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The blood antioxidant defence capacity during intermittent hypoxic training in elite swimmers

AUTHORS: Poprzęcki S[1], Czuba M[2], Zając A[2], Karpiński J[3], Wilk R[3], Bril G[2], Maszczyk A[4], Toborek M[5]

[1] Department of Biochemistry, The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland
[2] Department of Sports Training, The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland
[3] Department of Swimming, The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland
[4] Department of Statistics and Methodology, The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland
[5] Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, USA

Corresponding author:
Milosz Czuba
Department of Sports Training
The Jerzy Kukuczka Academy of Physical Education in Katowice, Poland
E-mail: m.czuba@awf.katowice.pl

ABSTRACT: The main objective of this study was to examine the chronic effect of simulated intermittent normobaric hypoxia on blood antioxidant defence capacity in swimmers. The study included 14 male and 14 female competitive swimmers performing part of land training under simulated intermittent normobaric hypoxia (O_2 = 15.5%) or in normoxia. Land interval training took place twice per week, with a total of 8 training units during the study, performed with individualized intensity. The activities of blood antioxidant enzymes did not change significantly during the first and last training unit in the hypoxic and normoxic group. However, when comparing individual variables a significant effect of exercise was observed on GPx an CAT activities, whereas training units significantly differentiated GPx and GR activities. The oxygen conditions and gender had a significant influence on CAT activity. The total antioxidant capacity was not significantly affected. Only in male swimmers from the hypoxic group did the training significantly increase resting levels of MDA. In conclusion, training in normobaric hypoxia was not an adequate stimulus for the excessive response of the antioxidant defence system, despite increased oxidative stress in these conditions.

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INTRODUCTION

It is well known that physical activity, regardless of the type and intensity, is a cause of oxidative stress. This phenomenon is due to the faster accumulation of reactive oxygen species (ROS) than their removal [1,2]. Even during prolonged low intensity exercise, a significant increase in oxidative stress markers was reported [3]. In addition, the production of free radicals and induction of oxidative stress can further increase with an escalation in exercise intensity [4]. The mechanisms of this phenomenon are complex and not completely understood; however, they are linked to the increased oxidative potential of heterogeneously modified forms of oxygen and nitrogen [5]. The reactions of the mitochondrial respiratory chain, especially complex I and III [5, 6], the oxidation of catecholamines, reactions involving xanthine oxidase, which catalyses the transfer of electrons from NADPH to oxygen [7], and 'oxygen burst' in leukocytes [8] are among frequent sources of ROS.

The consequences of oxidative stress under hypoxic conditions, i.e., when physical effort is limited by the availability of oxygen to the working muscles, are of great interest to sport science [9, 10, 11]. Indeed, physical training under hypoxic conditions is frequently used to improve physical performance. At the same time, hypoxia can reduce the antioxidant capacity of the body, which results in an increase in oxidative stress and the occurrence of fatigue [12]. Oxidative stress in athletes during exposure to hypoxia appears to result from a reduction in the partial pressure of oxygen and metabolic activation of the mitochondrial respiratory chain [13]. Hypoxia can lead to an adaptive proliferation of mitochondria in muscle fibres, increased consumption of delivered oxygen, and the promotion of ROS generation [12]. At the same time, increased tissue oxidative stress and elevated physical activity are strong inducers of the antioxidative system [13]. The system consists of antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPx]; catalase [CAT], glutathione reductase [GR]) and non-enzymatic mediators (e.g., vitamin C, E, retinol, bilirubin, uric acid [UA], reduced glutathione [GSH], thiols, coenzyme Q, transport stress proteins, and albumin) [10].

Training in hypoxic conditions has become an important element of preparing elite athletes, including swimmers, for major competitions [14, 15]. The training is thought to be most effective when it is performed at an altitude of 2,000 to 2,500 m [16, 17, 18, 19, 20, 21]. Therefore, the aim of this study was to evaluate the effects

of intermittent normobaric hypoxia, mimicking reduced oxygen availability at such altitudes, during land training on antioxidant potential in the blood of elite swimmers.

MATERIALS AND METHODS

Subjects. The subjects were 14 competitive female and 14 male swimmers, divided into the following groups: male normoxic group (MNG) (n=6), male hypoxic group (MHG) (n=8), female normoxic group (FNG) (n=7), and female hypoxic group (FHG) (n=7). The subjects were divided into the hypoxic (HG) and normoxic (NG) groups according to their VO_{2max} values. Table 1 presents the physical characteristics of the subjects. The swimmers participating in the study maintained their sea level training regime throughout the duration of the study. The athletes from the HG trained twice per week for 4 weeks on land in simulated hypoxia; however, they conducted swimming in normoxic conditions. In contrast, swimmers from the normoxic groups performed both swimming and land training under normoxia. All swimmers participating in the study were in a similar phase of the annual training cycle, maintaineed a mixed diet, did not take any supplements, and did not smoke. The research programme was approved by the Bioethics Committee of the Academy of Physical Education in Katowice. All study participants signed written consent to participate in the research project.

Experimental design

Before the training procedure began, all subjects underwent a series of examinations to determine optimal training loads. Body mass and body composition were evaluated by electrical impedance (Inbody 220, Biospace Co., Japan) after an overnight fast. Two hours after a light breakfast, a ramp ergocycle test was administered to determine maximal oxygen uptake (VO_{2max}) (MetaMax 3B, Cortex, Germany). VO_{2max} was used to establish individual training loads for interval training sessions in normoxia ($\%VO_{2max}$). The ramp test was performed on an Excalibur Sport ergocycle (Lode BV, Netherlands), beginning with a workload of 50 W, which was then increased linearly by 25 W per minute (0.42 W per second), until volitional exhaustion. The criteria of reaching VO_{2max} included a plateau in values of VO_2 or a gradual decrease in peak VO_2 during the maximal workload. After 24 h of recovery, the athletes in the hypoxia groups performed the same ramp test in normobaric hypoxic conditions, in order to establish individual training loads for the intermittent hypoxic training sessions ($\%VO_{2max}hyp$).

Throughout the study, both the hypoxic and normoxic groups continued the same training programme of 4 weekly microcycles with individualized training intensity. The only differentiating factor between these two groups was related to the conditions under which the land interval training took place. The hypoxic group performed training in normobaric hypoxic chamber, where the prevalence of O_2 was 15.5%, corresponding to 2,500 m a.s.l. altitude. The normoxic group performed the same training programme under normoxic conditions, where the concentration of O_2 equaled 21%. Land interval training took place twice per week, with a total of 8 training units during the study. Relative intensity of training units was selected individually, based on the results of initial aerobic capacity tests. Each training session consisted of a 10 min general warm-up, 45-55 min exercise, and a 10 min cool-down. The main part of the circuit consisted of exercise performed on an upper limb rotator (50 W) with a cadence of 80 rpm lasting 60 s. Then the swimmers performed a 30 s maximum effort on the rotator from a flying start with a load of 0.4 $Nm \cdot kg^{-1}$ (Brachumera Sport, Lode). After a 30 s rest period, the athletes performed a 3 min ride on a cycle ergometer (Cyclus 2) with the intensity set at 50% $VO_{2max}hyp/VO_{2max}$. Following a 3 min period of active rest on the cycle ergometer, the swimmers performed another 2 min exercise bout at an intensity of 95% $VO_{2max}hyp/VO_{2max}$. After the high intensity phase of training for the lower limbs, the athletes peddled for 3 min at the intensity of 50% $VO_{2max}hyp/VO_{2max}$ (ITU – initial training unit in Tables 2, 3, and 5). This circuit was repeated four times in the first 4 interval training sessions, after which a 5^{th} circuit was added to increase the overall training load. During the last workout, the programme was conducted under the same hypoxic conditions as during the initial training, which included 4 circuits (FTU – final training unit in Tables 2, 3, and 5). The swimming training was the same for athletes in both the normoxic and hypoxic groups and consisted of nine units per week, with a volume of approximately 50 km per week. The experiment was conducted during the precompetitive period.

TABLE I. Physical characteristics of swimmers.

Variables	Hypoxia groups		Normoxia groups	
	Males (n = 8)	Females (n = 7)	Males (n = 6)	Females (n = 7)
Age (years)	18.6 ± 1.84	21.0 ± 1.31	23.2 ± 3.40	22.0 ± 1.83
Body height (cm)	183.5 ± 3.26	172.4 ± 3.37	183.0 ± 6.98	169.5 ± 2.63
Body mass (kg)	76.5 ± 5.48	63.9 ± 6.32	79.1 ± 8.22	58.4 ± 3.69
BMI ($kg \cdot m^{-2}$)	22.7 ± 1.92	21.5 ± 1.55	23.7 ± 1.57	20.4 ± 1.58
$VO_2max(ml \cdot kg^{-1} \cdot min^{-1})$	56.9 ± 7.24	48.3 ± 4.72	52.2 ± 5.56	44.0 ± 3.89

Note: BMI, body mass index; VO_2max, maximal oxygen consumption.

Analytical procedures

Blood (5 mL total) was drawn from the antecubital vein before and after the initial and the final training units, and collected into test tubes containing an anticoagulant (EDTA). The blood was centrifuged (3400 rpm for 20 min) to obtain plasma and erythrocytes using routine procedures. The plasma was frozen at -70°C for a period of about 10 days until analysis, but with separated erythrocytes and whole blood, which were prepared from lysates according to routine methods described in diagnostic kits. One of the objectives of the study was to compare training-induced changes in the total capacity of the blood antioxidant defence system, while taking into account its individual components (enzymatic and low molecular weight antioxidants). For this purpose, an index of antioxidant potential (POTAOX) was calculated as the sum of activities of enzymatic and non-enzymatic antioxidants standardized against respective baseline values. The activity levels of superoxide dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GPx, EC 1.11.1.9) were determined in red blood cell (RBC) lysates using Randox diagnostic kits (Ransod SD125 and Ransel RS504, respectively). The activity of catalase (CAT, EC 1.11.1.6) was measured in RBC lysates by measuring the composition of hydrogen peroxide at 240 nm according to the method described by Aebi [22], and that of glutathione reductase activity (GR, EC 1.6.4.2) was measured in RBC lysates by the Glatzle et al. [23] method. Reduced glutathione (GSH) was assessed spectrophotometrically in whole blood lysates by a routine colorimetric method of Beutler et al. [24] with Ellman's reagent. Plasma total antioxidant status (TAS) was quantified with the Randox TAS kit NX2332 (UK) and expressed as mmol/L. In this assay, methaemoglobin reacts with H_2O_2 to form the radical species ferrylmyoglobin. The chromogen ABTS (2,2'-azino di-(ethylbenzothiazoline sulfonate)) is incubated with the ferrylmyoglobin to produce the radical cation species $ABPS^{+\cdot}$. Plasma concentration of uric acid (UA) was assessed with the Randox UA 230 kit with the colorimetric method, where uric acid was converted by uricase to allantoin and hydrogen peroxide. The activities of creatine kinase (CK; EC.2.7.3.2), and lactate dehydrogenase (LDH; EC.1.1.1.27) were measured spectrophotometrically in fresh plasma using Randox RX 110 and Randox RX 401 diagnostics kits respectively. The index for antioxidant potential (POTAOX) was calculated as the sum of activities and concentration of antioxidants, standardized against respective pre-experiment values according to the formula: $X_{iST}=(xi-X_{ref})/SD_{ref}$. The index of total antioxidant potential (POTAOX) was calculated using the equation: $POTAOX= \Sigma[SOD_{ST}]+[GPx_{ST}]+[CAT_{ST}]+[GR_{ST}]+[UA_{ST}]+[GSH_{ST}]$. Plasma concentration of malondialdehyde (MDA), as a biomarker of oxidative stress, was estimated by the thiobarbituric acid method, according to Buege and Aust [25], with extraction of the chromogen formed with n-butanol and reading the absorbance of the organic layer at 532 nm. The levels of lipid peroxides were expressed as μmol of malondialdehyde per litre of plasma, which was calculated from the calibration curve prepared with 1,1,3,3-tetraethoxypropane (Sigma) as an external standard.

TABLE 2. Antioxidant enzyme activity during training under hypoxic and normoxic conditions.

Group	Enzyme (unit)	Sex	Training period			
			ITU (before)	ITU (after)	FTU (before)	FTU (after)
HG	SOD	M	1184.5 ± 316.9	1176.9 ± 241.1	1293.9 ± 208.1	1378.9 ± 145.1
	(U·gHb-1)	F	1364.5 ± 210.4	1437.3 ± 209.8	1429.2 ± 163.7	1356.1 ± 251.9
	GPx	M	43.1 ± 12.41	44.9 ± 15.36	51.7 ± 14.2	52.8 ± 10.9
	(U·gHb-1)	F	38.4 ± 6.8	45.2 ± 14.8	58.9 ± 15.7	58.9 ± 10.6
	CAT	M	205.8 ± 29.3	195.4 ± 21.8	186.9 ± 37.4	217.2 ± 48.0
	(U·gHb-1)	F	213.7 ± 21.6	233.4 ± 33.3	239.6 ± 35.9	241.5 ± 27.6
	GR	M	40.7 ± 7.8	37.9 ± 5.6	30.9 ± 4.8	32.9 ± 8.8
	(U·gHb-1)	F	34.6 ± 6.9	35.4 ± 6.1	30.4 ± 3.8	29.2 ± 4.3
NG	SOD	M	1227.6 ± 98.6	1236.0 ± 108.3	1262.8 ± 50.3	1494.6 ± 73.4
	(U·gHb-1)	F	1300.0 ± 345.7	1207.9 ± 324.8	1375.1 ± 77.5	1339.6 ± 28.8
	GPx	M	49.1 ± 8.0	49.6 ± 8.1	40.8 ± 7.0	51.4 ± 9.1
	(U·gHb-1)	F	49.9 ± 22.9	54.9 ± 15.9	55.8 ± 12.5	69.4 ± 17.9
	CAT	M	172.6 ± 24.2	207.1 ± 37.1	172.3 ± 25.1	203.0 ± 27.9
	(U·gHb-1)	F	202.3 ± 23.6	214.3 ± 23.3	223.2 ± 28.7	220.3 ± 28.3
	GR	M	35.1 ± 4.7	36.7 ± 2.6	26.1 ± 2.1	29.2 ± 7.9
	(U·gHb-1)	F	38.4 ± 13.1	36.6 ± 7.3	31.2 ± 5.1	34.9 ± 7.9

Note: Abbreviations: SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; GR, glutathione reductase; HG, hypoxic group; NG, normoxic group; M, males; F, females. ITU, initial training unit; FTU, final training unit.

Statistical analysis

The data are presented as means and standard deviations (±SD). The Kolmogorov-Smirnov test of normality and Levine's test of homogeneity of variance were performed, assessing the normality of the distribution. To determine the effect of independent variables (i.e., oxygen availability (hypoxic vs normoxic conditions), sex (male vs female), time (pre- vs post-training), and effort) on the examined parameters, a 4-way analysis of variance was used with repeated measures using the general linear model test. The measurements were repeated for the variables training (the initial training unit and the last training unit) and effort (before and after the first and last training unit). These analyses were applied to investigate chronic and acute effects of the intervention, respectively. When a statistically significant effect was found, the post hoc Bonferroni test with correction for multiple comparisons was used to determine the significance level. The data were analysed using Statistica v. 10 software, and significance was set at $p<0.05$.

RESULTS

The presented results contain novel data regarding the antioxidant status of athletes exposed to intermittent hypoxic training with high intensity.

Alterations of antioxidant enzymes activities

The activities of antioxidant enzymes in the swimmers' blood did not change significantly during the first and last training unit in the hypoxic or normoxic groups (Table 2). However, when comparing individual variables, a significant effect of exercise was observed on GPx ($F_{1,24}=9.46$, $p \leq 0.001$) and CAT ($F_{1,24}=8.27$, $p \leq 0.001$) activities, whereas training units significantly differentiated GPx ($F_{1,24}=4.97$, $p<0.05$) and GR ($F_{1,24}=16.39$, $p \leq 0.001$) activities. The oxygen conditions and gender alone had a significant effect only on CAT activity ($F_{1,24}=4.60$, $p \leq 0.05$ and $F_{1,24}=17.02$, $P \leq 0.001$, respectively) (Table 3). A significant interaction effect of training and gender ($F=4.36$, $p = 0.047$) was observed for SOD activity. In addition, a significant interaction effect of sex and oxygen availability was noted for activity of GR ($F=5.05$, $p = 0.034$) (Table 3).

Sex and hypoxia - specific impact on induction of oxidative stress and stress enzymes activities

In the hypoxic groups, the land training significantly increased resting levels of MDA ($p = 0.003$) in male athletes. This effects was not observed in females, under either hypoxic or normoxic conditions (Table 4). Overall, a significant effect of sex on the level of MDA ($p=0.001$) was observed. Other independent variables, such as the training cycle and exercise (before or after training), also significantly modulated plasma MDA levels, respectively $p=0.001$ and $p=0.001$ (Table 5). A significant interaction effect was also observed for gender and oxygen availability on MDA levels ($p = 0.005$) (Table 5).

Intense oxidative stress is frequently related to tissue injury; therefore, we also evaluated the activity of the stress-related enzymes CK and LDH. A significant decrease in resting CK activity was found in male athletes before the last training unit under both hypoxic and normoxic conditions (Table 4). In female swimmers, these values were unchanged under hypoxic conditions. In contrast, resting activities of both CK ($p = 0.003$) and LDH ($p = 0.006$) enzymes were significantly elevated before the last training unit under normoxia, suggesting elevated susceptibility of female athletes to tissue injury. Overall, a significant effect of gender, training units, and exercise was observed on both CK and LDH activities (Table 5). CK activity was altered by a combination of gender and exercise ($p = 0.001$), while LDH activity was altered by gender and training ($p = 0.047$).

Sex and hypoxia - specific responses in alterations of non-enzymatic antioxidant levels

In male athletes resting plasma levels of GSH were insignificantly decreased before the last training unit under hypoxic conditions (Table 6). These changes corresponded to elevated levels of MDA (Table 4), suggesting an inverse correlation. The levels of UA and TAS were not altered as a result of training and/or oxygen availability. No enzymatic antioxidants measured in the present study were altered in female swimmers (Table 6). When comparing the effects of variables evaluated in this study, a significant effect of training on plasma levels of TAS ($p \leq 0.01$), GSH ($p \leq 0.01$), and UA ($p \leq 0.05$) was observed (Table 5). Exercise also significantly differentiated

TABLE 3. Results of ANOVA for independent variables: sex, oxygen availability, training, exercise and antioxidant enzyme activity.

Enzymes	Independent factors							
	Sex (males and females)		Oxygen availability (hypoxia or normoxia)		Training (initial and final training units)		Exercise (before and after)	
	F(1.24)	p=	F(1.24)	p=	F(1.24)	p=	F(1.24)	p=
SOD	2.483	0.128	0.255	0.617	4.246	0.0503	0.791	0.382
GPx	3.897	0.059	1.123	0.277	4.974	0.035	9.459	0.005
CAT	17.023	0.001	4.598	0.042	1.110	0.302	8.266	0.009
GR	0.008	0.926	7.181	0.736	16.392	0.001	0.550	0.465

Note: Abbreviations: SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; GR, glutathione reductase.

these parameters, except for plasma GSH levels. Gender had a highly significant effect on UA levels, but none on GSH and TAS (Table 5). In addition, a significant interaction was observed when the combined effect of gender and training cycle on GSH levels was analysed (p=0.009).

The POTAOX index was recorded before and after ITU and FTU in male and female swimmers under hypoxic and normoxic conditions. The total antioxidant capacity was not affected significantly. Only in male swimmers did we observe a tendency of the POTAOX index of antioxidant capacity to decline, yet it tended to increase in female athletes (Table 6). The POTAOX index revealed a significant effect only in the exercise variable (p=0.008) (Table 5). In addition, significant interactions were found between: training and sex (p=0.005), training and oxygen availability (p=0.041), training and exercise (p=0.013), training x exercise x oxygen availability (p=0.042).

DISCUSSION

The present study explores the interrelationship between training, exercise, oxygen availability, sex, and antioxidant capacity and its effect on blood antioxidant defence capacity in competitive swimmers. Based on previous [12, 13] reports, we hypothesized that exercise under hypoxic conditions may aggravate oxidative damage. As a study model, we evaluated the impact of land training of swimmers carried out under conditions of intermittent normobaric hypoxia and normoxia, and evaluated the occurrence of oxidative stress and induction of the antioxidant defence system. This system counteracts the damaging effects of oxidative stress. It includes several

TABLE 4. Impact of training under hypoxic and normoxic conditions on oxidative stress and activity of stress enzymes.

	Parameter	Sex	Training period			
			ITU (before)	ITU (after)	FTU (before)	FTU (after)
HG	MDA	M	4.18 ± 1.07	4.97 ± 1.16	6.39# ± 1.32	7.40 ± 1.57
	(nmol·ml-1)	F	4.39 ± 1.36	3.00 ± 1.50	5.74 ± 1.80	7.22 ± 1.62
	CK	M	243.19 ± 79.41	289.06 ± 101.29	208.8* ± 88.70	243.19 ± 87.36
	(U·L-1)	F	106.13 ± 66.46	128.62 ± 70.29	91.29 ± 54.22	100.29 ± 56.03
	LDH	M	306.94 ± 59.57	340.95 ± 52.11	385.52 ± 42.00	418.24 ± 35.59
	(U·L-1)	F	267.14 ± 54.79	313.01 ± 43.70	339.54 ± 44.49	369.22 ± 71.06
NG	MDA	M	5.42 ± 0.52	5.93 ± 0.99	7.47 ± 0.76	8.28 ± 1.02
	(nmol·ml-1)	F	2.82 ± 1.14	3.30 ± 0.72	4.84 ± 0.94	6.38 ± 1.08
	CK	M	218.57 ± 99.17	268.21 ± 100.33	182.5# ± 46.72	231.35 ± 34.27
	(U·L-1)	F	71.28 ± 28.62	91.23 ± 18.66	107.60# ± 88.70	243.19 ± 87.36
	LDH	M	305.49 ± 50.15	357.26 ± 71.46	316.12 ± 29.60	370.63 ± 12.53
	(U·L-1)	F	249.60 ± 36.36	319.75 ± 64.55	393.65# ± 24.44	398.11 ± 85.50

Note: Abbreviations: MDA, malondialdehyde; CK, creatine kinase; LDH, lactate dehydrogenase; ITU, initial training unit; FTU, final training unit. *Statistically significant versus the initial training unit p≤0.05; # statistically significant versus initial training unit p≤0.001

TABLE 5. Results of ANOVA for independent variables: sex, oxygen availability, training, exercise and oxidative stress marker, stress enzyme activity, the level of non-enzymatic antioxidants, total antioxidant status and total antioxidants potential.

Parameter	Independent values							
	Sex (male and female)		Oxygen availability (hypoxia or normoxia)		Training (initial and final training units)		Exercise (before and after)	
	$F_{(1,24)}$	p=	$F_{(1,24)}$	p=	$F_{(1,24)}$	p=	$F_{(1,24)}$	p=
MDA	27.83	0.001	0.239	0.630	82.970	0.001	19.210	0.001
CK	69.78	0.001	0.139	0.712	7.558	0.011	9.689	0.005
LDH	1.96	0.174	0.077	0.783	42.249	0.001	56.476	0.001
GSH	0.09	0.762	3.597	0.069	10.226	0.004	0.584	0.452
UA	69.77	0.001	0.139	0.712	7.558	0.011	9.689	0.005
TAS	1.15	0.292	3.021	0.095	11.849	0.003	20.727	0.001
POTAOX	2.62	0.119	1.106	0.303	0.256	0.617	8.409	0.008

Note: Abbreviations as in Tables 2 and 3.

TABLE 6. Impact of training under hypoxic and normoxic conditions on non-enzymatic antioxidants.

Group	Parameter/unit and sex		Training period			
			ITU (before)	ITU (after)	FTU (before)	FTU (after)
HG	GSH	M	3.32 ± 1.04	3.07 ± 0.76	2.26* ± 0.35	2.44 ± 0.22
	($mg \cdot mgHb^{-1}$)	F	2.85 ± 0.75	2.86 ± 0.23	2.81 ± 0.61	2.70 ± 0.53
	UA	M	6.14 ± 1.02	6.61 ± 1.12	5.97 ± 1.02	6.44 ± 1.17
	(g·dl-1)	F	4.52 ± 0.41	5.28 ± 0.50	4.04 ± 0.79	4.59 ± 0.72
	TAS	M	1.00 ± 0.05	1.13 ± 0.12	1.16 ± 0.08	1.27 ± 0.16
	($mmol \cdot l^{-1}$)	F	0.99 ± 0.13	1.12 ± 0.11	1.21 ± 0.34	1.31 ± 0.23
	POTAOX	M	0.00 ± 2.63	-0.40 ± 2.27	-2.10 ± 1.63	0.22 ± 2.73
	index	F	0.00 ± 1.71	-2.41 ± 2.28	2.69 ± 4.58	1.61 ± 2.73
NG	GSH	M	3.28 ± 0.99	3.50 ± 0.53	2.67 ± 0.24	2.56 ± 0.19
	($mg \cdot mgHb^{-1}$)	F	2.86 ± 0.61	3.23 ± 0.30	2.96 ± 0.22	3.16 ± 0.48
	UA	M	6.66 ± 0.57	6.68 ± 0.81	6.39 ± 0.85	6.60 ± 0.80
	(g·dl-1)	F	4.18 ± 0.94	4.75 ± 1.22	3.69 ± 0.55	3.77 ± 0.84
	TAS	M	1.05 ± 0.10	1.16 ± 0.11	1.15 ± 0.09	1.35 ± 0.07
	($mmol \cdot l^{-1}$)	F	1.19 ± 0.20	1.30 ± 0.18	1.21 ± 0.14	1.25 ± 0.21
	POTAOX	M	0.00 ± 2.67	2.18 ± 2.36	-3.66 ± 1.33	2.17# ± 0.87
	index	F	0.00 ± 1.85	1.54 ± 1.43	0.52 ± 2.89	1.44 ± 1.32

Note: GSH, reduced glutathione; UA, uric acid; TAS, total antioxidant status; POTAOX, index of antioxidant potential; ITU, initial training unit; FTU, final training unit.*Statistically significant versus the initial training unit $p \leq 0.05$; #Statistically significant versus the rest FTU $p \leq 0.05$.

antioxidant enzymes, macromolecules and an array of small molecules, such as antioxidant vitamins, thiol groups, uric acid, etc. [26].

Antioxidant enzymes play specific functions in the organism. The cupro-zinc SOD in red blood cells is irreversibly inactivated by its product hydrogen peroxide in a concentration-dependent manner [27]. The haem enzyme CAT is inhibited by superoxide anions due to a reversible reaction of O_2 radicals with native enzymes, which involves a univalent reduction of the ferric centre of oxyferrous haem (Fe^{2+})-O_2 [28]. SOD and CAT to protect erythrocytes against peroxidation [29] and their progressive inactivation during a competition can clearly account for substantially enhanced oxidative stress. It is known that GPx and CAT have the ability to break down hydroxyl peroxide into water and oxygen. GPx catalyses the reduction of H_2O_2 and lipid peroxides to water and oxidized glutathione, using the reduced glutathione (GSH) as the electron donor [13]. The oxidized glutathione is recycled back to GSH in a reaction catalysed by the enzyme glutathione reductase (GR), yet uric acid has powerful antioxidant properties in human biological fluids, and its concentration in plasma is almost 10-fold higher than other antioxidants, such as vitamins C and E.

In this study we observed that the changes in individual levels of non-enzymatic antioxidants throughout the study were individually tailored towards the targeted variables. For example, gender and training significantly altered the concentrations of UA, while GSH levels were significantly affected only by training. On the other hand, the TAS was significantly modulated by both training and exercise. A relatively minor alteration in the levels of TAS, GSH, and UA correspond to a mild induction of oxidative stress in these athletes, and is consistent with adaptive processes to ensure antioxidative protection [30]. Based on our results, it appears that a combination of TAS, UA, and GSH more adequately reflects the antioxidant status in relation to gender differences and the training regimen under hypoxic conditions, as opposed to measurement of a single antioxidant. Moreover, the concentration of blood GSH under hypoxic conditions was decreased, which was confirmed by the experiment of Joanny et al. [31] during Operation Everest III.

Among antioxidant enzymes, catalase activity was significantly modified by gender, the availability of oxygen, and exercise, therefore indicating high sensitivity as an antioxidant marker in athletes trained under hypoxic conditions. In addition, activity of GPx was differentiated by training and effort. On the other hand, the activities of GR and SOD showed only minor and insignificant changes, suggesting their limited applicability in the setting of hypoxia and exercise-driven tissue oxidation. Therefore our alternative approach to use the POTAOX index to quantify blood antioxidant capacity, which yielded information about the combined effectiveness of enzymatic and non-enzymatic components of the antioxidant defence system, seems to be also a reliable measure. It seems justified to presume that these dynamic changes in the POTAOX index better reflect the combined impact of major contributing elements to the overall blood antioxidant defence. In this work land training of swimmers under hypoxic conditions did not affect the blood antioxidant capacity. It is worth noting that the rest POTAOX index decreased only in male swimmers

training in hypoxia, as well as in normoxia. An explanation of this phenomenon is quite difficult. Several authors have investigated the total antioxidant capacity during exercise [32, 33, 34], but only during normoxia. Wozniak et al. [35] evaluated the influence of exercise in high-altitude conditions (about 2000 m a.s.l.) on SOD and CAT activities in 10 kayakers and 10 rowers. They found a significant increase of SOD and CAT activities in erythrocytes after the 4th, 10th and 18th day of training. Increased activities of these enzymes were probably responsible for the generation of oxygen-derived free radicals.

It is known that continuous and intermittent efforts under hypoxic conditions increase oxidative stress [10, 11, 36]. Bailey et al. [11] demonstrated that 60 min of simulated training under hypoxic conditions significantly increased the levels of serum lipid peroxides, with a simultaneous reduction of antioxidant enzyme activities. Gonzalez et al. [37] and Pialoux et al. [38] reported diminished MDA levels in the plasma of swimmers after an acute hypoxic swimming test (10 min at 4,800 m) as well as in cyclists who spent 13 days at an altitude of 2,500 to 3,000 m, and trained at 1200 m above sea level.

We observed that the resting levels of MDA were highly elevated before the last training unit in male athletes; however, they did not further increase as the result of a training cycle. This could be partially explained by the relatively short exposure to simulated hypoxia (4 h/week), which was not accompanied by additional stress factors such as exposure to cold, ultraviolet rays, or acute mountain sickness (i.e., factors encountered at high altitude). The elevated baseline levels of MDA in male athletes were accompanied by decreased levels of CK under both hypoxic and normoxic conditions, suggesting that the aggravated oxidative stress in male swimmers was not linked to tissue damage. In contrast, female swimmers did not have elevated plasma MDA levels; however, they did exhibit elevated activities of CK and LDH under normoxic conditions. This discrepancy may be related to the fact that MDA levels reflect primarily products of lipid peroxidation and other products of this reaction. On the other hand, in females the role of estrogens should be considered, as it is known that these hormones increase the antioxidant defence capacity [39]. Additionally, different forms of ROS may escape this measurement [4, 40, 41, 42]. Overall, the results of our study demonstrated a statistically significant effect of gender, training, and exercise on the baseline level of plasma MDA.

CONCLUSIONS

In conclusion, the intermittent hypoxic training did not affect the antioxidant defence system in competitive swimmers. Individually was observed, to slightly increased activities of antioxidant enzymes after endurance and a high intensity training programme. Oxygen availability and training significantly differentiated the activities of CAT, GPx and GR. Hypoxic land swimming training did not affect the total antioxidant potential (POTAOX). In this study the POTAOX index proved to be a good measure of the blood antioxidant defence capacity during land training of swimmers in hypoxic conditions. Our results indicate that intermittent hypoxic training, conducted twice per week in young athletes, does not significantly increase the oxidative stress, and may be considered a relatively safe approach for improving sports performance.

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