Long-term Effects of Oxidative Stress in Volleyball Players

Authors
J. Martinovic[1], V. Dopsaj[1,2], M. J. Dopsaj[3], J. Kotur-Stevuljevic[1], A. Vujovic[1], A. Stefanovic[1], G. Nesic[3]

Affiliations
[1] Faculty of Pharmacy, University of Belgrade, Institute of Medical Biochemistry, Belgrade, Serbia
[2] Clinical Centre of Serbia, Institute of Medical Biochemistry, Belgrade, Serbia
[3] Faculty of Sport and Physical Education, University of Belgrade, Belgrade, Serbia

Key words
- volleyball
- oxidative stress
- superoxide-dismutase
- ROMs, multiple discriminant analysis

Abstract

The aim of this study was to determine the impact of long-term training on elite female volleyball players and to determine parameters that could discriminate them according to the level of oxidative stress-associated adaptation. Fifty-four elite female volleyball players were divided into 3 groups (1: below-average training experience <8.0 years, 2: average training experience between 8.0 and 10.5 years and 3: above-average training experience >10.5 years). The measured parameters were reactive oxygen metabolites, biological anti-oxidative potential, superoxide anion, malondialdehyde, advanced oxidation protein products, lipid hydroperoxides, paraoxonase activity, superoxide-dismutase activity and sulphydryl groups. Multiple discriminant analysis of the oxidative stress status parameters between the three groups of athletes indicated a statistically significant difference (Wilks' lambda = 0.458, $X^2 = 35.898$, p = 0.031). The most important discriminant variables, superoxide-dismutase and superoxide anion, were the best indicators of differences between groups with different training experience. The significantly higher values were found in Group 3 compared with Group 1 in superoxide-dismutase activity (141 ± 32 vs. 86 ± 46; p = 0.002), sulphydryl groups (p = 0.031), and reactive oxygen metabolites (p = 0.042). The significantly lower superoxide anion was found between Group 3 and Group 1 (377 ± 187 vs. 1183 ± 905; p = 0.001). Oxidative stress status parameters adequately discriminated 68.5% of athletes with different training experience.

Introduction

The main goal of athletic training is to allow the body to adapt to an intensive stimuli, in turn elevating the capacity of various physiological systems to perform at increased work-loads, ultimately resulting in enhanced performance [9,15]. Elite level athletes require more ATP and display increased metabolism. This results in a high level of reactive oxygen species (ROS) generation [16,19]. The main sources of ROS during exercise are the mitochondrial respiratory chain, xanthine oxidase-catalysed reactions and neutrophil activation [6]. In addition, iron generates ROS in conditions of lactic acidosis during exercise, as mechanisms evolved to handle essential iron safely are compromised enabling iron ions to enter into Fenton chemistry [5,28,29]. The deleterious effects of free radicals are overcome via the activity of two complex internal protective mechanisms. Firstly enzymatic (superoxide dismutase, catalase, paraoxonase and glutathione peroxidase) and secondly non-enzymatic (vitamin C, vitamin E, retinol and reduced glutathione) [36]. Defence mechanisms against ROS-induced oxidative damage also include the binding of proteins (such as transferrin) to pro-oxidant metal ions [27]. ROS generation during exercise is associated with adaptation processes that involve redox-sensitive transcription, induction of anti-oxidant enzymes and more effective repair of ROS-related molecular insults [38]. The concept of hormesis [10], a dose-response relationship in which a low dose of a substance is stimulatory and a high dose is inhibitory, can be applied to ROS generation during exercise [24,37]. Within this context, exercise could be considered beneficial since trained athletes demonstrate an improved intracellular anti-oxidant status [11,44]. Volleyball is a team sport that involves both aerobic (fast movement at different intensity) and anaerobic (different types of jumps) metabolism in a balanced ratio [39]. Numerous studies both in animals and in humans

accepted after revision
July 17, 2009

Bibliography
DOI 10.1055/s-0029-1238289
Int J Sports Med 2009; 30: 851–856 © Georg Thieme Verlag KG Stuttgart · New York
ISSN 0172-4622

Correspondence
Prof. V. Dopsaj
Clinical Centre of Serbia
Institute of Medical Biochemistry
Visegradska 26
11000 Belgrade
Serbia
Tel.: +381/11/3970 379
Fax: +381/11/3972 840
violetap@eunet.rs

Table 1 Anthrophomorphological characteristics of the athletes.

Parameter	Group 1 Training experience: <8.0 years, (n=15)	Group 2 Training experience: 8.0–10.5 years, (n=23)	Group 3 Training experience: >10.5 years, (n=16)	Total, all players, training experience: 4.5–19.0 years, (n=54)
age (years)	19.1±1.9	21.1±1.6	23.3±4.1	21.1±3.7
training experience (years)	7.1±0.58	8.8±1.0	13.2±3.1	9.7±3.2
weight (kg)	68.9±7.0	71.6±7.0	71.6±7.1	70.7±6.8
height (cm)	180.3±3.8	183.4±7.5	186.8±6.7	183.4±6.7
body mass index	21.2±1.8	21.3±1.4	20.5±1.0	21.0±1.4
body fat (%)	16.9±3.7	14.8±3.5	14.7±2.6	15.5±3.4
lean body mass (kg)	57.1±5.1	61.7±3.4	60.1±6.0	59.6±5.2

Data are presented as means±SD

have demonstrated that anti-oxidant enzyme activity increases in the blood or in tissues after both aerobic and anaerobic exercise [17, 30, 45].

We sought to investigate the long-term effect of training on the anti-oxidative adaptation mechanism and its relationship with the number of years of training experience in elite female volleyball players. Three aims were defined as follows: 1) To determine the time period required for oxidative stress-induced adaptation, 2) To examine variation in oxidative stress status parameters between athletes with different training backgrounds i.e. years of training experience, and 3) To introduce and evaluate a new index of oxidative stress/anti-oxidant defence.

Materials and Methods

Subjects

Fifty-four adult elite female volleyball players from similar backgrounds participated in this study. The athletes were members of the top four senior female volleyball teams in Serbia in the 2007/2008 competition season. They were a representative sample derived from the best performers in the volleyball training system that is applicable in the Republic of Serbia. As training causes a stable influence on adaptive mechanisms in all athletes, careful study methodologies were selected (the dependent variable was training experience while independent variables were oxidative stress status parameters). All athletes were of senior age. We divided players according to tertile values into three groups (Group 1: below-average training experience <8.0 years, Group 2: average training experience between 8.0 and 10.5 years, and 3: above-average training experience >10.5 years). Above-average training experience value defined as the higher tertile in Group 3 was >10.5 years and below-average training experience value as the lower tertile in Group 1 was <8.0 years. The anthrophomorphological characteristics of the three study groups are shown in **Table 1**.

All training data for all the tested volleyball teams were recorded and processed using identical techiques by the assistant trainers on a daily basis for a pretest previous training mesocycle – basic mesocycle. In general, all the tested teams participated in between 8 and 10 training sessions per week with a single average duration of 104.24±12.50 min. The structure of the training process and the training load revealed an aerobic/anaerobic training index of 65.30±6.56%. According to standard training loads, within which most sport teams participate in 6–12 h of training per week over the entire year [9], we can state that the tested players received above-average training loads (14–16 training hours per week at last mesocycles).

All study participants were informed about the purpose and demands of the study before giving their written consent to participate. The whole study was planned according to the ethical standards following the Declaration of Helsinki, as revised in 1983. The Faculty of Pharmacy Ethics Committee (University of Belgrade, Belgrade, Serbia) approved the study protocol. Blood samples and athrophomorphological characteristics were collected at the beginning of the pre-competitive mesocycle training period. To avoid any confounding effects of individualized nutrition, subjects strictly followed a standardized diet, containing 55% carbohydrates, 15% protein and 30% fat. The average daily energy intake was 2784±150 Kcal during the six-week study. The head and assistant trainers supervised the appropriate nutrition of the athletes on a daily basis [43]. Four weeks prior to blood sampling the participants were instructed to abstain from any vitamin or anti-oxidant dietary supplementation. All participants were in good health, did not report any eating disorders, had no ongoing or previous (last year) injuries, not on any medication known to affect oxidative stress and were non-smokers.

Sample collection and analysis

Blood collection from the athletes was performed between 7 and 8 am at the beginning of the pre-competitive training period at the end of a 48 h total rest period and after a 12-h overnight fast. Venous blood was collected into evacuated tubes (Vacutainer, Becton Dickinson, USA) from the antecubital vein with minimal stasis. Blood samples were transported and stored in the laboratory where analyses were performed strictly following international guidelines [7]. Plasma and serum were separated by centrifugation and multiple aliquots of each sample were stored at −80 °C until analysis. The superoxide anioncontent of heparinised plasma was measured immediately. The following parameters were measured: oxidative stress status parameters [(reactive oxygen metabolites (ROMs), superoxide anion ($O_2^{\cdot-}$), malondialdehyde (MDA), advanced oxidation protein products (AOPP) and lipid hydroperoxides (LOOH)] and anti-oxidative defence parameters [biological anti-oxidative potential (BAP), PON1: paraoxonase activity toward paraoxon (POase), paraoxonase activity toward diazoxon (DZOase), superoxide-dismutase (SOD) and sulphydryl groups (-SH)]. By calculating the LOOH/BAP ratio we proposed a new oxidative stress status index. Measurements were performed in duplicate and the results were averaged. Quality control was provided by using quality control samples (pooled plasma).

Table 2 Multiple discriminant analysis. Oxidative stress/anti-oxidative defence parameters in the blood of three groups of female volleyball athletes according to Wilks' Lambda, F and p values.

	Mean ± SD			Tests of equality of group means		
Parameter	**Group 1**	**Group 2**	**Group 3**	**Wilks' Lambda**	**F value**	**p value**
ROMs, carr U	291 ± 51	286 ± 59$^{\blacklozenge\blacklozenge}$	344 ± 63*	0.830	5.205	0.009
BAP, µmol L^{-1}	2445 ± 296	2413 ± 290	2544 ± 276	0.962	1.011	0.371
SOD, U L^{-1}	86 ± 46	116 ± 44	141 ± 32**	0.793	6.659	0.003
MDA, µmol L^{-1}	0.838 ± 0.118	0.819 ± 0.131	0.876 ± 0.221	0.977	0.610	0.574
(-SH) groups, mmol L^{-1}	0.462 ± 0.049	0.512 ± 0.075	0.537 ± 0.102*	0.873	3.699	0.032
$O_2^{\cdot-}$, µmol $min^{-1}L^{-1}$	1183 ± 905	688 ± 430†	377 ± 187**	0.758	8.125	0.001
POase, IU L^{-1}	103 ± 27	198 ± 111††	165 ± 97	0.836	4.986	0.011
DZOase, IU L^{-1}	12654 ± 2253	12291 ± 1981	13183 ± 2572	0.983	0.451	0.640
AOPP, µmol L^{-1}	49 ± 20	47 ± 19	42 ± 17	0.978	0.570	0.596
LOOH, µmol L^{-1}	170 ± 38	162 ± 21	164 ± 19	0.982	0.458	0.635
LOOH/BAP index	1.67 ± 0.84	1.36 ± 0.74	1.41 ± 0.87	0.970	0.778	0.465

ROMs-Reactive oxygen metabolites, BAP-biological anti-oxidative potential-, $O_2^{\cdot-}$ -superoxide anion, SOD-superoxide-dismutase, MDA- malondialdehyde, (-SH)- total sulphydryl groups concentration, POase-paraoxonase (PON1) activity toward paraoxon, DZOase-paraoxonase activity toward diazoxon, AOPP-advanced oxidised protein products, LOOH-lipid hydroperoxides. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, Group 1 vs. Group 2. $^{*}p<0.05$, $^{**}p<0.01$, Group 1 vs. Group 3. $^{\blacklozenge}p<0.05$, $^{\blacklozenge\blacklozenge}p<0.01$, Group 2 vs. Group 3

A ROS analytical system (FRAS 4, H&D, Parma, Italy) incorporating a spectrophotometric device reader and a thermostatically-regulated mini-centrifuge was used to measure the ROMs and the BAP following instructions supplied by the manufacturer (Diacorn, Parma, Italy). The ROMs test was performed using capillary blood and expressed as CARR units (U), where one CARR U is equivalent to 0.08 mg × dL^{-1} of an aqueous solution of hydrogen peroxide. The test relies on the ability of transition metals [mainly iron (released when a condition of ischemia with micro-acidosis occurs)] to catalyse blood hydrogen peroxide breakdown generating ROS. The latter oxidise an aromatic amine causing the formation of a relatively stable coloured cation radical spectrophotometrically detectable at 505 nm.

The BAP test provides an estimation of the global antioxidant capacity of blood plasma, measured as its reducing potential against ferric ion. The BAP test is based on the ability of a coloured solution containing ferric ions bound to a chromogenic substrate (a thiocyanate derivate) to decolour when its ferric ions are reduced to ferrous after adding herarinised plasma. Solution discolouration was detected spectrophotometrically at 505 nm and was directly proportional to the concentration of all substances able to reduce ferric ion (expressed in µmol × L^{-1}). The intra-assay coefficients of variation were 3.2% and 5.8% for ROMs and BAP, respectively and the inter-assay CVs were 3.6% and 6.1% for ROMs and BAP, respectively.

Plasma MDA was measured using the thiobarbituric acid-reactive substances (TBARS) assay employing the molar absorption coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ at 535 nm, as previously described (the intra-assay CV was 5.8% and the inter-assay CV was 6.2%) [22]. The rate of nitroblue tetrazolium reduction was used to measure the level of superoxide anion [4]. Plasma superoxide-dismutase activity was measured according to a previously published method [34]. One unit of superoxide-dismutase activity is defined as the activity that inhibits the auto-oxidation of adrenalin by 50% (the intra-assay CV was 4.5% and the inter-assay CV was 7.1%). POase and DZOase were measured spectrophotometrically in serum according to Richter and Furlong [40]. Both paraoxon and diazoxon were purchased from Chem Service (West Chester, PA, USA). The activities are expressed as µmol × $min^{-1}L^{-1}$ (noted as IUL^{-1}). The intra-assay coefficients of variation were 5.2% and 6.9% for POase and DZOase, respectively and the inter-assay CVs were 7.7% and 8.5% for POase and DZOase, respectively. The concentration of sulphydryl groups in plasma was determined using 0.2 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reported by Ellmann (the intra-assay CV was 5.0% and the inter-assay CV was 8.1%) [18]. Advanced oxidation protein products were spectrophotometrically detected at 340 nm and expressed as chloramine-T equivalents (µmol × L^{-1}) (the intra-assay CV was 4.6% and the inter-assay CV was 7.8%) [41]. Lipid hydroperoxides were quantitated by the xylenol orange method (the intra-assay CV was 4.3% and the inter-assay CV was 7.9%) [2].

Statistical analysis

Multiple discriminant analysis was employed to investigate differences between the three study groups. In the first step an F-test (Wilks' lambda) was used to test if the discriminant model as a whole was significant. In the second step the co-variance matrices, coefficients of canonical correlation and the standardised canonical discriminant function coefficients were used to classify the dependent variable. The standardised canonical discriminant function coefficients were used to compare the relative importance of the independent variables (oxidative stress/anti-oxidative stress status parameters) [26]. The result was considered significant when $p < 0.05$.

Results

Oxidative stress and anti-oxidative defence parameters were measured and the results (means ± SD) with specific Wilks' Lambda F and p values are presented in **Table 2**.

In general, significant differences were found in all parameters that were investigated (Wilks' Lambda = 0.004, F value = 1.368, p = 0.011). Using multiple comparisons (Bonferroni *post hoc* criteria), significant higher superoxide-dismutase activity (p = 0.002) and SH groups concentration (p = 0.031) (Group 3 vs. Group 1) were observed. Significantly lower superoxide anion (p = 0.001) (Group 3 vs. Group 1) and (p = 0.031) (Group 2 vs. Group 1) were found. ROMs were significantly higher in Group 3 compared with Groups 1 and 2 (p = 0.042, p = 0.010, respectively). A significantly higher paraoxonase activity was observed in Group 2 compared with Group 1 (p = 0.008). The differences in

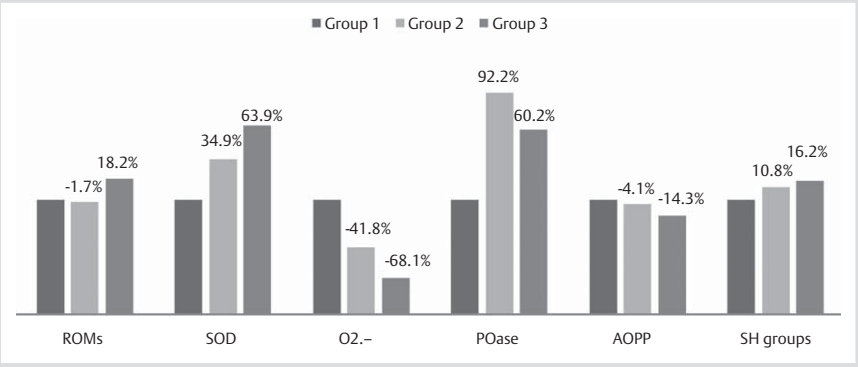

Fig. 1 Inter-group differences in oxidative stress status/antioxidative defence parameters. Group 1's (black bars) parameters were set at 100 %. Group 2 (light grey bars) and group 3 (dark grey bars) parameters were calculated as a percentage of group 1. Reactive oxygen metabolites ROMs and superoxide-dismutase (SOD) activity were higher (Group 3 compared to Group 1). Superoxide anion ($O_2^{\cdot-}$) was lower (Groups 2 and 3 compared to Group 1). In Group 2, paraoxonase activity toward paraoxon (POase) was greatest compared to Group 1.

Table 3 Classification of results of multiple discriminant analysis in all the elite female volleyball players.

	Predicted group membership			
Original count	**Group 1**	**Group 2**	**Group 3**	**Total**
1	10	4	1	15
2	5	14	4	23
3	0	3	13	16
%				
1	66.7	26.7	6.7	100
2	21.7	60.9	17.4	100
3	0	18.8	81.3	100

68.5 % of original grouped cases were correctly classified

Table 4 Structure matrix.

	Functions	
	Function 1 – $O_2^{\cdot-}$, SOD, AOPP and SH concentration	**Function 2 – ROMs, PON1, BAP, MDA, LOOH and LOOH/BAP**
$O_2^{\cdot-}$	**−0.627***	−0.094
SOD	**0.562***	0.180
SH groups	**0.424***	0.001
AOPP	**−0.153***	−0.130
d-ROMs	0.326	**0.754***
PON	0.368	**−0.643***
BAP	0.111	**0.378***
MDA	0.073	**0.307***
DZO	0.053	**0.272***
LOOH/BAP	−0.168	**0.194***
LOOH	−0.131	**0.141***

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. Variables ordered by absolute size of correlation within function*

the measured parameters were calculated as percentages and are shown in ◉ **Fig. 1**.

The results of multiple discriminant function analysis are shown in ◉ **Table 2**. Discriminant analysis of oxidative stress status [superoxide anion, ROMs, advanced oxidation protein products, malondialdehyde and lipid hydroperoxides] and anti-oxidative defence parameters [superoxide-dismutase, paraoxonase activity toward paraoxon, paraoxonase activity toward diazoxon, BAP, sulphydryl groups concentration and LOOH/BAP] between the three groups of athletes indicated a statistically significant difference only at the first discriminant function [superoxide anion, superoxide-dismutase, AOPP and sulphydryl groups concentration] (Wilks' lambda = 0.458, X^2 = 35.898, p = 0.031). When interpreting each function, the larger the standardised regression coefficient the greater the contribution to the total function score. The first discriminant function primarily characterised differences in anti-oxidative defence between the three groups of volleyball athletes according to their training experience. This discriminant function highlighted the difference between Groups 1 and 2 and Group 3 and elegantly described anti-oxidative defence via superoxide-dismutase activity (standardised canonical discriminant function coefficient = 0.562) and superoxide anion (standardised canonical discriminant function coefficient = −0.627) as the most important discriminant variables (Wilks' lambda = 0.793, F = 6.659 and p = 0.003) and (Wilks' lambda = 0.758, F = 8.125 and p = 0.001) (◉ **Tables 2** and **4**). Covariance matrices were significantly different between the three groups (Box's M = 168.21, F = 1.503 and p = 0.029) indicating that dependent variables were equal across the groups. The oxidative stress/anti-oxidative stress variables defined the three groups of athletes. Centroids for each group were calculated (Group 1: −1.317, Group 2: 0.205 and Group 3: 0.940) and are depicted graphically in ◉ **Fig. 2**. Functions at group centroids and Z Score differences for Function 1 – [superoxide anion, superoxide-dismutase, advanced oxidation protein products and sulphydryl groupconcentration] are shown in ◉ **Table 5**.

The accuracy of classification of the study groups based on the measured parameters and calculated index was 66.7 % for Group 1, 60.9 % for Group 2 and 81.3 % for Group 3. A total of 68.5 % of the original grouped cases were correctly classified (◉ **Table 3**).

Discussion

The current study is the first to demonstrate differences in the oxidative stress/anti-oxidative defence status between groups of professional female volleyball players differing in their number of years of training experience. Oxidative stress and anti-oxidative defence parameters measured in the elite-level trained athletes allowed us to visualise dynamic shifts in oxidative stress-related adaptation processes.

Superoxide-dismutase is the major protector from superoxide anion. The enzyme plays an important role in anti-oxidant defence during prolonged physical exercise [20]. Higher superoxide-dismutase activity in the third group of athletes appears to be consistent with published studies [21, 35]. The higher superoxide-dismutase activity was accompanied by lower superoxide anion over several years. Approximately ten years of training permitted superoxide-dismutase activity to reach its maximum, while paraoxonase reached its maximum earlier, between eight and ten years. In the second and third group of athletes, in addition to higher anti-oxidant enzyme activities,

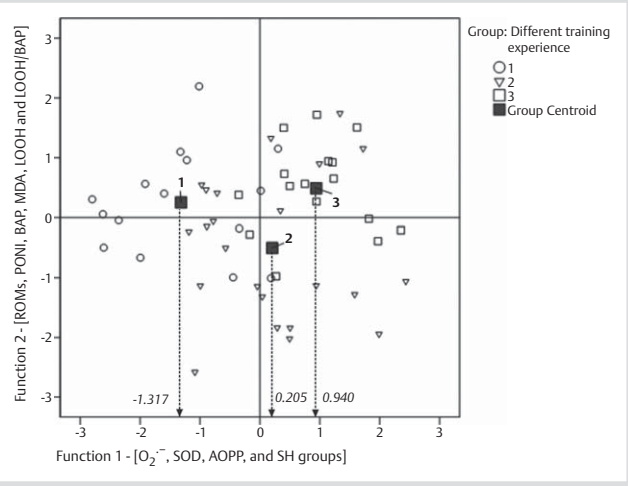

Fig. 2 Two-dimensional plot of the centroid belonging to each of the three experimental groups. Differences in training experience between the three groups of athletes and differences in their oxidative stress status and anti-oxidative defence parameters are evident. First discriminant function [$O_2^{\cdot-}$, SOD, AOPP and SH concentration] is shown as significant and characterised the differences in anti-oxidative defence between the three groups of volleyball players according to their training experience.

Table 5 Unstandardized canonical discriminant functions and Z score differences.

Functions at Group Centroids			Z Score differences for Function 1 – [$O_2^{\cdot-}$, SOD, AOPP and SH concentration]	
Group	Function			
	1	2		
1	−1.317	0.252	group 1 vs. group 2	1.522
2	0.205	−0.504	group 1 vs. group 3	2.257
3	0.940	0.489	group 2 vs. group 3	0.735

we observed higher sulphydryl groups as a further contributor to the total increase in the anti-oxidant defence capacity. Sulphydryl groups prevent deleterious effects such as protein misfolding, catalytic inactivation, decreased anti-oxidant capacity and loss of some specific functions [42]. The higher anti-oxidant defence resulted in unchanged AOPPs. The latter is a very positive attribute to participating in sport. Advanced oxidation protein products, apart from being markers of oxidative imbalance, are involved in oxidative stress progression and inflammation by the activation of immune cells [31,46].

One of the adaptive mechanisms that develops over years in athletes is the acquisition of a rapid state of greater energy consumption. This is a consequence of increased mitochondrial volume and the amount of enzymes involved in the citric acid cycle and electron-transport chain [3]. The ROMs calculation has been found to be a reliable parameter for oxidative stress status [8,12]. By comparing ROMs and superoxide anion we can conclude that the ROMs was higher while superoxide anion was lower over time. We can assume that the decline in superoxide anion was associated with increased superoxide-dismutase activity. Other types of radicals (hydroxyl radicals, hydrogen peroxide, alkoxyl radicals, peroxyl and hydroperoxyl radicals) that do not have a specific anti-oxidant enzyme may have caused a higher ROMs level. In addition, we can infer that the increase in ROMs was due to aerobic/anaerobic exercise-associated substantial lactic acidosis that enhanced ROS production via an increase in both free iron ions and Fenton and Haber–Weiss chemistry [1,3,5,13,39]. The increase in free radicals in the second group, compared to the first group, was not significant.

We can assume that the higher ROMs level was associated with the mitochondrial content of muscle. However, because of the well-developed anti-oxidant defence, particularly superoxide-dismutase activity, the amount of oxidised proteins was unmodified. The potential role of anti-oxidant supplementation should be investigated in the future, particularly in the light of some studies that documented inhibitory effects of antioxidant substances on cellular adaptations to exercise [23]. On the other hand, rigorous training which can deplete endogenous anti-oxidant defence in addition to promoting lipid peroxidation, DNA damage and protein oxidation, can lead to fatigue during muscular contraction and post-exercise muscular damage [32,33]. In cases where oxidative damage biomarkers are detected, anti-oxidant supplementation could be examined in order to reduce the negative effects of free radicals but not exercise-induced adaptation.

The BAP test provides a measurement of many anti-oxidants (uric acid, ascorbic acid, α-tocopherol, bilirubin) based on their ability to reduce the iron from its ferric (Fe^{3+}) to ferrous form (Fe^{2+}). The rate constants for reactions of non-enzymatic anti-oxidants with superoxide anion are much lower than the rate constant for the reaction of superoxide anion with superoxide-dismutase [14,21]. As a consequence it is likely that increased SOD activity resulting from training has a much greater effect on anti-oxidative status than non-enzymatic anti-oxidants.

The lack of significant differences in paraoxonase and superoxide-dismutase activity between the second and third study groups indicated that the athletes with between 8.0 and 10.5 years of training experience had already developed their enzyme anti-oxidative defence system. Based on the Z score difference (2.257), we can assume that athletes from the third group, with an average of 13.2 training experience years, achieved a substantial adaptation in relation to group 1, which had an average training experience of 7.1 years (◉ **Table 5**). For the groups 1 and 2 (with average of 8.8 training experience years), Z score difference was lower (1.552), which means that adaptation mechanism had not yet reached its maximum. For the groups 2 and 3, Z score difference was 0.735, we can assume that is about 4.5 years of additional training and participation in sports activities, which is the difference between the average training experience years of these two groups, the period in which the enzyme anti-oxidative defence system is continually and slightly improved. The significant difference (18.2%) in ROMs between the first and the third study groups indicated that, regardless of the level of adaptation, oxidative stress had increased.

Discriminant analysis of athletes according to their training experience indicated that out of all the oxidative stress/anti-oxidant defence parameters analysed the most important variable was superoxide-dismutase activity and superoxide anion which is its natural substrate. Oxidative stress biomarkers adequately defined athletes with different years of training and, generally, discriminated them in 68.5% of cases while in 31.5% of cases similarities existed between them. In addition to superoxide-dismutase and superoxide anion, significant absolute correlations between sulphydryl groups and advanced oxidation protein products with corresponding discriminant functions were found. Higher activity of superoxide-dismutase and paraoxonase as well as significantly higher SH content should provide information to doctors and coaches about relevant levels of anti-oxidant defence.

In conclusion, our study confirmed that there are differences in the oxidative stress/anti-oxidant defence status between athletes that have a different number of training years behind them. The duration of playing volleyball must be sufficiently long to trigger a sequential adaptive response of the anti-oxidant defence system. Differences between the three experimental groups were mainly characterised by superoxide anion reduction and higher superoxide-dismutase activity. Therefore, superoxide-dismutase activity and superoxide anion could be useful for monitoring athletes' adaptation. Based on our results we hope to predict anti-oxidant defence system development and accordingly adjust the intensity of training to obtain optimal results.

References

1 *Alderton WK*, *Cooper CE*, *Knowles RG*. Nitric oxide synthases: structure, function and inhibition. Biochem J 2001; 357: 593–615
2 *Arab K*, *Steghens JP*. Plasma lipid hydroperoxides measurment by an automated xylenol orange method. Anal Biochem 2004; 325: 158–163
3 *Astrand PO*, *Rodahl K*, *Dahl H*, *Stromme SS* (4th Ed.). Physical Training. In: Astrand PO, Rodahl K, Dahl HA, Stromme SS (eds) Textbook of work physiology: Physiological Bases of Exercise. Champaign IL: Human Kinetics; 2003; 313–368
4 *Auclair C*, *Voisin E*. Nitroblue tetrazolium reduction. In: Greenwald RA (ed) CRC Handbook of Methods for Oxygen Radical Research. Boca Raton FL: CRC Press; 1985; 123–132
5 *Balla G*, *Vercellotti GM*, *Eaton JW*, *Jacob HS*. Iron loading of endothelial cells augments oxidant damage. J Lab Clin Med 1990; 116: 546–554
6 *Banerjee AK*, *Mandal A*, *Chanda D*, *Chakraborti S*. Oxidant, antioxidant and physical exercise. Mol Cell Biochem 2003; 253: 307–312
7 *Banfi G*, *Dolci A*. Preanalytical phase of sport biochemistry and haematology. J Sports Med Phys Fitness 2003; 43: 223–230
8 *Banfi G*, *Malavazos A*, *Iorio E*, *Dolci A*, *Doneda L*, *Verna R*, *Corsi MM*. Plasma oxidative stress biomarkers, nitric oxide and heat shock protein 70 in trained elite soccer players. Eur J Appl Physiol 2006; 96: 483–486
9 *Bompa T*, *Carrera M*. Periodization Training for Sports. 2nd ed. Champaign IL: Human Kinetics; 2005
10 *Calabrese EJ*, *Baldwin LA*. Hormesis: the dose-response revolution. Annu Rev Pharmacol Toxicol 2003; 43: 175–197
11 *Cazzola R*, *Russo-Volpe S*, *Cervato G*, *Cestaro B*. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. Eur J Clin Invest 2003; 33: 924–930
12 *Cornelli U*, *Terranova S*, *Luca S*, *Cornelli M*, *Alberti A*. Bioavailability and antioxidant activity of some food supplements in men and women using the d-ROMs test as a marker of oxidative stress. J Nutr 2001; 131: 3208–3211
13 *Cooper CE*, *Vollaard NBJ*, *Choueiri T*, *Wilson MT*. Exercise, free radicals and oxidative stress. Biochem Soc Trans 2002; 30: 280–285
14 *Darley-UsmarV*, *Wiseman H*, *Halliwell B*. Nitric oxide and oxygen radicals: a question of balance. FEBS Lett 1995; 369: 131–135
15 *David JS*, *Delia R*. Effects of high volume and/or intense exercise on selected blood chemistry parameters. Clin Biochem 1994; 27: 435–440
16 *Davies KJA*, *Quintanilha AT*, *Brooks GA*, *Packer L*. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982; 107: 1198–1205
17 *Di Meo S*, *Venditti P*. Mitochondria in exercise-induced oxidative stress. Biol Signals Recept 2001; 10: 125–140
18 *Ellman E*. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70–77
19 *Fatouros IG*, *Jamurtas AZ*, *Viliotou V*, *Pouliopoulou S*, *Fotinakis P*, *Taxildaris K*, *Deliconstantinos G*. Oxidative stress responses in older men during endurance training and detraining. Med Sci Sports Exerc 2004; 36: 2065–2072
20 *Finaud J*, *Lac G*, *Filaire E*. Oxidative Stress. Relationship with exercise and training. Sports Med 2006; 36: 327–358
21 *Fukai T*, *Siegfried MR*, *Ushio-Fukai M*, *Cheng Y*, *Kojda G*, *Harrison DG*. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000; 105: 1631–1639
22 *Girotti MJ*, *Khan N*, *McLellan BA*. Early measurement of systemic lipid peroxidation products in plasma of major blunt trauma patients. J Trauma 1991; 31: 32–35
23 *Gomez-Cabrera MC*, *Domenech E*, *Romagnoli M*, *Arduini A*, *Borras C*, *Pallardo FV*, *Sastre J*, *Viña J*. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008; 87: 142–149
24 *Gomez-Cabrera MC*, *Domenech E*, *Viña J*. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radic Biol Med 2008; 44: 126–131
25 *Groussard C*, *Rannou-Bekono F*, *Machefer G*, *Chevanne M*, *Vincent S*, *Sergent O*, *Cillard J*, *Gratas-Delamarche*. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. Eur J Appl Physiol 2003; 89: 14–20
26 *Hair FJ*, *Anderson ER*, *Tatham LR*, *Black CW*. (5th Ed.) Multiple discriminant analysis and logistic regression. In: Multivariate Data Analysis. New Jersey: Prentice Hall; 1998; 239–274
27 *Halliwell B*, *Gutteridge JMC*. In: Halliwell B and Gutteridge J, (eds) Free Radicals in Biology and Medicine. Oxford University Press, New York; 1999; 105–245
28 *Hassan W*, *Ibrahim M*, *Deobald AM*, *Braga A*, *Nogueira C*, *Rocha J*. pH-Dependent Fe (II) pathophysiology and protective effect of an organoselenium compound. FEBS Lett 2009; 583: 1011–1016
29 *Hentze MW*, *Muckenthaler MU*, *Andrews NC*. Balancing acts: molecular control of mammalian iron metabolism. Cell 2004; 117: 285–297
30 *Inal M*, *Akyüz F*, *Turgut A*, *Getsfrid WM*. Effect of aerobic and anaerobic metabolism on free radical generation swimmers. Med Sci Sports Exerc 2001; 33: 564–567
31 *Kalousová M*, *Zima T*, *Tesar V*, *Dusilova-Sulkova S*, *Skrha J*. Advanced glycoxidation end products in chronic diseases-clinical chemistry and genetic background. Mutat Res 2005; 579: 37–46
32 *Laursen PB*. Free radicals and antioxidant vitamins: optimizing the health of the athlete. Strength Cond J 2001; 23: 17–25
33 *Margonis K*, *Fatouros GI*, *Jamurtas ZA*, *Nikolaidis GM*, *Douroudos I*, *Chatzinikolaou A*, *Mitrakou A*, *Mastorakos G*, *Papassotiriou I*, *Taxildaris K*, *Kouretas D*. Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. Free Radic Biol Med 2007; 43: 901–910
34 *Misra HP*, *Fridovich I*. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170–3175
35 *Ookawara T*, *Haga S*, *Ha S*, *Oh-ishi S*, *Toshinai K*, *Kizaki T*, *Ji LL*, *Suzuki K*, *Ohno H*. Effects of endurance training on three superoxide dismutase isoenzymes in human plasma. Free Radic Res 2003; 37: 713–719
36 *Powers SK*, *Lennon SL*. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. Proc Nutr Soc 2000; 58: 1025–1033
37 *Radak Z*, *Chung HY*, *Goto S*. Exercise and hormesis: Oxidative stress-related adaptation for successful aging. An Hypothesis. Biogerontology 2005; 6: 71–75
38 *Radak Z*, *Chung HY*, *Goto S*. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 2008; 44: 153–159
39 *Rajic B*, *Dopsaj M*, *Abella PC*. Basic and specific parameters of the explosive force of leg extensors in high trained Serbian female volleyball players: characteristics of the isometric force-time curve model. Serb J Sports Sci 2008; 2: 131–139
40 *Richter R*, *Furlong CE*. Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics 1999; 9: 745–753
41 *Selmeci L*, *Seres L*, *Antal M*, *Lukács J*, *Regöly-Mérei A*, *Acsády G*. Advanced oxidation protein products (AOPP) for monitoring oxidative stress in critically ill patients: a simple, fast and inexpensive automated technique. Clin Chem Lab Med 2005; 43: 294–297
42 *Sohal RS*. Role of oxidative stress and protein oxidation in the aging process. Free Radic Biol Med 2002; 33: 37–44
43 *Sotiropoulos A*, *Papapanagiotou A*, *Souglis A*, *Giosos G*, *Kotsis G*, *Bogdanis CG*. Changes in hormonal and lipid profile after a soccer match in male amateur players. Serb J Sports Sci 2007; 2: 31–36
44 *Sureda A*, *Ferrer MD*, *Tauler P*, *Romaguera D*, *Drobnic F*, *Pujol P*, *Tur JA*, *Pons A*. Effects of exercise intensity on lymphocyte H_2O_2 production and antioxidant defences in soccer players. Br J Sports Med 2009; 43: 186–190
45 *Thompson D*, *Williams C*, *Garcia-Roves P*, *McGregor SJ*, *McArdle F*, *Jackson MJ*. Post-exercise vitamin C supplementation and recovery from demanding exercise. Eur J Appl Physiol 2003; 89: 393–400
46 *Witko-Sarsat V*, *Friedlander MT*, *Nguyen Khoa T*, *Capeillere-Blandin C*, *Nguyen AT*, *Canteloup S*, *Dayer JM*, *Jungers P*, *Drueke T*, *Descamps-Latscha B*. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immun 1998; 161: 2524–2532