

Evaluation of the Blood Antioxidant Capacity in Two Selected Phases of the Training Cycle in Professional Soccer Players

by

Małgorzata Michalczyk¹, Barbara Kłapcińska¹,
Ewa Sadowska-Krępa¹, Sławomir Jągsz¹, Wiesław Pilis²,
Izabela Szoltysek-Bołdys³, Jan Chmura⁴, Elżbieta Kimsa¹,
Katarzyna Kempa¹

The aim of this study was to evaluate the effects of a regular pre-season training on the aerobic performance and the blood antioxidant defense capacity in soccer players from the Polish Premier League club (n=19) and IVth League team (n=15). The players participated in an incremental treadmill running exercise to volitional fatigue twice (i.e., at the beginning (Trial A) and the end (Trial B) of the pre-season spring training). In venous blood samples, taken at rest and 3 min post-test, the activities of antioxidant enzymes (SOD, GSH-Px, CAT, GR) and concentrations of non-enzymatic antioxidants (GSH, tocopherols, retinol, uric acid) and malondialdehyde as a lipid peroxidation biomarker were measured. With the aim of between-group comparisons and possible conclusions on training-induced changes in the capacity of the blood antioxidant defense, the POTAX index was calculated as a sum of standardized activities of antioxidant enzymes and concentrations of non-enzymatic antioxidants. The results of the present study indicate that the players from the Premier League club were characterized by only slightly higher maximal oxygen uptake rates, the differences compared to IVth League team, as assessed in both trials, were statistically insignificant. Participation in the pre-season training resulted in a moderate improvement of aerobic performance, although only a few players were characterized by VO₂max comparable

¹ - Academy of Physical Education, Department of Biochemistry, Katowice,

² - Katowice School of Economics,

³ - Department of General and Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Sosnowiec,

⁴ - University School of Physical Education in Wrocław

to the international-class elite performers. No distinct differences were observed in the level of aerobic performance between higher- and lower-classified players.

Pre-season training led to an improvement in the global blood antioxidant capacity expressed in terms of POTAOX indices, although the changes in the activities and concentrations of individual components of the antioxidant system were less pronounced. Training-induced level of antioxidant conditioning was higher among the Premier League players, which may be related to differences in the training schedule and nutritional preparation of the athletes.

Key words: antioxidant defense, blood, soccer

Introduction

Physical exercise is characterized by a marked increase in oxygen consumption by the whole body and, in particular, by contracting skeletal muscles, which is associated with enhanced production of reactive oxygen species (ROS) and impairment of the oxidant/antioxidant balance (Viña et al., 2000; Vollaard et al., 2005). The primary sources of ROS within the exercising muscle include leakage of electrons from the mitochondrial electron transport chain to oxygen, reactions catalyzed by xanthine oxidase localized in vascular endothelial cells or membrane-bound NAD(P)H oxidases, and autoxidation of myoglobin (Sjödín et al., 1990; Pattwell and Jackson, 2004; Finaud et al., 2006). ROS are known to exert many harmful effects as they can modify macromolecules in the cell including lipids, proteins, and nucleic acids (Mastaloudis et al., 2006) thus leading to cell damage. Exercise-induced enhanced generation of ROS is implicated in muscular fatigue (Ferreira and Reid, 2008; Reilly et al., 2008). On the other hand, it has been evidenced that ROS play an important role in cellular redox signaling, as they have been recognized as mediators of muscle adaptation to exercise through up-regulation of the expression of genes for functional proteins and antioxidant enzymes (Pattwell and Jackson, 2004; Reid, 2001). The main sources of ROS in the circulation are autoxidation of hemoglobin within the erythrocytes and reactions catalyzed by endothelial xanthine oxidase (Sjödín et al., 1990) or neutrophil NADPH oxidase (Dahlgren and Karlsson, 1999). The harmful effects of ROS may be limited due to protection conferred by a complex system of tissue specific antioxidant defense. This system consists of antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic antioxidants (e.g., tocopherol, retinol, reduced glutathione, ascorbic and uric acids).

In a game such as soccer, lasting 90 minutes with a 15-minute half-time break, the energy production is mainly dependent upon aerobic metabolism (Stølen et al., 2005; McMillan et al., 2005). Players cover 8-12km during a match consisting, on average, of 24% walking, 36% jogging, 20% coursing, 11% sprinting, 7% moving backwards and 2% moving while in possession of the ball (Bangsbo et al., 1991). The distance covered in a match varies with positional roles in the team with the greatest distances being covered by midfield players. The game is played at an average intensity close to the lactate threshold, approximately 80-90% of maximum heart rate or 75% of the maximum oxygen uptake (VO_2max) (Bangsbo, 1994). During this kind of exercise, large amounts of oxygen are inhaled into the body, which increases oxidative stress due to enhanced generation of ROS in the blood and muscle (Viña et al., 2000). Several

studies have found that regular endurance training may reduce post-exercise oxidative stress and attenuate muscular damage, mainly due to the increased capacity of enzymatic and non-enzymatic antioxidant defense system (Ji, 1999; Jenkins et al., 1994). Only a limited number of reports are available on the training-induced changes in the blood antioxidant status in professional soccer players (Banfi et al., 2006; Brites et al., 1999; Schippinger et al., 2002; Cazzola et al., 2003). Therefore, the aim of this study was to evaluate the changes in the capacity of enzymatic and non-enzymatic antioxidant defense system induced by regular pre-season training in the blood of professional soccer players from the Premier and IVth Polish League teams.

Material and methods

Subjects

Nineteen players from the Polish Premier League club and fifteen athletes from the IVth domestic league team volunteered to participate in this study. Their physical characteristics are presented in Table 1. They were all engaged in a controlled physical pre-season spring training program that consisted of 6 to 7 training sessions (each lasting 90 min) in a single week microcycle. The training sessions were aimed at integration of different elements of soccer fitness, such as building aerobic and anaerobic endurance, strength, power, speed, flexibility, technical, and tactical skills. All subjects were informed of the purpose and possible risks associated with the experiment before signing an informed consent document. The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and the experimental protocol was approved by the local Ethics Committee at the Academy of Physical Education.

Table 1

Physical characteristics (mean \pm SD) of the players in two consecutive trials (A and B)

Variable	Premier League team (n=19)		IV th League team (n=15)	
	Trial A	Trial B	Trial A	Trial B
Age, yr	26.05 \pm 3.56		27.66 \pm 5.98	
Height, cm	181.7 \pm 5.8		176.7 \pm 5.7	
Body mass, kg	75.5 \pm 3.3	74.9 \pm 2.7	74.6 \pm 6.0	74.0 \pm 5.6
BMI, kg/m ²	22.8 \pm 1.0	22.7 \pm 1.4	23.8 \pm 1.2	23.7 \pm 1.2
VO ₂ max, mlO ₂ /kg/min	53.9 \pm 4.1	54.8 \pm 4.1	52.2 \pm 4.2	53.2 \pm 5.1

Study design

The subjects reported to the laboratory in the morning, between 8 and 10 a.m., in order to perform an incremental running exercise until volitional fatigue on a treadmill (LE 300C, Jaeger). The initial running speed was set at 6 km/h, with increments of 2 km/h every 3 min to a level that brought the subjects to $\dot{V}O_2$ max, defined as leveling off of oxygen uptake despite increased exercise intensity. Oxygen uptake ($\dot{V}O_2$) was measured using Oxycon Alpha (Jaeger) diagnostic system. Venous blood samples were drawn from the antecubital vein at rest and 3-4 min. after the end of exercise. Each subject participated in two identical trials (A and B) performed at the beginning and at the end (7 weeks later) of the pre-season training program. The participants were instructed to refrain from strenuous exercise the day before the running test.

Analytical procedures

Fresh whole blood samples, with heparin as an anticoagulant, were immediately assayed for reduced glutathione (GSH) by the colorimetric method (Beutler et al., 1963) with DTNB (5,5'-dithiobis-2-nitrobenzoic acid) and the activity of glutathione peroxidase (GPx; E.C.1.11.1.9) by commercially available kit RANSEL (Nr.RS505, Randox Laboratories, U.K.). The remaining blood was centrifuged for 10 min. at 1000 x g at 4°C to separate plasma and erythrocytes that were washed three times with cold (4°C) saline and kept frozen at -20°C, until they were assayed for activities of antioxidant enzymes (i.e., superoxide dismutase (SOD, EC.1.15.1.9) with commercially available RANSOD kit (Nr. SD125; Randox Laboratories Ltd., UK), catalase (CAT, EC. 1.11.1.6) by the method of Aebi (1984), and glutathione reductase (GR, EC.1.6.4.2) according to Glatzle et al. (1970). The activity of CAT was expressed as the rate constant of a first order reaction of H_2O_2 decomposition related to the hemoglobin content (k_{Hb}^{-1}). One GR activity unit was defined as the reduction of 1 μ mol of GSSG per minute, monitored by a decrease in absorbance at 340 nm due to oxidation of NADPH consumed per each molecule of GSSG reduced. The activities of all antioxidant enzymes were measured at 37°C and expressed per 1 g of hemoglobin as assessed by standard cyanmethemoglobin method using a diagnostic kit (Nr.HG980, Randox Laboratories, UK).

Plasma α - and γ -tocopherols, and retinol were measured using reverse-phase-high-performance-liquid-chromatography (LaChrom HPLC; Merck Hitachi) with a L-7100 pump, D-7000 interface and L-7485 fluorescence detector. The system was controlled by HPLC System Manager (Merck Hitachi Model D-7000 Chromatography Data Station Software v.4.1). Lipid extraction was per-

formed using a method elaborated by Sobczak et al. (1999). Samples, blanks, and standards (Sigma) were injected, using the Rheodyne Model 7125 injector and a 50 µl sample loop, onto a reverse-phase LiChrospher 100RP18 column (4.0 mm x 25 cm, 5 µm particle size; Merck) at room temperature. The mobile phase was acetonitrile- n-butanol (95:5, v/v). The fluorescence was read at 285 nm (excitation) and 325 nm (emission) for α- and γ-tocopherols, or at 300 nm (Ex) and 480 nm (Em) for retinol. Lipid peroxides in plasma were assayed by the thiobarbituric acid test (Buege and Aust, 1978) with extraction of the chromogen formed with n-butanol and reading the absorbance of the organic layer at 532 nm. The level of lipid peroxides were expressed as µmol of malondialdehyde (MDA) per liter of plasma, which was calculated from the calibration curve prepared with 1,1,3,3-tetraethoxypropane (Sigma) as an external standard. Plasma uric acid concentration was measured by using a commercially available diagnostic kit UA230 (Randox). Whole blood selenium (Se) concentration was measured by spectrofluorometric method according to Danch and Drózdź (1996).

Statistical analysis

The results are expressed as means ± SD. All data were tested for normal distribution with the Kolmogorov-Smirnov-Lilliefors test and for homogeneity of variances using the Levene test and then analyzed using a two-way ANOVA with body condition (pre- and post-test) and time period (trial A and trial B) as independent factors, followed by the post-hoc Bonferroni test where appropriate. For skewed data the Wilcoxon signed rank and the Mann-Whitney non-parametric tests were used.

In order to elucidate between-group differences in the capacity of the antioxidant defense system, an index of antioxidant potential (POTAOX) was calculated as a sum of standardized values of activities of antioxidant enzymes (SOD, CAT, GSH-Px, GR) and non-enzymatic antioxidants (retinol, α- and γ-tocopherols, uric acid and GSH). Standardized value means that a value is expressed in terms of its difference from the mean, recorded under resting conditions in the first trial (Trial A) completed by the Premier League players and taken as reference, divided by the standard deviation. The following formula was used:

$$\text{POTAOX} = \Sigma \left[\{ \text{SOD} + \text{CAT} + \text{GSH-Px} + \text{GR} \}_{\text{ST}} + \{ \text{C}_{\text{Ret}} + \text{C}_{\alpha\text{-Tok}} + \text{C}_{\gamma\text{-Tok}} + \text{C}_{\text{KM}} + \text{C}_{\text{GSH}} \}_{\text{ST}} \right]$$

The multiple linear regression modeling was applied to determine the main contributors to variations in POTAOX measurements. All statistical analyses

were performed using STATISTICA 6.0 (StatSoft, Inc.) software. The level of significance was set at $p < 0.05$.

Results

Although both groups of soccer players were matched for age, height, body mass and BMI, the athletes from the Premier League team were characterized by slightly higher aerobic capacity defined as a maximum oxygen uptake rate ($VO_2\max$) (Table 1). The pre-season training program resulted only in a small increase in the mean $VO_2\max$ values in both teams. As presented in Table 2, the baseline activities of all antioxidant enzymes (SOD, CAT, GSH-Px, and GR) were comparable in both groups of players. The post-exercise changes in activities of antioxidant enzymes were mostly small and insignificant. However, significant increases in CAT and GR activities and a significant decrease in GSH-Px were recorded at the end of the pre-season training program, but only among the Premier League players (Table 2).

Table 2
Activities of antioxidant enzymes (mean \pm SD) in soccer players

Variable	Premier League team (n=19)		IV th League team (n=15)		
	Trial A	Trial B	Trial A	Trial B	
SOD, U/gHb	pre- test post- test	877.3 \pm 226.6 849.6 \pm 177.4	799.6 \pm 145.3 776.5 \pm 157.4	837.8 \pm 220.4 798.2 \pm 191.6	781.0 \pm 128.9 799.8 \pm 102.9
CAT, U/gHb	pre- test post- test	166.3 \pm 24.7 170.2 \pm 30.7	227.1 \pm 43.6# 223.1 \pm 57.2#	165.0 \pm 22.9 158.8 \pm 24.4	165.7 \pm 25.8 191.5 \pm 47.3
GSH- Px, U/gHb	pre- test post- test	48.5 \pm 12.1 48.1 \pm 12.7	37.8 \pm 9.6# 35.8 \pm 10.1#	44.2 \pm 11.9 42.1 \pm 10.4	43.3 \pm 17.8 37.7 \pm 12.4#
GR, U/gHb	pre- test post- test	22.8 \pm 2.7 22.3 \pm 3.4	28.3 \pm 5.7# 28.4 \pm 4.8#	23.5 \pm 4.1 22.9 \pm 3.9	28.9 \pm 5.5 29.2 \pm 4.6

Note: #- significantly ($p < 0.05$) different from the respective values recorded during trial A (ANOVA)

Plasma concentration of non-enzymatic antioxidants (i.e., α - and γ - tocopherols, retinol, uric acid- (UA), and reduced glutathione- (GSH)), as well as whole blood selenium (Se), are presented in Table 3. As compared to the baseline data, a tendency toward higher resting concentrations of both tocopherols and the contrary trend toward lower uric acid level, were observed during the second trial (Trial B) but only among the Premier League players, while practically no changes in most parameters (except for selenium content) were found

among the IVth League athletes. The post-test changes in both groups of the players and the between-group differences in most parameters studied were mostly small and non-significant. It is noteworthy that whole blood selenium concentration was comprised within the range of 60 to 80 µg/L (i.e., below the lower limit of the nutritional adequacy range (80 µg /L)) sufficient for maximization of activity of GSH-Px and other selenoproteins (Thomson, 2004).

Table 3

Plasma or whole blood concentrations of non- enzymatic antioxidants (mean ± SD)

Variable		Premier League team (n=19)		IV th League team (n=15)	
		Trial A	Trial B	Trial A	Trial B
α- tokoferol, mg/L	Pre- test	10.23 ± 2.2	11.56 ± 2.0	10.38 ± 2.7	10.83 ± 2.0
	post- test	9.56 ± 1.9	12.17 ± 2.2#	13.25 ± 6.7	10.74 ± 2.4
γ- tokoferol, mg /L	Pre- test	0.86 ± 0.3	0.92 ± 0.2	0.92 ± 0.3	0.86 ± 0.2
	post- test	0.83 ± 0.3	0.97 ± 0.3	1.07 ± 0.6	0.79 ± 0.2
Retinol, mg/ L	Pre- test	0.43 ± 0.1	0.44 ± 0.1	0.52 ± 0.1	0.51 ± 0.1
	post- test	0.41 ± 0.1	0.51 ± 0.1#	0.66± 0.4	0.43 ± 0.1
GSH, µg/mg Hb	Pre- test	2.33 ± 0.4	2.33 ± 0.3	2.11 ± 0.3	2.41± 0.3
	post- test	2.28 ± 0.4	2.12 ± 0.4*	2.14 ± 0.3	2.34 ± 0.3
Uric acid, mg /dL	Pre- test	4.34 ± 1.0	3.83 ± 0.8	4.67 ± 0.6	4.96 ± 1.10
	post- test	4.60 ± 1.1	3.87 ± 0.8#	4.62 ± 0.7	4.98 ± 1.0
Se, ng/ml	Pre- test	60.38 ±16.7	68.84 ±17.9	56.07±17.2	71.08 ± 6.8#

*Note: *- significantly (p<0.05) different from the pre- test values by ANOVA (the Bonferroni test); #- significantly (p<0.05) different from the respective values recorded during the first trial (ANOVA)*

With the aim of finding out whether and how the blood antioxidant defense capacity was affected by training, the plasma MDA (a lipid peroxidation biomarker) and POTAOX index were compared with the baseline values recorded during the first trial (Trial A). The results are presented in Table 4.

Table 4

Plasma concentrations of malondialdehyde (MDA), as biomarker for oxidative stress, and POTAOX index, as biomarker of the plasma total antioxidant capacity (mean ± SD)

Variable		Premier League team (n=19)		IV th League team (n=15)	
		Trial A	Trial B	Trial A	Trial B
MDA.	pre- test	2.85 ± 0.86	2.89 ± 1.01	2.66 ± 0.66	3.84 ± 1.19\$
	post- test	3.66 ± 1.15*	3.16 ± 1.15	2.75 ± 0.57\$	4.14 ± 1.36#\$
POTAOX	pre- test	0.00	0.420 ± 0.400	-0.029 ± 0.340	0.257 ± 0.490
	post- test	-0.064 ± 0.41	0.438 ± 0.520	0.229 ± 0.840	0.482 ± 0.520

Note: *- significantly ($p < 0.05$) different from the pre- test values by ANOVA (the Bonferroni test); #- significantly ($p < 0.05$) different from the respective values in the trial A (ANOVA)

\$- significantly ($p < 0.05$) different from the respective values in the Premier League team (the Mann-Whitney U-test)

Discussion

This study attempted to observe the effects of pre-season soccer training on the aerobic performance and the blood antioxidant defense capacity among players from the Polish Premier League club and IVth League team. The aerobic performance was evaluated on the basis of direct measurements of the maximal oxygen uptake rates (VO₂max) during the standardized laboratory test (treadmill running). Although there are alternative means of soccer-specific testing of maximal oxygen uptake (Hoff, 2005; Kemi et al., 2003), laboratory treadmill running test is considered a useful tool in the assessment of soccer players' aerobic capacity (Stølen et al., 2005; Svensson and Drust, 2005). Noteworthy, VO₂max does not always appear to be a sensitive measure of performance in important aspects of soccer match-play (Bangsbo and Lindqvist, 1992), which is due to differences between the activity patterns in soccer match-play and laboratory test (intermittent *vs.* continuous). According to the data reported in the literature (Wisløff et al., 1998), maximal oxygen consumption rate may be useful in differentiating between successful and unsuccessful teams, as teams who perform better in a specific league or at a higher standard possess higher VO₂max levels. Additionally, improved endurance also increases the number of sprints completed in a game (Helgerud et al., 2001). Maximal oxygen uptake also varies with playing position (the midfield players have higher maximal oxygen uptake compared with defense players) (Wisløff et al., 1998), as well as of the quality of training and standard of competition. Average maximum oxy-

gen uptake (VO_2max) reported in elite performers is comprised within the range of 55-68 mL $\text{O}_2/\text{kg}/\text{min}$ (Strøyer et al., 2004; Hoff and Helgerud, 2004) and in several cases, even beyond 70 mL $\text{O}_2/\text{kg}/\text{min}$ (Helgerud et al., 2001).

The results of the present study indicate that the players from the Premier League club were characterized by only slightly higher maximal oxygen uptake rates, the differences compared to IVth League team, as assessed in both trials, were insignificant. Interestingly, only in two players from each team, the VO_2max exceeded the level of 60 mL $\text{O}_2/\text{kg}/\text{min}$. Several authors reported that maximal oxygen uptake rate is sensitive to soccer-specific endurance training program (Helgerud et al., 2001), which seems to be supported by results of the present study (Table 1). It should be stressed, however, that the increases in VO_2max in both groups of players were relatively small.

The main objective of the present study was the evaluation of training-induced changes in the capacity of antioxidant defense in the blood of soccer players. The major finding of this study was that the pre-season training program positively affected the overall blood antioxidant status, as evidenced by increases in POTAOX indices recorded during the second trial (trial B) in both groups of players. It is noteworthy to recall that the antioxidant defense system in the blood is complex in nature and represents an interaction of different components, including antioxidant enzymes and non-enzymatic antioxidants. Therefore, in the present work, two approaches were applied to quantify the capacity of the blood antioxidant defense system, i.e.: (1) the activities or concentrations of the individual components of the antioxidant system were measured, and (2) an overall ("total") capacity of the antioxidant defense system was quantified to yield information about the combined effectiveness of all co-operative interactions. The latter approach takes into account the fact that various antioxidants have additive effects on its antioxidant status in human blood and the cooperation of these antioxidants protects the organism against the attacks of ROS.

In the present study, training-induced changes in activities of antioxidant enzymes (except for SOD) assessed in the Premier League players, appeared to be significant, although different in direction and extent (Table 2) (i.e., positive in the case of CAT and GR, but negative for GSH-Px). The opposite directions in training-induced changes in CAT and GSH-Px activities can be explained by differences in the affinities of these enzymes to H_2O_2 as substrate (Agar et al., 1986). Interestingly, the activities of the same enzymes (CAT and GSH-Px) in the blood of the IV league players remained practically unchanged. In both groups of players the activities of GR recorded during the second trial tended to be higher, whereas the opposite trend was apparent in the case of SOD activi-

ties, that tended toward lower values as compared to the baseline levels. The latter response seems to support our presumption that in the final phase of the pre-season training, characterized by progressively increasing physical loads and a growing exposition of the players to exercise-induced oxidative stress, the red blood cell SOD may be oxidatively modified by its product (H_2O_2) and then directed to proteolytic degradation (Salo, 1990). Our observations conform to those reported by other authors (Banfi et al., 2006; Ohno et al., 1988; Poprzęcki, 2003) who have also found that aerobic training had a positive effect on antioxidant processes in red blood cells. Resting concentrations of non-enzymatic antioxidants, such as retinol, α - and γ -tocopherols and uric acid in the plasma, and reduced glutathione (GSH) in whole blood, appeared not to be affected by training (Table 3). There was a tendency toward higher whole blood selenium content which might be due to dietary factors associated with higher meat intake. It should be stressed, however, that most players were characterized by a sub-optimal selenium status, which conforms to our previous finding of selenium inadequacy among a substantial number of the local population (Kłapcińska et al., 2005).

The most widely used method for quantifying exercise-induced oxidative stress relies on the measurement of lipid peroxidation products, such as malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), or conjugated dienes. One of the most frequently used biomarkers is the plasma concentration of MDA (Nielsen et al., 1997). As expected, the mean MDA concentrations recorded at rest in both groups of players during the first trial (Trial A) were comparable, whereas the significant between group differences were identified in the second trial (Trial B), in which the lower-classified players (IVth League team) were characterized by higher plasma MDA content (Table 4). A comparison of between group differences in training-induced changes in POTAOX indices revealed that more pronounced improvement in the total capacity of the blood antioxidant defense was gained in higher-classified Premier Division players (Table 4). It is difficult to speculate what was the reason of these discrepancies, however the impact of differences in the training schedule and nutritional preparation of players should be taken into account.

It is noteworthy that our approach to apply POTAOX index, as a measure suitable for between-group comparisons of total antioxidant capacity of the blood, appeared to be a useful and reliable tool for gaining insight into the role of the antioxidant defense systems in physiological, but possibly also in pathological processes, where oxidative stress is implicated. By using multiple linear regression modeling, we attempted to estimate the impact of individual components of the blood antioxidant system on POTAOX. It was evidenced that in

the case of Premier Division performers, the significant contributors to POTAOX measure at baseline (Trial A) were retinol (0.63), γ -tocopherol (0.59), GSH-Px (0.59), and α -tocopherol (0.56), whereas at the end of the pre-season training (Trial B), these were GR (0.80), α -tocopherol (0.58), and retinol (0.45). In the case of lower-classified IVth League players –the main contributors to POTAOX were SOD (0.75), CAT (0.55), uric acid (0.55) and GR (0.52) in Trial A and α -tocopherol (0.63), uric acid (0.56) and CAT (0.51) in Trial B. The results of this analysis are in general accordance with those reported by Maxwell et al. (2006) who also attempted to quantify the contribution made by individual, non-enzymatic plasma antioxidants to global antioxidant defense and found that vitamins A (retinol), C (ascorbic acid), E (tocopherols), and uric acid were significant predictors. It should be added that there are a number of assays of “total” antioxidant capacity (TAC) for measuring the capacity of the biological systems (tissues, plasma and body fluids) to withstand oxidative stress. The most widely used are Total Radical-trapping Parameter (TRAP), Ferric Reducing Plasma Ability (FRAP), or methods based on reduction of free radical ABTS⁺ (Janaszewska and Bartosz, 2002).

Conclusions

1. Participation in the pre-season training resulted in a moderate improvement of aerobic performance, although only a few players were characterized by VO₂max, comparable on international-class of elite performers.
2. No distinct differences were observed in the level of aerobic performance between higher- and lower-classified players.
3. Pre-season training led to an improvement in the global blood antioxidant capacity, although the changes in the activities and concentrations of individual components of the antioxidant system were less pronounced.
4. Training-induced level of antioxidant conditioning was higher among the Premier League players, which may be related to differences in the training schedule and nutritional preparation of the athletes.

References

- Aebi H. (1984) Catalase in vitro. *Methods in Enzymology*. 105:121
- Agar N.S., Sadrzadeh M.H., Hallaway P.E., Eaton J.W. 1986. Erythrocyte catalase. A somatic oxidant defense? *J Clin Invest*. 77: 319-321
- Banfi G., Malavazos A., Iorio E., Dolci A., Doneda L., Verna R., Corsi M.M. (2006) Plasma oxidative stress biomarkers. nitric oxide and heat shock protein 70 in elite soccer players. *Eur J Appl Physiol*. 96(5):483-486

- Bangsbo J., Nørregaard L., Thorsøe F. (1991) Activity profile of competition soccer. *Can J Sport Sci.* 16:110–61
- Bangsbo J., Lindqvist F. (1992) Comparison of various exercise tests with endurance performance during soccer in professional players. *Int J Sports Med.* 13:125-132
- Bangsbo J. 1994. Energy demands in competitive soccer. *J Sports Sci.* 12: 183-189
- Brites FD., Evelson PA., Christiansen M.G., Nicol M.F., Basilico M.J., Wikiński R.W., Llesuy S.F. (1999) Soccer players under regular training show oxidative stress but an improve plasma antioxidant status. *Clin Sci.* 96:381-385
- Buege J.A., Aust S.D. (1978) Microsomal lipid peroxidation. *Methods in Enzymology.* 52:302-310
- Beutler E., Duron O., Kelly B.M. (1963) Improved method for the determination of blood glutathione. *J Lab Clin Med.* 61:882-888
- Cazzola R., Russo-Volpe S., Cervato G., Cestaro B. (2003) Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. *Eur J Clin Invest.* 33(10)924-930
- Dahlgren C., Karlsson A. 1999. Respiratory burst in human neutrophils. *J Immunol Methods.* 232:3-14
- Danch A., Drózdź M. (1996) Uproszczona metoda fluorometrycznego oznaczania selenu w materiale biologicznym. *Diagn Lab.* 32:5729-5734
- Ferreira L.F., Reid M.B. 2008. Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol.* 104:853-860
- Finaud J., Lac G., Filarie E. (2006) Oxidative stress. Relation with exercise and training. *Sports Med.* 36(4):327-358
- Glatzle D., Korner W.F., Christeller S., Wiss O. (1970) Method for the detection of a biochemical riboflavin deficiency. Stimulation of NADPH 2-dependent glutathione reductase from human erythrocytes by FAD in vitro. Investigations on the vitamin B2 status in healthy people and geriatric patients. *Int Vit Res.* 40(2):166-183
- Helgerud J., Engen L.C., Wisløff U., Hoff J. (2001) Aerobic endurance training improves soccer performance. *Med Sci Sports Exerc.* 33: 1925-1931
- Hoff J., Helgerud J. (2004) Endurance and Strength Training for Soccer Players. *Sports Med.* 34(3): 165-180

- Hoff J. (2005) Training and testing physical capacities for elite soccer players. *J Sports Sci*, 23(6): 573-582
- Jenkins R., Friedland J., Holland H. (1994) The relationship of oxygen uptake to superoxide dismutase and catalase activity in human skeletal muscle. *Int Sci Sports Med*. 5:11-14
- Janaszewska A., Bartosz G. 2002. Assay of total antioxidant capacity: comparison of four methods as applied to human plasma. *Scand J Clin Lab Invest*. 62: 231-236
- Ji LL. (1999) Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med*. 222(3): 283-292
- Kemi O.J., Hoff J., Engen L.C., Helgerud J., Wisløff U. (2003) Soccer specific testing of maximal oxygen uptake. *J Sports Med Phys Fitness*. 43:139-144
- Kłapcińska B., Poprzęcki S., Danch A., Sobczak A., Kempa K. (2005) Selenium levels in blood of Upper Silesian population: evidence of suboptimal selenium status in a significant percentage of the population. *Biol Trace Elem Res Winter* 108: 1-15
- Mastaloudis A., Traber M.G., Carstensen K., Widrick J.J. (2006) Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Med Sci Sports Exerci*. 38(1):72-80
- Maxwell S.R., Dietrich T., Chapple I.L.C. (2006) Prediction of serum total antioxidant activity from the concentration of individual serum antioxidants. *Clin Chim Acta*. 372: 188-194
- McMillan K., Helgerud J., Macdonald R., Hoff J. (2005) Physical adaptations to soccer specific endurance training in professional youth soccer players. *Br J Sports Med*. 39:273-277
- Nielsen F., Mikkelsen B.B., Nielsen J.B., Andersen H.R., Grandjean P. 1997. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem*. 43:1207-1214
- Ohno H., Yahata T., Sato Y., Yamamura K., Taniguchi N. (1988) Physical training and fasting erythrocyte activities of free radical scavenging enzyme system in sedentary men. *Eur J Appl Physiol Occup Physiol*. 57:173-176
- Pattwell D.M., Jackson M.J. (2004) Contraction-induced oxidants as mediators of adaptation and damage in skeletal muscle. *Exerc Sport Sci Rev*. 32: 14-18
- Poprzęcki S. (2003) The effect of omega-3 fatty acid and α -tocopherol supplementation on physical work capacity and blood antioxidant status in male subject. *J Hum Kinetics*. 10:121-136

- Reid M.B. (2001) Plasticity in skeletal, cardiac, and smooth muscle. Invited review: Redox modulation of skeletal muscle contraction: what we know and what we don't. *J Appl Physiol.* 90: 724-731
- Reilly T., Drust B., Clarke N. (2008) Muscle fatigue during football match-play. *Sports Med.* 38: 357-367
- Rice-Evans C.A., Diplock A.T., Symons M.C.R. (1991) *Techniques in Free Radical Research*, Elsevier, Amsterdam 148-149
- Salo D.C., Pacifici R.E., Lin S.W., Giulivi C., Davies K.J. (1990) Superoxide dismutase undergoes proteolysis and fragmentation following oxidative modification and inactivation. *J Biol Chem.* 265: 11919-11927
- Shipfinger G., Wonish P.M., Fankhauser F., Winklhofer-Roob B.M., Halwachs G. (2002) Lipid peroxidation and antioxidant status in professional American football players during competition. *Eur Journal of Clin Invest.* 32:686-692
- Sjödin B., Hellsten Y., Apple F.S., (1990) Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Med.*10: 236-254
- Sobczak A., Skop B., Kula B. (1999) Simultaneous determination of serum retinol and alpha- and gamma- tocopherol levels in type II diabetics patients using high- performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl.* 730:265-271
- Stølen T., Chamari K., Castagna C., Wisløff U. 2005. Physiology of soccer. An update. *Sports Med.* 35: 501-536
- Strøyer J., Hansen L., Klausen K. (2004) Physiological profile and activity pattern of young soccer players during match play. *Med Sci Sports Exerc.* 36:168-174
- Svensson M., Drust B. (2005) Testing soccer players. *J Sports Sci.* 23:601-618
- Thomson C.D. 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. *Eur J Clin Nutr.* 58: 391-402
- Viña J., Gomez-Cabrera M.C., Lloret A., Marquez R., Minana J.B., Pallardo F.V., Sastre J. (2000) Free radicals in exhaustive physical exercise: mechanism of production. and protection by antioxidant. *IUBMB Life* 50(4-5):271-277
- Vollaard N.B.J., Sherman J.P., Cooper C.E. (2005) Exercise –Induce Oxidative Stress. Myths. Realities and Physiological Relevance. *Sports Med.* 35(12):1045-1062
- Wisløff U., Helgerud J., Hoff J. (1998) Strength and endurance of elite soccer players. *Med Sci Sports Exerc.* 30(3):462-467

Acknowledgments

This work was supported by grant 6P05D 058 20 from the State Committee for Scientific Research (Warsaw, Poland) to the Department of Physiological and Medical Sciences, Academy of Physical Education in Katowice.

Corresponding author:

Barbara Kłapcińska

Department of Biochemistry

Academy of Physical Education

72A Mikołowska Str., 40- 065 Katowice. Poland

E- mail: b.klapcinska@awf.katowice.pl

Phone: +48 32 207 5158

Fax. +48 32 2075200

Authors submitted their contribution of the article to the editorial board.

Accepted for printing in Journal of Human Kinetics vol. 19/2008 on April 2008.