Stress hormonal analysis in elite soccer players during a season

Yiannis Michailidis
Department of Physical Education and Sport Sciences, Democritus University of Thrace, Komotini, 69100, Greece

Received 22 October 2013; revised 5 December 2013; accepted 11 March 2014
Available online 14 July 2014

Abstract

Purpose: The purpose of this study was to evaluate the changes in some hormonal parameters (cortisol, testosterone, ratio of testosterone/cortisol (T/C)) in professional soccer players during a season.

Methods: Fifteen professional players from a soccer club of the first division of the Greek soccer league participated. All sport medical examinations were conducted four times: before the re-building period, post re-building period, mid-season, and after finishing the competition phase.

Results: For testosterone, significant differences were observed between the end season and post re-building period (11.6%; \( p < 0.05 \)) and mid-season (12.1%; \( p < 0.05 \)). The cortisol concentration increased at mid-season by approximately 23%, and this change differed significantly from all other measurements for this hormone. The T/C ratio increased at the post re-building period and decreased at the middle of the season.

Conclusion: These hormones and their ratios could be used as stress and recovery state indicators. Coaches can use these parameters in combination with other indicators to optimize workloads, and to avoid overreaching and overtraining.

Copyright © 2014, Shanghai University of Sport. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.

Keywords: Cortisol; Ratio testosterone/cortisol; Soccer; Testosterone

1. Introduction

Two very important hormones in the biochemical assessment of athletes are testosterone and cortisol. Testosterone is a steroid hormone that is the most potent naturally-occurring androgen, and regulates the development of the male reproductive system and secondary sex characteristics. Testosterone is produced mainly in the testes.\(^1\) Cortisol is a principal steroid hormone produced by the adrenal cortex. It regulates carbohydrate metabolism and the immune system, and promotes gluconeogenesis, glycogen synthesis, and protein synthesis in the liver.\(^2\) Cortisol belongs to the group of glucocorticoid hormones, and testosterone to the androgen family. A high cortisol concentration may cause inhibition of the immune system and proteolysis, and for this reason it is related to the control of catabolic processes in the body.\(^2\) Testosterone has anabolic effects in the body, and thus it has been related to the control of anabolic state.

Exercise has been used in many studies as a stress factor to activate various body systems like the endocrine system.\(^2\) It has been reported in the literature that plasma cortisol increased after acute exercise which exceeded 60% of the \( \text{VO}_2\text{max} \), and also after intense resistance exercise.\(^2,4\) Systematic training has resulted in higher concentrations of cortisol at rest as compared to non-exercisers. This measurement of cortisol can be used as an indicator of physical stress.

If one wanted to increase the concentration of testosterone, the exercise intensity and duration should be increased.\(^3,5,6\) The effect of regular exercise on the concentrations of testosterone is less clear, but also in this case, the intensity and duration play a significant role. In some cases, runners had lower rest values of testosterone than non-athletes.\(^1\) Testosterone promotes protein synthesis\(^7–9\) and therefore is used as an indicator of anabolic processes in the body. However, it has been reported that during exhaustive exercise, the concentration of this hormone decreases.\(^10\)

Another parameter used to evaluate athletes is the resting ratio of testosterone/cortisol (T/C). This ratio has been
associated with overtraining syndrome. In this syndrome, an accumulation of training and/or non-training stress results in a long-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation, in which the restoration of the performance capacity may take several weeks or months.\textsuperscript{11,12} There are studies that indicate overtraining athletes to this decreased T/C ratio was associated with increased proteolysis and decreased protein synthesis.\textsuperscript{13,14} However, there are studies that found no correlation between this ratio and overtraining syndrome.\textsuperscript{15—17} Despite the controversial role of this ratio, regular measurement is an indicator of the balance between catabolic and anabolic processes,\textsuperscript{13,14,18} and suggests possible changes required in the training program of the athlete.

Soccer at a high level is a demanding sport. Apart from technique and tactics knowledge, the participants should also be sufficiently developed in all physical abilities. The training season in soccer lasts about 11 months. Approximately 2 months are the preparation period, 8 months represents the competitive season, 1 month is a transitional period and 1 month is the players’ holiday. In the re-building period, the players try to improve their physical abilities. In the next phase (in season), they have to maintain these abilities at the highest level. To improve through training, the process should be a proper balance between the volume and intensity of training with rest periods.\textsuperscript{19} A long recovery from training may not lead to optimal adjustments whereas limited recovery for a long time will probably lead to overtraining syndrome with all of its negative effects on the player’s performance and health.\textsuperscript{16—20} Furthermore, the psychological stress that a player experiences during the season is an additional factor that can influence his physiological state. One way to protect the players is to regularly monitor the concentrations of testosterone and cortisol and their ratio during the course of the season.\textsuperscript{21—25}

The aim of this study was to analyze the testosterone and cortisol responses in a professional soccer team that participates in a professional Greek league throughout the season. To our knowledge, this research is the only study performed in professional soccer players over an entire season.

### 2. Methods

#### 2.1. Experimental approach

We studied the changes in testosterone, cortisol, and their ratio throughout a soccer season. In this way, we studied the stresses provoked by exercise through a season of competition in professional players. The team participated in 30 matches for the championship, and five matches for the cup. Samples were collected before the beginning of the re-building period, just after the re-building period, at the middle of the season, and at the end of the season (Fig. 1). The samples were collected 24 h after different matches, at 8:00 am, in the fasting state. The blood samples were collected at rest. The measurements were assessed as part of the standard anthropometric/physical conditioning testing.

#### 2.2. Subjects

At the beginning of the study, 25 male professional soccer players were enrolled. During the season, some of them were injured and lost some training sessions. In total, 15 players participated in the study who followed the team program without having any serious injuries. All of the players had participated for at least 3 years in the first division of the Greek league. On average, the players trained 6—7 times per week and participated in a match every week.

After receiving a detailed explanation of the study’s benefits and risks, each subject signed an informed consent document that was approved by the local ethics committee.

#### 2.3. Anthropometrics

Body mass was measured to the nearest 0.1 kg (BC-418 Segmental Body Composition Analyzer, Tanita, Japan) with the subjects wearing their underclothes and barefoot. The body fat percentage was calculated from seven skinfold measurements (average of two measurements from each site) using a Harpenden calliper (John Bull, British Indicators, St Albans, UK) on the right side of the body as described by Jackson and

**Fig. 1.** Procedure for the sampling and anthropometric measurements. BC = body composition.
Fat free mass values were obtained from the measurements of the estimated body fat and body mass. Standing height was measured to the nearest 0.1 cm (Stadiometer 208, Seca, Vogel Halke, Hamburg, Australia).

2.4. Blood collection, testosterone, and cortisol measurements

Blood samples (8 mL) were collected via venipuncture from an antecubital arm vein using a safety butterfly set with the participants always in a semi-recumbent position. Blood was collected into Vacutainer tubes containing SST-Gel and Clot Activator. The blood was allowed to clot at room temperature, and subsequently centrifuged (1500 g, 4 °C, 15 min) for serum separation. The resulting serum was used for the measurements. The samples were stored and frozen at −75 °C until analyzed. Testosterone and cortisol were analyzed with assay kits from DRG diagnostics (DRG, International Inc., New York, USA; Research Use Only, Testosterone CLA-4660, Cortisol CLA-4651, New York, USA). The intra- and inter-assay coefficients of variation (CVs) for testosterone were 3.7% and 5.6%, respectively. The intra- and inter-assay CVs for cortisol were 4.0% and 5.7%, respectively.

2.5. General training program in each period

During the re-building period, the trainers were trying to improve the physical abilities of the players. The players also participated to six friendly games. Table 1 presents the general characteristics of the training session.

A general program for the competitive period is presented in Table 2. Until the 26th week, the team participated in 17 games and after this week, 18 games. The maintenance period was 6 weeks and the players participated in some kind of exercise 2–3 times per week. The first 3 weeks they took part in some sports like swimming, tennis, or basketball and the next 3 weeks they performed running, general strength and flexibility exercises, and individual soccer techniques.

2.6. Statistical analysis

All data are presented as mean ± SEM. Data normality was verified with the One-sample Kolmogorov–Smirnov test; therefore, a nonparametric test was not necessary. The data were analyzed by a one-way repeated measures analysis of variance (ANOVA) to examine changes in the mean values of the hormones over the course of the soccer season. When a significant effect was found, a post hoc Bonferroni test was performed. The level of significance was set at p < 0.05. The data were analyzed using SPSS, PC program, version 13 (SPSS Inc., IBM, Armonk, NY, USA).

3. Results

No significant differences were revealed by the ANOVA in the estimated body composition indicators. Table 3 presents the mean values of these variables.
The statistical analyses for testosterone demonstrated differences \( F(3, 42) = 4.267, p < 0.05 \) between the measurements. The concentration of testosterone increased at the end of the re-building period (11.6%), and remained at the same level (12.1%) in the next measurement (mid-season). However, at the end of the season, the concentration of the hormone decreased to below the initial levels (−1.5%). A statistical difference was observed between the measurement at the end of the re-building period and at the mid-season with that at the end of the season \( (p < 0.05) \) (Table 4).

Significant differences in the cortisol concentration were found by ANOVA \( F(3, 42) = 7.782, p < 0.001 \). The cortisol concentration decreased at the end of re-building period (−5.3%), then increased during mid-season (23.4%), and at the end of the season, the concentration reached the initial values (2.8%). The mid-season value of the hormone differed significantly from the first two measurements (pre and post re-building period) \( (p < 0.05) \). Furthermore, the measurement at the end of the season differed from that of the mid-season \( (p < 0.05) \) (Table 4).

T/C ratio also showed significant changes along the season \( F(3, 42) = 6.147, p = 0.001 \). The initial value increased by 12.1% at the end of the re-building period \( (0.37 ± 0.03) \). At the mid-season measurement, the ratio decreased by 15.2% compared with the initial measurement \( (p < 0.05) \). Finally, at the end of the season, the value of the ratio was 9.1% less than the first measurement (Table 4).

### Table 2
Program during the competitive period.

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning Activity</th>
<th>Afternoon Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>Recovery training</td>
<td>—</td>
</tr>
<tr>
<td>Tuesday</td>
<td>Aerobic capacity</td>
<td>—</td>
</tr>
<tr>
<td>Wednesday</td>
<td>Strength</td>
<td>Technical and tactical training</td>
</tr>
<tr>
<td>Thursday</td>
<td>—</td>
<td>Speed and power</td>
</tr>
<tr>
<td>Friday</td>
<td>—</td>
<td>Speed reaction</td>
</tr>
<tr>
<td>Saturday</td>
<td>Specific tactics</td>
<td>—</td>
</tr>
<tr>
<td>Sunday</td>
<td>—</td>
<td>Game</td>
</tr>
</tbody>
</table>

### Table 3
Variables of body composition at sample collecting time (mean ± SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre re-building</th>
<th>Post re-building</th>
<th>Mid-season</th>
<th>End season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>76.70 ± 1.82</td>
<td>77.60 ± 1.72</td>
<td>76.70 ± 1.74</td>
<td>76.90 ± 1.72</td>
</tr>
<tr>
<td>% body fat</td>
<td>10.32 ± 0.73</td>
<td>10.21 ± 0.63</td>
<td>11.25 ± 0.63</td>
<td>11.30 ± 0.61</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>68.76 ± 1.65</td>
<td>69.69 ± 1.58</td>
<td>68.09 ± 1.59</td>
<td>68.25 ± 1.67</td>
</tr>
</tbody>
</table>

### Table 4
Hormone concentrations and their ratio value (mean ± SEM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre re-building</th>
<th>Post re-building</th>
<th>Mid-season</th>
<th>End season</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (μg/dL)</td>
<td>5.90 ± 0.38</td>
<td>6.59 ± 0.36</td>
<td>6.62 ± 0.38</td>
<td>5.82 ± 0.34</td>
</tr>
<tr>
<td>C (μg/dL)</td>
<td>19.18 ± 1.22</td>
<td>18.16 ± 0.95</td>
<td>23.67 ± 0.57</td>
<td>19.71 ± 0.95</td>
</tr>
<tr>
<td>T/C</td>
<td>0.33 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>0.28 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

Abbreviations: T = testosterone; C = cortisol.

\( a \ p < 0.05 \), compared with the values at pre re-building period.

\( b \ p < 0.05 \), compared with the values at post re-building period.

\( c \ p < 0.05 \), compared with the values at mid-season period.

### 4. Discussion

It is important to note that the major aim of the team was to remain in this division. At the end of the season, the players had accomplished this purpose.

The secretion of cortisol is related to stress. As mentioned above, the exercise functioned as a stress factor, and the amount of hormone produced depends positively on the intensity and duration of exercise. \(22,23\) In this study, the largest change in the hormone was an increase of cortisol concentration (23.4%) in the mid-season. The increased concentration of cortisol in the mid-season in team sports has been reported by other researchers. \(28\)

When athletes follow a properly designed exercise program, the cortisol that is produced after each workout is removed from the body within 24 h. Therefore, the changes in the concentration of cortisol may be associated with stress accumulated during the season. \(29\) In this study, according to the concentration of cortisol, the players experienced a time point during the season with intense stress. This measurement was at the middle of the season, where the matches, the training load, the psychological pressure to perform, and the difficulty of soccer pitches (the mid-season coinciding with the middle of winter) exhausted the players. Furthermore, after the end of the season when all of the above aggravating factors disappeared, the hormone levels decreased.

Testosterone as an anabolic hormone has been reported as an indicator of the rate of regeneration of the body, \(7,9\) and stress caused by training. \(22,23,30\) In this study, the concentration of testosterone after an intense re-building period was increased by 11.6%, and returned to baseline levels at the end of the season. These findings partly reflect the good management between the training load and the rest periods. It is also apparent that increased levels of this hormone are desirable for long seasons with intensive competition. The increase in the testosterone concentration during the season of team sports has been reported by other investigators. \(28\)

The T/C ratio has been reported as an indicator of the homeostasis between anabolic and catabolic processes in the body, \(13,14\) and overtraining. \(31\) In the present study, the ratio increased after the re-building period by 12.1%, whereas the next measurement (mid-season) decreased by 24.3%, in comparison with post re-building period. These findings suggest that during the pre-season, the players were not tired and could respond adequately to the coaching without accumulating fatigue. However, in the mid-season, the significant decline in this ratio is probably a result of the aggravating factors mentioned above, and led to the increased cortisol concentration. \(19\) A significant decrease in the ratio was also reported by Handziski et al. \(32\) after half of the soccer season. The end of the season resulted in a dramatic reduction in total stress related to the season, and restored the ratio to baseline. The fact that there was a decline in the ratio of about 24% does not necessarily mean that the players were in an overtraining
state, since for such a finding several other factors should be taken into account.\textsuperscript{31,33}

If a player had overtraining symptoms, the trainers would have to protect him and prepare individualized training sessions. A player with this problem has to abstain from vigorous activities. A player with this problem has to abstain from vigorous activities. A player with this problem has to abstain from vigorous activities. A player with this problem has to abstain from vigorous activities. A player with this problem has to abstain from vigorous activities.

In exercise, many hormones play a significant role.\textsuperscript{1} In this study we investigate the changes of testosterone and cortisol in a complete soccer season. Soccer coaches and sport scientists have to observe regularly the changes of these hormones for improving players’ performance.

5. Conclusion

The concentrations of these hormones and their ratio are an indicator of the functional status of an athlete, and should be measured during the soccer season so that the coaches can individualize the training as needed. For the measurement of these hormones to be useful, they should not be isolated but should be timed regularly so that the medical team can observe these changes. Proper cooperation between the medical team and the coaches will help to maximize the performance of the players, and limit any cases of overtraining.

Acknowledgment

The author thanks the soccer players for their cooperation.

References