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PHYSICAL PERFORMANCE AND ANTIOXIDANT EFFECTS IN TRIATHLETES

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Abstract: Exercise results in an increased production of reactive oxygen species. Two major classes of endogenous protective mechanisms work together to ameliorate the harmful effects of oxidants in the cell: (1) components of the enzymatic scavenging system such as superoxide dismutase, glutathione-peroxidase and catalase and (2) nonenzymatic antioxidants. The purpose of this study was to identify any relationship between duration and intensity of prolonged physical exercise and markers of oxidative stress with the primary antioxidant system. Eleven triathletes performed a field test, which consisted of 1.9 km swimming, 60 km cycling and 21 km running. Venous and arterialized blood enzymatic activities of SOD, CAT, GPX, and creatine kinase and concentrations of glucose, lactate, malondialdehyde and bilirubin were determined. Athletes were divided into two groups: the more efficient group (A), and the less efficient group (B), according to their duration of the field test. The activity of GPX was significantly higher in Group A than Group B, irrespective of the duration of the exercise, but bilirubin concentration was lower. For Group B, SOD activity increased during running while CAT activity decreased after cycling and after running. Upon completion of the test, CK activity was elevated in both groups. The free radical scavenging system appears to be directly related to individiual physiological efficiency with prolonged submaximal physical exercise. According to our estimation of the individual training status and the adequate adaptation level, it is important to take into consideration the markers of free radical production and the activities of the scavenging compounds. Abbreviations: SOD - superoxide dismutase, GPX - glutathione peroxidase, CAT - catalase, MDA - malondialdehyde, CK - creatine kinase.

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Introduction

Physical exercise can increase the production of many kinds of free radicals, which can result in damage to individual cells. However, the antioxidant system of these cells can effectively protect them against severe damage. Two major classes of endogenous protective mechanisms work together to ameliorate the harmful effects of oxidants in the cell. The primary enzymatic antioxidant system found in cells includes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Many nonenzymatic antioxidants also exist in the cell. One member of this family is bilirubin, which is a product of hemoprotein catabolism and can be found in both the intracellular and extracellular space. It appears that bilirubin is capable of protecting albumin-bound fatty acids from lipid peroxidation [25,26]. The measurement of the markers of lipid peroxidation, in humans, is determined by the production of free radicals. One marker is malondialdehyde (MDA), which is a product of the oxidation of polyunsaturated fatty acids that contain more than two methylene-interrupted double bonds [5]. Another marker is creatine kinase (CK), an intramuscular protein, which leaks into the serum after membrane damage [2].

Antioxidant supplementation can decrease the extent of free radicals but has not been shown to enhance performance [22]. Physical training has been also ambiguously shown to result a reduction in lipid peroxidation and in an augmented antioxidant system because it depends on the intensity of physical activity and the individual training status [2]. Many studies have examined the effects of acute physical exercise on the antioxidant scavenging system, using laboratory tests, but few have observed the effects in a field condition [4,19,30]. Therefore, the aim of this study is to examine the relationship between exercise performance in a field condition, using markers of oxidative stress, the primary enzymatic scavenging system and a nonenzymatic antioxidant marker.

Materials and Methods

Subjects: The subjects for the study were 11 male triathletes (age=29.8±6.4 years; body height=176.5±4.5 cm; body weight=73.8±6.9 kg). The athletes performed a field test, which consisted of 1.9 km swimming, 60 km cycling and 21 km running. Blood samples were taken from the index finger at rest (R), after

swimming (S), at the half way point in the cycling phase (C1), at the completion of the cycling phase (C2), halfway through the running phase (R-1), at the completion of the running phase (R-2), as well as 30 minutes into recovery (R30). Heart rate was monitored during each phase of the physical activity. Athletes were divided into two groups according to their performance: Group A performed below the mean value (mearured in duration time), whereas Group B performed above the mean value (Table 1).

Table 1
Duration of phases of a field test for more (Group A) and less efficient (Group B) triathletes

Time (min) (mean±SD)	S	C1	C2	R-1	R-2	total
Group A	28.1	50.2	50.8	46.3	47.9	223.9
n=7	±2.5	±2.1	±2.9	±3.4	±3.9	±8.9
Group B	40.6	54.9	56.9	48.9	56.3	257.7
n=4	±4.1***	±4.4*	±5.8*	±3.5	±10.1	±20.6**

S=swimming; C1=first stage of cycling; C2=second stage of cycling; R-1=first stage of running; R-2=second stage of running
*p<0.05;**p<0.01;***p<0.001

Analytical methods: Assay kits (purchased from RANDOX), for quantitative determination of SOD in whole blood, were used to measure enzyme activity by spectrophotometry. In this method, xanthine and xanthine oxidase were used to generate superoxide radicals which react with I.N.T. (2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride) to form a red formazan dye when measured at 505 nm [27].

The method of determination of GPX enzyme activity is based on that of Paglia and Valentine [20,28]. GPX catalyzes the oxidation of glutathione (GSH) using cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured.

CAT was measured by the method of Góth *et al.* [7]. The yellow complex formation between hydrogen peroxide and ammonium molybdate was measured at 405 nm.

Lactate was assayed by the method of Hohorst [8]. L-lactate is oxidised to pyruvate by NAD, in a reaction catalysed by lactate dehydrogenase. The NADH formed during the reaction is equivalent to the L-lactate present. NADH is the reaction indicator and its absorbance at 340 nm was measured.

MDA was determined by a modification to Yagi's fluorometric assay [3]. MDA is treated with diethylthiobarbituric acid (DETBA) in an acid medium, and the fluorescence compound is then extracted with butanol and measured between 515 and 553 nm by fluorescence spectroscopy.

CK was measured by Mathieu's method [14]. CK catalyzes the dephosphorylation of creatine phosphate produced by ATP. Hexokinase assisted with the conversion of ATP to ADP as D-glucose is transformed to glucose-6-phosphate. In the presence of glucose-6-phosphate dehydrogenase and $NADP^+$, this compound oxidized and immediately formed NADPH. Change in absorbance was measured at 340 nm.

The most widely used method for determining bilirubin has been that of Jendrassik-Grof [10], by which a five minute color development is employed. In the presence of a caffeine accelerator, total bilirubin couples with sulfanilic acid to form a red azobilirubin dye, the color intensity of which is proportional to the bilirubin concentration. Determination of direct bilirubin is performed without caffeine additive.

Hematocrit value was determined indirectly (counted from the number and the volume of the cells) on a pHOx PLUS L blood gas analyser. Concentration of glucose was determined using same equipment.

Statistical analyses: All of the data are presented as means ± SD. Student's t-test for dependent and independent samples was used for comparison between groups. Bivariate associations were assessed by Pearson correlation coefficients. Principal component analysis (PCA) was used to evaluate the prediction factors of the performance. PCA consists of an eigenanalysis of a correlation matrix calculated on the original measurement data. It can be described as a rotation of a swarm of data points in multidimensional space so that the longest axis (the axis with the greatest variance) is the first PCA axis, the second longest axis, perpendicular to the first, is the second PCA axis, and so forth. Thus these first few PCA axes represent the greatest amount of variation in the data set. Calculations were performed with STATISTICA 6 statistical package (StatSoft, Inc.) and MVSP Version 3.13m (Kovach Computing Services). The level of significance was set at $p<0.05$.

Results

The members of Group A performed the field test in 223.9±8.9 min (n=7) while the members of Group B finished it in 257.7±20.6 min (n=4). The significant difference in duration was found to be in the swimming and cycling phases (Table 1). Heart rate was similar for both groups in each stage of the exercise (Table 2).

Table 2
Heart rate at phases of a field test for more (Group A) and less efficient (Group B) triathletes

Heart rate (bpm) (mean±SD)	R	S	C1	C2	R-1	R-2	mean value from S to R-2
Group A	77	142	153	146	152	154	150
n=7	±5	±20	±10	±10	±7	±7	±8
Group B	75	141	150	146	156	150	149
n=4	±2	±13	±11	±9	±4	±9	±7

R=rest, S=swimming; C1=first stage of cycling; C2=second stage of cycling; R-1=first stage of running; R-2=second stage of running

Group A demonstrated higher lactate concentrations than Group B after swimming but not after cycling or running. Maximal lactate concentration was reached after swimming in Group A and after the first stage of cycling in Group B. The average lactate concentration was below 4 mmol/l after cycling for both groups. Maximal glucose concentration was reached post-test in Group A and after running for Group B. The mean glucose concentration was below 10 $mmol \cdot l^{-1}$ after all phases of the exercise (Table 3). Hematocrit did not change during work as liquid supplementation was provided throughout the exercise.

The primary antioxidant enzyme system varied minimally during the exercise. The only significant differences between the two groups have been observed after cycling (C2) for SOD (Fig. 1), where Group B was higher than Group A, and for GPX where Group B, after swimming (Fig. 3), had levels that were still well below those of Group A.

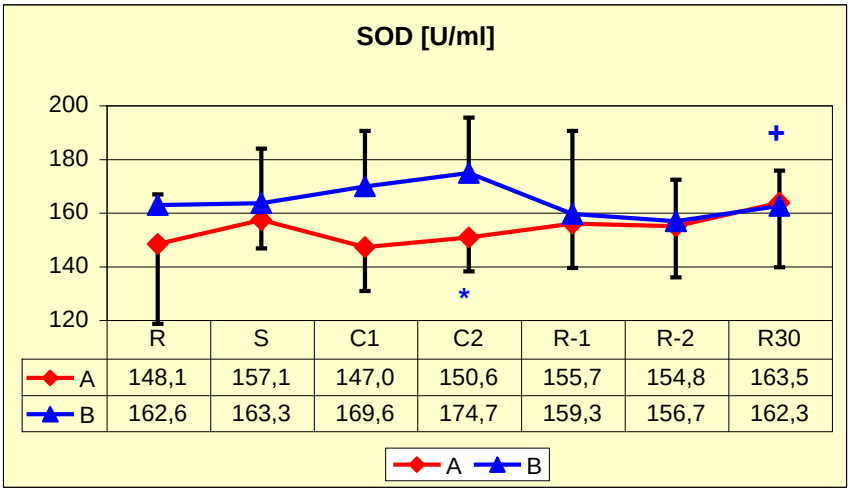

Fig. 1
Activity of superoxide dismutase during a triathlete race
*=Group A vs Group B; +=vs before stage)

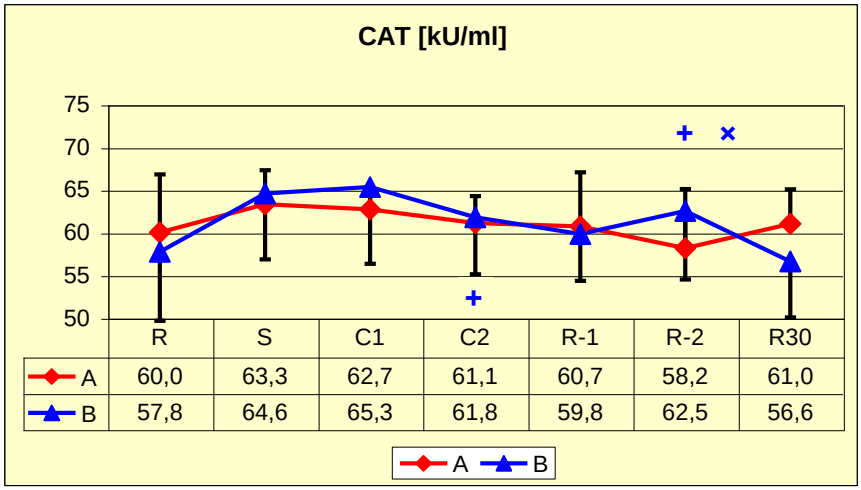

Fig. 2
Activity of catalase during a triathlete race
×=vs rest; +=vs before stage

Table 3
Concentrations of lactate and glucose during a field test for more (GroupA) and less (Group B) efficient triathletes

Mean±SD	Group	R	S	C1	C2	R-1	R-2	R30
Lactate ($mmol \cdot l^{-1}$)	A n=7	2.47 ±1.02	6.02 ±1.89[a]	4.44 ±2.13[a]	2.80 ±0.66[b]	2.79 ±0.84[a]	3.00 ±1.11	3.46 ±1.10[a]
	B n=4	2.03 ±0.33	4.18 ±1.82	5.00 ±1.88[a]	3.40 ±0.94[a]	3.88 ±1.42	3.60 ±0.59[a]	3.35 ±1.19
Glucose ($mmol \cdot l^{-1}$)	A n=7	6.69 ±0.88	8.28 ±2.32	6.27 ±0.79	6.78 ±0.76	7.21 ±0.85	8.08 ±1.55	9.97 ±2.77
	B n=4	6.32 ±0.42	7.04 ±1.56	7.15 ±1.50	6.41 ±0.48	8.64 ±1.68[b]	9.10 ±2.02	8.29 ±1.57

R=rest, S=swimming; C1=first stage of cycling; C2=second stage of cycling; R-1=first stage of running; R-2=second stage of running; R30=30th min of restitution
$p<0.05$ a=vs rest; b=vs before stage

Table 4
Concentration of bilirubin and malondialdehyde and activity of creatine kinase before and after the exercise in Groups A and B

Mean±SD	Group A		Group B	
	Before exercise	After exercise	Before exercise	After exercise
Bilirubin ($\mu mol \cdot l^{-1}$)	15.7 ±4.3	13.3 ±3.3[a]	13.8 ±3.3	22.0 ±6.2[b]
Creatine kinase ($U \cdot l^{-1}$)	200.1 ±107.9	422.0 ±157.5[a]	172.5 ±81.8	427.8 ±147.4[a]
Malondialdehyde (mmol/l)	2.62 ±0.83	3.40 ±0.82	3.47 ±0.67	3.71 ±0.87

a=after exercise vs before exercise; b=Group A vs Group B $p<0.05$

Significant differences were noted for Group B during exercise and recovery compared to the previous stages: SOD activity was significantly higher in R30 than in R2, CAT activity (Fig. 2) was significantly lower in C2 than in C1 and significantly higher in R2 than in R1.

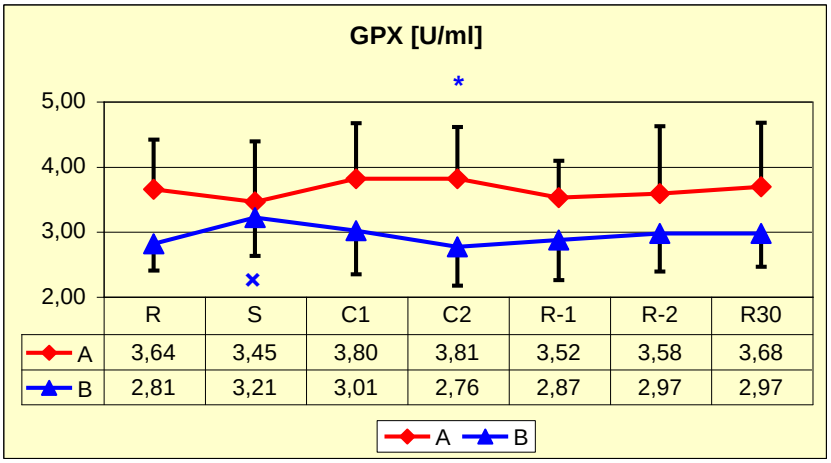

Fig. 3
Activity of glutathione peroxidase during a triathlete race
*=Group A vs Group B; ×=vs rest

Bilirubin concentration decreased in Group A ($p<0.03$) but increased in Group B after the exercise, and a significant difference ($p<0.02$) was also revealed between the two groups with exercise. CK activity increased ($p<0.05$) in both groups with exercise but no differences were noted between groups. Similarly, malondialdehyde concentration increased after exercise but no differences were observed between groups (Table 4).

The PCA have shown a similarity between the duration of exercise, changes in lactate concentration, changes in SOD activity (venous), changes in CAT activity (arterialized and venous), changes in MDA concentration, and changes in bilirubin concentration, when Group A and B are pooled (Table 5). The first three PCA axes could account for 64% of the total variance.

Table 5
Results of the first five PCA (principal component analysis) axis (the biochemical parameters represent the changes of its activity or concentration on the effect of exercise)

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Duration of exercise	0.326	0.058	0.384	-0.204	-0.327
Mean value of heart rate	-0.332	0.399	0	0.057	-0.034
Glucose	0.102	0.483	-0.047	0.309	0.025
Lactate	0.143	0.194	0.372	0.507	0.057
SOD	-0.365	0.046	0.017	-0.155	-0.453
SOD (venous)	0.367	-0.081	-0.015	0.381	0.105
CAT	0.367	0.167	-0.239	-0.39	-0.077
CAT (venous)	0.412	0.124	-0.013	-0.239	0.289
GPX	0.089	-0.311	0.144	0.353	-0.483
GPX (venous)	0.292	-0.424	-0.055	-0.012	-0.041
MDA	-0.225	-0.151	0.351	-0.035	0.571
CK	-0.086	-0.195	0.602	-0.255	0.033
bilirubin	0.17	0.421	0.381	-0.182	-0.139
Eigenvalues	3.724	2.706	1.938	1.897	1.322
Percentage	28.646	20.812	14.907	14.591	10.17
Cumulative Percentage	28.646	49.458	64.365	78.957	89.127

Discussion

Exercise appears to increase reactive oxygen species, which can result in damage to cells. Exercise also results in increased concentration of malondialdehyde in the blood, which serve as indirect indicators of lipid peroxidation. However, not all studies report these increases because of a large intersubject variability in, or the nonspecificity of the assays [22]. In the present study, increases in lipid peroxidation, with exercise, were not seen, as indicated by the unchanged concentrations of MDA. Exercise training seems to reduce the oxidative stress of exercise, such that trained athletes show less evidence of lipid peroxidation for long-term endurance exercise and an enhanced defense system than untrained subjects. In both groups no significant differences, in every stage of exercise, in heart rate, lactate or glucose values were observed. However, the

results of this study show higher serum CK activity after exercise in both groups but, in Group B the increase is greater. Increased CK activity indicates production of free radicals in skeletal muscle, primarily in the cytosolic fraction of the cells. It is generally believed that this enzyme is released into the bloodstream even when there is only a small change in the cell membrane permeability [30].

The body's natural antioxidant defense system is sufficient to counteract the increase in reactive oxygen species with exercise, and the effects of an additional exogenous supplement are not known. Generally, the athletes who received antioxidant supplements have shown reduced oxidative stress. Susceptibility of lipids to peroxidation is reduced by training and this effect is independent of antioxidant supplement use, and, as well, may be mediated by induction of endogenous antioxidants [6]. Supplementation with vitamins C, E and other antioxidants, or antioxidant mixtures can reduce lipid peroxidation or other indicators of oxidative stress as a result of exercise. However, these supplements appear to have no beneficial effect on performance [22]. Liu *et al.* [17] have observed, that with prolonged physical exercise, the total peroxyl radical trapping antioxidant capacity of plasma and uric acid concentrations are increased after a race, but concentrations returned to normal within four days. Uric acid could provide antioxidant protection to ascorbate by chelating transition metals. Plasma thiol concentrations were reduced after exercise, which indicated a shifted oxidative balance. However, no significant changes were observed in plasma ascorbic acid, α-tocopherol, ß-carotene, and retinol concentration after the marathon race [17]. After antioxidant supplementation, seven males triathletes demonstrated no effect on maximal oxygen uptake or muscle energy metabolism as measured by 31-phosphorus nuclear magnetic response spectroscopy [18].

The magnitude of the antioxidant defense system enhancement depends on training loads in various sport disciplines [13] and the changes of plasma levels of antioxidant substances must be explained as a consequence of the different training status of sportsmen, including intensity and duration of the exercise [1]. Antioxidant status, at rest, could characterize the individual training status. Glutathione levels have been shown to be correlated with relative aerobic capacity which correlates well with training volume and race results [13]. Overload training in triathletes would also induce oxidative stress and could compromise the antioxidant defense mechanism. Overload training could thus result in higher levels of thiobarbituric acid reactive substances with exercise and an induced plasma GPX activity increase in the resting condition [21].

Long-term endurance training does not greatly alter hematological status [23]. During a short course of sprint triathlon a significant decrease in weight, and an increase in white blood cell count and platelet count have been reported [12].

Physiological responses to laboratory tests may be relatively similar to field tests in triathletes. Millet [15] has established that the overall triathlon time is significantly correlated to relative aerobic capacity ($r=-0.80$; $p<0.001$) and peak power output ($r=-0.85$; $p<0.001$) in cycle ergometry. Maximal and submaximal laboratory measures can account for 93% of the variance in times during a triathlon race [9]. The performance on a shorter triathlon race can also be adequately predicted from a laboratory test [29]. Millet [16] has found that maximal and submaximal physiological characteristics do not depend on the distance of the cycling and running section of triathlon. Nontheless, it can be concluded that the duration of a triathlon race characterizes well the endurance activity of a triathlete.
Multiple regression analysis (namely principal component analysis) has shed light as to the exact correlation between the performance and the enzymatic, non-enzymatic and free radical systems. Single comparisons between the above mentioned variables show a significant positive correlation between performance and the changes in bilirubin concentration ($r^2=0.41$; $p<0.05$). This could be an indication of the importance of one of the nonenzymatic antioxidant defenses against free radicals. The decreased bilirubin concentrations with exercise ($p<0.05$) for Group A could indicate the excess production of peroxil radicals and therefore, the bilirubin can be oxidized into bilirubin free radicals which cause further cell damage [31].

Taking into consideration multiple parameters in determining the performance of a triathlon race, Van Schuylenbergh [29] *et al.* have revealed that running speed and swimming speed at maximal lactate steady state (MLSS), together with blood lactate concentrations in running at MLSS, yielded the best prediction of performance. Other components such as some morphological factors (like robustness, adiposity, segmental length and skeletal mass) account for 47% of the variance in total triathlon duration, and proportionally longer segmental length contributed to a successful swimming outcome [11]. Schabort *et al.* [24] have concluded that the five most significant predictors of triathlon performance are blood lactate during steady state workload, blood lactate during running, peak power output, peak treadmill running velocity, and relative aerobic capacity in laboratory exercise test. To the best of our knowledge there is no publication which examines the correlation between performance and antioxidant and free radical systems using multiple analyses. Principal component analyses have revealed a similarity between the duration of exercise and the changes in lactate

concentration, changes in SOD activity (venous), changes in CAT activity (arterialized and venous), changes in MDA concentration as well as changes in bilirubin concentration. Future research must take into account multiple parameters and multiple data analyses in order to establish the influence of the antioxidant scavenging and free radical producing systems on different exercises.

In conclusion, the free radical scavenging system appears to correspond to individiual efficiency with prolonged submaximal physical exercise. It is important to take into consideration the markers of free radical production and the activity of the scavenging compounds. Until research fully substantiates that the long-term use of antioxidants is safe and effective, the prudent recommendation for physically active individuals is to ingest a diet rich in antioxidants only for short periods of time [22].

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