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ARTICLE

Biochemical impact of soccer: an analysis of hormonal, muscle damage, and redox markers during the season

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Abstract: This study aimed to analyze changes in performance, muscle function, and stress-related biochemical markers in professional soccer players (n = 14) at 4 timepoints (3 for performance and 4 for stress-related biochemical markers) during the soccer season — preseason (E1), midseason (E2), end of the season (E3) — and after the end of the recovery period (E4). Performance in 5- and 30-m sprints, countermovement jump, and agility, and maximal isokinetic knee extension and knee flexion strength were measured (E1 to E3). We observed increased in-season levels of myoglobin (E2 > E1 and E4; $p < 0.05$), a higher testosterone/cortisol ratio (T/C), and increased levels of creatine kinase (CK), C-reactive protein, superoxide dismutase (SOD), protein sulfhydryls (–SH), and malondialdehyde (E2 and E3 > E1 and E4; $p < 0.05$). Lower cortisol concentrations (E3 < E1 and E4; $p < 0.05$) and glutathione reductase activity (E3 < E2 and E4; $p < 0.05$) were observed at the end of the season. T/C, CK, SOD, –SH, and malondialdehyde decreased during the off-season, and cortisol and glutathione reductase increased (E3 < E4; $p < 0.05$). Agility increased in E2 and E3 ($p < 0.01$). Significant correlations were found during the season between hormonal and muscle function parameters (r = 0.56–0.86; $p < 0.05$). In addition, in E2, significant associations were observed between match-accumulated time (MAT_{E2}; minutes played by each player during the competition period), performance, and hormonal and redox parameters (r = 0.456–0.615; $p < 0.05$). In conclusion, this study shows that soccer players face significant changes in biomarkers of physiologic strain (muscle damage and oxidative stress-related markers) during the season, but values return to normal during the off-season. Additionally, MAT influences physical, hormonal, and oxidative stress-related parameters in professional soccer players.

Key words: performance monitoring, biochemical monitoring, testosterone, cortisol, muscle damage, inflammation, antioxidant system, oxidative stress, match exposure.

Résumé : Cette étude analyse les variations de la performance, de la fonction musculaire et des marqueurs biochimiques associés au stress (3 variables pour la performance et 4 pour les marqueurs biochimiques associés au stress) chez des joueurs de soccer professionnel (n = 14) tout au long de la saison (avant-saison (E1), milieu de la saison (E2), fin de la saison (E3) et après la fin de la période de récupération (E4)). On évalue (E1–E3) aussi la performance au sprint de 5 et 30 m, au saut avec contremouvement préparatoire et à un test d'agilité ainsi que la force isocinétique maximale à l'extension du genou et à la flexion du genou. Durant la saison, on observe une augmentation des valeurs de myoglobine (E2 > E1 et E4; $p < 0,05$), du ratio testostérone/cortisol (T/C), de la créatine kinase (CK), de la protéine C-réactive, de la superoxyde dismutase (SOD), des groupes sulfhydryles des protéines (–SH) et du malonaldéhyde (E2 et E3 > E1 et E4; $p < 0,05$). À la fin de la saison, on observe une plus faible concentration de cortisol (E3 < E1 et E4; $p < 0,05$) et une diminution de l'activité de la glutathion réductase (GR) (E3 < E2 et E4; $p < 0,05$). Hors saison, on observe une diminution de T/C, CK, SOD, –SH et de malonaldéhyde et une augmentation de C et de GR (E3 < E4; $p < 0,05$). L'agilité augmente à E2 et à E3 ($p < 0,01$). En saison, on enregistre des corrélations significatives entre les variables hormonales et musculaires (r = 0,56–0,86; $p < 0,05$). De plus, dans E2, on observe des corrélations significatives entre le nombre de minutes de compétition accumulées au milieu de la saison par un joueur (« MAT_{E2} ») et la performance, les variables hormonales et de redox (r = 0,456–0,615; $p < 0,05$). En conclusion, cette étude révèle des modifications significatives des biomarqueurs du stress physiologique (lésions musculaires, stress oxydatif) durant la saison, mais les valeurs reviennent à la normale hors saison. En outre, le MAT a un effet sur les variables physiques, hormonales et le stress oxydatif chez les joueurs de soccer professionnel. [Traduit par la Rédaction]

Mots-clés : suivi de la performance, suivi biochimique, testostérone, cortisol, lésion musculaire, inflammation, système antioxydant, stress oxydatif, exposition à la compétition.

Introduction

Accumulated levels of physiologic stress during the playing season might predispose soccer players to an inability to cope with training and competitive demands, thus compromising performance. In fact, the metabolic and mechanical stress imposed on soccer players might induce physiologic disturbances that could be exacerbated during periods of prolonged or intense exposure. There are reports of alterations in the anabolic and catabolic hormonal environments (Kraemer et al. 2004; Handziski et al. 2006), muscle damage markers (Heisterberg et al. 2013; Meister et al. 2011), and immunologic (Rebelo et al. 1998) and redox states after periods of high-intensity soccer training and competition

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(Magalhães et al. 2010; Andersson et al. 2010). Interestingly, it has recently been observed that more match exposure during the season might influence muscle strength levels and the performance of different muscle-power actions (Silva et al. 2011; Sporis et al. 2011). Moreover, when players are exposed to high volumes of training and (or) competition interspersed with insufficient recovery, they can show signs of fatigue (Filaire et al. 2003); ultimately, players might transition from a functional to a nonfunctional over-reaching state and, in more severe cases, to an overtraining state (Kraemer and Ratamess 2005; Schmikli et al. 2012).

However, despite some controversy, distinct methodologic approaches to the analysis of longitudinal alterations in the physical fitness of soccer players during the season (Kraemer et al. 2004; Reinke et al. 2009; Faude et al. 2011; Meyer and Meister 2011; Silva et al. 2011, 2013*b*; Heisterberg et al. 2013; Rebelo 1999) challenge the concept that soccer players can accumulate fatigue during periods of heavy match commitments and (or) as the season progresses (Reilly and Ekblom 2005). Moreover, little is known about the integrative analysis of individual physical performance and biochemical parameters during the season. In fact, data regarding the putative relations between physical fitness, match exposure, and biochemical markers (e.g., hormonal and oxidative stress parameters) during competitive soccer stress conditions are limited. Therefore, longitudinal designs aimed to study stress-related alterations induced during a soccer season should use a multidimensional test panel, consisting of sport-specific performance and biochemical-related parameters (Kraemer and Ratamess 2005; Faude et al. 2011). This type of analysis might help us understand the physiologic demands of professional soccer. In addition, given the increased frequency of match commitments in professional soccer, a clear understanding of the biochemical impact of training and competition will help in the design and development of more effective strategies to accelerate recovery (Andersson et al. 2008). Ultimately, these might help prevent situations of accumulated stress in professional competitive contexts.

This study was designed to examine, for the first time, alterations in neuromuscular parameters and hormonal, oxidative stress, muscle damage, and inflammatory markers in a cohort of professional soccer players during a season. We also wanted to determine whether individual match-accumulated time is associated with certain neuromuscular and biochemical parameters in professional soccer players.

Materials and methods

Subjects

A cohort of 14 male professional outfield soccer players (mean ± standard deviation: age, 25.7 ± 4.6 years; body mass, 76.5 ± 9.2 kg; height, 178.1 ± 5.7 cm; fat mass percentage, 9.8% ± 3.7%) from a team competing in the Portuguese professional soccer league were involved in this study. All subjects were fully informed about the aims, experimental protocol, and procedures associated with the study prior to giving their consent to participate. The study was approved by the Ethics Committee of the Faculty of Sport Sciences, University of Porto, Portugal, and followed the Declaration of Helsinki for research with humans.

Experimental design and procedures

Measurements were taken in the same order and in the same period for the 4 different evaluation timepoints: E1, conducted at the beginning of the preseason period; E2, conducted in the middle of the competitive season (January); E3, conducted at the end of the competitive season; and E4, conducted after the end of the transition period and before the beginning of the preparation period for the next season.

In E1, E2 and E3, blood samples were collected and performance was evaluated. In E4, only blood samples were collected. All in-season evaluations (E2 and E3) were performed 72 h after an official match. The performance tests were conducted in indoor facilities to exclude the effect of possible ground-surface variations during the season. Players were instructed to maintain normal routines for daily food intake. All players followed the same dietary recommendations, which were defined by the medical staff of the club. In addition, during the physical test days, the players were instructed to refrain from drinking beverages containing caffeine or alcohol. In the 2 days preceding in-season evaluations, the players had a day off (first day) and a training session (second day), which involved low-intensity exercise designed to enhance postmatch recovery.

To match the circadian rhythms of the different variables, all evaluations were done in the morning (0800 to 1200 h). The procedures were conducted in the following order: blood sample collection; countermovement jump (CMJ); 5-m sprint (T5) and 30-m sprint (T30); agility ability (T-test); and isokinetic strength assessment. In the laboratory, a resting blood sample was taken after subjects had been standing for at least 15 min. After this, subjects consumed a light standardized meal and drink, and rested for 2 h. The meal consisted on 1.7 $g \cdot kg^{-1}$ body mass of white bread and 0.3 $g \cdot kg^{-1}$ body mass of low-fat spread (Thompson et al. 2003). Before the physical tests, players performed a 10- to 15-min warm-up that consisted of light jogging, specific mobility exercises and a stretching routine, and 10-m sprints. With the exception of the isokinetic evaluation, players completed 2 trials of each test interspersed with a 1-min recovery period; the best result was used in the analysis. Each subject was allowed a minimum rest period of 5 min between tests to ensure adequate recovery. Additionally, individual accumulated time during competitive matches was recorded and used to analyze the association between performance and biochemical variables.

Playing schedule and training program

The generic training and competition plan followed by the team was developed by the technical staff. Training during the preseason consisted of 2 sessions per week of high-intensity aerobic training and strength training plus 2 "friendly" matches from week 1 to week 3; and 1 session per week of aerobic and strength training plus 6 friendly matches (3 matches a week) in weeks 4 and 5. Between the start and the middle of the season (first 25 weeks), the team performed 150 training sessions (6 per week) and played 26 officials matches (1.04 matches per week). Between E2 and E3 (17 weeks), players were engaged in 90 training sessions (5.3 per week) and played 26 official matches (1.5 per week). In the off-season (E3 to E4; 5.5 weeks) players were advised not to abstain completely from physical activity.

Physical performance tests

Countermovement jump

The CMJ was performed on an Ergojump Digitime 1000 jump mat (Digitest; Jyväskylä, Finland), in accordance with the methods of Bosco et al. (1983), in which the highest vertical jump was recorded.

Sprint

Sprint measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175; Draper, Utah, USA) mounted on tripods, positioned approximately 0.75 m above the floor and situated 3 m apart, facing each other on either side of the starting line (0 m) at 5- and 30-m distances. The players stood 0.3 m behind the starting line, started at their own discretion (timing was activated when players crossed the first pair of photocells), and ran as fast as they could to complete the 30-m sprint.

Agility

Agility was evaluated using a modified version of the agility T-test (Semenick 1990). Subjects began with both feet 0.3 m be-

hind the starting line (point A). At their discretion, each subject sprinted forward 9.14 m (to point B) and touched the base of the cone with his right hand. Next, each sprinted to the left 4.57 m and touched the base of a cone (point C) with his left hand. Each subject then sprinted to the right 9.14 m and touched the base of a cone (point D) with his right hand, sprinted to the left 4.57 m back to point B, and touched the base of a cone with his left hand. Each then turned 270° and ran to point A, crossing the finish line. As in the sprint ability test, measurements were carried out using telemetric photoelectric cells. Each player stood 0.3 m behind the starting line, the timer was activated when he passed the electronic sensors, and the clock was stopped the instant he crossed the finish line. Two test trials were performed, and times were recorded to the nearest 0.001 s.

Strength

Maximal gravity-corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement at an angular velocity of 90°·s^{-1}, using the Biodex System 2 isokinetic dynamometer (Biodex Medical Systems; Shirley, N.Y., USA), as described by Magalhães et al. (2004). After individual self-report, the dominant leg was determined with routine visual inspection of a simple target-kicking test that required accuracy. Briefly, subjects performed a standardized warm-up, consisting of 5 min on a cycle ergometer (Monark model E-824, Vansbro, Sweden) with a fixed load corresponding to 2% of body weight. This was followed by a specific submaximal warm-up, consisting of 5 submaximal repetitions at 90°·s^{-1} on the Biodex device, for familiarization purposes. Recovery time between the warm-up on the isokinetic device and the maximal test was 1 min. Three maximal repetitions were carried out, and the higher peak torque value was used for data analyses.

Blood sampling

All the venous blood samples were taken from an antecubital vein while players were in a seated position. Blood (10 mL) was collected in vacutainer tubes, using EDTA as the anticoagulant. No tourniquet constriction was used; this minimized the possible enhancement of oxidative stress induced by an ischemia–reperfusion maneuver. The freshly drawn blood was immediately centrifuged at 3000 r·min^{-1} (825*g*) for 10 min to remove the plasma. Plasma was separated into several aliquots and was rapidly frozen at –80 °C for later biochemical analysis.

Biochemical assays

Immunoenzymatic plasma testosterone (T) and cortisol (C) measurements were taken with VIDAS testosterone (Ref. 30418) and VIDAS cortisol S (Ref. 30451) commercial test kits (bioMerieux, Carnaxide, Portugal).

Plasma creatine kinase (CK) activity was determined spectrophotometrically with a commercial test kit (ABX A11A01632; Montpellier, France).

Plasma myoglobin (Mb) concentration was assessed with a commercial test kit (myoglobin bioMérieux 30446; Carnaxide, Portugal).

C-reactive protein (CRP) was measured with an enzyme-linked immunosorbent assay system (PENTRA 400, Horiba ABX; Montpellier).

Total antioxidant status (TAS) was measured spectrophotometrically with a commercial kit (Randox NX 2332; Crumlin, UK). Uric acid was determined with an enzymatic method, using a commercial kit (Horiba ABX A11A01670; Montpellier).

Regarding enzyme activities in plasma, superoxide dismutase (SOD) activity was measured spectrophotometrically at 550 nm, using a commercial RANSOD kit from Randox (catalogue No. SD 125, Crumlin). The activity of glutathione peroxidase (GPX) was assayed with a spectrophotometric technique at 340 nm, using a commercial RANSEL kit from Randox (catalogue no. RS 505). The activity of glutathione reductase (GR) was measured with a spectrophotometric procedure at 340 nm, using a commercial GR kit from Randox (catalogue no. GR 2368).

Plasma malondialdehyde (MDA) was assayed in accordance with the method described by Rohn et al. (1993), with some modifications, and measured using the formation of thiobarbituric acid reactive substances at 535 nm. Plasma protein sulfhydryls (–SH) were spectrophotometrically evaluated at 414 nm, in accordance with the method described by Hu (1990). Protein content was spectrophotometrically assayed using bovine serum albumin as the standard, in accordance with the method described by Lowry et al. (1951). Samples were analyzed in duplicate, and the mean of the 2 values was used for statistical analysis.

Table 1. Changes in muscle and performance parameters during the soccer season.

	Evaluation timepoint		
Parameter	E1	E2	E3
5-m sprint time (s)	1.01±0.06	1.00±0.05	1.02±0.05
30-m sprint time (s)	4.19±0.17	4.15±0.14	4.18±0.17
T-test (s)	8.76±0.27	8.29±0.23*	8.42±0.27*
Countermovement jump (cm)	42.4±4.0	42.8±4.4	42.2±4.4
KED (N·m^{-1})	256±25	265±14	274±19
KEND (N·m^{-1})	230±23	239±31	241±34
KFD (N·m^{-1})	139±12	143±10	146±10
KFND (N·m^{-1})	133±11	141±8	138±7

Note: Values are means ± SD. E1, beginning of the preseason period; E2, middle of the season; E3, end of the season; KED, knee extensors in the dominant leg; KEND, knee extensors in the nondominant leg; KFD, knee flexors in the dominant leg; KFND, knee flexors in the nondominant leg.

*$p < 0.01$ for E1 vs. E2 and E3.

Statistical analysis

Descriptive statistics (means ± standard deviation) were calculated. The biochemical, performance, and muscle function parameters, and the match-accumulated time showed no significant deviations from a normal distribution (Shapiro–Wilk test). One-way analysis of variance (ANOVA) with repeated measures was used to establish whether there were differences between evaluations. The Bonferroni post hoc test was performed to compare testing. Pearson's correlation coefficients (*r*) were used to assess the associations between within-player changes in biochemical, performance, and muscle function parameters and match-accumulated time. The Cohen (1988) criteria were adopted to interpret the magnitude of the correlation between the different measures: ≤0.1, trivial; >0.1–0.3, small; >0.3–0.5, moderate; >0.5, large. IBM SPSS version 18.0 (SPSS Inc.; Chicago, Ill., USA) was used for all analyses. Statistical significance was set at 0.05.

Results

Performance parameters

Performance tests scores are presented in Table 1. With the exception of agility, which improved significantly in E2 and E3, compared with E1 ($p < 0.01$), all neuromuscular parameters were unaltered during the season.

Biochemical parameters

Changes in biochemical parameters during the season are presented in Table 2. No significant changes were observed in plasma testosterone concentrations. Plasma cortisol concentrations were significantly lower in E3 than in the other timepoints ($p < 0.05$). The T/C ratio increased progressively during the competitive season, compared with E1 ($p < 0.05$), but decreased in the recovery period ($p < 0.05$).

Mb content was higher in E2 than in any other timepoint ($p < 0.05$). Plasma CK activity was higher in E2 than in E1 and E4 ($p < 0.01$), and higher in E3 than in E4 ($p < 0.05$). Plasma CRP content was higher in E2 and E3 than in E1 ($p < 0.05$).

Table 2. Changes in biochemical parameters during the soccer season.

	Evaluation timepoint			
Parameter	E1	E2	E3	E4
T (ng·mL^{-1})	6.7±1.3	7.2±0.6	6.8±0.3	6.3±1.6
C (ng·mL^{-1})	176.5±35.3	150.6±33.2	90.5±41.3*	157.3±47.2
T/C (%)	3.9±1.3	4.7±1.9†	8.6±5.3†	4.6±1.6
Myoglobin (μg·L^{-1})	17.0±5.1	28.6±16.4†	20.4±11.0	15.9±4.5
CK (U·L^{-1})	166.3±96.5	406.6±202.4†	286.5±141.7†	187.6±130.3
CRP (mg·L^{-1})	0.39±0.25	0.90±0.69‡	0.69±0.50‡	0.45±0.32
TAS (nmol·L^{-1})	1.27±0.35	1.26+0.30	1.17±0.13	1.05±0.33
Uric acid (mg·dL^{-1})	5.41±1.24	5.44±0.82	4.96±0.49	5.57±1.01
SOD (U·L^{-1})	643.3±23.6	665.3±21.9†	689.1±37.3†	660.4±26.8
GPX (U·L^{-1})	996.9±99.4	921.3±81.7	858.1±169.5	964.1±99.2
GR (U·L^{-1})	67.8±10.1	67.2±3.7	60.9±5.5§	68.8±11.5
–SH (μmol·g^{-1})	1.5±0.9	2.9±1.5†	2.8±1.3†	1.8±0.9
Malondialdehyde (μmol·g^{-1})	6.9±1.7	11.3±2.0†	12.3±4.9†	7.7±1.1

Note: Data are means ± SD. C, cortisol; CK, creatine kinase; CRP, C-reactive protein; E1, beginning of the preseason period; E2, middle of the season; E3, end of the season; E4, after the end of the transition period and before the beginning of the preparation period for the next season; GPX, glutathione peroxidase; GR, glutathione reductase; –SH, sulfhydryl groups; SOD, superoxide dismutase; T, total testosterone; T/C, testosterone cortisol ratio; TAS, total antioxidant status.

*$p < 0.01$–0.05 vs. E1, E2, and E4.

†$p < 0.01$–0.05 vs. E1 and E4.

‡$p < 0.05$ vs. E1.

§$p < 0.01$–0.05 vs. E2 and E4.

Plasma TAS, uric acid content, and GPX activity were unaltered during the season. Plasma SOD activity was higher in E2 and E3 than in E1 ($p < 0.05$). However, a decrease in SOD activity was observed from E3 to E4 ($p < 0.05$). Plasma activity of GR was lower in E3 than in E2 or E4 ($p < 0.05$). Plasma –SH content was higher in E2 and E3 than in E1 and E4 ($p < 0.05$). Moreover, increased plasma MDA content was observed in E2 and E3, compared with E1 and E4 ($p < 0.05$).

Associations between match-accumulated time, performance, and biochemical parameters

We observed that changes in the T/C ratio from E2 to E3 ($T/C_{E2–B3}$) were associated with changes in the knee flexors in the dominant leg ($KFD_{E2–E3}$; $r = 0.858$; $p = 0.003$), knee flexors in the nondominant leg ($KFND_{E2–E3}$; $r = 0.848$; $p = 0.004$) from E2 to E3. Also, in E3, associations between the T/C_{E3} ratio and knee extensors in the dominant leg (KED_{E3}; $r = 0.563$; $p = 0.015$) and $KFND_{E3}$ ($r = 0.636$; $p = 0.005$) were found. We also observed a negative association between plasma concentrations of C_{E3} and KED_{E3} ($r = -0.646$; $p = 0.009$), $KEND_{E3}$ ($r = -0.662$; $p = 0.004$), KFD_{E3} ($r = -0.677$; $p = 0.004$), and $KFND_{E3}$ ($r = -0.636$; $p = 0.006$) in E3.

In the midseason (E2), we observed that match-accumulated time (MAT_{E2}) was associated with T30 sprint ($r = -0.531$; $p = 0.019$) and CMJ ($r = 0.615$; $p = 0.009$) performances. An association between MAT_{E2} and C_{E2} ($r = 0.560$; $p = 0.024$) and T/C_{E2} ($r = -0.553$; $p = 0.033$) was also detected in E2. Additionally, correlations with MAT_{E2} were observed in E2 for TAS_{E2} ($r = 0.647$; $p = 0.003$), GPX_{E2} ($r = 0.544$; $p = 0.016$), and SOD/GPX_{E2} ($r = -0.456$; $p = 0.050$).

Discussion

Performance and biochemical alterations

The overall analysis of our data highlights significant alterations in the biochemical parameters (e.g., muscle damage and oxidative stress markers) of professional players during the season but not during the off-season.

As described in a previous report (Silva et al. 2011), an improvement in agility ability was observed from preseason to midseason and the end of the season; however, no significant alterations in other neuromuscular parameters (e.g., sprint, strength) were detected in the pre- and midseason timepoints. Despite the fact that straight sprinting has been shown to be the most frequent action in goal situations (Faude et al. 2012), enhancement in agility is an important in-season adaptation. During matches, players perform a large number of nonlinear high-intensity running-based activities (~726 + 203 turns) (Bloomfield et al. 2007). Moreover, the acceleration and deceleration performed during changes in direction produce high levels of mechanical and metabolic stress, which increase metabolic power demands and overall energy expenditure during training and matches (Osgnach et al. 2010).

Regarding the analyzed anabolic and catabolic hormonal regulation, testosterone concentration was unaltered during the in- and off-season periods. Similar (Kraemer et al. 2004; Filaire et al. 2003) and contrary (Handziski et al. 2006) findings have been reported elsewhere. However, our cortisol kinetics were different from the unchanged observations of Kraemer et al. (2004) and the in-season increments reported elsewhere (Filaire et al. 2003; Handziski et al 2006). Additionally, our results highlight the contradictions in T/C kinetics during the soccer season (Handziski et al. 2006; Filaire et al. 2003; Kraemer et al. 2004). Differences between studies might be partially explained by distinct experimental protocol designs. For example, Handziski et al. (2006) and Kraemer et al. (2004) analyzed the soccer season at different timepoints (e.g., half-season and 11-week competitive season, respectively). The different degree of competition during the in-season cycles — characterizing distinct timepoints — might explain the different results. However, the distinct evidence reported for soccer players (Filaire et al. 2003; Gorostiaga et al. 2004; Kraemer et al. 2004; Handziski et al. 2006) raises the question of the relevance of T/C kinetics as a sign of overtraining or neuroendocrine dysfunction. In fact, a negative association has been observed between changes in performance and T/C (Gorostiaga et al. 2004), and improved performance has been reported in players with higher T/C values (Kraemer et al. 2004).

Along with the lower levels of cortisol and higher T/C ratios in E3 than in the other timepoints, we observed less muscle damage (CK and Mb) and inflammation (CRP) in E3 than in E2 (altough not significant). The kinetics of muscle damage and inflammation markers suggest that muscle demands were higher in E2. CK has been associated with higher cortisol concentrations, and increased interleukin-6 cytokine release (mixed cytokine) has been associated with increased CRP and cortisol concentrations (Fragala et al. 2011; Brancaccio et al. 2007; Baird et al. 2012; Fischer 2006). In line with our observations, studies with high-level basketball players have shown, alternately, increases and decreases in catabolic- and anabolic-related

hormone concentration during the season (Martinez et al. 2010). Interestingly, the kinetics of hormone-related parameters suggest that different training strategies could be implemented to correct or prevent situations of accumulated stress in professional competitive contexts (Martinez et al. 2010). Several factors could explain the unexpected higher cortisol levels seen in E1 and E4. The psychophysiologic factors associated with this period of the season (Hoffman et al. 2005) and (or) the hypothesis of a stress reaction related to autoimposed initial physical loads during the preseason should be considered to be the most likely.

The increase in muscle damage markers in the middle of the season might be associated with residual levels of muscle damage related to regular training sessions (McLellan et al. 2010). It is reasonable to presume that an increase in the volume of training devoted to recovery during E2, due to a congested match schedule, had been adopted. This suggests a possible decrease in weekly training load, contributing to decreased skeletal muscle aggression and (or) lesion. Consequently, lower levels of CK and CRP, a higher T/C ratio, lower Mb and cortisol concentrations, and no changes in oxidative stress parameters were observed from E2 to E3.

Interestingly, significant associations between CK responses during preseason and in-season periods were observed. This suggests that CK responses are very individual (e.g., high and low responders tend to maintain this specific characteristic throughout the season). However, this physiologic characteristic was not observed in other biochemical markers of tissue aggression, such as Mb, CRP, –SH, and MDA. It should also be noted that the CK and CRP values observed during the season are within the reference ranges defined for elite soccer players (Meyer and Meister 2011). Furthermore, muscle enzyme release might not always reflect the degree of muscle damage; it is possible that the efflux of intracellular proteins results from both damage and transient changes in membrane permeability (Brancaccio et al. 2007). Other mechanisms (e.g., AMP-activated protein kinase) might explain the appearance of cytoplasmic enzymes and (or) proteins in serum after physical exercise, as opposed to structural damage arising from muscle trauma (Baird et al. 2012). Moreover, increases in serum enzyme leakage without impairments in muscle performance have been reported (Nedelec et al. 2012; Silva et al. 2013*a*). Therefore, some caution should be taken when trying to predict muscle recovery and (or) impairments from changes in serum CK concentrations.

To the best of our knowledge, no reports have been published on chronic alterations in the endogenous antioxidant system during the soccer season. Match uric acid and inosine monophosphate responses suggest that a soccer match is associated with a significant degradation in purine nucleotides (Krustrup et al. 2006; Magalhães et al. 2010). Despite the fact that we observed no changes in uric acid concentrations during the season, a compensated hypoperfusion (and hypoxia) during the playing season, resulting in decreased muscle resting and postischemic blood flow, has been suggested (Reinke et al. 2009). Consequently, this hypoxia-related condition could increase xanthine oxidase activity in endothelium cells and predispose them to oxidative and inflammatory damage (Terada et al. 1992). This is in line with in-season increases in plasma CRP and MDA concentrations.

The consistent upregulation of SOD activity might have occurred from a continuous pro-oxidant insult. In fact, SOD is a major defense against superoxide radicals, and is the first line of defense against oxidative stress (Finaud et al. 2006). Taking into account the critical physiologic role of the balance between first- (SOD) and second-step (GPX and (or) catalase) antioxidant enzymes and the function of GR in the glutathione cycle (e.g., GPX activity was unchanged, and GR activity decreased from E2 to E3), the progressive increase in the plasma SOD/GPX ratio observed during the season might favor H_2O_2 accumulation, and could indicate a low plasma scavenging efficiency, contributing to enhanced oxidative stress (Gaeta et al. 2002). This is consistent with the increased levels of MDA we found. Despite the fact that increased lipid peroxidation markers were observed (e.g., MDA), our results show a decrease in disulphide linkages (–S–S–) concentration from both proteins and reduced glutathione, which suggest less evidence of protein oxidation during the season. The homeostatic disturbances (e.g., changes in temperature, pH, and oxidative balance) that soccer players experience during activity might induce a higher expression of plasma heat shock proteins (Banfi et al. 2006). A higher heat shock protein response is thought to offer some degree of protection against further physiologic stress, which contributes, at least in part, to the higher –SH concentration found during the season. In this regard, athletes with a better training background and from a higher standard seem to show better oxidative stress-associated adaptations, manifested by a higher concentration of sulfhydryl groups (Martinovic et al. 2009; Nakagami et al. 2009).

Associations of match-accumulated time with performance and biochemical parameters

Our data suggest that match-accumulated time during the season is associated with physical, hormonal, and oxidative stress-related parameters. Associations between match-accumulated time and physical (Silva et al. 2011; Sporis et al. 2011) and hormonal responses have been described (Kraemer et al. 2004). Meister et al. (2011) observed that professional players with more match exposure presented better but nonsignificant scores in neuromuscular parameters (i.e., CMJ, drop jump height, and contact time) than players with less match exposure. In our study, higher match exposure in the midseason was associated with higher cortisol levels, a lower T/C ratio, and better physical performance (T30 sprint and CMJ). Again, this raises the question of the extent to which the anabolic/catabolic ratio can be used as a sign of overtraining or neuroendocrine dysfunction. Notwithstanding that, changes in $T/C_{E2–E3}$ were positively associated with changes in $KFD_{E2–E3}$ and $KFND_{E2–E3}$. Furthermore, a positive association of T/C_{E3} with KED_{E3} and $KFND_{E3}$, and a negative association of plasma C_{E3} with KE_{E3} and KF_{E3} force production were observed at the end of the competitive season.

Besides physical performance and hormone-related parameters, match exposure during the season might influence the endogenous enzymatic and nonenzymatic antioxidant system. Considering the potential mechanisms of increased reactive oxygen and nitrogen species (RONS) production during and after soccer training and competition, some adaptation of cellular and blood antioxidant status can be expected (Magalhães et al. 2010). The performance of eccentric contractions inducing muscle damage and inflammation (e.g., increasing neutrophil oxidative burst), ischemia-reperfusion events (augmenting xanthine oxidase free-radical-generation system activity) associated with power-related actions, the excessive trauma (causing the disruption of iron-containing protein), and increased oxygen consumption are all sources of RONS production related to the metabolic and mechanical demands of soccer that can favor increased oxidative stress and muscle damage (Magalhães et al. 2010; Fisher-Wellman and Bloomer 2009). Therefore, the demands of soccer can lead to an upregulation of the antioxidant defense system that favors a more reducing environment, protecting players with more match exposure from excessive oxidative damage during subsequent competitions and training sessions (Fisher-Wellman and Bloomer 2009; Andersson et al. 2010).

In conclusion, this study shows that soccer players face significant changes in biomarkers of physiologic strain (muscle damage and oxidative stress-related markers) during the season, but values revert to normal during the off-season. Additionally, our results suggest that match-accumulated time influences physical,

hormonal, and oxidative stress-related parameters in professional soccer players.

Disclosure statement

The authors have no conflict of interest and nothing to disclose.

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