ALTERACIONES INDUCIDAS POR DOS DIFERENTES TIPOS DE ENTRENAMIENTO DE FÚTBOL EN NIVELES SÉRICOS DE CK, CORTISOL Y TESTOSTERONA

ALTERATIONS INDUCED BY TWO DIFFERENT SOCCER WORKOUTS IN CK, CORTISOL AND TESTOSTERONE SERUM LEVELS

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RESUMEN
El objetivo de este estudio fue examinar los efectos de dos sesiones diferentes de entrenamiento de fútbol sobre los valores basales de cortisol, testosterona y creatina kinasa (CK) en jugadores profesionales de fútbol un día después del esfuerzo.
Veintitrés jugadores profesionales de fútbol (edad: 25,2 ± 3,1 años, talla: 175,6 ± 6,2 cm, peso: 72,8 ± 6,4 kg), realizaron 2 tipos de tareas (W1 y W2). Veintidós horas después de los entrenamientos se tomaron muestras de sangre en ayuno de la vena antecubital para verificar los cambios de los biomarcadores seleccionados.
El cortisol aumentó significativamente de 278,8 ± 19,8 nmol.l-1 (promedio de los valores basales fuera de temporada) a 527,8 ± 76,4 nmol.l-1 (p <0,001) y 747,7 ± 121,1 nmol.l-1 (p <0,0001) tras W1 y W2, respectivamente. La testosterona no modificó significativamente (p> 0,05) [19,8 ± 7,2 nmol.l-1 (fuera de temporada), 24,4 ± 5,6 nmol.l-1 después de W1, 23,4 ± 6,1 nmol.l-1 después de W2]. La creatina kinasa (CK) incrementó significativamente de 101,5 ± 30,2 U / L (fuera de temporada) a 178,4 ± 88,5 U / L (p <0,01) después de W1 y 419,8 ± 372,0 U / L (p <0,0001) tras W2.
Se concluyó que W2 indujo un aumento más significativo en los niveles séricos de CK y cortisol. Estos aumentos son probablemente debidos a la cantidad relativamente superior de las contracciones excéntricas en W2.

Palabras clave: Hormonas; Enzimas; Control del entrenamiento

ABSTRACT
The purpose of the study was to examine the effects of two different soccer workouts on basal values of cortisol, testosterone and creatine kinase (CK) in professional soccer players one day after exertion.
Twenty three professional soccer players (age: 25.2±3.1 years) realised two different workouts (W1 and W2). Twenty-two hours after the workouts blood samples were collected in fasting state from the antecubital vein for verifying changes of the selected biomarkers.
Cortisol increased significantly from 278.8±19.8 nmol.l⁻¹ (average basal values out-season) to 527.8±76.4 nmol.l⁻¹ (p<0.001) and 747.7±121.1 nmol.l⁻¹ (p<0.0001) after W1 and W2, respectively. Testosterone was not significantly (p>0.05) altered [19.8±7.2 nmol.l⁻¹ (out-season); 24.4±5.6 nmol.l⁻¹ after W1; 23.4±6.1 nmol.l⁻¹ after W2]. Creatine Kinase (CK) increased significantly from 101.5±30.2 U/L (out-season) to 178.4±88.5 U/L (p<0.01) after W1 and 419.8±372.0 U/L (p<0.0001) after W2.
It was concluded that W2 induced a more significant increase in CK and cortisol serum levels. These increases are probably due to the relative higher number of eccentric contractions in W2.

Keywords: Hormones; Enzymes; Training control
INTRODUCTION

Strenuous physical exertion alters serum concentration of several hormones and enzymes. Decreased levels of testosterone and increased levels of cortisol are suggested to be indicative for a disturbance in the anabolic-catabolic balance, which may induce performance impairment (Hoogeveen & Zonderland, 1996). Training volume is related to serum testosterone and cortisol (Purge et al., 2006). Serum cortisol is increased after intense prolonged exercise (Murray & Hackney, 2000) while in response to short-term sub-maximal workload testosterone concentration was significantly increased, returning to pre-exercise levels after 40 min of recovery (Sutton et al., 1973). However, if exercise is prolonged, there is an initial rising followed by decline as the activity continued (Galbo et al., 1977). It seems that during exercise to exhaustion, testosterone concentrations increase proportionally to exercise intensity (Hacney, 1996) while basal values are reduced inversely to training load intensity (Murray & Hackney, 2000).

High basal cortisol levels can result in performance impairment due to the catabolic effect of this hormone. Exercise-induced increasing in cortisol can contribute for the lower resting testosterone levels verified in some endurance-trained athletes (Cumming et al., 1986; Wheeler et al., 1991), affecting muscle repair after strenuous exercise.

Muscle actions during soccer training are very disruptive because the large participation of eccentric contractions. Elevation of serum CK following various forms of exercise are well documented and are used as indicator of muscle damage after continuous and especially maximal eccentric exercise (Nosaka et al., 2002).

The purpose of this study was to assess, in the day after exertions, Cortisol, Testosterone and CK responses after two workouts characterised by identical training volume and different intensities. These assessments can be important to define recovery strategies (e.g. planning of resting times) to avoid overreaching which can affect the competitive level of the soccer players.

METHODS

Twenty-three professional male soccer players (age: 25.2±3.1 years; height: 175.6±6.2 cm; weight: 72.8±6.4 Kg) participated in this study. These players belong to the team finalist of the European Cup 2010-2011, and achieved the third place in the Portuguese Premier league in the same season in which the data were collected. Goal-keepers were excluded. The subjects were informed about the design of the study and, after informed about the eventual risks and discomfort, they gave a signed consent prior to the start of the study. The study was conducted according to the declaration of Helsinki and was approved by the Scientific Council of the Sport’s Faculty of the University of Porto, Portugal.

Biochemical data were recoiled in three moments: Out-season (in the first day after vacations), and in the third month (W1) and fourth month (W2) of the competitive season.

Biochemical Procedures

After an overnight fast, at the same time of the day (9A.M.) to avoid diurnal variation of the hormones, blood samples were drawn from antecubital vein using standard venotomy techniques with the subjects in the sitting position, 22 hours after workouts, starting point for the next workout.

The samples were clotted at room temperature and then centrifuged. Separated serum was divided into aliquots and stored at 2-8ºC for the determination of Cortisol, and Testosterone. Serum levels of Cortisol and Testosterone were quantified within 13 hours using a commercial automated enzyme immunoassay kit (ADVIA Centaur ® from Bayer Diagnostics). CK determination was made by an automated device Olympus AU2700.

Training sessions

The workouts were performed in the same day of the microcycle after 40 hours of rest. W1 and W2 were included in the third and fourth month within the competitive season, respectively, because these were the only two consecutive weeks without important matches in the middle of the week.

Workout 1 (W1)

Slow continuous running (warm-up) – 20 min
Active Stretching – 5 min.
Technical skills – 17 min
Small sided game (half-court) 11x11 – 30 min
Slow continuous running (cool-down) – 10 min
Passive Stretching – 5'

**Workout 2 (W2)**

Slow continuous running (warm-up) – 10 min
Active Stretching – 5 min.
Small sided game (30 m side triangle) 3 x 3 (only ball possession); 3 min (maximum intensity) 3 min rest - each player worked 18 min
Small sided game (penalty area) 3 x 3 (trying to score in the goal) – 20 min
Small sided game (lateral reduced half-court) 6 x 6 with 6 lateral supporters (2 min of effort with 30 s of recovery) – each player worked 12 min
Slow continuous running (cool-down) – 7 min
Passive Stretching – 5'

**Statistical Analysis**

Values are expressed as mean ± SE. Shapiro-Wilk was used to test for normality of the distribution. As the normality was not verified, it was used Test T-Wilcoxon to establish the differences between moments. Statistical significance was accepted at p < 0.05 level.

**RESULTS**

Table 1 summarises the results of this study. From out-season values, testosterone was not significantly (p>0.05) altered after W1 and W2. Cortisol increased after W1 and experienced a more marked increase after W2. The same pattern was verified for CK alterations.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Out-season</th>
<th>W1</th>
<th>W2</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nmol.l⁻¹)</td>
<td>278.8±19.8</td>
<td>527.8±76.4 *</td>
<td>747.7±121.1 **</td>
<td>138 - 635</td>
</tr>
<tr>
<td>Testosterone (nmol.l⁻¹)</td>
<td>19.8±7.2</td>
<td>24.4±5.6</td>
<td>23.4±6.1</td>
<td>4.1 - 55.2</td>
</tr>
<tr>
<td>Creatine Kinase (U/L)</td>
<td>101.5±30.2</td>
<td>178.4±88.5 ***</td>
<td>419.8±372.0 **</td>
<td>38 - 174</td>
</tr>
<tr>
<td>Testosterone / Cortisol ratio</td>
<td>0,2</td>
<td>0,05</td>
<td>0,03</td>
<td>-</td>
</tr>
</tbody>
</table>

* Significant different from out-season (p<.001)
** Significant different from W1 (p<.0001)
*** Significant different from out-season (p<.01)

**DISCUSSION**

In daily sport’s practice is normally difficult to assess the overall changes induced by training loads what can derive in chronic stressful situations that can overpass body capability to cope with physical exertion. High level soccer competition is conditioned by training level. High game’s quality demands high physical and mental intensity during training. Physiological strain imposed by stressful workouts demands at least 72 h to full recovery (Reilly & Ekblom, 2005). So, to improve competitive conditions the alleviation of load’s intensity is fundamental what is often neglected by coaches.

Biochemistry studies in soccer are sparse and the regulation of load intensity on a daily base is usually made by other indicators easier to manage (e.g. heart rate, rate of perceived exertion and motor tests).

The main difficulty with studies relating exercise to hormonal and enzymatic changes is the control of variables such as emotional stress, sleep loss, diet, weight loss and hereditary factors. In a professional soccer team others factors can be added as the individual training level, specific motor pattern and actual physical condition. However, and overpassing these limitations, the measurement of some hormones and enzymes can give us some insights to manage with physical loads during training.

Endogenous hormones are essential for physiological reactions and adaptations during physical work and influence the recovery phase after exercise by modulating anabolic and catabolic processes. Decreased levels of testosterone and increased levels of cortisol are suggested to be indicative for a disturbance in the anabolic-catabolic balance (Hoogeveen & Zonderland, 1996) which can affect performance.

From our results it can be verified that daily basal cortisol variations are function of training intensity. In this study, serum cortisol after workout 1 (W1) was significantly higher than basal level achieved out-season after the transition period. However the values were within normal laboratory references. After workout 2 (W2), cortisol values showed a more pronounced increase which is related to the greater physical intensity induced by the precedent training session. Our high values contrast with those measured by Hoogeveen and Zonderland (1996), after an intensified period of training with cyclists,
who increased resting cortisol levels from 272±110 nmol/l to 379±242 nmol/l. These authors considered these values as an increased catabolic state. We think that our higher values result from the more intense muscle contractions mainly derived from the large number of eccentric contractions which characterise soccer skills. Our data are similar to those obtained by Gaul et al. (1995) with long distance runners and swimmers during a high-volume and high-intensity training period. It seems that systematic intensive training induces a temporary elevation of basal serum cortisol (Hoogeveen & Zonderland, 1996; Rodrigues dos Santos, 2002) that returns to basal values after some days of tapering (Cou tts et al., 2007). Although, high basal values of cortisol and reduction in the testosterone/cortisol ratio verified in this study can be an index of overreaching, we refuse this hypothesis. This behaviour is normal is healthy subjects experimenting strenuous physical loads and are indicative of temporary incomplete recovery from precedent loads. However, if this situation is sustained during large periods can impair players' physical capability and disturb their mental readiness for training (Pivac et al., 1997) because high levels of plasmatic cortisol are related with physiological stress and depression (Carroll et al., 2007).

As normal response to short-term sub maximal exercise, there is an increase of serum testosterone concentration, returning to basal values within 40 minutes (Hackney, 1996). It appears that basal testosterone suffers little variations between intense-training and tapering phases (Gaul et al., 1995), however endurance training is likely to induce a temporary decrease of basal testosterone (Hoogeveen & Zonderland, 1996). In this study, basal testosterone concentrations are similar after the two workouts and not significantly different from out-season what reinforces the daily stability of this hormone. Our data are in accordance with the results of Hackney (1996).

Some authors suggest that testosterone/cortisol (T/C) ratio indicates the overall training stress and the reduction of such ratio is an early index of an imbalance between anabolic and catabolic metabolism (Adlercreutz et al., 1986). Banfi and Dolci (2006) hypothesised the possibility of overtraining syndrome when is verified a 30% decrease in T/C ratio in comparison with one previous value. Albeit our results have verified a 40% decrease in the T/C ratio after W2 in comparison with W1, these changes are linked to acute alterations rather to overtraining syndrome. Only when T/C ratio is continuously decreased and maintained in time, we can hypothesise about the eventual entrance in a situation of overtraining. The scientific data correlating variations on testosterone/cortisol ratio with performance indicators are often conflictual (Vervoorn et al., 1991). The decrease verified on T/C ratio in M2 is the direct outcome of load's intensity, and must be seen as index of acute stress rather as indicator of chronic anabolic/catabolic imbalance. These data give us information about the adaptations induced by the training loads and must be considered as means to adjust training strategies to promote full recovery before competition.

Soccer training is characterised by high-intensity intermittent exercises that rely predominantly on aerobic energy pathways (Ekblom, 1986), however, highest performances are usually supported by anaerobic metabolism and are linked to short-term high intensity contractions with special emphasis in the eccentric phase of the movement. Soccer training exercises have a lot of eccentric contractions which are more prone to induce fibre disruption (Clarkson & Hubal, 2002) independently to be high- or low-intensity eccentric contractions (Nute et al., 1992). Exercise-induced muscle injury is expressed by significant rise on serum CK (Rodrigues dos Santos, 2004). It seems that training attenuates CK rise provoked by eccentric exercise (Evans et al., 1986). With the same subjects, 16 hours after the first workout of the season (free game during 30 minutes developed at high intensity) we verified CK values (918.8 ± 1769.5 UI/l) higher than those obtained after much more intense and prolonged workouts during the season (W1 and W2). However, from our data, even in a more fitted condition different mode and/or exercise intensity induce different and significant changes in enzymatic response to training.

The selected workouts promoted different CK responses which must be seen as indicators of different levels of muscular aggression. Even if athletes are prone to usually have higher levels of basal plasma CK (Mougios, 2007), that situation can impair physical performance when assume chronic condition. High daily plasma CK levels is an index of intracellular destruction and is mainly directed related to the intensity of the physical loads.
It’s known that recovery time is not significantly altered when a second maximal eccentric exercise is performed two days after the first. However, the second exercise induces a further impairment in isometric torque and reduction in total work and peak eccentric torque values (Paddon-Jones et al., 2005), what can decrease capability for maximal physical performance. This must be taken in account for soccer players training which exercise in a daily basis and be well recovered in the match day.

CONCLUSIONS

It can be concluded that in well trained soccer players plasma cortisol and CK changes are dependent on the type of exercise performed. High intensive soccer exercises, performed in reduced spaces, in comparison with those performed in larger spaces, induce a more disruptive cellular destruction which is reflected by high levels of cortisol and CK. Biochemical soccer characterisation not only can be an important tool to avoid overreaching, but also a mean for establishing logic for load’s administration during the microcycle.

Limitations of the study:

The constraints imposed by the coach did not allow us to assess consecutive days of training. Probably, CK resting levels before exertion were higher than out-season values what reduce the validity of changes verified in this study.

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REFERENCES


