Introduction

The anaerobic threshold (AT₄) is used for assessment of athletes’ adaptation to exercise, level of trainedness (in endurance athletes in particular) and the intensity of training loads. It is defined as the lowest training load during progressively increased exercise, above which the increase of lactate concentration in blood becomes rapid and continuous [1]. Although the exercise model used in studies of athletes does not reflect the progression of intensity or the time of starting exercise, the anaerobic threshold is currently one of the basic diagnostic methods used for assessment of rowers’ aerobic capacity in laboratory conditions [2]. An analysis of the threshold power in an annual training cycle can be useful in management of training through the choice of optimal training loads. It can also be a useful index of effectiveness of endurance training [3].

The exercise-induced oxidative stress leads to excess production of reactive oxygen species. The increased amount of free radicals, along with the insufficient antioxidant adaptation to exercise, leads in turn to significant changes in cellular and tissue functions [4]. The muscles can be protected against the harmful effects of oxygen free radicals thanks to the activity of muscular antioxidant enzymes as well as antioxidants in blood [5].

A number of researchers [6, 7] show that exercise can affect the body’s antioxidative defenses. According to some authors [8], physical training, especially endurance training, can alter the adaptability of enzymatic antioxidants by increasing their activity. Mena et al. [9] compared the blood level of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in amateur cyclists, professional cyclists and non-training subjects. The lowest SOD level was noted in the group of amateur cyclists and the lowest in the non-training individuals. The highest levels of CAT and GPx were observed in the group of professionals. Jenkins et al. [10] noted that in vitro oxygen uptake in
skeletal muscle was correlated with the levels of CAT and SOD, which was an indication of the growing muscle antioxidative potential along with increasing muscle metabolism.

In consideration of the above results the present study was designed to examine the threshold power and exercise-induced changes in the blood antioxidative stress parameters in rowers between two measurements (terms of the study)

Material and methods

The sample for the study were 15 rowers from the Polish National Youth Rowing Team. The subjects’ basic anthropometric characteristics are presented in Tab. 1. The tests took place at the end of the preparatory phase of an annual training cycle: at the beginning of May 2003 (Term I) and in May 2004 (Term II) at a training camp in the Olympic Games Training Centre in Walcz, Poland.

The rowers performed an ergometer test with graded intensity until refusal. The maximal power was set during a controlled run at a distance of 2,000 m. The exercise was performed on a Concept 2 rowing ergometer (USA). A rower started the test at 50% of the maximal power, and then the intensity was increased every three minutes to 60, 70, 80 and 90%, respectively. The test at 90% of the maximal power was performed by the rowers until refusal. The consecutive three-minute exercise sessions were separated with 30-second breaks, during which capillary blood samples were taken from the subjects to mark the blood lactate level and the anaerobic threshold (AT₄) [1].

Before the test, within one minute after its completion and after a 24-hour recovery the blood samples were drawn from the ulnar vein. The blood was taken on an anticoagulant containing dipotassium versenate (K₂EDTA), and centrifuged to separate red blood cells from the blood plasma. The red blood cells were irrigated three times with normal saline (0.9% w/v of NaCl) and haemolyzed with ice-cold double distilled water. The hydrolysate was frozen at a temperature of –28°C. The following concentrations were marked in the hemolysate: superoxide dismutase (SOD) using the RANSOD assay (SD 125) (UK), glutathione peroxidase (GPx) with the RANSEL assay, (RS 505) with the Paglia and Valentine assay [11], tiobarbiturate reactive lipid peroxidation products (TBARS) following chromogen extraction with n-butanol with the Buege and Aust assay [12], and hemoglobin with the Drabkin hemoglobin assay. The lactate concentration (La) was marked in the capillary blood using the Dr Lange enzymatic assay (Germany).

The obtained results were statistically processed with the use of Mann-Whitney test. All calculations were made using the Statistica 6.0. software package. The subjects expressed their consent to participate in the study and the tests were granted approval of the Local Research Ethics Committee of the Poznań University of Medical Sciences.

Results

The obtained results are presented in Tab. 1–3. Tab. 1 contains the subjects’ anthropometric characteristics, Tab. 2 includes the levels of measured stress parameters. The comparative analysis of the antioxidant enzymes (Tab. 3) at both terms of the study revealed a significantly higher SOD activity at rest, and lower GPx activity following exercise (p < 0.05) at the second term. No significant changes in the concentration of enzymes were found after the 24-hour recovery. The results of the rowing ergometer test at the second term of the study also revealed smaller changes in TBARS concentration.

At the second term of the study the threshold power was 12 W lower, and the maximal power 17 W lower, whereas the total exercise time was 15 s longer than the time at the first term (p = n.s.). The post-exercise blood lactate concentration at the second term was more than 7 mmol/l higher; however, this increase was significantly smaller than that from the first term (p < 0.05).

Discussion

The values of threshold power achieved by the rowers at the end of the preparatory phase of their annual training cycle did not differ significantly from the results obtained a year earlier (Tab. 2). A small decrease in the threshold power – a recognized index of endurance training tests – was, however, noted. This tendency could have resulted from insufficient training, but it
also confirms earlier observations by Klusiewicz et al. [13] in their study of a six-year training cycle of Olympic rowing champions. The anaerobic threshold values of the Olympic rowers’ in the consecutive years did not change significantly. Their threshold power did not increase significantly even in the years of the rowers’ greatest sports successes. In training practice the absolute value of threshold power AT4, is, in fact, far less important than the slightest fluctuations and differences between the values achieved in the preparatory and competitive periods of training.

The progression of intensity of training loads used in the test at the first and second terms of the study led to an increased production of reactive oxygen species (ROS) measured with the level of thiobarbiturate reactive substances (TBARS). At the first term of the study the increased ROS level in the blood sample taken immediately after exercise amounted to about 0.5 µmol/gHb, and at the second term to about 0.3 µmol/gHb. The increased ROS production leads, first of all, to oxidative modification of phospholipids and disrupts the cell membrane integrity [14]. An introduction of polar peroxide, ketone, aldehyde or hydroxyl groups in the areas of phospholipid molecules decreases the hydrophobicity of the lipid bilayer and increases the permeability of not only hydrogen protons but also of other polar molecules into the extracellular environment. According to many authors [10, 15–17] physical exercise, especially of high intensity, leads – on the one hand – to production of ROS, but on the other, to adaptation of the body’s defenses against free radical-induced damage. In the opinion of Lu et al. [18] this adaptation consists of a direct and indirect stimulation of synthesis of multiple proteins, including antioxidants. This observation is confirmed by Marzatico et al. [19], and Ji et al. [20]. Marzatico et al. revealed that the activity of superoxide dismutase at rest was two times higher in sprinters and three times higher in marathon runners than in their healthy non-training counterparts. They also noted an increase in glutathione peroxidase for 58% and 100% in the marathon runners in comparison with the control group. Evelo et al. [16] in their study of the effects of endurance training on the antioxidative glutathione system of human erythrocytes noted, for the first time, that

Table 2. Levels of oxidative stress parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Term I</th>
<th>Term II</th>
<th>Statistical significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD Min Max</td>
<td>x ± SD Min Max</td>
<td></td>
</tr>
<tr>
<td>AT4 (Watt)</td>
<td>319.5 ± 30.41 283 346 307 ± 80.02</td>
<td>249 376</td>
<td></td>
</tr>
<tr>
<td>HRAT4 (beats/min)</td>
<td>175.4 ± 10.92 159 192 174.2 ± 11.95</td>
<td>155 193</td>
<td></td>
</tr>
<tr>
<td>t (s)</td>
<td>969 ± 74.21 780 1080 984.6 ± 85.34</td>
<td>900 1080</td>
<td></td>
</tr>
<tr>
<td>Maximal power (Watt)</td>
<td>426.9 ± 20.74 400 450 409.4 ± 30.86</td>
<td>370 456</td>
<td></td>
</tr>
<tr>
<td>HRmax (beats/min)</td>
<td>194.3 ± 7.52 187 211 191.7 ± 5.96</td>
<td>180 204</td>
<td></td>
</tr>
</tbody>
</table>

AT4 – threshold power, HRAT4 – heart rate at the anaerobic threshold, t – exercise time, HRmax – maximal heart rate

Table 3. Comparative analysis of the parameters studied at rest, after exercise and after 24-hour recovery at the two terms of the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Term I</th>
<th>Term II</th>
<th>Statistical significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>1139.5 ± 85.84</td>
<td>1248.1 ± 117.39</td>
<td>n.s.</td>
</tr>
<tr>
<td>GPx</td>
<td>1239.3 ± 126.51</td>
<td>1369.5 ± 167.15</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBARS</td>
<td>1409.8 ± 89.89</td>
<td>1448.8 ± 113.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>La</td>
<td>1.3 ± 0.22 1.2 ± 0.09</td>
<td>1.5 ± 0.27 2.1 ± 0.37</td>
<td>n.s.</td>
</tr>
<tr>
<td>SOD</td>
<td>1.6 ± 0.32 1.6 ± 0.32</td>
<td>8.8 ± 3.31 8.8 ± 3.31</td>
<td>n.s.</td>
</tr>
<tr>
<td>GPx</td>
<td>41.8 ± 12.76 45.1 ± 10.89</td>
<td>48.3 ± 12.5 45.1 ± 7.19</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBARS</td>
<td>45.8 ± 11.49 45.1 ± 11.49</td>
<td>45.8 ± 11.49 45.1 ± 11.49</td>
<td>n.s.</td>
</tr>
<tr>
<td>La</td>
<td>44.4 ± 10.53 48.3 ± 12.5</td>
<td>45.8 ± 11.49 45.8 ± 11.49</td>
<td>n.s.</td>
</tr>
<tr>
<td>La</td>
<td>45.8 ± 11.49 45.1 ± 11.49</td>
<td>45.8 ± 11.49 45.1 ± 11.49</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

SOD – superoxide dismutase level, GPx – glutathione peroxidase level, TBARS – level of lipid peroxidation products, La – lactate blood concentration, * statistically significant at p ≤ 0.05, n.s. – statistically non-significant.
the level of exercise-induced oxidative stress is related to the character of an annual training cycle. The exercise-induced adaptation of the athlete’s body depends on the type, duration and intensity of exercise as well as the level of the body’s “trainedness.” According to Sen et al. [21], long-term training of moderate intensity increases the body’s physiological antioxidant capacity. Ji et al. [20] observed that exercise-induced increase of ROS production stimulates the gene expression of antioxidant enzymes with NF-κB as the signaling pathway.

The intense physical effort involved in rowing training is related to an increase in the number and size of mitochondria and it simultaneously enhances the activity of mitochondria enzymes in the electron transport chain [6]. As shown by Mader et al. [22] from 93 to 99% of rowing training loads are exercises in which the lactate concentration in blood does not exceed 4 mmol/l, and in 80% is not higher than 2 mmol/l. Data from literature show that endurance training, in particular, increases the level of reduced glutathione and glutathione peroxidase, a decrease in catalase concentration and a stable level of superoxide dismutase were observed in animal muscles in response to training [25]. Gunduz et al. [26] noted improved antioxidant capacity in many tissues, including muscles, in aged rats in response to one-year swimming exercise.

In our study, an increase in superoxide dismutase in red cells (p < 0.05) and glutathione peroxidase (Tab. 3) was noted at rest in rowers after one-year training. The ergometer test at the second term of the study contributed to the increase in the activity of both enzymes. It should be added that the increase in GPx activity at the second term of the study was significantly lower than at the first term of the study (p < 0.05).

The assessment of the status of antioxidant capacity can be difficult due to the fact that the expression of antioxidants can be induced in response to oxidative stress. Thus a high activity of antioxidants can be an indication of a good state of antioxidative defence mechanism as well as of increased level of oxidative stress. The high activity of both enzymes at rest at the second term of the study points to the positive influence of physical exercise. On the other hand, their higher activity during 24-hour recovery indicates an increasing ROS production but it can also be a proof of facilitation of the body’s antioxidative defenses. The concurrent higher level of TBARS can also be a manifestation of expression of antioxidant proteins in response to oxidative stress. Błaszczyk et al. [27] report that submaximal exercise increases the concentration of antioxidant enzymes (SOD, GPx,) and lowers the level of malon dialdehyde (MDA) in red cells. The results of our study show that the applied exercise test after a year of training brought about different responses of antioxidant enzymes and a lower increase of TBARS, right after the exercise and after a 24-hour recovery (Tab. 3).

**Conclusion**

It should be concluded that physical training induces positive changes in the body’s antioxidant adaptation, as confirmed by the lower post-exercise level of thiobarbiturate reactive substances. It can be assumed that among the many factors affecting the sports result, the capability of maintaining the prooxidant-antioxidant equilibrium is one of the key components of biological restitution and the capacity to take up new training and competitive loads.

**References**

HUMAN MOVEMENT
P. Basta, A. Skarpańska-Stejborn, Ł. Pilaczyńska-Szcześniak, Influence of annual training cycle


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