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# COMPARISON OF THE EFFECTS OF CONTINUOUS VERSUS INTERMITTENT EXERCISES ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN RAT TISSUES

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**Abstract.** The aim of the present study was to investigate the effects of adaptation to continuous as compared to intermittent endurance exercises performed with low and high intensities, respectively, on the activity of superoxide dismutase in the mitochondrial (MnSOD) and post-mitochondrial (CuZnSOD) fractions of the rat liver, heart, and red (RG) and white (WG) portions of the gastrocnemius muscle. The adaptation protocol consisted of running daily for four weeks, five days per week, the 600 meter-distance on a mechanical treadmill slanted at 15°, at the speed of 10 m/min (EE) or of 30 m/min with a four-minute-break every two minutes (IE). The results demonstrate that in the RG the activity of MnSOD increased after the intermittent exercises $p<0.001$), whereas that of CuZnSOD raised following both types of the exercises ($p<0.05$). Concurrently, neither intermittent nor continuous exercises triggered any changes in the CuZnSOD or MnSOD activities in the WG. After the continuous exercises, the elevated activities of MnSOD ($p<0.001$) and CuZnSOD ($p<0.05$) were found in the liver and heart, respectively. The MnSOD activity in the heart muscle was reduced after both the IE and EE ($p<0.05$). The obtained results suggest that adaptation to intermittent versus continuous endurance exercises differently affects the activity of superoxide dismutase. ***(Biol.Sport 22:341-352, 2005)***

*Key words:* Superoxide dismutase - Intermittent exercise - Continuous exercise

## Introduction

Oxidative metabolism of a cell inherently produces reactive oxygen species (ROS) which, through their prompt interaction with cellular lipids, carbohydrates, proteins, and nucleic acids, play an important role in various physiological

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processes. Consequently, when the concentration of ROS in a cell overwhelms the scavenging capacity of the anti-oxidative mechanisms, damages to cell membranes, nucleic acids, and other cellular structures ensue [37]. One of the factors leading to the enhanced production of ROS is an increase in oxidative metabolism. About 2% of oxygen metabolised in mitochondria is only partially reduced and released as superoxide radical anions [4] which are further transformed into very reactive hydroxyl radicals. During an intense exercise, the oxygen uptake may increase between ten- and twenty-fold, and the aerobic metabolism in the working muscles even 200-fold, the phenomena associated with elevation of the concentration of ROS in the oxygen metabolic chain. However, adaptation to physical effort is associated with a significant increase in the efficiency of the antioxidant defence system relying on, e.g., the activities of a number of antioxidant enzymes. One of such enzymes is superoxide dismutase (SOD) which catalyses the conversion of a superoxide radical anion into hydrogen peroxide triggering thereby a decay of this form of oxygen. In mammals, the cytoplasmic SOD contains copper and zinc (CuZnSOD), whereas in mitochondria the metal component of the isoform (MnSOD) is manganese. Thus far, the results of studies of the effect of physical training on the activity and tissue contents of SOD have been inconsistent. In fact, even though most reports demonstrated increased SOD activity in the oxidative fibres of skeletal muscles [7,14,31], some authors were unable to confirm these results [23]. In the glycolytic-oxidative fibres both the increase [13,16,26,29] and no change [3,7,17,35] in the enzyme's activity were detected. Training did not appear to elevate the activity of SOD in the liver and heart [9,14,17,18,19,26], although the intensity of the exercise-related peroxidation processes tended to decrease [8,11,20,22].

Enhanced activity of the antioxidant enzymes in tissues was previously demonstrated after both the continuous [13,26,28] and intermittent [7,25,29] exercises. Notably, only one report directly compared the effect of both types of training on the activity of antioxidants in the skeletal muscles [7] but no clear-cut differences were found. However, the authors examined the total activity of SOD without differentiating between CuZnSOD and MnSOD, and they did not separate the gastrocnemius muscle into the red (composed mainly of the glycolytic-oxidative fibres) and white (containing the glycolytic fibres) portions [2].

Hence, the aim of the present study was to compare the effects of adaptation to continuous, low intensity endurance exercises versus intermittent exercises of higher intensity on the activity of superoxide dismutase in the mitochondrial (MnSOD) and post-mitochondrial (CuZnSOD) fractions obtained from the rat liver, heart, and red and white portions of the gastrocnemius muscle.

## Materials and Methods

For the experiments, twenty four male Wistar rats fed ad libitum the special feed for rodents (Murigran, Motycz, Lublin) and water were used. The animals were kept in standard conditions of 22±2°C ambient temperature and 40-70% humidity, and at the 12/12-hour light-darkness cycle. Mean body mass of the rats before and after the training equalled to 140±10 g and 270±12 g, respectively, After two days of adaptation, the animals were randomly divided into the control, resting group (untrained, U), the continuous endurance exercise group (EE), and the intermittent exercise group (IE). Over the period of four weeks, five days per week, the animals from the two latter groups ran daily the 600 meter-distance on the electrically-stimulated mechanical treadmill slanted at 15°. The running speed equalled to 10 $m \cdot min^{-1}$ (EE) or 30 $m \cdot min^{-1}$ with the four-minute-break every two minutes (IE). After completion of the adaptive exercising the rats were kept at rest for two days, then sacrificed by cervical dislocation and samples of the liver, heart, and red (RG) and white (WG) portions of the gastrocnemius muscle were collected and kept in -70°C until the analysis. Mitochondrial fractions of the collected organs were prepared by differential centrifugation, modified according to Casstorin *et al.* [6]. Briefly, the tissue samples were homogenised in cold 1/15M KCl (10-20% w/v) and centrifuged for 10 min. at 600xg, 4°C; the collected supernatant was then centrifuged for 15 min at 12000xg. The resulting supernatant was regarded as the post-mitochondrial fraction containing CuZnSOD. The mitochondrial pellet was washed and resuspended in a phosphate buffer solution, pH=8.6, and lysed with digitonin. The lysates were centrifuged for 15 min at 12000xg and the mitochondrial fraction containing MnSOD was collected. The SOD activity in both fractions was estimated with use of the tests obtained from RANDOX (catalog No SD 125); the control RANDOX tests were used to check the correctness of the estimations. Protein content was measured using the method of Lowry *et al.* [24]. Student's t and Cochran-Cox tests were utilised for the statistical analysis of equal and different variances, respectively.

## Results

As indicated in Fig. 1, the continuous exercise only led to the significant ($p<0.001$) elevation of the activity of MnSOD in the liver, whereas neither intermittent nor continuous exercises exerted any effects on CuZnSOD. After the EE, the activity of CuZnSOD enhanced ($p<0.001$) in the heart muscle (Fig. 2),

whereas no changes in this activity were demonstrated after the IE. The MnSOD activity was reduced after both the IE and EE ($p<0.05$).

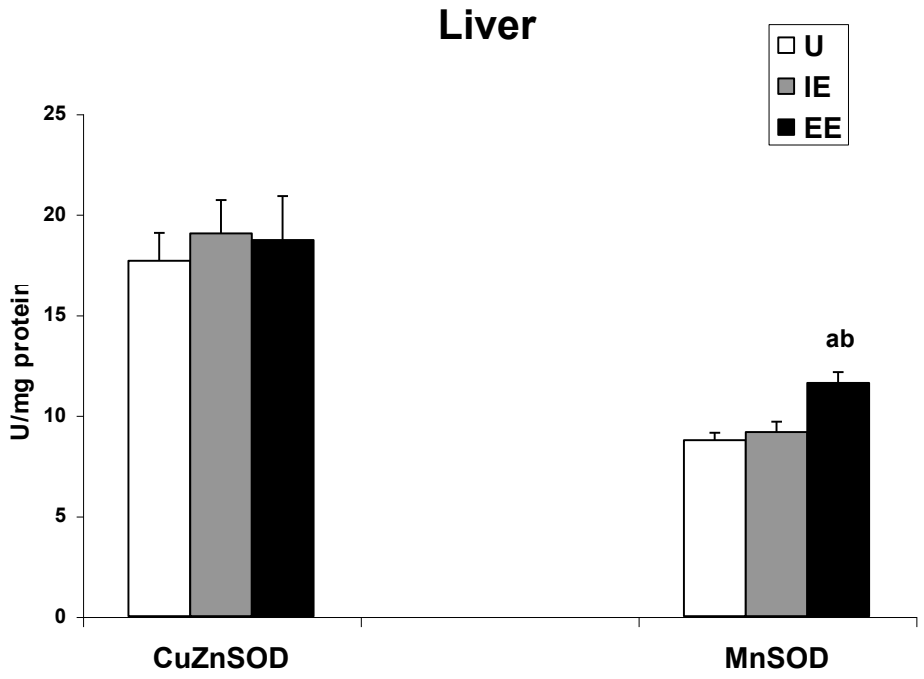


**Fig. 1**
Effects of the intermittent (IE) and continuous endurance (EE) exercises on the activity of CuZnSOD and MnSOD in the rat liver. U represents untrained animals. Presented are means±SE (bars). a - indicates statistically significant ($p<0.05$) difference from the results obtained in the U group; b - indicates statistically significant ($p<0.05$) difference from the results obtained in the IE group

Fig. 3 shows the elevated activity of CuZnSOD in the red portion of the gastrocnemius muscle following both types of the exercises ($p<0.05$) while the MnSOD activity rose only after the IE ($p<0.001$). No effect of the execises on the activities of MnSOD and CuZnSOD in the white portion of gastrocnemius could be detected (Fig. 4).

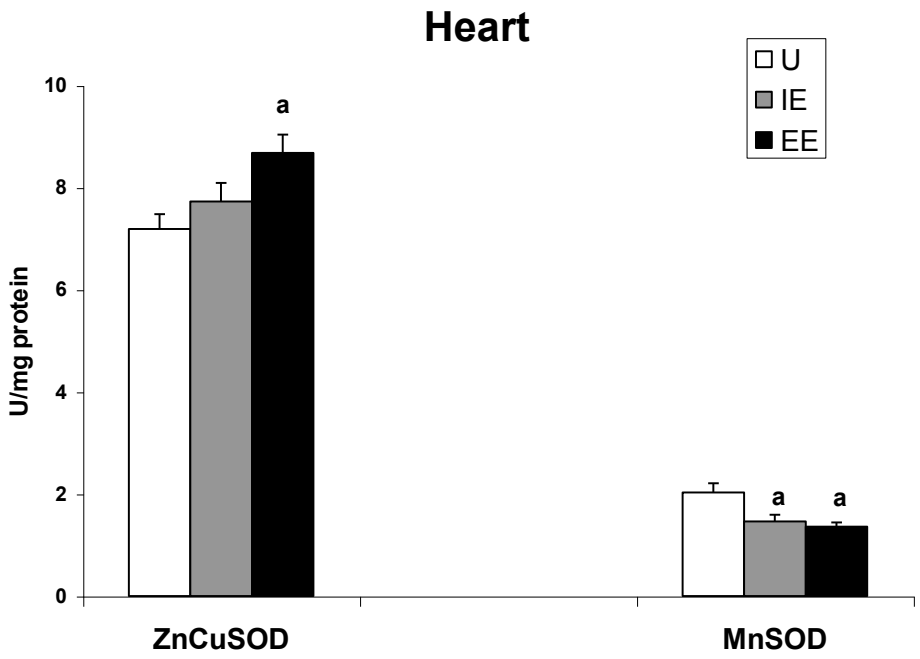


**Fig. 2**
Effects of the intermittent (IE) and continuous endurance (EE) exercises on the activity of CuZnSOD and MnSOD in the rat heart muscle. U represents untrained animals. Presented are means±SE (bars). a - indicates statistically significant ($p<0.05$) difference from the results obtained in the U group

**Disscussion**

The obtained results demonstrate different effects of adaptation to the intermittent versus continuous endurance exercises on the tissue activity of superoxide dismutase. In the red portion of the gastrocnemius muscle, which consists predominantly of the glycolytic-oxidative fibres [2], the significant increase in CuZnSOD was recorded after both types of the endurance adaptation, whereas the elevated activity of MnSOD occurred only after the intermittent exercises. These results differ from the previously reported findings of Criswell *et*

*al.* [7] who did not detect any changes in the gastrocnemial SOD following either the intermittent or continuous training, even though the training cycle was longer

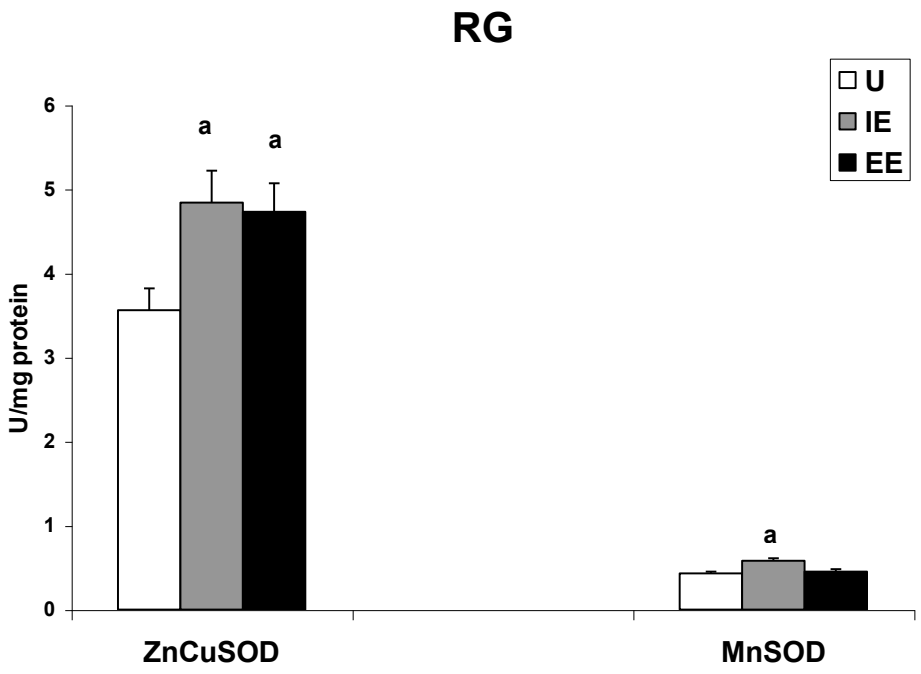


**Fig. 3**
Effects of the intermittent (IE) and continuous endurance (EE) exercises on the activity of CuZnSOD and MnSOD in the red portion of the gastrocnemius muscle in rats. U represents untrained animals. Presented are means±SE (bars). a - indicates statistically significant ($p<0.05$) difference from the results obtained in the U group

and more intense than that used in the present study. The difference may, to some extent, be explained by the fact that Criswell *et al.* examined the enzyme activity in the whole gastrocnemius muscle. As a result, the lack of changes in the white portion of the muscle, determined also in the present investigation, might have "fogged" the possible alterations occurring in the red portion. The same authors demonstrated the training-induced increase in the SOD activity in the soleus muscle, although the effect was independent of the type of training. In the present

study, the enhanced gastrocnemial activity of MnSOD occurred only after the intermittent exercises. These discrepancies may relate to different types of the

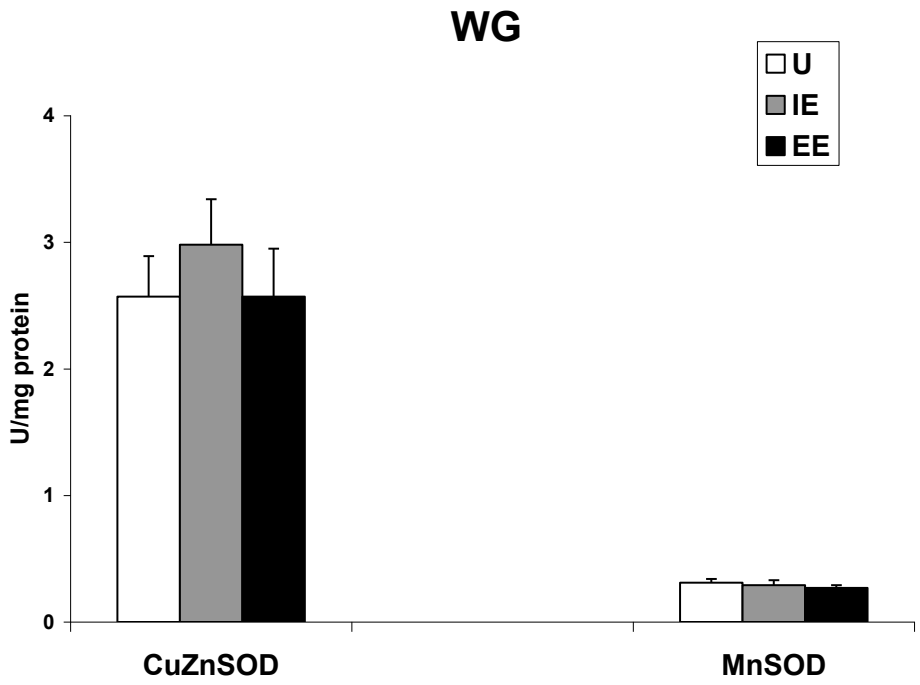


**Fig. 4**
Effects of the intermittent (IE) and continuous endurance (EE) exercises on the activity of CuZnSOD and MnSOD in the white portion of the gastrocnemius muscle in rats. U represents untrained animals. Presented are means±SE (bars)

fibres present in the soleus (oxidative) versus the gastrocnemius (glycolytic-oxidative) muscles and, to some extent, to the lesser intensity of exercises used in the present investigation. Noteworthy, in our hands the activity of MnSOD equalled to merely a dozen or so percent of the total SOD activity in the muscle. Therefore, when Criswell *et al.* [7] measured the total activity of SOD, which included both MnSOD and CuZnSOD, the presently detected by us training type-dependent differences with respect solely to MnSOD could have remained obscured. The more pronounced effect of the intermittent versus continuous exercise on the activity of MnSOD is probably associated with the more abrupt periodical increase in the aerobic metabolism during the former type of training. As

suggested by Starnes *et al.* [33], the ratio of the released ROS to the consumed oxygen increases with the elevation of the tissue oxygen uptake. Consequently, at the comparable general energy expenditure on external work the amount of ROS released in the mitochondria may be higher during the intermittent than during the continuous exercises. Noteworthy, about 80% of ROS are scavenged by MnSOD [27]. The studies of the effects of training on the activity of SOD in the glycolytic-oxidative fibres of skeletal muscles have yielded inconsistent results. For example, in humans Atalay *et al.* [3], Tonkonogi *et al.* [35], and Tiidus *et al.* [34] found no increase in SOD in the gastrocnemius and the quadriceps femoris muscles after the sprint training, in the quadriceps femoris muscle after the endurance training, and in the vastus lateralis muscle, respectively. On the other hand, Ortenbald *et al.* [29] demonstrated that the activity of SOD in vastus lateralis of the volleyball players is higher than that in non-athletes. Similar relationship was detected by Jenkins [16] in the physically active versus sedentary subjects. In experimental animals, Ji [17] could not demonstrate any endurance training-related increase in the CuZnSOD or MnSOD activities in the deep layer of vastus lateralis which, like RG, consists of the glycolytic-oxidative fibres. Nakao *et al.* [26] found the enhanced MnSOD activity in gastrocnemius after the six-week-training of mice, whereas the CuZnSOD content remained unchanged. In contrast, Gore *et al.* [13], similarly to the result of the present study, demonstrated the elevated activity of CuZnSOD in the glycolytic-oxidative fibres from the red portion of vastus lateralis with no changes in the MnSOD activity in rats subjected to the 12-week-endurance training (although the increased level of the enzymatic protein of the isoenzyme was detected by these authors).

In the present investigation, the applied exercise did not affect the activity of SOD in the white portion (glycolytic fibres) of the gastrocnemius muscle. This finding confirms the observations of other authors who studied the effects of training on the activity of SOD in the glycolytic fibres [1,7,23,31]. Noteworthy, as indicated by Powers *et al.* [31] the activity of SOD in these muscle structures not only increased but was significantly reduced after the very intense training.

In contrast to the red portion of gastrocnemius, in the present investigation only mitochondrial and postmitochondrial fractions of SOD were elevated in the rat liver and heart, respectively. In both cases, however, the increases were small and detectable exclusively after the continuous exercise. Admittedly, these effects are difficult to explain. Indeed, other authors did not demonstrate any influence of the even more intense training on the activity of SOD in the rat liver [17,18]. In mice, the exercise-induced decrease rather than increase in the levels of both MnSOD and CuZnSOD were detected [26]. Moreover, as indicated by the results of the

majority of the published studies no changes in the SOD activity could be detected in the heart of the trained rats [9,14,17,19]. In fact, as demonstrated by Powers *et al.* [31], elevated activity of this enzyme was found only in the left atrium and only after intense exercise. This finding, however, could not be confirmed by other investigators from the same laboratory [8] who, using identical animals, did not detect any significant changes in the SOD activity in either the left or the right atriums of the heart.

The markedly less pronounced increase in the SOD activity in the liver and heart, as compared to the red portion of gastrocnemius, may be related to a significant, training-induced enhancement of metabolism in the locomotor muscles accompanied by a more abundant release of ROS in the muscles compared to the heart and liver. As indicated by Kayatekin *et al.* [21], the intermittent exercise-induced increase in lipid peroxidation was much higher in the gastrocnemius muscle than in the liver. Furthermore, the 'resting' level of SOD in the liver is significantly higher than in the muscles, whereas the turnover of proteins in the latter is incomparably faster than that in the heart which may explain the less evident effect of exercising on the SOD content in the heart muscle. Perhaps, the SOD activity in the liver and heart of untrained animals suffices to effectively scavenge the excess of a superoxide radical. This results in accumulation of the product of the scavenging which is further metabolised by other enzymes leading thereby to inhibition of the activity of SOD [15]. Such a chain of events could explain our present finding of the decreased activity of MnSOD in the heart muscle. Likewise, we demonstrated previously [10] that a decrease of the SOD content in the blood induced by the training identical to the one used in the present investigation was accompanied by elevation of the activity of glutathione peroxidase in blood. It is noteworthy that training, in spite of its insignificant effect on SOD in the heart muscle, markedly reduces this muscle's sensitivity to peroxidation [8,11,20,22], the phenomenon probably related to an increased concentration of the reduced glutathione [22].

In the present study the rats were trained for four weeks. Presumably, this would be enough for the induction of potential alterations in the activity of SOD. However, the effect of the time of training on changes of SOD has not been fully recognized. Generally, in the investigations of the authors cited in the present report the applied trainings lasted between four and 12 weeks. The analysis of the therein described alterations in the activity of SOD indicates their independence of the time of training when it exceeded four weeks. Indeed, only Kanter *et al.* [20] reported that the activity of SOD in the liver and heart did not change after nine weeks of training and eventually increased at the 21st week post-exercise. In

contrast, in another study [36] elevation of the SOD activity in the rat diaphragm was detected as soon as after five days of training. Interestingly, modifications of the enzyme's activity detected in the present investigation in the glycolytic-oxidative fibres of gastrocnemius were similar to or higher than those recorded by other authors [7,13,17,31], even though the applied by us exercises were less intense. Notably, however, Gambelunghe *et al.* [12] demonstrated that the level of reduced glutathione in the serum rapidly declined after the slightly lower running speed of 8 $m \cdot min^{-1}$, suggesting that release of the reactive oxygen species was up-regulated. According to the formula based on the findings of Shepherd and Gollnick [32], oxidative metabolism in rats running at 10 $m \cdot min^{-1}$ on a slightly ascending treadmill corresponds to about 63% $VO_{2max}$ which, as indicated by Popovic *et al.* [30], corresponds to over a two-fold increase in the blood lactate concentration. Considering the slant of the treadmill used in the present study (15°), it is possible that the energy expenditure in the EE group slightly exceeded 63% $VO_{2max}$ [5] and, consequently, sufficed to bring about the described adaptive changes in the activity of SOD.

In conclusion, the original findings of the present investigation are that only the intermittent exercise induced elevation of the activity of MnSOD in the glycolytic-oxidative fibres of the gastrocnemius muscle and that, even when the CuZnSOD and MnSOD isoenzymes were examined separately, the endurance adaptation had no effect on the SOD activity in the white portion of the muscle. Moreover, the obtained results support the previous findings of some other authors demonstrating the training-induced increase in the activity of CuZnSOD in the red portion of gastrocnemius. Noteworthy, we were able to detect the increase despite the fact that the applied intensity of the exercises was lower than that used by other authors.

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