Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues

CHITOSE Nakao,1,2 TOMOMI OOKAWARA,2 TAKAKO KIZAKI,2 SHUJI OH-ISHI,2 HIROMI MIYAZAKI,3 SHUHKO HAGA,3 YUZO SATO,1 LI LI JI,4 AND HIDEKI OHNO2

1Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya 464-0814; 2Department of Hygiene, National Defense Medical College, Tokorozawa 359-8513; 3Institute of Health and Sport Science, University of Tsukuba, Tsukuba 305-0006, Japan; and 4Department of Kinesiology, School of Education, University of Wisconsin, Madison, Wisconsin 53706

Nakao, Chitose, Tomomi Ookawara, Takako Kizaki, Shuji Oh-Ishi, Hiromi Miyazaki, Shuhkoh Haga, Yuzo Sato, Li Li Ji, and Hideki Ohno. Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues. J. Appl. Physiol. 88: 649–654, 2000.—The purpose of the present study was to investigate the effects of swimming training on the changes in three superoxide dismutase (SOD) isoenzymes in mice. The trained mice underwent a 6-wk swimming program (1 h/day, 5 days/wk) in water at 35–36°C. Immunoreactive extracellular SOD (EC-SOD), copper- and zinc-containing SOD (CuZn-SOD), and manganese-containing SOD (Mn-SOD) contents and their mRNA abundance were determined in serum, heart, lung, liver, kidney, and gastrocnemius muscle. EC-SOD content in liver and kidney was significantly increased with training. After training, CuZn-SOD content rose significantly only in kidney but decreased significantly in heart, lung, and liver. Mn-SOD content showed a significant increase in lung, kidney, and skeletal muscle but a significant decrease in liver. In most tissues, however, the changes in SOD isoenzyme contents were not concomitant with those in their mRNA levels. The results obtained thus suggest that, except for kidney, the responses in mouse tissues of three SOD isoenzymes (protein levels and mRNA abundance) to swimming training are different and that kidney may be one of the most sensitive organs to adapt to oxidative stress during physical training, although the mechanism remains vague.

extracellular superoxide dismutase; mRNA; affinity for heparin

MANY STUDIES HAVE INDICATED the possibility that strenuous or endurance exercise causes oxidative stress to the body because of the increased oxygen consumption and the enhanced generation of reactive oxygen species (ROS). Superoxide dismutase (SOD), which serves to convert superoxide anion to oxygen and hydrogen peroxide, is one of the important enzymes in the antioxidant defense system.

Three types of SOD isoenzymes have been identified in mammals. They are characterized by prosthetic metal ions and cellular localization. Copper- and zinc-containing SOD (CuZn-SOD) is found predominantly in the cytoplasm (23). Manganese-containing SOD (Mn-SOD) is located in the mitochondria (41). Copper- and zinc-containing extracellular SOD (EC-SOD) is located mainly in the extracellular space (20, 22, 29). Moreover, the three SOD isoenzymes are differently distributed among various tissues, with CuZn-SOD found in erythrocytes and liver in a high concentration and Mn-SOD mainly in heart, kidney, and liver. EC-SOD is rich in lung and kidney (22, 34).

There is growing evidence that physical exercise training may affect antioxidant enzymes, such as CuZn-SOD and Mn-SOD (27). Controversy, however, exists as to the effect of physical training and acute exercise on the regulation of these antioxidant enzymes. In addition, it is rather difficult to measure the activity of EC-SOD because it is the least abundant form of SOD, with the ratio of EC-SOD to the total SOD in each tissue being only 5–15% (22). Therefore, the measurement of EC-SOD has been disregarded for studying the effects of physical training.

The recent development of ELISA for EC-SOD (29) in addition to CuZn-SOD and Mn-SOD (39) has facilitated accurate and reproducible determination of the protein levels of these isoenzymes. It appears that the measurement of immunoreactive SOD protein using the specific antibody against each SOD isoenzyme is more reliable and reproducible compared with enzymatic methods (27).

In the present study, to study the mutual interaction between physical exercise and SOD isoenzymes in mice, we selected swimming as a model for exercise performance, because swimming appears to belong to the natural behavior of rodents (16). In addition, it is not easy to run a mouse on a treadmill; thus exercise research of the mouse has used swimming. It is less stressful and can avoid electric shock and foot injury, which may generate ROS unrelated to exercise.
The present study is the first to investigate the effect of physical training on the immunoreactive levels of the three SOD isoenzymes in some tissues of mice. To obtain further information about the response of SODs, relative abundance of each SOD isoenzyme mRNA was also determined.

METHODS

Animals and exercise. Twelve male C57BL/6J mice, at 6 wk of age, were obtained from the Jackson Laboratory (Bar Harbor, ME). The mice were housed under barrier conditions at National Defense Medical College (Tokorozawa, Japan). They were reared at 25°C under artificial lighting for 12 h from 7 AM to 7 PM daily and were given standard laboratory diet (Oriental MF, Oriental Yeast, Tokyo, J apan) and tap water ad libitum. The animals were cared for in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of J apan, based on the Declaration of Helsinki, 1964. After 2-wk acclimation to the new environment, they were divided randomly into sedentary (n = 6) and swimming-trained (n = 6) groups. The mice in the trained group were accustomed to swimming training for a period of 6 wk, 5 days/wk, during which exercise was for 10 min initially and then was extended by 10 min daily until the animals swam continuously for 1 h. Water temperature was controlled every 10 min at 35–36°C. Mice swam as a group in a bath measuring 51 × 71 cm (diameter, height). Group swimming was used because it promotes more vigorous exercise than when mice are allowed to swim alone (40).

Tissue preparations. About 48 h after the last session (to attenuate acute exercise effect), mice were killed by decapitation and were exsanguinated, and then five tissues (heart, lungs, liver, kidneys, and gastrocnemius muscles) were quickly excised and frozen in liquid nitrogen. Subsequently, a portion of the tissues was homogenized in 9 vol of phosphate-buffered saline (50 mM sodium phosphate and 150 mM NaCl, pH 7.4) with a Polytron homogenizer (Kinematika, Lucerne, Switzerland) and then centrifuged at 5,000 g (4°C) for 10 min, and the supernatant was used for various assays. Protein content was determined by a bichinchoninic acid protein assay kit (Pierce, Rockford, IL) with BSA used as a standard.

Enzyme activity. Citrate synthase (CS) activity, as an index of physical training, was measured according to the method of Shepherd and Garland (35). ELISA. EC-SOD was purified from mouse lungs, and the rabbit antiserum against EC-SOD was obtained according to our previous study (28). The antibodies for rat CuZn-SOD and Mn-SOD were supplied by N. Taniguchi (Osaka University, Osaka, J apan). The specificity of these antibodies to mouse SODs was judged by Western blotting analysis (data not shown). To estimate the protein contents of EC-SOD, CuZn-SOD, and Mn-SOD, an ELISA was developed using each antibody with a sandwich method (29, 39).

Northern blot analysis. Total RNAs were isolated from each mouse tissue by using a TRIzol reagent (Life Technologies, Gaithersburg, MD). Total RNAs (11 µg) were fractionated by electrophoresis on a denaturing formaldehyde 1% agarose gel and transferred onto a positively charged nylon membrane (Hybond N+, Amersham Life Science, Arlington Heights, IL) by capillary action in 20× SSC overnight (1× SSC: 150 mM NaCl-15 mM trisodium citrate), followed by ultraviolet irradiation fixation. The cDNA probes for each isoenzyme were prepared according to former reports (24, 29) and labeled with [32P]dCTP according to the random-priming method (4) through use of a DNA labeling kit (Wako, Tokyo, J apan). The blotted membrane was prehybridized in the prehybridization solution [50% formamide, 3× SSC, 5× Denhardt, 0.1% SDS, 10 µg/ml denatured shared salmon sperm DNA, 5% (wt/vol) dextran sulfate (Pharmacia Biotech, Bromma)] at 42°C for 4 h, followed by the hybridization in the same buffer containing 1 × 10⁶ cpm/ml of the [32P]-labeled probe at 42°C overnight. The membrane was washed twice in 1× SSC-0.1% SDS at room temperature and once each in 0.1× SSC-0.1% SDS for 15 min at 42, 50, or 60°C, and then it was exposed to an imaging plate (Fujix, Tokyo, J apan) at room temperature for 30–60 min.

Autoradiographic signals were quantified with the use of a BAS 2000 Bioimaging Analyzer (Fujix). To ascertain the integrity and amount of the RNA sample, the same blots were rehybridized later to the β-actin probe. The degree of SOD isoenzyme mRNA was calculated after normalization to levels of β-actin mRNA (an internal control).

Statistical analysis. Data are expressed as means ± SE. The statistical significance of the data on the five tissues of each group was assessed by an ANOVA, followed by the Bonferroni post hoc comparison. Comparisons between untrained and trained groups were assessed by Student’s t-test for unpaired observations. The significance was set at P < 0.05.

RESULTS

Body weight. The body weight of trained mice was not significantly different from that of untrained mice (26.3 ± 0.8 and 27.9 ± 0.7 g, respectively).

CS activity in skeletal muscle. CS activity in the gastrocnemius muscle from trained mice (0.465 ± 0.010 U/mg protein) was significantly higher than that from untrained mice (0.340 ± 0.020 U/mg protein).

EC-SOD. As shown in Fig. 1, of the five tissues examined, lung showed the highest EC-SOD content, with no significant difference in the enzyme content between untrained and trained groups. EC-SOD in kidney had the second highest values and increased significantly with training. EC-SOD values in lung and kidney were significantly higher than those in other tissues. EC-SOD content in liver was also significantly higher in trained mice than in untrained mice. In contrast, no effect of training was noted for heart or gastrocnemius muscle. In addition, trained mice did not likewise differ from untrained mice in serum EC-SOD concentration (91.5 ± 11.4 and 85.0 ± 9.5 µg/ml, respectively). After training, the level of EC-SOD mRNA in gastrocnemius muscle decreased significantly but did not change substantially in other tissues (the mRNA level was not detectable in liver).

CuZn-SOD. As shown in Fig. 2, CuZn-SOD content in liver of untrained mice was significantly higher than that in any other tissues. The CuZn-SOD content in heart, lung, and liver was significantly decreased by swimming training; conversely, that in kidney was significantly increased. The CuZn-SOD content in skeletal muscle was a relatively lower level in the tissues investigated, and it remained unchanged with training. After training, the CuZn-SOD mRNA levels were decreased in lung but enhanced in liver. On the other hand, the mRNA level in other tissues was unchanged.

Mn-SOD. As shown in Fig. 3, Mn-SOD content in heart was markedly higher than that in any other
tissues. Training significantly increased Mn-SOD content in lung, kidney, and skeletal muscle but significantly decreased that in liver.

No overt effect of training on Mn-SOD mRNA abundance was noted for of the tissues examined.

**DISCUSSION**

Tissue distributions of SOD isoenzymes. There appeared to be no definite relationship among the three SOD isoenzyme contents in tissues (Figs. 1–3). As depicted in Fig. 1A, lung showed the highest EC-SOD level, probably because it is a unique organ that is always exposed to oxygen and subsequent constant capillary occurrence of ROS (15). Moreover, lung is an organ rich in vessels and connective tissues. This seems to be the reason for the high level of EC-SOD in lung, because the EC-SOD exists amply in the connective tissues and vascular smooth muscles with its affinity for heparin analogs (30). Liver had the highest CuZn-SOD level (Fig. 2A), a finding that is in approximate agreement with that of ji (11) regarding the enzyme activity. On the other hand, Mn-SOD content was much higher in heart than in all the other tissues (Fig. 3A), probably because heart is abundant in mitochondria. Heart is a unique muscle that works continuously, unlike skeletal muscles. Thus heart may always be exposed to some degree of oxidative stress.

Skeletal muscle is one of main working organs during exercise, but the contents of all SOD isoenzymes in this tissue are known to be relatively lower compared with those in other tissues (11, 18, 22).

Effects of swimming training. As already described, we selected swimming as an endurance training mode. For instance, there is evidence that acute and chronic hemodynamic responses to swimming, such as hypercapnia and acidosis, are different from responses to other types of exercise such as running (38). Collectively, Geenen et al. (6) suggested a possible sympathoadrenal role in the differences observed in cardiac adaptations between swimming and running rats. Probably such differences would be true for other tissues including locomotor musculature.

Compared with running, swimming leads to a wide difference of physical responses and mechanical stresses because of effects of water pressure, utilization of different muscles, and reduced effects of gravity and so on. For instance, Flaim et al. (5) reported the redistribution of blood flow among tissues without a marked change in cardiac output or heart rate during swimming. In the present study, however, CS activity in gastrocnemius muscle was significantly enhanced with swimming training, thereby suggesting that the swim-
mice. Values are means ± SE; n = 6 in each group. Values with different alphabetical letters are significantly different (P < 0.05) (comparisons among Mn-SOD contents in untrained mouse tissues). *P < 0.05.

Aerobic exercise, superoxide production increases parallel to mitochondrial respiration. During vigorous training, the activity of the enzyme is present in the extracellular fraction (90–99%) (12, 22). Accordingly, the change in EC-SOD levels in plasma during physical training might be trivial when compared with the much higher levels in tissues. In contrast, EC-SOD contents in liver and kidney were significantly increased with swimming training without obvious changes in those mRNA levels. It is plausible that such increases were due to the increased synthesis of EC-SOD; however, another possibility might also exist that the affinity of EC-SOD for heparin analogs was potentiated after training, resulting in high retention of the enzyme in the tissues. The question as to whether physical training alters the heparin affinity, however, cannot be answered at present. Despite its decreased mRNA level, EC-SOD content in skeletal muscle did not vary significantly after training.

Longo et al. (19) suggested that a major source of ROS is mitochondrial respiration. During vigorous aerobic exercise, superoxide production increases particularly in the mitochondria and microsomes (2, 10). Several investigators (8, 24) have revealed that the Mn-SOD level in soleus muscle is significantly higher in treadmill-trained rats than in untrained rats, whereas the CuZn-SOD level is not different. The results of the present study were in approximate agreement with their findings, albeit in a different skeletal muscle, presumably because the primary diversion of the circulating blood to both muscles during exercise led to increased mitochondrial respiration. Also, Mn-SOD content in lung and kidney was significantly increased with swimming training. Unexpectedly, however, the accumulation of Mn-SOD appeared not to be due to the enhancement of mRNA level. Although the mechanisms by which training may change SOD content without altering its mRNA level have not been elucidated yet, the present results may indicate the possibility that exercise training induces an excessive store of Mn-SOD in some tissues. On the other hand, this study failed to observe a training-induced increase in heart Mn-SOD content. Powers et al. (31) have demonstrated that total SOD activity in ventricular myocardium is enhanced with rigorous training, with much higher training intensity than that in the present study.

By contrast, it is generally accepted that CuZn-SOD activity is unaffected by exercise training (9). In the present study on immunoreactive CuZn-SOD content, however, there were significant decreases in heart, lung, and liver but significant increases in kidney after training, with such changes being accompanied by parallel changes in the mRNA level only in lung. In the liver, CuZn-SOD mRNA levels were elevated, possibly to compensate for the decline in liver protein levels.

The contents of all the SOD isoenzymes in kidney were significantly increased with training. Renal work is dependent on blood flow: the greater the flow, the greater the amount of filtrate and the more NaCl to be reabsorbed. Kidneys receive 25% of the heart stroke volume (i.e., 1 l oxygen/min) at rest, but the renal blood flow during exercise may be decreased to 20% of its normal value (27). So far, however, there has been no direct evidence of true ischemia or ischemia-reperfusion in the kidney with exercise. In the present study, thus, all the SOD isoenzymes in kidney might accumulate significantly after training to cope with exercise-induced oxidative stress, most likely due to other factors besides ischemia-reperfusion.

One may deduce that, during exercise, liver would presumably undergo a reduction in blood flow similar to that in the kidney and also show an increase in EC-SOD content. As already described, however, the responses of the three SOD isoenzymes to swimming training were not concomitant in liver and kidney. In our previous study on rats, we have revealed that the level of thiobarbituric acid-reactive substances (TBARS), an indirect sign of lipid peroxidation, in liver is significantly elevated immediately after acute running exercise, whereas that in kidney increases markedly 3 days after running exercise (32), suggesting that the kidney may undergo oxidative stress longer than does liver.
this holds true for the present study, such different observations in liver and kidney might be due, in part, to the differences in the time of occurrence and the term of oxidative stress during and after each swimming training.

In most cases, alterations of tissue SOD isoenzyme levels were not accompanied by concomitant changes in the respective expression of mRNA. Various exogenous and endogenous factors play a regulatory role in antioxidant enzymes. Gene expression-independent enhancement of protein that is blocked by some reagents such as glucocorticoids and nicotinamide has been demonstrated in interleukin-1-induced nitric oxide synthase (1). Harris (7) has also summarized that hormones and metal ion cofactors impose pre- and posttranslational control over the genetic expression of antioxidant enzymes. Moreover, the environmental toxic metal HgCl₂ enhances Mn-SOD protein independent of its gene expression (17). From these facts, we may expect involvement of translational and/or posttranslational mechanisms of the regulation of antioxidant enzymes even under physical exercise (37). Indeed, in rat skeletal muscles, we have previously shown a discordance between the levels of CuZn-SOD and Mn-SOD proteins and their mRNA levels both under acute exercise (24, 26) and under physical training (24, 25). As for EC-SOD, in our previous study mouse lung showed a markedly higher value of EC-SOD than other tissues, whereas kidney showed the strongest expression of EC-SOD mRNA (29), suggesting similar mechanisms. Otherwise, because EC-SOD is a secretory enzyme, secretion and/or relocalization in extracellular space may be involved in the regulation of tissue EC-SOD level. At all events, such discrepancies observed in the present study require further investigation.

Collectively, swimming training had different effects on SOD isoenzymes. The differences in the response of SOD isoenzymes to training among the tissues investigated might depend, in part, on differences in their subcellular localization and/or respective properties. Moreover, another potential mechanism involved in the oxidative stress response to exercise training could possibly be the redistribution of the blood flow, that is, reduced blood flow in liver and kidney, presumably resulting in an increase in certain ROS-producing factor(s), and elevated blood flow in heart, lung, and gastrocnemius muscle, leading to increased mitochondrial respiration, which results in an elevated production of ROS. Only in kidney was the content of all SOD isoenzymes increased by training, suggesting that kidney is one of the organs most sensitive to exercise-induced oxidative stress. In most organs, including kidney, the changes in SOD isoenzyme contents were not concomitant with changes in their mRNA levels. Therefore, the levels of SOD isoenzymes appeared to be regulated by translational and/or posttranslational mechanism(s) during physical training, although the mechanisms remain to be clarified. Moreover, whether or not increases in SOD isoenzyme levels actually reduce exercise-induced oxidative stress also remains vague. Thus the precise mechanism and physiological significance of the changes in SOD isoenzymes during physical training should await further study.

We thank Dr. Keiichiro Suzuki and Prof. Naoyuki Taniguchi (Osaka University, Osaka, J apan) for providing us with polyclonal antibodies for rat Mn-SOD and CuZn-SOD and Masahiko Segawa (National Defense Medical College, Tokorozawa, J apan) for excellent technical help.

The present study was supported by a grant from Kawano Memorial Foundation for Promotion of Pediatrics.

Address for reprint requests and other correspondence: H. Ohno, Dept. of Hygiene, Kyorin University, School of Medicine, 6-20-2, Shinkawa, Mitaka 181-8611, J apan (E-mail: ohno2o@kyorin-u.ac.jp).

Received 3 J uly 1998; accepted in final form 14 October 1999.

REFERENCES


19. Longo, V. D., E. B. Gralla, and J. S. Valentine. Superoxide dismutase activity is essential for stationary phase survival in
EFFECTS OF SWIMMING TRAINING ON SOD ISOENZYMES


