Effects of chronic strenuous physical exercise on oxidative stress and antioxidant capacity in Sub-Saharan African professional soccer players

R.D.M. Takam1, V.J.A Moor2,3,4, J.R.N. Nansseu5,6*, C.A. Pieme3, M. Azabji-Kenfack3, B.M. Moukette4, F. Tankeu4, C.M. Tchoula4, J.Y. Ngogang1,2,3

1University of Mountains, Bangangté, Cameroon
2Laboratory of Biochemistry, Yaoundé University Teaching Hospital, Yaoundé, Cameroon
3Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon
4Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
5Sickle Cell Disease Unit, Mother and Child Centre, Chantal Biya Foundation, Yaoundé, Cameroon
6Department of Public Health, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon

Correspondence author: Dr. Jobert Richie N. Nansseu; Department of Public Health, Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, PO Box 1364 Yaoundé, Cameroon; Tel: 00237 674359276; Fax: 00237 242230504; e-mail: jobertrichie_nansseu@yahoo.fr

Institute where the work was conducted: The Yaoundé University Teaching Hospital, Yaoundé, Cameroon

Objective: To evaluate the oxidative stress status of professional soccer players and its evolution during part of a competition season in Cameroon, a Sub-Saharan African country.

Material and methods: We conducted a cohort study in 2012 among a team of the Championship. Participants were 18 healthy male soccer players. Three samplings were performed in March (T1), May (T2), and July 2012 (T3) to measure malonaldehyde (MDA), the ferric reducing antioxidant power (FRAP), reduced glutathione (GSH), superoxide dismutase (SOD), oxidized low density lipoprotein antibodies (ox-LDL-Ab) and uric acid.

Results: Ages ranged from 16 to 28 years old with a mean (Standard deviation) of 20.6 (3.1) years old. MDA values decreased from T1 to T3, though non-significantly (p = 0.092). Titers of ox-LDL-Ab significantly increased from T1 to T3 (p = 0.006). FRAP values diminished significantly from T1 to T3 (p = 0.033). We found an overall increment in levels of GSH from T1 to T3, but the difference was not statistically significant (p = 0.713). SOD activity significantly rose up from T1 to T3 (p = 0.030). Uric acid levels decreased un-significantly from T1 to T3 (p = 0.383). On the whole, pro-oxidants were not correlated or very weakly correlated to antioxidant products throughout the three samplings.

Conclusion: Chronic exposure to strenuous exercise may be marked by an increased production of pro-oxidants alongside a relative increase in antioxidants. However, this latter increment may not be sufficient enough to counterbalance the overproduction of pro-oxidants.

Key words: oxidative stress status, pro-oxidants, antioxidants, professional soccer players, Cameroon.

ABSTRACT

Objective: To evaluate the oxidative stress status of professional soccer players and its evolution during part of a competition season in Cameroon, a Sub-Saharan African country.

Material and methods: We conducted a cohort study in 2012 among a team of the Championship. Participants were 18 healthy male soccer players. Three samplings were performed in March (T1), May (T2), and July 2012 (T3) to measure malonaldehyde (MDA), the ferric reducing antioxidant power (FRAP), reduced glutathione (GSH), superoxide dismutase (SOD), oxidized low density lipoprotein antibodies (ox-LDL-Ab) and uric acid.

Results: Ages ranged from 16 to 28 years old with a mean (Standard deviation) of 20.6 (3.1) years old. MDA values decreased from T1 to T3, though non-significantly (p = 0.092). Titers of ox-LDL-Ab significantly increased from T1 to T3 (p = 0.006). FRAP values diminished significantly from T1 to T3 (p = 0.033). We found an overall increment in levels of GSH from T1 to T3, but the difference was not statistically significant (p = 0.713). SOD activity significantly rose up from T1 to T3 (p = 0.030). Uric acid levels decreased un-significantly from T1 to T3 (p = 0.383). On the whole, pro-oxidants were not correlated or very weakly correlated to antioxidant products throughout the three samplings.

Conclusion: Chronic exposure to strenuous exercise may be marked by an increased production of pro-oxidants alongside a relative increase in antioxidants. However, this latter increment may not be sufficient enough to counterbalance the overproduction of pro-oxidants.

Key words: oxidative stress status, pro-oxidants, antioxidants, professional soccer players, Cameroon.
INTRODUCTION

During aerobic metabolism, much of the oxygen consumed is bound to hydrogen during oxidative phosphorylation, thus forming water. However, it is clearly figured out that almost 2 to 5% of the oxygen consumed during respiration is not completely reduced to water, instead converted into byproducts, free radicals also known as reactive oxygen species (ROS)\(^1\)\(^-\)\(^3\). A free radical is defined as a molecule containing one or several unpaired electrons in its outer orbit. Free radicals of biological importance include nitric oxide, dioxygen, superoxide, and lipid hydroperoxyl radicals\(^2\),\(^4\). During exercise, when oxygen uptake is increased to 10–20-fold above that at rest, it is very likely that free radicals are concurrently produced to a greater extent than at rest. Indeed, evidence has accumulated that free-radical generation is enhanced during strenuous or even moderate exercise\(^1\),\(^5\)\(^-\)\(^10\).

The high production of ROS is responsible for a series of physiological and biochemical changes capable of inducing adverse effects on health and wellbeing\(^11\). In fact, ROS have been shown to induce damage in all cellular macromolecules such as lipids, proteins and DNA\(^12\). Irreversible oxidative damage to certain vulnerable molecules contributes to the degenerative process associated with cell breakdown and aging\(^3\). Moreover ROS are incriminated in the generation of diseases such as cancer, atherosclerosis, myocardial ischemia, and hepatic diseases\(^13\). Thus, chronic augmented ROS generation associated with strenuous aerobic performance might promote a wide range of injuries, illnesses, and probably, premature aging and death of the athletes\(^14\).

However, and happily, in order to combat the deleterious effects of ROS, the human body contains an elaborate exogenous antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals (zinc and selenium for instance), alongside endogenous antioxidant compounds. These include anti-oxidative enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase, and non-enzymatic defenses such as vitamin C, vitamin E, carotenoids, flavonoids, albumin, uric acid, bilirubin, ubiquinol coenzyme Q-10, reduced glutathione (GSH), and estrogens\(^2\),\(^3\),\(^6\),\(^15\),\(^16\). During exhaustive exercise, the pro-oxidant/antioxidant balance shifts in favor of the former, with the rate of ROS production exceeding their rate of removal by antioxidant defense mechanisms, leading to the development of oxidative stress defined as an increase above physiological values of the steady-state concentrations of ROS\(^1\),\(^3\),\(^6\),\(^15\).

Oxidative stress status can be assessed by measurement of pro-oxidant markers such as malondialdehyde (MDA) and oxidized low-density lipoproteins (ox-LDL) on one hand, and that of anti-oxidant compounds such as reduced glutathione (GSH), superoxide dismutase (SOD), uric acid and vitamins C & E on the other hand, along with dosage of the ferric reducing ability of plasma now renamed as ferric reducing antioxidant power (FRAP). FRAP may express the total antioxidant capacity of blood\(^17\). Until now, there is a lot of controversy on whether excess production of pro-oxidants is efficiently counter-balanced by a proportional and adaptive production of anti-oxidants during acute or specifically chronic strenuous training. This has being well reviewed by Clarkson and Thompson\(^2\).

In order to better elucidate this concern, the present study was designed to evaluate the oxidative stress status of professional soccer players and its evolution during part of a competition season in a Sub-Saharan African (SSA) country where there is dearth of research on the topic. We hypothesized that pro-oxidants may increase over time, and that anti-oxidant production may be positively correlated to them so as to counteract negative effects of these deleterious byproducts.

PARTICIPANTS AND METHODS

Study design and participants

This was a prospective and cohort study carried-out from May to July 2012 among a team of the
Cameroon Elite one Football Championship, namely “Renaissance de Ngoumou”. This is a football club founded in 2000 and based in Ngoumou, a town situated 60 km from Yaoundé, the capital city of Cameroon. All the procedures used in this study were in keeping with the current revision of the Helsinki Declaration. The study received approvals from the administrative staff of the team and from the Cameroon Football Federation, and was delivered an ethical clearance by the Cameroon National Ethics Committee.

Participants were male soccer players belonging to the aforementioned team, enrolled if they were in good health and irrespective of their age, body mass index and region of origin. Before the beginning of the Championship in early March 2012, these players went through 14 weeks of intensive physical preparation consisting of at least two hours of daily trainings plus regular friendly matches. Our study period covered the first leg of the Elite One Championship, a part of the return phase, and a truce during which the team was engaged in qualification matches counting for the Cameroon Male Soccer National Cup. During this period, players were weekly subjected to 10 hours of workouts plus a match. Obviously, before enrolling a player in the study, we obtained from him (or his guardian for those aged below 18 years old) a written and signed informed consent form.

**Blood sampling**

Three samplings were performed during the study period, occurring at an 8-weeks constant interval: March (T1), May (T2), and July 2012 (T3). Players had to be free of any training for at least 24 hours prior to blood collection which was performed early in the morning after a 12-hours overnight fast. Blood was aseptically collected from each participant by venipuncture of the brachial vein in a 5 ml EDTA tube and in a 5 ml dry tube, without a tourniquet or fist clenching. Samples were afterwards put on ice and immediately transported to the Biochemistry Laboratory where plasma and serum specimens were separated accordingly by centrifugation at 3000 rpm during 5 minutes and kept at -20°C for further biochemical analyses.

**Biochemical determinations**

For the determination of malonaldehyde (MDA) concentrations, 100 μl of plasma were added to 2 ml of reagent [Thiobarbituric acid (0.37%) / Hydrochloric acid (0.25N) / Trichloroacetic acid (15%)] and boiled at 100°C for 15 minutes. After cooling, the solution was centrifuged at 3000 rpm for 5 minutes, and the optical density of the supernatant collected was measured at 535 nm against the blank (i.e. plasma replaced by distilled water) using a spectrophotometer (BioMate3S UV-Visible, Thermo Scientific™ Manufacturer, Wohlen, Switzerland). Eventually, the concentration of MDA was calculated and expressed in μmol/l.

The ferric reducing antioxidant power (FRAP) concentration was assayed with regard to Benzie and Strain’s procedure (17). Indeed, fifty μl of plasma diluted twice were added to 900 μl of FRAP reagent and incubated for 25 minutes at 37°C. The optical density of the mixture was afterwards measured at 593 nm against the blank. The desired concentration was derived using a standard curve and expressed in mmol/l.

In order to determine the concentration of reduced glutathione (GSH), a volume of 20 μl of serum was added to 3ml of Ellman’s reagent, then mixed and incubated at 37°C for 60 minutes. The optical density of the solution was subsequently read at 412 nm against the blank, and the concentration of GSH expressed in μmol/l.

The activity of superoxide dismutase (SOD) and titers of oxidized low density lipoprotein antibodies (ox-LDL-Ab) were assessed using commercial ELISA kits respectively from Cayman® (Cayman Chemical Company, Ann Arbor, USA) and Cusabio Biotech® (CusabioBiotech Company, Hubei, China). Their concentrations were expressed in IU/ml and mIU/ml respectively.
**Statistical methods**

Statistical analyses used SPSS version 20.0 (IBM SPSS®Inc, Chicago, Illinois, USA). Results are presented as count (proportion), mean (SD) or median (inter quartile range, IQR) where appropriate. Variable comparisons were performed by means of the Student’s t-test for paired samples, and the Pearson correlation test was utilized to search for any association between quantitative variables. Results were considered statistically significant each time the p value was less than 0.05.

**RESULTS**

From 30 players at the first sampling, only 18 players attended the last measurement, meaning that 12 players (40%) have abandoned the study. Age of participants ranged from 16 to 28 years with a mean of 20.6 (3.1) years. The mean BMI was equal to 23.4 (1.3) kg/m², 2 subjects (11.1%) being slightly overweight (25 < BMI < 26 kg/m²). The mean values of MDA, ox-LDL-Ab, FRAP, GSH, SOD and uric acid from the first to the third sampling are displayed in Table 1, and their variations are more depicted by Figures 1, 2, 3, 4, 5 and 6 respectively.

We observed a significant increase in MDA values between the first and the second sampling (p = 0.022), but contrarily a significant decrease in these values from the second to the third sampling.

**TABLE 1. Profile of the study participants (N = 18)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16</td>
<td>28</td>
<td>20.6</td>
<td>3.1</td>
<td>19.5</td>
<td>19 - 23</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4</td>
<td>25.8</td>
<td>23.4</td>
<td>1.3</td>
<td>23.3</td>
<td>22.4 - 24.4</td>
</tr>
<tr>
<td>Uric acid 1 (mg/L)</td>
<td>32.45</td>
<td>115.47</td>
<td>61.67</td>
<td>20.05</td>
<td>58.11</td>
<td>49.05 - 70.00</td>
</tr>
<tr>
<td>Uric acid 2 (mg/L)</td>
<td>26.46</td>
<td>63.38</td>
<td>48.44</td>
<td>9.58</td>
<td>48.51</td>
<td>41.98 - 54.27</td>
</tr>
<tr>
<td>Uric acid 3 (mg/L)</td>
<td>42.84</td>
<td>86.81</td>
<td>57.54</td>
<td>10.87</td>
<td>56.74</td>
<td>48.39 - 63.97</td>
</tr>
<tr>
<td>MDA 1 (μmol/L)</td>
<td>0.115</td>
<td>0.340</td>
<td>0.168</td>
<td>0.048</td>
<td>0.160</td>
<td>0.155 - 0.173</td>
</tr>
<tr>
<td>MDA 2 (μmol/L)</td>
<td>0.102</td>
<td>0.544</td>
<td>0.236</td>
<td>0.105</td>
<td>0.224</td>
<td>0.170 - 0.264</td>
</tr>
<tr>
<td>MDA 3 (μmol/L)</td>
<td>0.057</td>
<td>0.243</td>
<td>0.139</td>
<td>0.049</td>
<td>0.135</td>
<td>0.102 - 0.182</td>
</tr>
<tr>
<td>FRAP 1 (mmol/L)</td>
<td>0.405</td>
<td>0.658</td>
<td>0.539</td>
<td>0.082</td>
<td>0.537</td>
<td>0.469 - 0.613</td>
</tr>
<tr>
<td>FRAP 2 (mmol/L)</td>
<td>0.285</td>
<td>0.597</td>
<td>0.441</td>
<td>0.084</td>
<td>0.451</td>
<td>0.397 - 0.488</td>
</tr>
<tr>
<td>FRAP 3 (mmol/L)</td>
<td>0.204</td>
<td>0.616</td>
<td>0.470</td>
<td>0.126</td>
<td>0.503</td>
<td>0.363 - 0.575</td>
</tr>
<tr>
<td>GSH 1 (μmol/L)</td>
<td>1.20</td>
<td>7.35</td>
<td>3.71</td>
<td>1.61</td>
<td>3.08</td>
<td>2.61 - 4.83</td>
</tr>
<tr>
<td>GSH 2 (μmol/L)</td>
<td>1.39</td>
<td>6.61</td>
<td>3.38</td>
<td>1.40</td>
<td>3.05</td>
<td>2.44 - 4.39</td>
</tr>
<tr>
<td>GSH 3 (μmol/L)</td>
<td>1.98</td>
<td>7.94</td>
<td>3.84</td>
<td>1.42</td>
<td>3.64</td>
<td>2.72 - 4.46</td>
</tr>
<tr>
<td>SOD 1 (IU/mL)</td>
<td>4.95</td>
<td>26.45</td>
<td>15.35</td>
<td>6.70</td>
<td>17.25</td>
<td>8.27 - 20.99</td>
</tr>
<tr>
<td>SOD 2 (IU/mL)</td>
<td>5.63</td>
<td>26.45</td>
<td>14.23</td>
<td>6.62</td>
<td>14.38</td>
<td>7.60 - 20.11</td>
</tr>
<tr>
<td>SOD 3 (IU/mL)</td>
<td>4.44</td>
<td>29.90</td>
<td>18.05</td>
<td>8.57</td>
<td>21.25</td>
<td>6.88 - 24.44</td>
</tr>
<tr>
<td>ox-LDL-Ab1 (mIU/mL)</td>
<td>379.52</td>
<td>1786.20</td>
<td>724.69</td>
<td>366.65</td>
<td>653.32</td>
<td>468.18 - 838.80</td>
</tr>
<tr>
<td>ox-LDL-Ab2 (mIU/mL)</td>
<td>197.52</td>
<td>1494.34</td>
<td>832.10</td>
<td>377.54</td>
<td>777.73</td>
<td>553.71 - 1150.74</td>
</tr>
<tr>
<td>ox-LDL-Ab3 (mIU/mL)</td>
<td>284.78</td>
<td>2640.76</td>
<td>1198.67</td>
<td>564.05</td>
<td>1037.68</td>
<td>901.74 - 1481.46</td>
</tr>
</tbody>
</table>

Min = Minimum; Max = Maximum; SD = Standard Deviation; IQR = Inter Quartile Range; BMI = Body Mass Index
measurement ($p = 0.002$). On the whole, as shown by table 2 and figure 1, there was a decrease in MDA values between the first and the third sampling even though it was not statistically significant ($p = 0.092$).

Concerning ox-LDL-Ab, we observed that its titers rose from the first to the second measurement, this result being the same from the second to the third measurement although the

**TABLE 2. Variable comparisons between the 3 measurements (T1, T2 and T3)**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid 1 – Uric acid 2</td>
<td>0.003*</td>
</tr>
<tr>
<td>Uric acid 2 – Uric acid 3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Uric acid 1 – Uric acid 3</td>
<td>0.383</td>
</tr>
<tr>
<td>MDA 1 – MDA 2</td>
<td>0.022*</td>
</tr>
<tr>
<td>MDA 2 – MDA 3</td>
<td>0.002*</td>
</tr>
<tr>
<td>MDA 1 – MDA 3</td>
<td>0.092</td>
</tr>
<tr>
<td>FRAP 1 – FRAP 2</td>
<td>0.003*</td>
</tr>
<tr>
<td>FRAP 2 – FRAP 3</td>
<td>0.389</td>
</tr>
<tr>
<td>FRAP 1 – FRAP 3</td>
<td>0.033*</td>
</tr>
<tr>
<td>GSH 1 – GSH 2</td>
<td>0.336</td>
</tr>
<tr>
<td>GSH 2 – GSH 3</td>
<td>0.249</td>
</tr>
<tr>
<td>GSH 1 – GSH 3</td>
<td>0.713</td>
</tr>
<tr>
<td>SOD 1 – SOD 2</td>
<td>0.230</td>
</tr>
<tr>
<td>SOD 2 – SOD 3</td>
<td>0.004*</td>
</tr>
<tr>
<td>SOD 1 – SOD 3</td>
<td>0.030*</td>
</tr>
<tr>
<td>ox-LDL-Ab 1 – ox-LDL-Ab 2</td>
<td>0.299</td>
</tr>
<tr>
<td>ox-LDL-Ab 2 – ox-LDL-Ab 3</td>
<td>0.010*</td>
</tr>
<tr>
<td>ox-LDL-Ab 1 – ox-LDL-Ab 3</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*p value < 0.05

FIGURE 1. Variation of Malondialdehyde (MDA) values between the three measurements. There was no statistical difference of MDA values between T1 and T3 ($p = 0.092$).

FIGURE 2. Variation of oxidized Low-Density-Lipoprotein antibodies (ox-LDL-Ab) titer between the three measurements. From T1 to T3, we observed a significant rise in oxidized LDL antibodies titer ($p = 0.006$).
significance was not the same ($p = 0.299$ and $0.010$ respectively). Figure 2 and Table 2 present an overall significant increment in ox-LDL-Ab titers between the first and the third sampling ($p = 0.006$).

FRAP values diminished significantly from the first to the second measurement ($p = 0.003$), but increased from the second to the third sampling, the difference being non-significant ($p = 0.389$). Overall, between the first and the third sampling, FRAP values decreased significantly ($p = 0.033$) (Table 2 & Figure 3). In relation to GSH, its values decreased from the first to the second sampling and increased contrariwise from the second to the third sampling (Table 2 & Figure 4), giving an overall increment values from the first to the third measurement, though

**FIGURE 3.** Variation of Ferric reducing antioxidant power (FRAP) values between the three measurements. Variable comparisons showed a significant decrease in FRAP values between T1 and T3 ($p = 0.033$).

**FIGURE 4.** Variation of reduced glutathione (GSH) levels between the three measurements. We observed no significant difference of GSH values between T1 and T3 ($p = 0.713$).

**FIGURE 5.** Variation of Superoxide dismutase (SOD) values between the three measurements. Using the Student t-test for paired samples, we found a statistically significant increase in SOD values between T1 and T3 ($p = 0.030$).

**FIGURE 6.** Variation of Uric acid levels from sampling 1 to sampling 3. From T1 to T3, uric acid values decreased without any statistical significance ($p = 0.383$).
none of these variations were of statistical significance ($p = 0.336, 0.249$ and $0.713$ respectively). Focusing on SOD, we noticed a non-significant lessening in its values from the first to the second measurement ($p = 0.230$), and a significant raise from the second to the third measurement ($p = 0.004$). On the whole, as depicted by Figure 5 and table 2, there was a significant increase in SOD values between the first and the third sampling ($p = 0.030$). Uric acid values on their own diminished significantly from T1 to T2 ($p = 0.003$), but contrarily increased significantly from T2 to T3 ($p = 0.001$), with an overall diminution of its values from T1 to T3 (table 2 & figure 6), though being of no statistical significance ($p = 0.383$).

While seeking associations between variables (data not shown), we found a positive and significant correlation between age and GSH at the first sampling ($r = 0.567, p = 0.007$). At the second measurement, we found a positive and significant correlation between SOD and ox-LDL-Ab ($r = 0.528, p = 0.012$). Lastly, GSH and ox-LDL-Ab were significantly correlated at the third sampling ($r = 0.424, p = 0.040$) as well as SOD and FRAP values ($r = -0.550, p = 0.009$). All other correlations were found statistically non-significant (all $p$ values $> 0.05$).

**DISCUSSION**

For most animals, mobility has always been essential for survival. In humans specifically, exercise is no longer a means of survival, but becomes a lifestyle, recreation, and sometimes, a means of therapeutic treatment. An elevated metabolic rate as a result of exercise can drastically increase oxygen consumption in the locomotive muscles and heart as well as other tissues. In the past decade, evidence has shown that unaccustomed and strenuous exercise may manifest an imbalance between ROS and antioxidant defense, resulting in an oxidatively stressful environment in the body. Our results reveal that, in a context of chronic exposure to exhaustive regular exercise, oxidative stress status is marked on one hand by an increased production of pro-oxidant substances (ox-LDL-Ab and MDA) over time. On the other hand, we noticed an increasing SOD activity over time and an elevation in GSH, though non-significant. But the total antioxidant capacity of blood assessed by FRAP decreased meanwhile. Further, uric acid did not vary significantly from the first to the third sampling, exhibiting paradoxically a tendency to decrease over time. Contrariwise to our initial hypothesis, there was an inconstant and very weak correlation between pro-oxidant and anti-oxidant substances at the different samplings (ox-LDL-Ab & SOD at T2; ox-LDL-Ab & GSH at T3), suggesting that the anti-oxidant defense system may be overwhelmed with pro-oxidant production in chronic exhaustive physical training, leading to lipid peroxidation and cell damage. Thus, indices of oxidative stress and the anti-oxidant capacity ought to be monitored in our athletes, and perhaps anti-oxidant supplements be added to their diets to enhance and strengthen their anti-oxidant capacity, thereby avoiding ROS-induced tissue damage.

Nowadays, it is fully accepted that oxidative stress may be implicated in the etiology of atherosclerosis. The risk of developing atherosclerosis is determined by the absolute levels of atherogenic lipoproteins, and by the relative tendency of such substances to undergo oxidation. Specifically, Low-density lipoproteins (LDL) are susceptible to oxidative processes initiated by oxygen free radicals. Oxidatively-modified LDL particles (ox-LDL) are strongly atherogenic and immunogenic; as a consequence, autoantibodies against ox-LDL (ox-LDL-Ab) are produced by the immune system. Consistent with previous reports, we found high titers of ox-LDL-Ab as well as an increment in these titers over time, suggesting the presence of in vivo LDL oxidation processes during chronic exhaustive exercise. A large number of epidemiological and clinical studies indicate that increased titers of ox-LDL-Ab correlated with the progression and intensity of atherosclerosis, hypertension, and the appearance of coronary artery disease. Consequently, there is an apparent paradox between the benefits of heavy aerobic exercise on cardiovascular risk factors and the potentially deleterious consequences of free radicals generated during heavy
exercise. According to such observation, we can hypothesize that atherosclerotic diseases among our professional athletes cannot be excluded during or at the end of their careers as it has been shown that atherogenic and cardiovascular risks are plausible in these categories of top athletes.

Reports from the literature regarding effects of exercise on MDA concentrations are equivocal. Indeed, one can read from the excellent review presented by Clarkson and Thompson\(^2\) that some studies have found a substantial increase in MDA while others found no increase or rather a decrease in MDA as compared with healthy sedentary individuals after an acute strenuous exercise. For instance, Sharifi et al.\(^8\) recently reported significant lower MDA concentrations among professional handball female players when compared with sedentary women of the same background at a steady-state, this finding being in line with ours, though the diminution in MDA over time among our participants was non-significant. This discrepancy observed from one study to another may be explained by the high inter-subject variability in MDA as well as the non-specificity of the assay\(^2\). Moreover, Maxwell et al.\(^25\) pointed out that the intensity of exercise and the training level of subjects may also affect the results.

In keeping with a large majority of surveys reviewed by Clarkson and Thompson\(^2\), we observed a significant increment in SOD activity over time, but the increase in GSH was not statistically significant. It was shown for instance that when SOD activity rise, no changes in glutathione reductase activity are found (26). Clarkson and Thompson revealed controversial results where GSH values increased, did not change or rather decreased in relation with exercise\(^2\). This variability may be due to the differences in the mode of exercise used, the time points examined, the training level of subjects as well as environmental factors (altitude for example) or lack of control for changes in plasma volume\(^2\). Our findings may be suggestive that exercise-induced changes in the redox status of tissues may initiate intracellular signal transduction processes that trigger and reinforce antioxidant defense protein expression, reducing thereby the oxidative stress of exercise\(^1,3\).

Uric acid as an antioxidant acts by binding iron and copper ions and also by directly scavenging ROS\(^27\). In addition, uric acid adds to the enhanced antioxidant profile by protecting ascorbic acid from oxidative reactions in plasma\(^28\). Previous studies\(^1,3,15,29\) have shown a significant increase in uric acid levels of professional athletes as compared with sedentary subjects. By contrast, we found a non-significant diminution in uric acid levels over time, while remaining in normal ranges. It is worth remembering however that we did not compare our soccer players to sedentary individuals, so the results could have corroborated those of previous surveys. Further, we did not investigate the protein-dietary intake of our subjects which could have interfered with our current results. On the whole, as previously stated by Ji LL\(^7\), understanding the unique characteristics and regulatory mechanisms of various antioxidants will guide in developing proper strategies to enhance cellular antioxidant capacity through physiological and nutritional means.

In order to assess the total antioxidant power, a number of tests have been proposed among which the total peroxyl radical trapping antioxidant capacity of plasma (TRAP), the ferric reducing/antioxidant power (FRAP), the trolox equivalent antioxidant capacity (TEAC) and the oxygen radical absorbance capacity (ORAC)\(^15-17\). If Benzie and Strain (17) found FRAP to be a reliable direct measure of total antioxidant activity of biological fluids like plasma, Prior et al.\(^16\) did not share this point of view. According to the latter, the FRAP assay involves neither a pro-oxidant nor an oxidizable substrate; what it really measures is the ability of a compound to reduce Fe\(^{3+}\) to produce Fe\(^{2+}\). Furthermore, the FRAP assay does not measure GSH, an important antioxidant in vivo\(^16\). These authors nevertheless agreed that the ferric-reducing ability measured for a biological sample may indirectly reflect the total antioxidant power of the sample. It was observed for instance a linear correlation between serum FRAP and serum ORAC (the substance they considered to be the real direct measure of total antioxidant activity), even if this was weak\(^30\). In accordance with our initial hypothesis, we would have expected FRAP values to increase over time and correlate both with pro- and antioxidants, but this was not the case as we observed
contrarily a significant decrease in these values from the first to the third sampling. Further, this variation is contrary to SOD activity which increased concomitantly, and we found a negative correlation between FRAP and SOD values at T3. Does FRAP really measure the total antioxidant activity is thereby a question needing more attention. Considering as Benzie and Strain\textsuperscript{17} that FRAP directly estimates the total anti-oxidant capacity, the present survey figures out an insufficient and in-adaptive response of antioxidant defense mechanism to pro-oxidants production.

A major limitation of the present work relies in the absence of a control group composed of healthy sedentary subjects to compare and contrast results that would have been obtained from both groups. Consequently, we are unable to conclude with strong evidence that changes observed with regard to pro and antioxidant substances measured over time are only due to the chronic exposure to strenuous exercise. Further well-designed studies with more subjects are warranted to better elucidate the effect of chronic exhaustive training on oxidative stress status of our professional athletes. Additionally, we did not investigate the nutritional profile of our participants which could have explained some of our results. Another limitation of this study could be the fact that we did not measure the other antioxidant byproducts such as vitamin C, vitamin E as well as catalase and glutathione peroxidase activities. Nonetheless, as all of the methods to assess lipid peroxidation and oxidative stress in humans may be affected by both the chemical composition of the tissue being studied and the presence or absence of metal ions, it is recommended that at least two techniques should be used for an accurate and consistent evaluation of oxidative stress in humans\textsuperscript{31}. In fact, because of lack of accuracy and/or validity, there is currently no single biomarker that can be considered as the “gold standard” of lipid or protein oxidation. In the present survey, we measured 6 biomarkers to evaluate the oxidative stress status of our players. Moreover, there are few studies that have followed-up professional athletes during a certain period of competition to seek modifications of their oxidative status over time, and to the best of our knowledge, this is one of the rare studies targeting this topic that has been held in SSA.

As we have seen from the present data that antioxidant defense response may not be so much sufficient to counteract concomitant increase in pro-oxidant production during chronic strenuous exercise, exposing therefore professional athletes to lipid peroxidation and cell damage, supplementation of antioxidants could be an effective alternative. Indeed, appropriate nutrition is likely to be vitally important in maintaining adequate antioxidant defense mechanisms\textsuperscript{29}. There is a body of evidence suggesting that bolstering antioxidant defenses may ameliorate exercise-induced damage and that the benefits of antioxidant intervention may be for the long term rather than the short one\textsuperscript{21,29,32}. It is true however that some authors have found that, except for carbohydrate beverages, none of these supplements are an effective countermeasure to exercise-induced immune suppression\textsuperscript{13,34}. Besides, there is, to date, little or no evidence showing that antioxidant supplementation can improve performance\textsuperscript{2,12}. Further studies advocated at better elucidating these issues are therefore warranted.

**CONCLUSION**

This prospective observational survey of 18 professional soccer players followed-up during part of a competition season showed that a context of chronic exposure to heavy exercise may be marked by an increased production of pro-oxidants along with a relative increase in antioxidant defense mechanisms. However, this latter increment may not be sufficient enough to counterbalance the overproduction of pro-oxidants, leading presumably to a chronic oxidative stress status with subsequent potential harmful effects on cellular functioning. We consequently join to Shippingeret et al.\textsuperscript{35} to recommend that indices of oxidative stress as well as the antioxidant capacity be monitored in athletes and, why not, antioxidants be supplemented to avoid ROS-induced tissue damage.
Competing interests

The authors do not declare any conflict of interest with this article. The authors alone are responsible for the content and writing of the paper, and they have benefited neither from any

Acknowledgments

The authors are grateful to all the football players who have accepted to take part in this study, and more so are they to the technical staff of “Renaissance of Ngoumou” for all the efforts made to help the authors conducting this piece of work.

REFERENCES


European Journal of Sports Medicine