

Contents lists available at ScienceDirect

Journal of Exercise Science & Fitness

journal homepage: www.elsevier.com/locate/jesf



Changes in blood antioxidant status in American football players and soccer players over a training macrocycle



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ARTICLE INFO

Article history: Received 1 October 2020 Received in revised form 2 August 2021 Accepted 4 August 2021 Available online 9 August 2021

Keywords: American football Antioxidant enzymes Non-enzymatic antioxidants Lipid peroxidation Training

ABSTRACT

Background: The effectiveness of sport training programs should be assessed regularly against biochemical indices. This study assesses changes in the antioxidant status indices in American football players (AF) and soccer players (SP) over a training macrocycle.

Methods: The study was carried out with Poland's American Football League players (AF, n=11, age 24.0 ± 3.7 years) and first-league soccer players (SP, n=11, age 26.5 ± 3.8 years). Resting venous blood samples were collected from the players at the beginning of the three periods (preparatory, competition, and transition) making up the training macrocycle to determine the activity levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), creatine kinase (CK), and lactate dehydrogenase (LDH), as well as the concentrations of non-enzymatic antioxidants (uric acid-UA and glutathione-GSH) and the levels of malondialdehyde (MDA).

Results: The period effect on SOD (p < 0.001), CAT (p < 0.05), GPx (p < 0.05), GSH (p < 0.0001) and UA (p < 0.0001), and the group \times period interaction effect on SOD, CAT and GPx (p < 0.05), GSH (p < 0.001), and UA (p < 0.01) proved to be significant. Also significant were the group effect on MDA (p < 0.001) and LDH (p < 0.001) and the period effect on MDA (p < 0.01) and LDH (p < 0.001). The activity of SOD and CAT and the concentration of GSH were higher in both AF (12%, 2%, and 15%, respectively) and SP (33%, 10%, and 42%) at the start of the competition period than in the preparatory period, but the concentration of MDA and the activity of CK and LDH was lower (0.8%, 29%, 5% (AF) and 2%, 11%, 5% (SP). The highest activity of GPx and LDH and the greatest concentrations of UA and MDA occurred in the early transition period.

Conclusion: The study revealed an association between American footballers' and soccer players' training loads in the preparatory period and moderate improvements in their blood antioxidant status at the beginning of the competition period.

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1. Introduction

Soccer (association football) and American football are speedand-endurance team sports involving physical contact between the players and requiring them to perform acyclical movements in response to the ever-changing game circumstances. The two sports are very similar in many ways, for instance, in the number of players, the field dimensions, and the structure of the training

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macrocycle. In soccer, it starts with a general conditioning (preparatory) period divided into an adaptation phase and a phase aimed to strengthen players' motor skills and aerobic capacity. 1–3 The training macrocycle in American footballers also starts with the adaptation phase, but the focus of the next phase is on building the players' maximum strength and muscle mass. The specialist training phases preceding the competition period are similar. Both emphasize technical exercises, with American footballers additionally undergoing aerobic training. 3 The competition periods are also much the same. In both sports, the volumes of general conditioning exercises are then reduced in favor of targeted and specialist exercises and high-intensity aerobic and speed/speed-

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endurance exercises. In the transition periods concluding the training macrocycle, training volume and intensity are decreased so that more time is available for specialist exercises addressing individual players' technical weaknesses.⁴

As has been observed, the response of the antioxidant defense system to physical effort varies depending on exercise type, intensity, volume, and duration. A single session of prolonged or high-intensity exercise can lead to oxidative damage and skeletal muscle injury, but regular sessions involving moderate-intensity exercises promote the production of reactive oxygen and nitrogen species (RONS) that improve endurance and the ability to generate muscle strength. Exercise-induced RONS can also make the endogenous protective mechanisms more effective in lessening of oxidative stress and muscle damage. 15,16

Oxidative stress is an adverse physiological reaction that activates blood antioxidant defense responsible for maintaining and restoring prooxidant-antioxidant balance. The defense consists of two complementary mechanisms, one of which uses enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) to prevent the initiation and propagation of a chain reaction leading to the generation of free radicals. The other mechanism makes use of reduced glutathione (GSH) and uric acid (UA). All the compounds prevent the peroxidation of lipids and proteins by reacting with free radicals or free-radical oxidation products. It has been found that dietary antioxidants such as vitamins A, E, and C, selenium, and polyphenols also help prevent damage due to oxidative stress.¹¹

The monitoring of the markers of prooxidant-antioxidant balance in the athletes gained in popularity following the discovery that its disturbance may lead to the overtraining syndrome or activate a signal pathway leading to post-exercise adaptive changes and super-compensation. ^{15–19}

Because of many similarities between American football and soccer and a limited number of studies investigating the biochemical aspects of American footballers' training, this study was undertaken to assess and compare changes in the blood antioxidant defense of American footballers and soccer players over the training macrocycle based on the activity of the main antioxidant enzymes and the concentrations of selected non-enzymatic antioxidants and oxidative stress markers.

2. Methods

2.1. Participants

American footballers (AF, n=11) and soccer players (SP, n=11) participating in the study were recruited from the same teams competing in the first (the second highest) leagues. The exclusion criteria included the use of tobacco, alcohol, any medicines, or antioxidant supplements in the four preceding weeks. The main characteristics of the study participants are presented in Table 1.

All participants were informed about the protocol and aim of the study and consented in writing to participate in it. The study protocol conformed to the ethical guidelines of the World Medical Association's Declaration of Helsinki and was approved by the Institutional Ethics Committee at the Jerzy Kukuczka Academy of

Table 1The basic characteristics of participants.

Variable	$AF\left(n=11\right)$	SP (n = 11)
Age, years	24.0 ± 3.7	26.5 ± 3.8
Height, cm	183.7 ± 10.3	182.7 ± 5.4
Body mass, kg	98.2 ± 15.5	81.1 ± 5.4
VO _{2max} , ml/kg/min	52.4 ± 3.7	56.7 ± 4.3

Physical Education, Poland (certificate No. 4/2013).

In order to assess changes in the blood oxidant status of the participants over the training macrocycle, biochemical measurements were performed at the start of each of the three periods making up the macrocycle, i.e., preparatory (PP), competition (CP), and transition (TP) (Table 2).

2.2. Training programs

The training programs of the AF and SP groups, which followed the general training principles, were aimed to help the players achieve the target level of performance (Table 2). In the preparatory period, the SP had 8-10 training sessions of 90 min in a week and played one test game while the AF trained 4–5 times in a week for 120–180 min in the gym and on the field. In the competition periods (running from the mid of the second week in March to the end of the second week in soccer and from the third week in April to the end of the second week for 90 min and played 15 league games, and the AF had 3-4 training sessions per week of between 60 and 150 min and played 8 games. In the transition periods (2 weeks and 4 weeks, respectively), the number of specific exercises and training volumes were lower compared with the two previous periods.

2.3. Biochemical analysis

At the start of each of the macrocycle periods, resting venous blood samples were taken from the participants and immediately assayed for reduced glutathione (GSH) by a calorimetric method²⁰ with 5.5'-dithiobis-2-nitrobenzoic acid. The remaining blood was centrifuged at 1,000 g for 10 min at 4 °C to separate plasma and erythrocytes, which were then washed three times with cold (4 °C) saline and kept frozen at -80 °C until analyzed for the activity of antioxidant enzymes, i.e. superoxide dismutase (SOD, EC 1.15.1.1, the commercially available RANSOD SD125 kit by Randox, UK); glutathione peroxidase (GPx, EC 1.11.1.9, the commercial RANSEL RS505 kit by Randox, UK), and catalase (CAT, EC 1.11.1.6, Aebi's method²¹). Fresh plasma samples were assayed for the activity of creatine kinase (CK, EC 2.7.3.2) and lactate dehydrogenase (LDH, EC 1.1.1.27) and for the concentration of uric acid (UA) using diagnostic kits by Randox Laboratories (CK522, LD3818, and UA230, respectively). The plasma level of malondialdehyde (MDA), a lipid peroxidation and oxidative stress biomarker, was estimated based on the thiobarbituric acid reaction according to Buege and Aust.²

Biochemical assays were performed by a laboratory certified as meeting the requirements of PN-EN ISO 9001:2015, in line with the recommendations of the testing kits manufacturers.

2.4. Statistical analysis

All data stated below represent means and standard deviation (M \pm SD). The normality of data distribution was tested using the Shapiro-Wilk test, the homogeneity of variance by the Levane test, and sphericity by the Mauchly's test. The significance of betweengroup differences and within-group differences was determined using a two-way mixed-design ANOVA with two groups (AF and SP) and three point-times (PP, CP, and TP) as the main factors, followed, when appropriate, by the HSD Tukey post-hoc test. The ANOVA effect size was assessed by calculating eta squared (η^2), whose values can range from 0 to 1 (0.01 – small effect; 0.06 – medium effect; 0.14 – large effect).²³ The level of significance was set at α < 0.05. All statistical analysis procedures were performed in Statistica 10.0 (StatSoft, Tulsa, OK, USA).

Table 2Characteristics of training programs for American football players and soccer players.

Sport	Period				
	Preparation		Competition	Transition	
	General	Special			
American football	- maximum strength building, - muscle mass building, - fitness training, - technical training -tactical training No. of sessions per week: $3 \times 2 \text{ h}$ (the gym) + 1 × 3 h (the field) Period length: 10 weeks	(the field) Period length: 4 weeks	- strength training with reduced volume - tactical training with speed, speed and endurance elements - plyometric exercises No. of sessions per week: $1\times 1\ h\ (\text{strength training}) + 2\times 2.5\ h\ (\text{the field})$ Period length: $8\ \text{weeks}$	- lower intensity exercises - special exercises addressing players' technical shortcomings No. of sessions per week: $1 \times 1 \text{ h (the gym)} + 1 \times 2 \text{ h}$ (the field) Period length: 4 weeks	
Soccer	- motor skills - endurance training No. of sessions per week: $3 \times 1.5 \text{ h (the gym)} + 5 \times 1.5 \text{ h (the field)}$ Period length: 6 weeks	- tactical and technical training - test matches No. of sessions per week: $3 \times 1,5 \text{ h (the gym)} + 5 \times 1,5 \text{ h (the field)}$ Period length: 4 weeks	- special exercises and high-intensity aerobic exercises, speed exercises, and speed-endurance exercises No. of sessions per week: $2\times1,5\ h\ (the\ gym)+4\times1,5\ h\ (the\ field)$ Period length: $14\ weeks$	- lower intensity exercises - special exercises addressing players' technical shortcomings No. of sessions per week: $1\times 1,5\ h\ (the\ gym) + 2\times 1,5\ h\ (the\ field)$ Period length: $2\ weeks$	

3. Results

3.1. Antioxidant enzymes

Table 3 contains values characterizing participants' antioxidant status by period of the training macrocycle and study group. According to the two-way mixed-design ANOVA, the period effect and the group \times period interaction effect were significant for SOD, CAT, and GPx. Between-group differences for SOD and CAT were statistically significant at the beginning of the TP (p < 0.05), and for GPx in the early CP. The highest activity of SOD and CAT occurred in both groups at the start of the CP. In the SP group, GPx was statistically significantly more active (p < 0.01) at the beginning of the TP than in the early PP and CP; in the AF group, its changes during the macrocycle were not significant.

3.2. Non-enzymatic antioxidants

The period effect and the group \times period interaction effect proved significant for the concentrations of GSH and UA (Table 3). GSH concentrations changed in both groups, but only in the SP its values at the beginning of the CP and TP were significantly higher than in the early PP (p < 0.01). The lowest concentrations of this antioxidant were recorded in both groups at the start of preparatory period. Changes in the UA concentration in the AF were not significant, unlike the increase in the UA concentration (p < 0.01) between the beginning of the CPO and the TP in the SP.

3.3. Lipid peroxidation and muscle-damage markers

The group effect and the period effect were significant for MDA

Table 3The indices of participants' blood antioxidant status by period of the training macrocycle.

Variable	Period	$AF\left(n=11\right)$	SP(n=11)	A mixed ANOVA	Eta squared
SOD, U/gHb	PP	1270.61 ± 174.42	1161.10 ± 264.50	GE: F _{1,20} = 3.39, p = 0.08	$\eta 2 = 0.15$
	CP	1426.30 ± 282.49	1545.00 ± 413.53*	PE: $F_{2.40} = 9.01$, p < 0.001	$\eta 2 = 0.31$
	TP	1346.86 ± 185.49	$988.15 \pm 182.51^{\# +}$	INT: $F_{2,40} = 4.37$, $p < 0.05$	$\eta 2 = 0.18$
CAT, U/gHb	PP	195.45 ± 26.42	188.44 ± 44.21	GE: $F_{1,20} = 1.03$, $p = 0.32$	$\eta 2 = 0.05$
	CP	199.49 ± 19.42	204.55 ± 35.43	PE: $F_{2,40} = 6.30$, p < 0.05	$\eta 2 = 0.24$
	TP	195.11 ± 13.37	$163.55 \pm 36.60^{\# + \uparrow \uparrow}$	INT: $F_{2,40} = 4.24$, p < 0.05	$\eta 2 = 0.18$
GPx, U/gHb	PP	56.60 ± 6.56	44.69 ± 14.29	GE: $F_{1,20} = 3.98$, $p = 0.06$	$\eta 2 = 0.17$
	CP	54.89 ± 8.72	$42.22 \pm 12.07^{\dagger}$	PE: $F_{2.40} = 8.73$, p < 0.001	$\eta 2 = 0.30$
	TP	59.08 ± 8.99	$60.35 \pm 17.49******$	INT: $F_{2,40} = 3.81$, $p < 0.05$	$\eta 2 = 0.16$
GSH, μg/mgHb	PP	2.35 ± 0.35	2.06 ± 0.12	GE: $F_{1,20} = 0.90$, $p = 0.36$	n2 = 0.04
	CP	2.70 ± 0.29	$2.94 \pm 0.36**$	PE: $F_{2.40} = 29.82$, p < 0.0001	$\eta^2 = 0.60$
	TP	2.56 ± 0.37	$2.89 \pm 0.35**$	INT: $F_{2,40} = 8.36$, p < 0.001	$\eta 2 = 0.29$
UA, mg/dl	PP	5.62 ± 0.78	4.28 ± 1.67	GE: $F_{1,20} = 0.63$, $p = 0.44$	$\eta 2 = 0.03$
	CP	5.91 ± 0.62	5.83 ± 1.65	PE: $F_{2.40} = 18.73$, p < 0.0001	$\eta^2 = 0.48$
	TP	6.29 ± 1.21	$6.85 \pm 1.93^{##}$	INT: $F_{2,40} = 6.42$, p < 0.01	$\eta^2 = 0.24$

SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase, GSH – glutathione, UA – uric acid, PP – preparatory period, CP – competition period, TP – transition period, GE – group effect, PE – period effect, INT – group × period interaction effect.

Values are presented as mean (SD). *p < 0.05, **p < 0.01, ***p < 0.0001 – significantly different vs. PP; *#*p < 0.01 – significantly different vs. CP; †p < 0.05, †p < 0.05, †p < 0.01 – significantly different between AF and SP.

and LDH (Table 4). As regards CK, the two-way mixed-design ANOVA did not show the group effect, the period effect, and the group \times period interaction effect to be significant. The concentration of MDA and the activity of LDH (p < 0.05) significantly differentiated the AF from the SP at the start of PP and CP. In the AF group, neither the concentration of MDA nor the activity of CK and LDH changed significantly during the training macrocycle; CK activity changes in the SP group were not significant, but the activity of LDH measured at the start of the TP was significantly higher (p < 0.05) than in the early PP and CP.

4. Discussion

This study provides an assessment of the changes in blood antioxidant defense in American footballers and soccer players recorded between the three periods making up the training macrocycle.

4.1. Changes in antioxidant enzymes over the training macrocycle

The effectiveness of antioxidant defense relies on antioxidant enzymes (SOD, CAT and GPx) and endogenous non-enzymatic antioxidants, mainly UA and GSH. The key antioxidant enzyme is SOD catalyzing the dismutation of superoxide radical anion into hydrogen oxide $(H_2O_2)^{.24-26}$ It is supported by synergistic enzymes CAT and GPx that decompose H_2O_2 to water and oxygen. During high-intensity physical exercise that stimulates the production of H_2O_2 and reduces the effectiveness of the glutathione system, the role of the main element of antioxidant defense is taken over by CAT.²⁷

A higher activity of SOD we observed in the early competition period (by 12% (AF) and 33% (SP)) was probably associated with the training loads applied to the players in the preparatory period. According to many reports, RONS production induced by physical training has an important effect on cell signaling by initiating mitogen-activated protein kinases (MAPKs) and nuclear factor- κ B (NF- κ B) stimulating the expression of genes coding for antioxidant enzymes. The large interaction effect ($\eta 2 = 0.18$) on the activity of SOD, CAT, and GPx ($\eta 2 = 0.16$) implies that the different effectiveness of the enzymatic antioxidant defense systems of American footballers and soccer players at the start of the transition period could be associated with the different length of their preparatory periods (see Table 2) and different numbers of games they played in the competition period (8 vs.15).

Table 4The indices of participants' muscle damage by period of the training macrocycle.

Variable	Period	AF (n = 11)	SP (n = 11)	A mixed ANOVA	Eta squared
MDA, μmol/l	PP	6.28 ± 1.02	$4.80 \pm 0.70^{\dagger}$	GE: F _{1.20} = 16.57, p < 0.001	$\eta 2 = 0.45$
	CP	6.26 ± 1.28	$4.70 \pm 0.78^{\dagger}$	PE: $F_{2.40} = 4.81$, p < 0.01	$\eta 2 = 0.20$
	TP	6.89 ± 1.85	5.91 ± 1.09	INT: $F_{2,40} = 0.58$, $p = 0.56$	$\eta 2 = 0.03$
CK, U/l	PP	306.92 ± 95.37	263.94 ± 122.76	GE: $F_{1,20} = 0.67$, $p = 0.42$	$\eta 2 = 0.03$
	CP	271.13 ± 102.17	293.89 ± 78.73	PE: $F_{2.40} = 1.95$, $p = 0.16$	$\eta 2 = 0.09$
	TP	293.11 ± 118.21	224.11 ± 126.37	INT: $F_{2,40} = 0.58$, $p = 0.57$	$\eta 2 = 0.03$
LDH, U/I	PP	401.78 ± 67.89	$289.99 \pm 67.54^{\dagger}$	GE: $F_{1.20} = 21.20$, p < 0.0001	$\eta 2 = 0.51$
	CP	382.60 ± 63.65	$276.14 \pm 77.22^{\dagger}$	PE: $F_{2.40} = 10.42$, p < 0.001	$\eta 2 = 0.34$
	TP	430.52 ± 48.29	$355.20 \pm 5.53*^{\#}$	INT: $F_{2,40} = 0.93$, $p = 0.40$	$\eta 2 = 0.04$

 $MDA-malondial dehyde, CK-creatine\ kinase, LDH-lactate\ dehydrogenase, PP-preparatory\ period, CP-competition\ period, TP-transition\ period, GE-group\ effect, PE-period\ effect,\ INT-group\ \times\ period\ interaction\ effect.$

The values in the table represent means (SD). *p < 0.05 - significantly different vs. PP; *p < 0.05 - significantly different vs. CP; †p < 0.05 - significantly different between AF and SP.

4.2. Changes in non-enzymatic antioxidants over the training macrocycle

GSH "scavenges" RONS and promotes the regeneration of other antioxidants as well as helping maintain the correct cellular redox potential involved in the regulation of intracellular metabolism. In our study, its concentration was lower in both groups in the early preparatory period than at the beginning of both competition and transition periods, which is consistent with variations in the redox status of the elite French league soccer players observed by Le Moal et al.⁸ over the training macrocycle.

Another marker we analyzed was UA, a product of the metabolic breakdown of purine nucleotides catalyzed by xanthine oxidore-ductase. UA accounts for 50% of blood antioxidant capacity when in the normal range but higher concentrations turn it into a prooxidant. 29 In our study, the plasma concentration of UA was rising over the training macrocycle in both groups, reaching its highest level in the early transition period. Accordingly, a mixed-design ANOVA confirmed by a large period effect ($\eta 2=0.48$). This finding is consistent with the results reported by other authors, according to whom the plasma concentration of UA increases after high-intensity exercise with a delay, probably due to a lower UA renal clearance. 30 While UA concentrations varied over the training macrocycle, they never fell outside the reference range (3–7 mg/dl), meaning that in both groups UA retained its function of a nonenzymatic antioxidant.

4.3. Changes in lipid peroxidation and muscle damage over the training macrocycle

High-intensity physical exercise increasing RONS production results in the peroxidation of membrane polyunsaturated fatty acids. One of the final products of this process is malondialdehyde (MDA).³¹ In our study, the highest, although statistically nonsignificant, increase in the concentration of MDA occurred in both groups at the beginning of the transition, probably because of increased lipid peroxidation and greater leakage of cellular enzymes into the bloodstream brought about by the competition period.^{32–34} The highest concentration of MDA was observed in both groups in the early transition period.

Biochemical monitoring for training effectiveness frequently involves the analysis of plasma CK and LDH. The post-exercise increase in the activity of these two enzymes depends on exercise intensity, duration, and the type of muscle contractions $^{35-37}$ and tends to be higher in individuals who exercise regularly. In this study, the group, period, and interaction effects for CK activity proved not significant as opposed to the group effect (p < 0.0001,

 $\eta 2 = 0.51$) and the period effect (p < 0.001, $\eta 2 = 0.34$) for the activity of LDH, which was probably associated with the athletes' training status.

5. Limitations

The main limitation of our study is that antioxidant defense changes in American footballers and soccer players in Poland during the training macrocycle are only considered with respect to the endogenous mechanisms, without taking account of the role of dietary antioxidants.

6. Conclusion

The study has shown an association between training loads in the preparatory period and moderate improvements in American footballers' and soccer players' blood antioxidant status, and their prooxidant-antioxidant balance continuing into the early competition period. It can be hypothesized that the use of dietary antioxidants or antioxidant supplements as an enhancement in the preparatory period would additionally improve the effectiveness of the athletes' antioxidant defense mechanisms and mitigate the oxidative stress impact.

Funding

This study was supported by the statutory funds of the Jerzy Kukuczka Academy of Physical Education, Katowice, Poland.

Declaration of competing interest

The authors declare no conflict of interest.

Author statement

E.S.K. and S.B. wrote the manuscript. E.S.K. and S.B. designed the research. S.B. and A.K. performed experiment. E.S.K., S.B. and J.I. analyzed data. All authors have read the manuscript and agreed to its publication.

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