1, and increased in Team 2.

Tsal response did not significantly change after training in accordance with several studies that measured the concentration of this hormone in saliva (López-Calbet et al., 1993) or in plasma (Hoogeveen et al., 1996; Kiilavuori et al., 1999; Urhausen et al., 1998; Uusitalo et al., 1998). Although not significant, the team with the highest volume of training did show a decrease in testosterone response, as reported in other studies (Fry et al., 1993; Vasankari et al., 1993). On the other hand, Csal response did not change significantly after training in Team 1, but it dropped in Team 2, this response being significantly higher for Team 2 in PS_1. Most studies have reported a decrease after periods of training of different duration, volume, and intensity in male students (Tabata et al., 1990) and in athletes, after measuring blood (Fry et al., 1993; Urhausen et al., 1998) and salivary cortisol responses (Filaire et al., 1998). Other studies have not found any modifications in professional cyclists (López-Calbet et al., 1993; Hoogeveen et al., 1996) or in patients with stable chronic heart failure (Kiilavuori et al., 1999). One study reported a different serum cortisol response depending on the fitness level, showing an increase in untrained but no changes in well-trained healthy men (Vasankari et al., 1993). In the present study, the response of both teams to the maximal cycle ergometry was different before the training, but did not differ after it. This could be due partially to uncontrollable variables during the season break; another possibility is that the differences in training volume characterizes these two teams season after the season (although no data about training background before PS_1 are available, the difference of training between teams displayed in this study could be presumably maintained throughout years). This in turn might be the cause of the high Csal in baseline levels in Team 1, which contrary to expectations could produce a buffered response to the ergometry in PS_1 (Hermus et al., 1984). Subtle differences in vigor between the teams and correlations between initial Csal levels and vigor changes suggest that vigor could modulate – at least in part – the initial hormonal levels and the way players cope with the sports season. Considering the pattern of relationships that initial cortisol levels showed with posterior responses to ergometry and training period, there is need for a deeper examination of the psychological characteristics in order to clarify the complex interactions between hormonal status and psychological well-being.

Furthermore, it is worth noting that the difference between the Csal response displayed in both sessions significantly correlated to the volume of training, albeit positively in opposition to our initial hypothesis. It is probable that more training volume and/or intensity would be necessary to provoke a reduced response, which has been described in overtrained subjects compared well-adapted runners (Barron et al., 1985). As the POMS scores indicated, subjects were not overtrained and had a positive mood in both periodical sessions, in accordance with the range proposed for professional athletes (Morgan et al., 1988). All subjects improved their mood after the training period, which could be related to the fact that habitual practice of physical activity produces a reduction of negative mood and depression (Petruzzello et al., 1997). Profiles of the POMS did not show important changes in either session, especially in Team 1 which displayed a smoother profile.

The Tsal and Csal responses to a training program were different. This fact makes it more important to take into account the results of the Tsal/Csal ratio, which gives information about the balance between anabolic and catabolic processes. There was a drop in Tsal/Csal response in Team 1 and an increase in Team 2 after training, the difference being negatively related to volume of training. These results suggest that Team 2 had a better adaptation to training, with a predominance of the anabolic processes, whereas Team 1 had a catabolic dominance. The Tsal/Csal ratio response to acute physical stress after training has been considered in a few studies. Our results are limited because of their not having been done in an experimental study involving the manipulation of factors and control of the initial differences. Further studies should consider how training moderates the responses of Tsal/Csal ratio to acute physical stressors in order to derive an overall perspective of the anabolic/catabolic balance and of the adaptation to training.

In conclusion, a 4-month training period did not significantly change the Tsal response to a maximal cycle ergometry in professional basketball players, but it did modify the Csal and Tsal/Csal ratio responses. Apart from baseline Csal values, neither team showed differences in anthropometric and motivational characteristics, in competition time or in success in the league which could explain the different responses. Hormonal changes were modulated by initial baseline values and by the volume of training, as demonstrated by the significant correlations obtained. Furthermore, the Tsal/Csal ratio is a useful index of adaptation to training since it includes anabolic and catabolic processes and the ratio response is sensitive to training volume. Low levels of this quotient has been associated with nonadaptive states, although they were not present in the sample of this study. The use of saliva sampling is especially suitable for the assessment of hormonal response as a functional view of the hormonal physiology, offering more information than a static picture of the processes. Finally, it is worth noting that the quantification of training facilitates the comparison among studies using samples of the same or different sports specialties.

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Alicia Salvador
Departament de Psicobiologia i Psicologia Social
Facultat de Psicologia
Apartado 22109
E-46071 Valencia
Spain
Tel. +34 963 864420
Fax +34 963 864668
E-mail Alicia.Salvador@uv.es