

Effects of competitive pentathlon training on the antioxidant defence components

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Summary

Study aim: To assess the effects of training on the activities of antioxidant enzymes in erythrocytes and on the total plasma antioxidant status in competitive pentathletes.

Material and methods: A group of 10 senior male pentathletes (P) and of 10 sedentary male subjects (S) participated in the study. Blood was withdrawn from the antecubital vein in the morning, in the preprandial state. The activities of superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) were determined in erythrocyte haemolysates, that of glutathione peroxidase (GPX) in whole blood haemolysate, and the total antioxidant status (TAS) in plasma.

Results: The activities of all enzymes were significantly ($p < 0.05 - 0.001$) higher in P than in S group while no significant between-group difference was found for TAS.

Conclusions: The pronounced enzymatic antioxidative potential and oxidative stress defence observed in athletes practicing modern pentathlon may be attributed to their extensive training.

Key words: Antioxidant enzymes - TAS – Pentathletes

Introduction

Under physiologic conditions, oxygen metabolism in body cells is associated with generation of reactive oxygen species (ROS), mainly during the mitochondrial electron transport in the respiratory chain [8]; ROS are known to damage proteins, lipids and DNA, as well as cell structures [9]. On the other hand, ROS play important roles in cell differentiation and apoptosis, and act as signalling entities that activate adaptive responses of cells to exertion stimuli [10,28].

Two main antioxidant systems that neutralise harmful effects of ROS are present in human cells: enzymatic (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) and non-enzymatic (low-molecular substances, e.g. reduced glutathione, uric acid, vitamins A, E and C) [16,30]. An augmented ROS generation, exceeding the antioxidative capacity, results in oxidative stress [5,11].

Physical exertions, especially the endurance ones, bring about increased generation of ROS [13,26]. Ultramarathon run (50 km) was reported to increase the level of ROS and the associated lipid peroxidation, together with a decreased concentration of vitamin E in plasma [20] and similar results were reported for a 24-h bike

racing [19]. However, increased lipid peroxidation following an intense training in downhill skiing suggested that also anaerobic exertions may lead to oxidative stress [29]. Systematic physical training may induce adaptive increases in the activities of antioxidant enzymes not only in liver and muscle cells but in the erythrocytes as well, the latter being susceptible to endogenous and exogenous ROS forms [26].

Modern pentathlon is particularly interesting in that respect as it consists of diverse activities: the 3-km run is a typical aerobic exertion while 200-m swimming is an anaerobic one as evidenced by the post-exertion lactate levels in blood [24]. On the other hand, a 12-obstacle horse show jumping calls for high muscle strength necessary for keeping right position on the horse, and fencing contest needs additionally technical skills [27]; shooting, combined with running, requires concentration and maintaining equilibrium. Since all 5 disciplines are performed on the same day, the pentathlon training ought to shape multiple adaptive processes. Since no reports on physiological specificities of modern pentathlon were found in the available literature, the aim of this study was to assess the effects of training on the activities of antioxidant enzymes in erythrocytes and on the total plasma antioxidant status in competitive pentathletes.

Material and Methods

Two groups of male subjects volunteered to participate in the study: 10 senior pentathletes (P) and 10 sedentary, age-matched ones (S). All subjects submitted their written consents to participate and the study was approved by the local Committee of Ethics. Basic somatic data (body height, body mass and BMI) were recorded and body fat content was determined from 4 skinfolds (biceps, triceps, subscapular and supriliac; left body side) according to Durnin *et al.* [4].

Blood was sampled from the antecubital vein in the morning, in preprandial state, into heparinised tubes. A portion of blood was centrifuged at 3000 rpm for 10 min and the erythrocytes were washed 3 times with cold saline, the rest was stored at -70°C until assayed. In erythrocyte hemolysates the activities of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX) and catalase (CAT) were assayed using commercial assay kits (Ransod SD125, Glut Red GR2368 and Ransel RS504 of Randox, UK, and Bioxytech Catalase 520, 21042 of Oxis, USA, respectively). The activities of enzymes were expressed as per g or mg of haemoglobin (Hb) determined in hemolysates by Drabkin's method. Total antioxidant status of plasma (TAS) was determined using assay kits NX2332 (Randox, UK).

Student's *t*-test for independent data was used to assess the between-group differences using Statistica® 6.0 software, the level of $p \leq 0.05$ being considered significant.

Results

As follows from data presented in Table 1, the pentathletes (Group P) had significantly lower BMI and lower body fat content compared with the sedentary subjects (Group S). On the other hand, the activities of all antioxidant enzymes were in Group P significantly ($p < 0.05 - 0.001$) higher than in Group S while TAS levels were in both groups alike (Table 2).

Table 1. Mean values (\pm SD) of basic characteristics of male sedentary subjects (S) and pentathletes (P)

Variable	Group	S (n = 10)	P (n = 10)
Age (years)		21.8 \pm 3.1	20.0 \pm 3.0
Body mass (kg)		89.0 \pm 7.8**	70.1 \pm 2.5
Body height (cm)		182.0 \pm 9.3	176.0 \pm 4.8
BMI		26.4 \pm 3.7*	22.6 \pm 0.8
Body fat content (%)		21.7 \pm 4.9***	9.5 \pm 1.3
Training experience (years)		–	9.0 \pm 2.9

Significantly higher than in Group S: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2. Mean values (\pm SD) of antioxidant enzyme activities and of total antioxidant plasma status (TAS) in male sedentary subjects (S) and pentathletes (P)

Variable	Group	S (n = 10)	P (n = 10)
SOD (U/g Hb)		1022 \pm 194	1483 \pm 168***
CAT (U/mg Hb)		216.9 \pm 46.4	295.0 \pm 40.0**
GPX (U/g Hb)		40.1 \pm 8.4	56.8 \pm 15.0*
GR (U/g Hb)		9.0 \pm 1.3	14.0 \pm 2.4***
TAS (mmol/l)		1.43 \pm 0.20	1.37 \pm 0.19

Legend: SOD – Superoxide dismutase; CAT – Catalase; GPX – Glutathione peroxidase; GR – Glutathione reductase; Significantly different from the S group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

The reports on adaptive changes in the activities of antioxidant enzymes in blood are ambiguous. Endurance athletes were reported to have a better antioxidant defence compared to the strength athletes which was attributed to differences in training structure and loads [15]. A training-induced increase in the resting activities of antioxidant erythrocyte enzymes was reported by Melikoglu *et al.* [21] for basketball players; similar increase in SOD was found in football players [3]. Mizayaki *et al.* [23] noted increases in SOD and GPX activities in men following a 12-week training and Banfi *et al.* [1] noted that resting activity of GR was significantly higher in highly fit handball players than in untrained subjects. Increases in GPX and CAT activities were also observed in men trained for Ironman competition [12]. On the other hand, Margaritis *et al.* [18] and Pallazzetti *et al.* [25] found no differences in SOD and GPX activities between trained triathletes and untrained men. Also, no differences in antioxidant erythrocyte enzymes were found between football players of Leagues I and IV [22].

The activities of antioxidant erythrocyte enzymes were found to be correlated with maximal oxygen uptake; Kostaropoulos *et al.* [14] found a higher activity of CAT in long-distance runners than in sprinters. In an earlier study [6], resting GPX activity was correlated with $\dot{V}O_2\text{max}$ while that of SOD was not [7]. Lutoslawska *et al.* [17] reported that resting CAT activity was lower and GPX activity higher in elite rowers compared with physical education students. Yet, the effect of high-intensity training loads, i.e. anaerobic ones, on adaptive increases in antioxidant enzyme activities cannot be ruled out [2,29].

No data on antioxidant enzymes in modern pentathlon athletes were found in the available literature. This study demonstrated remarkably higher activities of erythrocyte enzymes (SOD, CAT, GPX and GR) compared

with untrained subjects, no difference being found for plasma TAS; that latter, however, depended predominantly on the supply of antioxidant vitamins, especially Vitamin E. It is to note that both groups of subjects significantly differed in body fat content and BMI; however, a lack of reports on possible effects of those variables on the antioxidant status may support the presumption that the pronounced enzymatic antioxidative potential and oxidative stress defence observed in athletes practicing modern pentathlon could be attributed to their extensive, predominantly aerobic training.

References

- Banfi G., A.Malavazos, E.Iorio, A.Dolci, L.Doneda, R.Verna, M.M.Corsi (2006) Plasma oxidative stress biomarkers, nitric oxide and heat shock protein 70 in trained elite soccer players. *Eur.J.Appl.Physiol.* 96:483-486.
- Bloomer R.J., A.H.Goldfarb, L.Wideman, M.J.McKenzie, L.A.Consitt (2005) Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J.Strength Cond. Res.* 19:276-285.
- Brites F.D., P.A.Evelson, M.G.Christiansen, M.F.Nicol, M.J.Basilico, R.W.Wikinski, S.F.Llesuy (1999) Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. *Clin.Sci.* 96:381-385.
- Durnin J.V.G.A., J.Womersley (1974) Body fat assessed from total density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br.J.Nutr.* 32:77-97.
- Finaud J., G.Lac, E.Filaire (2006) Oxidative stress. Relationship with exercise and training. *Sports Med.* 36:327-358.
- Hübner-Woźniak E., G.Lutosławska, W.Sendecki, D.Sitkowski, L.Borkowski (1996) Resting glutathione peroxidase activity in whole blood in response to various modes of training. *Biol.Sport* 13:267-272.
- Hübner-Woźniak E., G.Lutosławska, W.Sendecki, K.Lerczak, L.Borkowski, A.Klusiewicz (1998) Effect of physical training on the resting activity of superoxide dismutase in erythrocytes. *Biol.Sport* 15:197-203.
- Jacob R.A., B.J.Burri (1996) Oxidative damage and defense. *Am.J.Clin.Nutr.* 63:985S-990S.
- Jenkins R.R. (2000) Exercise and oxidative stress methodology: a critique. *Am.J.Clin.Nutr.* 72(suppl):670S-674S.
- Ji L.L., M.C.Gomez-Cabrera, J.Vina (2006) Exercise and hormesis: activation of cellular antioxidant signalling pathway. *Ann.NY Acad.Sci.* 1067:425-435.
- Jones D.P. (2006) Redefining oxidative stress. *Antioxid. Redox Signal.* 8:1865-79.
- Knez W.L., D.G.Jenkins, J.S.Coombes (2007) Oxidative stress in half and full ironman triathletes. *Med.Sci.Sports Exerc.* 39:283-288.
- Knez W.L., J.S.Coombes, D.G.Jenkins (2006) Ultra-endurance exercise and oxidative damage. *Sports Med.* 36:429-441.
- Kostaropoulos I.A., M.G.Nikolaidis, A.Z.Jamurtas, G.V.Ikonomou, V.Makrygiannis, G.Papadopoulos, D.Kouretas (2006) Comparison of the blood redox status between long-distance and short-distance runners. *Physiol.Res.* 55:611-616.
- Koury J.C., de Oliveira A.V., Portella E.S., de Oliveira C.F., Lopes G.C., Donangelo C.M. (2004) Zinc and copper biochemical indices of antioxidant status in elite athletes of different modalities. *Int.J.Sport Nutr.Exerc.Metab.* 14:358-372.
- Laursen P.B. (2001) Free radicals and antioxidant vitamins: optimizing the health of the athlete. *Natl.Strength Cond. Assoc.* 23: 17-25.
- Lutosławska G., E.Hübner-Woźniak, B.Panczenko-Kresowska, D.Sitkowski (1999) The effect of submaximal cycling on blood antioxidants, lipid peroxidation and plasma total antioxidant status in subjects with different aerobic capacity. *Żywnie Człowieka i Metabolizm* 26:5-13.
- Margaritis I., F.Tessier, M.J.Richard, P.Marconnet (1997) No evidence of oxidative stress after a triathlon race in highly trained competitors. *Int.J.Sports Med.* 18:186-190.
- Martarelli D., P. Pompei (2009) Oxidative stress and antioxidant changes during a 24-hours mountain bike endurance exercise in master athletes. *J.Sports Med.Phys.Fitn.* 49:122-127.
- Mastaloudis A., S.W.Leonard, M.G.Traber (2001) Oxidative stress in athletes during extreme endurance exercise. *Med.Sci.Sports Exerc.* 31:911-922.
- Melikoglu M.A., M.Kaldirimci, D.Katkat, I.Sen, I.Kaplan, K.Senel (2008) The effect of regular long term training on antioxidant enzymatic activities. *J.Sports Med.Phys.Fitn.* 48: 388-90.
- Michalczyk M., B. Kłapcińska, E.Sadowska-Krepa, S.Jagasz, W.Pilis, I.Szołtysek-Boldys, J.Chmura, E.Kimsa, K.Kempa (2008) Evaluation of the blood antioxidant capacity in two selected phases of the training cycle in professional soccer players. *J.Hum.Kinetics* 19:93-108.
- Miyazaki H., S.Of-ishi, T.Ookawara, T.Kizaki, K.Toshinai, S.Ha, S.Haga, L. L.Ji, H. Ohno (2001) Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur.J.Appl.Physiol.* 84:1-6.
- Ogonowska A., E.Hübner-Woźniak, A.Kosmol, W.Gromisz (2009) Anaerobic capacity of upper extremity muscles of male and female swimmers. *Biomed.Hum.Kinetics* 1:79-82. DOI: 10.2478/v10101-009-0020-z
<http://versita.com/science/healthsciences/bhk>
- Palazzetti S., M.J.Richard, A.Favier, I.Margaritis (2003) Overloaded training increases exercise-induced oxidative stress and damage. *Can.J.Appl.Physiol.* 28: 588-604.
- Radak Z., H.Y.Chung, S.Goto (2008) Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radical Biol.Med.* 44:153-159.
- Roi G.S., D.Bianchedi (2008) The science of fencing. Implications for performance and injury prevention. *Sports Med.* 38:465-481.
- Sen C.K. (2001) Antioxidant and redox regulation of cellular signalling: introduction. *Med.Sci.Sports Exerc.* 33:368-370.
- Subudhi A.W., S.L.Davis, R.W.Kipp, E.W.Askew (2001) Antioxidant status and oxidative stress in elite alpine ski racers. *Int.J.Sport Nutr.Exerc.Metabol.* 11:32-41.
- Urso M.L., P.M.Clarkson (2003) Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 189:41-45.

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