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Original Article

Variation of oxidative stress of elite football players during pre-season and in-season

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Abstract
The elite players are constantly being exposed to multiple high physiological demands due to elevated number of training sessions and matches played in national and international competitions held several times during a week. The aim is to assess oxidative stress variation throughout pre-season and the first half of the season by comparing month-by-month free radicals. Secondly, we aimed to compare overall pre-oxidative stress to in season one. The methods is experimental. Blood samples were obtained per month from twenty-three elite football players, during seven-months season (2 months pre-season and five-months in-season). The *results show a* large negative correlation was observed between July and August and between November and December ($p=0.02$; $r=-0.54$; $p=0.03$; $r=-0.45$ respectively). Significant interaction was observed in ROS over the whole analysed period ($p<0.01$; $\eta 2=0.19$) in function of the monthly analysis. Monthly ROS increased ($p<0.05$) from November to October, and then decreased ($p<0.05$) from October to November. In conclusion the Oxidative stress vary over the season. Thus, a certain risk of non-contact injury and illness must be considered at a given seasonal stage, particularly in September, when competitive season is beginning. Further research may relate training load to oxidative stress over a given period to better explain the present results.
Key-words: Periodization, Physiology, Stress, Blood sampling, Monitoring

Introduction

The elite players are constantly being exposed to multiple high physiological demands due to elevated number of training sessions and matches played in national and international competitions held several times during a week (King & Duffield, 2009); furthermore also psychological demands, in order to situation awareness, have the role in the performance (Di Tore, 2015). Thus, high physiological capacities are required to elite football players for sustaining the stress imposed by training and matches, that could influence the subsequent performance and recovery over the season (Silva et al., 2014). Other aspects are involved in non elite soccer players (Raiola, Tafuri, 2015ab) and for intermitting training (Gaetano, Rago, 2014), that is not previous in this study, such as the stretching effects (Altavilla, 2014) and the postural aptitude (Guetano et al., 2015). In this way, the monitoring of work-load, stress and recovery in football plays utmost importance for the planning and the periodization of training (Little & Williams, 2007) to determine whether an athlete is adapting to the training program, also minimizing the risk of injury, overreaching and illness. Particularly, when recovery is uncompleted, impairments in performance and risk of injury must be strongly considered (Brink et al., 2010; Nédélec et al., 2012). Thus, several reasons have been suggested for testing athlete's status during training in order to predict performance during competition (Buchheit, Simpson, Al Haddad, Bourdon, & Mendez-Villanueva, 2011; Svensson & Drust, 2005). Oxidative stress indicators have been suggested for monitoring physiological stress imposed by football matches (Andersson, Karlsen, Blomhoff, Raastad, & Kadi, 2009; Ascensão et al., 2008a). Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defences (Betteridge, 2000). Moreover, it is highly remarkable that ROS have a high potential as signalling molecules within the cells as they regulate and can switch (on/off) several mechanisms that are redox-dependent (Betteridge, 2000; Gutteridge, 1995; Gutteridge & Halliwell, 1992). Under normal physiological conditions, the quantity of ROS is in a fine-tuned equilibrium with the antioxidant defence system (Andersson et al., 2009). However, despite well-trained athletes present an higher antioxidant defence (Brites et al., 1999), significant imbalances may be resulted following a football match or an intense training session. In this context, An official match implies several acute physiological changes such as increased cardiac output and blood flow, increasing markers of oxidative stress in plasma (Andersson et al., 2009; Ascensão et al., 2008a; Krustrup et al.,

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2006). Increased oxidative stress induced by a match or training may compromise performance throughout a given period. Particularly, a competitive football match increases the levels of oxidative stress and delayed onset muscle soreness throughout the 72 h-recovery period (Ascensão et al., 2008a). Although the levels of ROS can be measured directly, they can also be estimated by measuring the by-products resulting from their interaction with other molecules in the cells with which they have biochemical affinity. These include markers of lipid peroxidation, deoxyribonucleic acid (DNA) and protein oxidation (Gutteridge & Halliwell, 1992). Understanding how oxidative stress respond throughout a given period of the season may provide useful information on training adaptations, particularly providing an understanding of exercise-induced fatigue and recovery over the season, in order to design efficient recovery strategies. In regarding, Brink et al. (2010) suggested the individual monitoring of stress and recovery to provide useful information to prevent football players from injuries and illnesses. Recently, J. R. Silva et al. (2014) showed significant changes in oxidative stress markers of physiological strain during the season in elite football players. However, training prescription during pre-season seems to be more challenging than in-season period due the higher training load experienced by footballers (Buchheit et al., 2013). Pre-season training focuses on the fitness rebuilding following to the transition period (Jeong, Reilly, Morton, Sang-Won, & Drust, 2011; J. Silva, Brito, Akenhead, & Nassis, 2015). In contrast, the in-season focuses on the maintenance of the specific capacities, achieved during pre-season (Jeong et al., 2011). At our knowledge, little has been investigated in regarding oxidative stress over pre-season and in-period as well. The aim of this paper was to analyse changes in stress throughout the pre-season and the first half of the season by comparing month-by-month free radicals.

Methods and materials

Subjects

23 elite football players (age: 23.65 ± 2.70 years old; height: 182.43 ± 7.34 cm; body mass: 75.65 ± 6.36 kg; previous experience in elite football 9.50 ± 3.40 seasons) from the same team competing in the Italian 2nd league (*Serie B*) participated with consensus in the study. Goalkeepers were not considered for analysis. Since physical and physiological football demands imposed on goalkeepers considerably differ from that of the outfield players (Andersson et al., 2008), players from this playing position were not included in the study and in the data analysis.

Experimental design

Data collection was performed in the context of the players' regular pre-seasonal and in-seasonal work. All blood analysis were performed within the first week of each month. During pre-season, blood analysis were performed 48-hours after from a friendly match, played against a local sub-elite or amateur team. During the in-season period, the latter blood analysis were performed 48-hours from a domestic match within the first two weeks of the month.

Procedures

Diet The food intake was standardised for all players during the study period. All of them were provided to a meal plan designed by a nutritionist, and they ate all the meal together. Carbohydrate and protein intake was adjusted to the players' body weight (55/60/65/70 kg, respectively) to meet recommendations for daily recovery suggested by Maughan, Depiesse, and Geyer (2007) setting intake of ≥ 6g/kg body weight CHO and ≥ 1.2 g/kg body weight protein. The mean plan included a variation of bread, pasta, rice, cereals, milk/yogurts, vegetables and fruit to ensure appropriate intake of micro- and macro-nutrients.

Blood sampling Values of ROS were sampled at 9.00 hours, approximately one hour before the start of the training session. Blood samples were collected within 15-20 min (0 h) after breakfast.

Statistics

Data are presented as Mean ± SD. Normality was assessed thought *Shapiro-Wilks* test. A one-way repeated-measures analysis of variance (month x period) was performed. Significant interactions were subsequently determined by a *Bonferroni* post-hoc test. Magnitude of differences and explained variance are assessed by *d* values for interpreting effect size according to Cohen (1992) setting at 0.1, 0.3 and 0.5 trivial, moderate and large respectively. Correlation between variables was assessed trough Pearson' r. Magnitudes of correlation (*r*) are defined as: trivial ($r \leq 0.1$), small ($0.1 > r \leq 0.3$), moderate ($0.3 > r \leq 0.5$), large ($0.5 > r \leq 0.7$), very large ($0.7 > r \leq 0.9$) and almost perfect ($0.9 > r \leq 1.0$). If the 90% CI overlap small positive and negative values, the magnitude is deemed unclear (Hopkins, Marshall, Batterham, & Hanin, 2009). Statistical significance is set at $p<0.05$. Statistical significance was set as $\alpha \leq 0.05$. Data were analysed using SPSS 19.0 (IBM, Chicago, Illness, USA) for Windows.

Results

Large negative correlation was observed between July and August and between November and December (p=0.02; r=-0.54; p=0.03; r=-0.45 respectively). All correlations are illustrated in table 1. Significant interaction was observed in ROS over the whole analysed period ($p<0.01$; η^2=0.19) in function of the monthly analysis. Monthly ROS increased (p<0.05) from November to October, and then decreased (p<0.05) from October to November (Figure 1).

Table 1: Correlations between blood sampling collected over the considered period. Data are presented as magnitudes of correlation (*r*) and significance (*p*).

		Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Aug.	*r*	-0.54*					
	p	0.023					
Sep.	*r*	-0.01	0.21				
	p	0.94	0.40				
Oct.	*r*	-0.17	0.31	0.22			
	p	0.47	.199	0.32			
Nov.	*r*	0.10	-0.08	0.33	0.26		
	p	0.66	0.73	0.13	0.22		
Dec.	*r*	-0.15	0.14	-0.39	0.06	-0.45*	
	p	0.54	0.58	0.07	.780	0.03	
Jan.	*r*	-0.37	0.05	-0.14	-0.38	-0.26	-.290
	p	0.16	0.84	0.57	0.11	0.298	0.243

*: Significant correlation between variables (p<0.05).

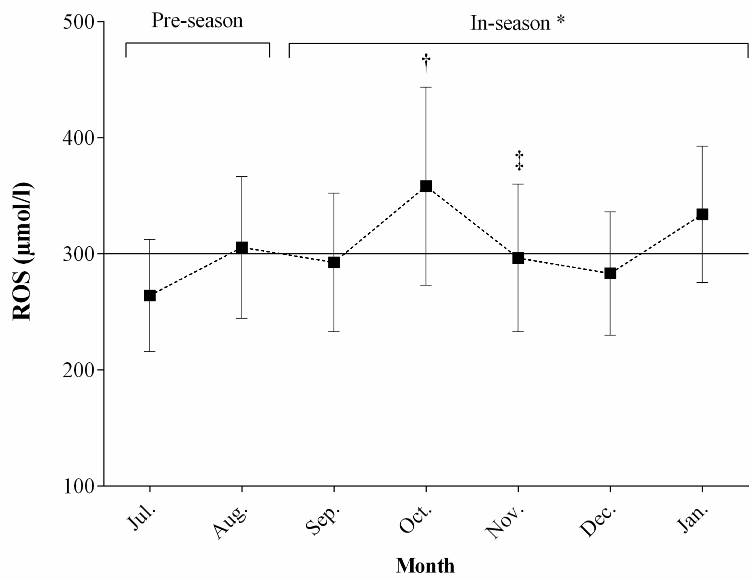

Figure 1: Repeated-measures comparisons of ROS over the considered period.
*: significant differences from Pre-season; †: significant increments from the previous month; ‡: significant decrements from the previous month; ROS: Reactive oxygen species. 300 µmol represent the ROS limit to be considered out of non-contact injury risk and/or illness.

Discussion

A football match involves several acute physiological changes such as increased cardiac output and blood flow, augmented catecholamine release, high contractile eccentric demands, mobilization of blood leukocytes and importantly relies on aerobic metabolism (Ascensão et al., 2008b). These demands seriously impact body systems causing important adaptations to exercise stimulus and, when inappropriate, may cause maladaptation that induce impairments of physical performance. Due to the above-referred relationship between body redox-related reactions and tissue adaptation and also between physical performance and oxidative damage and inflammation, we analysed the changes in stress throughout the pre-season and the first half of the season by comparing month-by-month free radicals. Pre-season vs in-season comparison. Month-by-month induced ROS was higher during pre-season compared to in-season. Our results may partially explain the conclusions of Jeong et al. (2011) where training load during pre-season appeared more intense (rating of perceived exertion and heart-rate based training load). Jeong and colleagues (2011) quantified the physiological loads of programmed mycrocycle during pre-season and in-season training in elite Japanese football players, observing higher training load values during pre-season. Month-by-month analysi. Monthly ROS increased from November to October, and then decreased from October to November. Further research is required to understand whether ROS changes are observed and its relationship to various contextual- and nutrition variables. Correlations. A large negative correlation was observed between July and August and between November and December. Further research is required to clarify and identify various factors associated to our magnitudes of correlation values. However, various limitations are implicit in the present study. Various factors related to either present subjects' shape or opponents among the various seasonal stage was not controlled. Thus, blood samplings were collected after a friendly match over the pre-season, whereas were collected after a competitive match. Another limitation was that nutritional intake was monitored only during pre-season.

Conclusions - Given the innumerable quantity of factors occurring during in-season influencing performance, a complete physical performance and recovery may not be achieved between-matches and trainings. Members of medical staff, fitness coaches should be aware of variation in free radicals over the competitive period in order to reduce the risk of non-contact injury and illness, and to ensure adequate between- matches and training recovery, providing and personalised diet and manipulate training load over the pre-season and the in-season period.

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