Redox Status of Professional Soccer Players is Influenced by Training Load Throughout a Season

Abstract

The purpose of this study was to follow-up the variation of pro-/antioxidant status throughout a whole season in elite professional soccer players from the French league (n=19, 18.3 ± 0.6 years) and to examine a possible link between these variations and training load. 5 time points (T1, T2, T3, T4, T5) were proposed to surround crucial periods of training during the whole season: the pre-season training/mid-season periods (T1–T2 and T3–T4), the championship or in-season periods (T2–T3 and T4–T5). At these times, blood samples were collected to measure pro-/antioxidant status (in erythrocytes: the ratio of reduced glutathione/oxidized glutathione, superoxide dismutase and glutathione peroxidase activities, in plasma: alpha-tocopherol, beta-carotene), and dietary intakes were also recorded. Training loads were quantified by the rating of perceived exertion method weekly throughout the season. Pro-/antioxidant-related measurements showed no modifications except for GSH/GSSG ratio, which evolved significantly between season periods: from 36.43 ± 4.15 (T1) to 115.99 ± 16.43 (T2) to 91.64 ± 21.24 (T3) to 202.29 ± 29.26 (T4) to 59.61 ± 14.61 (T5). We observed a significant correlation ($r^2=0.84$) between changes in GSH/GSSG ratio and cumulated mean training loads. In conclusion, these results suggest that the redox status of professional soccer players is altered according to training period (in-season periods) and that GSH/GSSG ratio variations are correlated with cumulated training loads.

Introduction

Soccer is a highly demanding activity that requires players to run about 10–12 km per game including many high-intensity running bouts, sprints and changes of direction that taxes both aerobic and anaerobic energy pathways [2]. These considerable physical demands have deleterious consequences on physical capacities, as extensively described elsewhere [23]. These impairments of physical capacities are notably explained by alterations of biological processes such as inflammation [16], muscle damage [6] and oxidative stress [6,16,26].

Oxidative stress, which refers to an imbalance between reactive oxygen species (ROS) molecules and the antioxidant system, may lead to DNA, protein and lipid damage, depending on the magnitude of stress [11]. Such an oxidative stress state has been highlighted in response to soccer games [6,16] and in response to any form of both aerobic and anaerobic exhaustive exercises [8]. In recent years, the understanding of the role of ROS in homeostasis has improved. It is now well accepted that ROS production plays a key role in many cellular functions within skeletal muscle tissue. Indeed, during exercise, ROS produced by skeletal muscle are known to contribute to both force and fatigue development [25], illustrating that ROS effects on skeletal muscle are regulated by the hormesis theory. Low levels of ROS (reduced state) do not allow optimal force production whereas high levels of ROS (oxidized state) impair skeletal muscle force production [25] and are also closely associated with overtraining syndrome [28]. Therefore, maintaining an optimal redox state is of fundamental importance for optimal muscle performance and hence for soccer performance throughout the season. One of the most commonly measured markers of cellular redox status is the ratio of reduced to oxidized glutathione (GSH/GSSG), which acts as a redox control node for training-induced adaptations and responses to acute exercise-induced oxidative stress [5].

In high-level soccer, the season is divided into 2 main categories of training periods: the pre-season and the in-season periods, which are com-
posed of distinct training cycles. During the in-season period, the number of games played [3] and as a consequence, the global training load can leave soccer players vulnerable to oxidative stress and oxidative stress-related maladaptation and underperformance [8]. Consequently, to avoid maladaptations, overtraining [8,29] and then to maintain performance, it is crucial to follow biomarkers related to oxidative stress and antioxidant status. Monitoring of pro-/antioxidant balance has been performed in a few studies [19,29,30]. It has been shown that intense training periods are associated with an increase in oxidative stress-related markers and a decrease in antioxidant markers, suggesting that training intensity may alter cellular integrity (see review in [8]). To our knowledge, very few studies have investigated the relationship between seasonal periods of training and oxidative stress [29]. In this study, authors have not associated oxidative stress with training load measurements. Such a characterization, with the use of rating of perceived exertion (RPE) methodology, could help to clarify how the pro-/antioxidant balance is modulated by training period and training load throughout a whole season. Therefore, the aim of this study is to characterize the evolution of pro-/antioxidant status in professional soccer players throughout a whole season and to explore a possible link between redox status and training load in professional players. We hypothesize that 1) redox status will vary during the soccer season and 2) redox status variations during the season are related to the training load.

Methods

Subjects

19 professional soccer players from the Stade Rennais Football Club (French League) (18.3 ± 0.6 years; 179.4 ± 6.2 cm; 73.0 ± 6.2 kg) participated in this study. 10 players of the 19 are international players in their age categories and respective country. We only included in the study cohort subjects for which we have collected both training loads and blood samples. In case of international competition, games, injury or specific training, training loads were also collected and taken into account for general quantification. Subjects were asked to not use antioxidant supplements during the season. All players were notified of the research procedures, requirements, benefits, and risks before giving informed consent. A university research ethics committee granted approval for the study. This study meets the ethical standards of this journal as detailed elsewhere [12].

Design

To study the evolution of pro-/antioxidant status of professional soccer players, we have addressed the design of a non-interventionist study (i.e., no intervention in training program) and performed monitoring during a whole season. 5 time points T1 (representing baseline conditions in July), T2 (in September), T3 (in December), T4 (in January) and T5 (in May) were proposed to surround crucial periods of training during the season: the pre-season training (T1–T2), the championship or in-season period (T2–T3 and T4–T5) and the mid-season period between the 2 periods of championship (T3–T4). Blood samples were collected at each time point to monitor pro-/antioxidant status. Dietary intakes were also recorded at the same time. Furthermore, training loads were recorded after each training sessions and games.

Methodology

Training program

During the studied period, players performed 6–7 training sessions and one game per week representing 15–18 h of training per week. The training program is briefly described in Supplement 1.

Training load assessment

Training loads were assessed using the Borg’s category ratio (CR) validated as a tool to control and monitor internal load in soccer activity by Impellizzeri and colleagues [15] (Supplement 2). To obtain a training load index, rating of perceived exertion obtained after each training session was multiplied by the training session duration in minutes. Data were collected within 15 min following the end of each training session and daily training loads were calculated as the sum of training load cumulated per day and then per week as illustrated in Fig. 1. All athletes had been familiarized with this scale for rating perceived exertion before the commencement of the study.

Dietary intake

The participants were asked to eat and drink normally and to not consume any antioxidant supplements. A quantitative assessment of dietary and antioxidant intake was provided by means of a diet record. The food records were completed by a nutritional expert and analyzed using a computer dietary analysis (Nutrilog 2.3, Nutrilog SAS, France) employing the Ciqual table of food composition as previously described [31]. This dietary intake assessment aims to evaluate antioxidant intake.

Pro-/antioxidant status-related measurements

To avoid exercise-related blood perturbations, blood samples were collected at rest after 48 h of recovery, in a fasted state from an antecubital vein. An EDTA vacutainer was used to evaluate α-tocopherol, retinol and β-carotene in plasma; superoxide dismutase (SOD) in erythrocytes [SOD] ery , glutathione peroxidase (GPX) in erythrocytes [GPX] ery , reduced and oxidized glutathione (GSH and GSSG) in whole blood. Preparation of blood was immediately carried out after collection. Before centrifugation, for GSSG measurement 100 μL of whole blood was added to 10 μl of...
scavenger molecules, to avoid oxidation, provided in the GSSG kit and immediately stored at −80 °C. For reduced GSH and GPx measurements, 50 μL and 150 μL of whole blood were collected, respectively and immediately stored at −80 °C. For SOD activity, 500 μL of the whole blood was centrifuged (1 500 g, 10 min, 4 °C) and 1 mL for plasma alpha-tocopherol, beta-carotene and retinol measurement was stored at − 80 °C. Analyses were performed on plasma obtained after centrifugation (1 500 g, 10 min, 4 °C) and measurements were shown to be valid and reliable as previously described [24, 31].

**Results**

**Training load measurements**

Training load varies significantly according to the training period. During the pre-season period (T1–T2), the training load is 2 510 ± 489 AU and significantly decreases by 12% during the first in-season period (T2–T3) (p < 0.05). During the mid-season period (T3–T4), the training load increases significantly by 10% as compared with the previous periods (T2–T3) (p < 0.05) and finally decreases by 4% as compared with the mid-season period during the last in-season period (T4–T5) (p < 0.05) (●▶ Fig. 1).

**Assessment of antioxidant intake**

No significant changes are observed concerning AO intake except for Vitamin E that significantly changes during the season. From 9.4 ± 4.0 mg in T1, it decreases by 10% in T2 (p < 0.05) and continues to significantly drop by 60% in T3 (p < 0.05). Despite a lack of significance, we observe an increase by 26% and 2% in T4 and T5, respectively, as compared with the previous time point (●▶ Table 1).

**Pro-/antioxidant status-related measurements**

The concentration of plasma antioxidant vitamins does not change significantly throughout the season. Similarly, there are no changes in enzymatic activities of either [GPX]_{ery} or [SOD]_{ery} throughout the season (●▶ Fig. 2).

The GSH/GSSG ratio significantly changes during the season. At baseline (T1), the ratio is 36.4 ± 15.2 and significantly increases (318%, p < 0.05) at the end of the pre-season period (T2). In T3, the ratio decreases by 21% as compared with T2 (n.s.). From T3 to T4, the ratio significantly increases by 221% (p < 0.05). Finally, the ratio significantly increases by 221% (p < 0.05). Finally, 

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**Table 1** Antioxidant dietary intake throughout the season.

<table>
<thead>
<tr>
<th>Vitamin Type</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (ug)</td>
<td>37.2</td>
<td>31.8</td>
<td>25.0</td>
<td>24.7</td>
<td>23.5</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>246</td>
<td>132.7</td>
<td>148.2</td>
<td>150.1</td>
<td>163.1</td>
</tr>
<tr>
<td>SEM</td>
<td>56.45</td>
<td>30.45</td>
<td>34.04</td>
<td>40.1</td>
<td>43.59</td>
</tr>
<tr>
<td>95% CI low</td>
<td>259.6</td>
<td>254</td>
<td>178.5</td>
<td>160.6</td>
<td>141.5</td>
</tr>
<tr>
<td>95% CI hi</td>
<td>496.8</td>
<td>381.9</td>
<td>321.5</td>
<td>333.9</td>
<td>329.2</td>
</tr>
<tr>
<td>Effect size</td>
<td>-0.32</td>
<td>-0.48</td>
<td>-0.02</td>
<td>-0.07</td>
<td></td>
</tr>
</tbody>
</table>

Antioxidant dietary intake was recorded 5 times during the season. Evolution of retinol intake (µg), beta-carotene intake (µg), Vitamin E intake (mg) and vitamin C intake (mg) throughout the season. Data are represented as mean ± SD.

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**Statistics**

Results are presented as mean±SD. A one-way analysis of variance (ANOVA) was performed to test the differences between variables according to the season period (time effect). Inferential statistics such as 95% of confidence interval and the effect size were also calculated. Pearson’s correlation was also used to correlate training load and changes in selected parameters. For the statistical analysis, GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA) was used. The significance level was set at p < 0.05.
the GSH/GSSG ratio significantly drops in T5 by 71% (p < 0.05) as compared with T4 (Fig. 3a).

**GSH/GSSG ratio and training loads relationship**

To investigate a possible link between redox status and training loads, we have plotted change in redox status mean values, expressed as a percentage (Tₙ–Tₙ⁻¹), and the cumulated mean training load values.

There is a significant correlation between mean change in GSH/GSSG and the mean of cumulated training load values over the period (p < 0.001; r² = 0.86) (Fig. 3b).

**Discussion**

This study is the first to monitor the pro-/antioxidant status of professional soccer players in relation to training periods and quantitative loads over an entire season. In this study, we showed for the first time that redox status changes (measured by GSH/GSSG ratio) of professional soccer players are significantly associated.

The RPE is one of the gold standard methods to quantify training load. This method is simple, validated, non-invasive, inexpensive and can be applied routinely and simultaneously in a large numbers of athletes [15]. Here, the use of the RPE method reveals a distinct distribution of training load according to the training periods. We report significant differences between the pre-season period and competitive period. The pre-season period focuses on the building of physical conditioning (high volume; moderate intensity) whereas the competitive season focuses on the maintenance of physical capacities required to perform at a high level such as sprinting or repeat high-intensity running bouts (moderate volume; high intensity) [2]. These differences in training program are illustrated by the disturbances in internal load perceived by players and training quantification estimates frequently made by the fitness coach. These psychometric data are in accordance with other data showing that psychometric markers such as stress indicators vary with seasonal changes [7]. Overall, these data strongly suggest that these different training programs have different effects that can be illustrated by both psychometric indices such as RPE or mood profile and also by biological and hematological responses in professional soccer players [7].

Blood samples were collected following a 48h period of total recovery. This fixed condition set by medical staff and coaches.
aims to avoid drastic changes in redox status following training sessions as observed [8] and thus strengthen our study design. No modifications of either antioxidant defenses in plasma or in erythrocytes and antioxidant dietary intake were observed throughout the whole season. Therefore, as reported earlier [14], our results show an inconsistent relationship between antioxidant dietary intake assessment and blood-related antioxidant measurements. These results may suggest that low antioxidant intake does not necessarily induce low plasma values when values of antioxidant intake are in accordance with French recommendations [20]. Collectively, it suggests that the investigation of the pro-/antioxidant profile requires not only dietary intake assessment but also blood exploration to obtain insights into pro-/antioxidant body homeostasis.

Antioxidant enzymes display no significant modifications throughout the season, probably due to the repeated training sessions that do not allow the body to adapt. In addition, antioxidant dietary intake may not be sufficient for soccer players to cope with repeated exercise-induced oxidative stress. These results suggest that the overall training load associated with the antioxidant dietary intake does not allow the increase of antioxidant enzymes activities. Interestingly, the only antioxidant-related biomarker that evolved implies in its expression an oxidized form of antioxidant. Indeed, we report that only the GSH/GSSG ratio displays large and significant variations during the studied period. Between T1 and T2, the ratio strongly increases, meaning that the body adapts to pre-season training [22]. It decreases significantly at the end of the first competitive period (T3) characterized by intense training and increases after the mid-season period between the 2 competitive periods (T4). Finally, the ratio decreases sharply at the end of the second competitive period (T5). GSH is the most important low molecular weight antioxidant synthetized in cells and its biologically active form, reduced GSH, plays a major role in the removal of many reactive species and is also involved in the maintenance of the intracellular redox state, in cellular signaling, regulation and activation of redox-sensitive transcription factors and thiol-disulfide exchange. GSSG is the oxidized form of GSH and reflects an intracellular redox imbalance. Therefore, the GSH/GSSG ratio is related to oxidative stress and is even considered the most sensitive marker of oxidative stress in response to training and the best indicator to reflect redox status [4]. The authors of the study have reported a significant increase in resting GSH/GSSG ratio and a significant decrease in GSSG levels after physical training that comprises aerobic training, circuit training or combined training. Generally, previous studies have reported that following regular aerobic training, antioxidant potential increases and oxidative stress decreases [5]. In contrast, high-intensity strenuous training results in a decrement in circulating antioxidant [18, 27, 29]. These data are in accordance with our measurements showing that pre-season and mid-season training (i.e., aerobic training and light resistance power training) have been found to increase the GSH/GSSG ratio, indicating a positive adaptation in comparison to competitive period (i.e., high-intensity training and moderate to heavy resistance power training) which is characterized by a striking decrease in the GSH/GSSG ratio. These data suggest that regular moderate training may enhance antioxidant capacities whereas repetitive intense training can lead to the depletion of antioxidant defenses and oxidative stress [8], illustrating the so-called hormesis concept. Hormesis is a dose-response phenomenon characterized by either a U-shaped or an inverted U-shaped dose response depending on the end point measured. This means that oxidative stress is needed for optimal exercise capacity (i.e., force production) [25] and for training-induced adaptations [10] but that a low or too high level of stress inhibits this process and can lead to performance impairment, overtraining [18, 29] and thus potential injury [8]. Similarly, an optimal level of antioxidant defenses exists. It should be neither too low to protect the body against oxidative stress nor too high to ensure optimal exercise capacity.

To our knowledge, only one study has performed longitudinal monitoring of pro-/antioxidant status in both sedentary and trained subjects [1]. Trained subjects did not display similar changes in pro-/antioxidant status, strengthening the fact that training is an important moderator of changes of pro-/antioxidant status [9, 10]. Because of different time points that are not related to training periods but rather to seasonal periods, we are not able to address a comparison of the changes of pro-/antioxidant status [1]. A few studies have reported that intense training may alter redox homeostasis and lead to oxidative stress [9, 17, 18, 29], however, the relationship between intense training and oxidative stress remains unclear. Interestingly, the most important result of this study is the link between training load quantification and redox homeostasis. Indeed, mean changes in GSH/GSSG ratio and the cumulated training loads of the period are correlated, and are so for a whole season. We have chosen to express training load as the cumulated training load to better reflect the cumulated effect of repeated loads over a prolonged period compared to mean training load. In our view, this index better reflects the repetitive nature of professional soccer training and its role in the modulation of pro-/antioxidant balance.

These results based on a unique numerical report strongly suggest that in professional soccer players, the more training loads are cumulated, the more the GSH/GSSG ratio decreases, suggesting the occurrence of oxidative stress. As a consequence, monitoring pro-/antioxidant parameters in elite athletes is of interest to reach and maintain the highest performance ability. Interestingly, training load quantification based on RPE methodology fits well with variations of biological outcomes related to redox status. Our results highlight a new function of RPE that have to be characterized and validated further.

Some blood-sample-related studies have been performed [13, 22] and point out the need for appropriate procedures (i.e., rest period before sampling) to properly explore hematological parameters and avoid misinterpretation. In contrast to the short-term study from Meister and colleagues [21] who have recently reported that a 3-week period of high match exposure in elite soccer players does not affect laboratory parameters, we show that the iterative nature of training and competitive program alters the pro-/antioxidant balance in professional soccer players during a whole season. Such alterations may have deleterious consequences for physical capacity and subsequent soccer performance. Therefore, monitoring should be integrated into a long-term individual procedure to observe training effects.

### Practical Applications

**Physical demand in professional modern soccer players is considerable** [2]. The balance between stress and recovery is a crucial issue to avoid such negative outcomes. Regular blood samples related to the pro-/antioxidant profile of professional soccer players appear to provide interesting information with regard to exercise capacity that should be considered by coaches and medical staff.

In this study, we point out that competitive periods are characterized by high training loads and are associated with an altered redox homeostasis. This illustrates the need for a recovery period following prolonged physical strain. The mid-season period allows players to recover and present lower oxidative stress, highlighting that coaches and physical trainers have to integrate a mid-season period to limit oxidative stress and maintain the physical integrity of professional soccer players. Consequently, we recommend that monitoring should be realized in close association with procedure recommendations and training periods, and training loads monitored to limit the risk of unbalanced stress and recovery state that may lead to injury. In future, it could be tempting to integrate more detailed information related to the training program (i.e., aerobic, resistance, high intensity intermittent training, etc.) to further explore the relationship between the long-term training program and redox homeostasis.

**Conclusion**

In conclusion, this study is the first to address an objective relationship between alterations of redox status and training load in professional soccer players. We demonstrate that training periods are specific in terms of training loads and biological redox outcomes. Interestingly, competitive periods are characterized by higher training loads and are associated with an altered redox homeostasis. We also report a new function of RPE methodology as a tool for detection of variations of biological outcomes related to redox status such as GSH/GSSG.

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**References**