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Responses of Antioxidant System to Acute and Trained Exercise in Rat Heart Subcellular Fractions

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SOMANI, S. M., S. FRANK AND L. P. RYBAK. *Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions.* PHARMACOL BIOCHEM BEHAV 51(4) 627-634, 1995.—The effects of acute and trained exercise on antioxidant enzymes (AOE), glutathione (GSH), and malondialdehyde (MDA) were compared in rat heart subcellular fractions and red blood cells. Fischer-344 rats were exercised acutely to 100% $\dot{V}O_2$ max and another group of Fischer-344 rats were given trained exercise for 10 weeks. The AOE and MDA were measured by spectrophotometry and GSH and oxidized GSH (GSSG) by high pressure liquid chromatography. Trained exercise significantly increased cytosol GSH to 131% of sedentary control (SC). Acute exercise significantly increased mitochondrial superoxide dismutase, catalase, and glutathione peroxidase by 167%, 358%, and 129% of SC, respectively, whereas enzyme activities following trained exercise were increased by 133%, 166%, and 128% of SC. The mitochondria/cytosolic ratio for superoxide dismutase, catalase, and glutathione peroxidase after acute exercise increased to 1.9, 2.7, and 1.7, respectively, whereas the respective ratios of these enzymes after trained exercise were 1.3, 1.6, and 1.3. Acute exercise contributed to oxidative stress more than trained exercise. Acute exercise resulted in a larger increase in enzyme activities than trained exercise, possibly as a compensatory mechanism to cope with the enhanced production of superoxides and oxyradicals during exhaustive exercise.

Heart mitochondria Antioxidant enzymes Glutathione Exercise

THE RATE of free radical or reactive oxygen species (ROS) generation in biological tissue is closely related to oxygen consumption, because under physiological conditions the majority of ROS are produced in mitochondria. Exercise may result in oxidative stress in mitochondria and it increases the oxygen consumption rate, resulting in increased production of ROS, which could be detrimental to tissues (2,20,21). The heart is an aerobic organ and has one of the highest oxygen consumption rates in the body. Most of the oxygen consumed by the myocardium is reduced to H_2O in the mitochondrial respiratory chain. However, a small amount of oxygen (2-5%) is converted to H_2O_2 and free radicals via stepwise one-electron reduction (9). Oxygen uptake increases 10-fold by the body during acute exhaustive exercise. The increased oxygen, which contributes to an increase in aerobic metabolism, primarily occuring in mitochondria, can increase ROS production, causing damage to mitochondria and tissues (11,12,50). However, at the same time, antioxidant enzymes (AOE) activity is stimulated to scavenge the ROS and to cope with the oxidative stress. The ROS are scavenged by AOE [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)] and glutathione (GSH). Superoxide anions are converted to hydrogen peroxide (H_2O_2) by SOD; this H_2O_2 is degraded to water and singlet oxygen by CAT. Hydrogen peroxide or the lipid peroxide formed are converted to water and singlet oxygen by GSH-Px. The copper-zinc isozyme of superoxide dismutase (CuZnSOD) is present in erythrocytes and cytosol. Manganese superoxide dismutase (MnSOD) is present in mitochondria and constitutes about 8% of total SOD.

Exhaustive exercise decreases the GSH concentration in liver and muscle (29). However, exercise training increases GSH concentration in plasma and tissues (28). After maximal exercise in rats, oxidation of GSH to oxidized glutathione (GSSG) increases in the liver. The single bout of exercise

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changes the GSH/GSSG ratio in plasma (17,29). GSH plays an important role in scavenging free radicals and hydroxyradicals and singlet oxygen generated during exercise. While scavenging free radicals, GSH is oxidized to GSSG. Thiol groups are involved in scavenging free radicals from mitochondria and cytosol. Mitochondrial thiol groups in the reduced state are important in preserving the integrity of mitochondrial membranes (34). Thus, the ratio of GSH to GSSG is a useful tool in determining oxidative stress in tissue or subcellular fractions (18,49).

The effect of exercise on the AOE activities in different tissues such as liver (5), skeletal muscle (21), cardiac muscle (27,37,48), lung (38), red blood cells (6,35), and muscle, liver, and blood (14,23,24,26) has been reported. Recently, we have shown the differences in brain regional AOE activities as well as GSH/GSSH ratios that are specially regulated during exercise training (46). The influence of exercise-induced oxidative stress on the central nervous system has been reviewed (42). Although AOE activity was determined in cardiac tissues after trained exercise in a previous study (25), no comparison was made in AOE activities after acute exercise and trained exercise. Furthermore, the differences between mitochondrial and cytoplasmic AOE activities after acute and trained exercise are not known. Heart tissue has four times less activity of SOD than liver, and CAT activity is also extremely low (30). Therefore, heart tissue may be more prone to peroxidative damage due to oxidative stress. The effect of treadmill exercise (acute or trained) on SOD, CAT, GSH-Px, and glutathione reductase (GR) in rat erythrocytes, heart homogenate, mitochondria, and cytosol has not been fully studied. Acute exercise causes oxidative stress, which in turn stimulates AOE activity, specifically in mitochondria to prevent mitochondrial and tissue damage. Any alterations in GSH, AOE, GR, and malondialdehyde (MDA) in heart subcellular fractions are reflective of oxidative stress.

Therefore, we investigated the effects of acute exercise (100% $\dot{V}O_2$ max) and trained exercise on the activities of the MnSOD, CnZnSOD, CAT, GSH-Px, and GR and on the levels of GSH as well as the effect of exercise on lipid peroxidation in the mitochondria, cytosol, heart homogenate, and erythrocytes of the rat.

METHOD

Epinephrine, NADPH, SOD, CAT, GSH-Px, GR, GSH, GSSG, *tert*-butylhydroperoxide, H_2O_2 iodoacetic acid, 1,1,3,3-tetraethoxypropane, and γ-glutamyl glutamate were obtained from Sigma Chemical Co. (St. Louis, MO).

Sanger's reagent (1-fluoro-2,4-dinitrobenzene) was obtained from Eastman Kodak Co. (Rochester, NY).

Animals

Male Fisher-344 rats from Harlan Industries (Indianapolis, IN) weighing 290–315 g were used for acute exercise and rats weighing 95–110 g were used for trained exercise for 10 weeks. Exercise-trained rats weighed 290–310 g at the time of sacrifice.

Acute Exercise

Four male Fisher-344 rats (300 g) were acutely exercised on an Omni-Pacer treadmill (Omnitech Electronics Inc., Columbus, OH) at 100% $\dot{V}O_2$ max. The speed of the belt and angle of inclination were increased as shown in Table 1. The oxygen consumption and heat production in individual rats undergoing different stages of exercise were recorded by an Oxyscan System (Omnitech Electronics Inc.). Measurement of maximal oxygen consumption (100% $\dot{V}O_2$ max) was considered valid only if the animal ran until it could no longer maintain pace with the treadmill (44). After exercise, both acute exercise (n = 4) and sedentary control (SC) rats (n = 7) were sacrificed immediately (between 0900 and 1100 h). The heart was removed, put into ice-cold TRIS buffer, and cut into pieces to remove blood. The heart was then processed for isolation of mitochondria and cytosol. Mitochondria were characterized by transmission electron microscope (38) and were stored at −80°C until analysis.

TABLE 1
ACUTE EXERCISE PROTOCOL FOR CONSTANT DURATION

Stage	Inclination/ Degrees	Speed (m/min)	Duration (min)
0*	0	2	5
1	0	8.2	5
2	5	15.2	5
3	10	19.3	5
4	10	26.8	5
5	12.5	30.3	5
0†	0	2	5

*Resting.
†Post Exercise.

Trained Exercise

Male Fisher-344 rats (weighing 95–110 g) were given trained exercise on the treadmill (Omnitech Electronics, Inc. Columbus, OH) utilizing an incremental exercise program 5 days a week for 10 weeks duration as shown in Table 2.

The exercise protocol was designed to induce optimal exercise training (7,43,47).

On the day of the experiment, SC rats (300 g) were sacrificed between 0900 and 1100 h. Exercise training was stopped 24 h prior to sacrifice in the exercise-trained group (n = 4) and the SC rats (n = 8) were sacrificed at the same time. Hearts from SC and trained exercise rats were removed, placed in ice-cold TRIS buffer, and cut into pieces to remove blood. The heart was processed for isolation of mitochondria and cytosol as given below. They were analyzed for SOD, CAT, GSH- Px, GR, GSH, GSSG, and MDA as described in enzyme assays.

Blood was collected in heparinized tubes. The plasma was separated by centrifugation at 3000 rpm at 4°C for 20 min in a Juan centrifuge. Red blood cells (RBC) were suspended in phosphate buffer (pH 7.4) and centrifuged. This procedure was repeated twice to wash RBC, which were then stored at −80°C.

Separation of mitochondria and cytosol. The heart tissue (about 500 mg) was weighed and placed in a beaker containing buffer and kept on ice. The mitochondrial fraction was separated by the differential centrifugation method of Murfitt et al. (32). The heart was sliced into several pieces and immersed in ice-cold isolation media consisting of 0.17 M KCl, 0.01 M EDTA, 0.01 M Tris-HCl, pH 7.4, and 1% BSA. The tissue samples were further diced into 5-mm cubes, removed from the buffer, and blotted dry. The minced tissue was weighed

TABLE 2
TRAINED EXERCISE PROTOCOL

Week	Inclination (Degrees)	Belt Speed (m/min)	Duration at Each Speed (min)	Exercise Total Time (min)
1-2	6°	8.2, 15.2, 19.3	5	15
3-4	6°	15.2, 19.3, 26.8	10	30
5-7	9°	15.2, 19.3, 26.8	10	30
8-10	9°	19.3, 26.8, 30.3	10	30

and homogenized with 4 ml/g of isolation media in a Thomas "C" Teflon-glass homogenizer. Tissue was subjected to 20 passes of homogenizer at approximately 180 rev/min, diluted to 10 ml/g, and the homogenate was centrifuged at 125 × *g* for 15 min to remove cell debris and nuclei. This process was repeated again to remove any remaining cellular debris and nuclei. The supernatant was centrifuged at 18,000 × *g* for 10 min and the mitochondrial pellet was isolated and the postmitochondrial supernatant was centrifuged at 105,000 × *g* to obtain the cytosolic fraction. The above mitochondrial pellet was washed by suspending in the media and centrifuging at 18,000 × *g* for 10 min to obtain the final mitochondrial pellet. The supernatant was discarded and the mitochondrial were suspended in a buffer and stored at −80°C until analysis. The intactness of mitochondria was seen under electron microscope (38) and maleic acid dehydrogenase was also determined in mitochondria and cytosolic fraction.

Enzyme Assays

Superoxide dismutase (SOD). SOD was determined according to Misra and Fridovich (31) at room temperature. Tissue extract (100 μl) was added to 880 μl (0.05 M, pH 10.2, 0.1 mM EDTA) carbonate buffer. Epinephrine (20 μl of 30 mM) (dissolved in 0.05% acetic acid) was added to the mixture and SOD was measured at 480 nm for 4 min on a Hitachi U 2000 spectrophotometer. The rate of the reaction was calculated where linearity occurred, usually between 90–100 s. SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to one unit.

Catalase (CAT). CAT was determined by a slightly modified version of Aebi (1) at room temperature. ETOH (10 μl) (100%) was added per 100 μl of tissue extract (dissolved in 0.5 M, pH 7.0, 0.1 mM EDTA, phosphate buffer), and then placed in an ice bath for 30 min. Then 10 μl of Triton X-100 RS was added per 100 μl of the tissue extract. Tissue extract (500 μl) was added to 500 μl (0.066 M) H_2O_2 (dissolved in phosphate buffer) and measured at 240 nm for 30 s on a Hitachi U2000 spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/min/mg of protein.

Glutathione peroxidase (GSH-Px). GSH-Px was determined by a modified version of Flohe and Gunzler (16) at 37°C. All reaction mixtures were dissolved in 0.05 M, pH 7.0, 0.1 mM EDTA phosphate buffer. A reaction mixture of 100 μl phosphate buffer, 100 μl 0.01 M GSH (reduced form), 100 μl 1.5 mM NADPH, and 100 μl GR (5 mg/μl of 120 U/mg). The tissue extract (500 μl) was added to the reaction mixture and incubated at 37°C for 10 min. Then 100 μl of 12 mM *t*-butyl hydroperoxide was added to the tissue reaction mixture and measured at 340 nm for 180 s on a Hitachi U 2000 spectrophotometer. The molar extinction coefficient of 6.22 mM cm^{-1} was used to determine the activity of GSH-Px. One unit of activity is equal to the mM of NADPH oxidized/min/mg protein.

Glutathione reductase (GR). GR was determined by a slightly modified method of Carlberg and Mannervik (8) at 37°C. NADPH (25 μl, 2 mM) in 10 mM Tris-HCl buffer (pH 7.0), 25 μl of GSSG (20 mM) in phosphate buffer (0.5 M, pH 7.0, 0.1 mM EDTA), and 250 μl of phosphate buffer were incubated at 37°C for 10 min. Tissue extract (200 μl) was added to the NADPH-GSSG buffered solution and measured at 340 nm for 3 min on a Hitachi U2000 spectrophotometer. The molar extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to determine GR activity. One unit of activity is equal to the mM of NADPH oxidized/min/mg protein.

Maleic acid dehydrogenase. Maleic dehydrogenase (MDH) was determined by a modified method of Englard and Siegal (13). Mitochondrial or cytosolic extract (15 μl) in phosphate buffer (0.5 M, pH, 0.70, 0.1 mM EDTA) was added to 470 μl (0.13 mM) β-NADH and 15 μl (4.5 mM) *cis*-oxalacetic acid. This reaction mixture was immediately mixed and measured at 340 nM, 25°C, on a Hitachi UV 2000 spectrophotometer for 5 min. The molar extinction coefficient of 6.22 mM/cm^{-1} was used to determine the activity of MDH. One unit of activity is equal to the millimoles of β-NADH oxidized/min/mg protein. This enzyme was determined in cytosolic fraction to determine the intactness of mitochondria.

Lipid peroxidation assay. This assay is used to determine MDA levels. The extent of lipid peroxidation was determined based on the method described by Ohkawa et al. (33). Tissue (200 μl) was added to 50 μl of 8.1% sodium dodecyl sulfate (SDS), vortexed, and incubated for 10 min at room temperature; 375 μl of 20% acetic acid (pH 3.5) and 375 μl of thiobarbituric acid (0.6%) were added to the tissue-SDS and placed in a boiling water bath for 60 min. The samples were allowed to cool to room temperature. Butanol : pyridine (1.25 ml) (15 : 1) was added, vortexed, and centrifuged at 4000 rpm for 5 min. The colored layer (500 μl) (largely MDA) was measured at 532 nm on a Hitachi U 2000 spectrophotometer. 1,1,3,3-Tetraethoxypropane was used as a standard, with an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Glutathione determination. The high pressure liquid chromatography method for reduced (GSH) and oxidized (GSSG) GSH was determined by a modified method of Reed et al. (15,36). The tissue-acid extract (500 μl) containing internal standard (γ-glutamyl glutamate) was mixed with 50 μl of 100 mM iodoacetic acid in a 0.2 mM *m*-cresol purple solution. This acidic solution was brought to basic conditions (pH 8.9) by the addition of approximately 500 μl of 2 M KOH 2.4 M $KHCO_3$. The sample was placed in the dark at room temperature for 1 h. Rapid *S*-carboxymethyl derivatization of GSH,

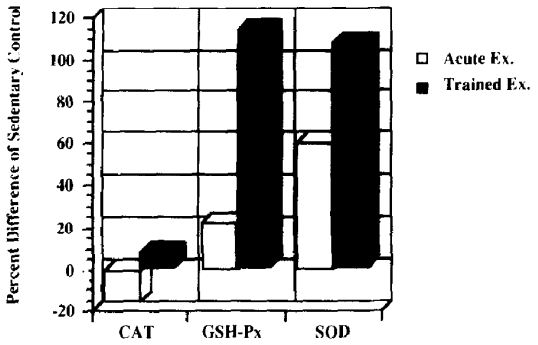

FIG. 1. Catalase, glutathione peroxidase, and superoxide dismutase activities after acute (n = 4) and trained (n = 4) exercise in rat red blood cells. The activities are shown as percent of difference of sedentary control rat (n = 8). Both acute and trained exercise show significantly (*$p < 0.05$) higher values of GSH-Px and SOD in red blood cells compared to sedentary control.

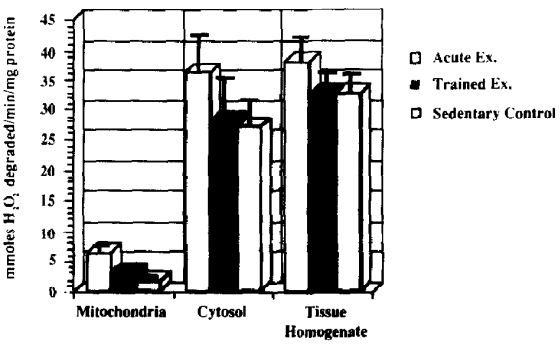

FIG. 3. Catalase activity in mitochondria, cytosol, and heart tissue homogenate of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 8) rats. Values are means ± SEM. *$p < 0.05$ compared to the respective control group.

GSSG, and γ-glutamyl glutamate occurs soon after the change in pH. *N*-Dintrophenyl derivatization of the samples was obtained by incubation for 12 h at 4°C in the presence of 1% fluoro-*d*-nitrobenzene. The internal standard accurately corrects for alterations in derivatization, efficiency, stability, chromatographic conditions, and injection volumes.

Protein. Protein content of M, cytosol, and tissue homogenate was measured by the Bradford method (4) using a Hitachi U2000 spectrophotometer.

Mitochondrial/cytosol ratio. The ratio of percent of sedentary control enzyme activity after acute exercise and trained exercise in mitochondria vs. cytosol is defined as the M/C ratio.

Statistical analysis. All data were expressed as means ± SEs. Data were analyzed in a pairwise manner within a one-factor analysis of variance (ANOVA). Data were compared between groups using standard repeated-measures ANOVA. Differences were considered statistically significant at $p < 0.05$ level.

RESULTS

Changes in percent of SC AOE activity in RBCs due to exercise are depicted in Fig. 1. The activity of SOD (U/mg Hb) in RBC of SC, acute exercise, and trained exercise rats were 2.13 ± 0.27, 3.40 ± 0.71, and 4.44 ± 1.04, respectively. CAT activities (mmol H_2O_2 degraded/min/mg Hb) were 36.88 ± 3.56, 31.02 ± 6.13, and 39.87 ± 9.08 in SC, acute exercise, and trained exercise rats, respectively. GSH-Px activity (μmol NADPH oxidized/min/mg Hb) increased consistently with exercise in RBC for SC 8.72 ± 1.32, for acute exercise 10.62 ± 0.74, and for trained exercise 18.62 ± 1.33.

MnSOD (U/mg protein) activity of acutely exercised rat increased significantly from SC 6.08 ± 2.36 to 10.17 ± 1.96 (Fig. 2). However, there is little change in CuZn SOD activity in cytosol and tissue homogenate due to acute exercise. Trained exercise increased MnSOD activity from 6.08 ± 2.36 to 8.4 ± 1.65. CuZnSOD activity also increased due to trained exercise.

The CAT activity (mmol H_2O_2 degraded/min/mg protein) increased significantly from SC 1.80 ± 0.73 to acute exercise 6.46 ± 1.12; 27.30 ± 8.24 to 36.5 ± 8.51 and 32.91 ± 3.68 to 38.24 ± 4.30 in heart mitochondria, cytosol, and tissue homogenate, respectively (Fig. 3). Trained exercise also in-

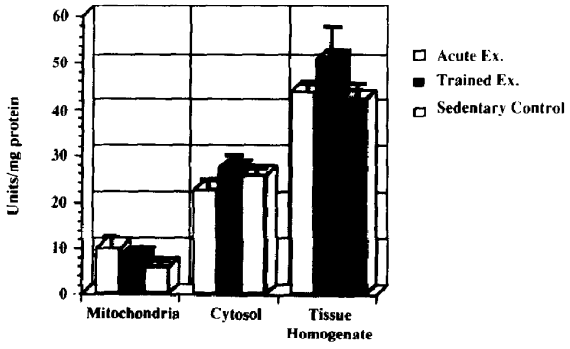

FIG. 2. Manganese superoxide dismutase activity in mitochondria and copper-zinc superoxide dismutase activity in cytosol and superoxide dismutase activity in heart tissue homogenase of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 8) rats. Values are means ± SEM. *$p < 0.05$ compared to the respective control group.

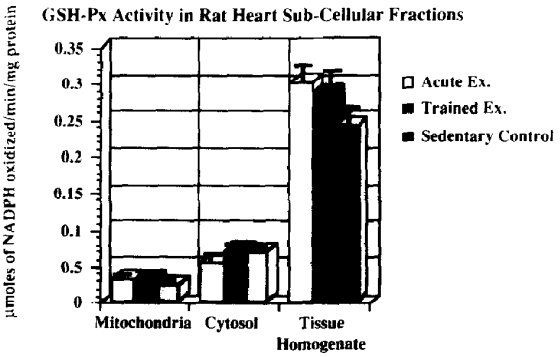

FIG. 4. Glutathione peroxidases activity in mitochondria, cytosol, and heart tissue homogenate of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 8) rats. Values are means ± SEM. *$p < 0.05$ compared to the respective control group.

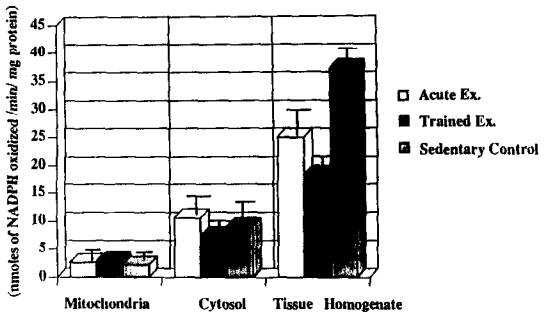

FIG. 5. Glutathione reductase activity in mitochondria, cytosol, and heart tissue homogenate of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 8) rats. Values are means ± SEM.

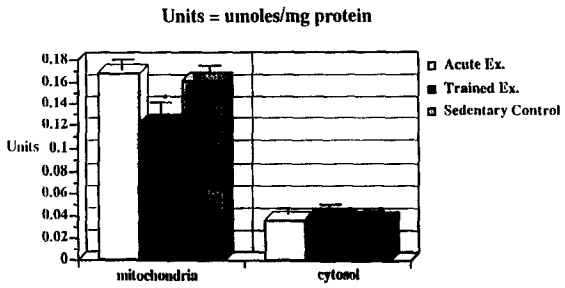

FIG. 7. Malondialdehyde levels in heart mitochondria and cytosol of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 5) rats. Values are means ± SEM. *$p < 0.05$ compared to the sedentary control group.

creased CAT activity in mitochondria, cytosol, and tissue homogenate.

The GSH-Px activity (nmol of NADPH oxidized/min/mg protein) increased in mitochondria due to exercise and the activities were 25.56 ± 9.57, 33.04 ± 3.63, and 32.73 ± 16.53 in SC, acute exercise, and trained exercise, respectively (Fig. 4). The enzyme activities in the cytosolic fraction were 71.02 ± 4.96 (SC), 55.24 ± 20.97 (acute exercise), and 71.98 ± 11.37 (trained exercise). In tissue homogenate, GSH-Px activity was 224.48 ± 53.48 (SC), 301.39 ± 19.67 (acute exercise), and 286.95 ± 37.11 (trained exercise).

The GR activity (nmol of NADPH oxidized/min/mg protein) in mitochondria increased due to exercise and the activity was 2.12 ± 0.41 (SC), 2.59 ± 0.49 (acute exercise), and 2.77 ± 0.16 (trained exercise) (Fig. 5). In cytosol, these values were 9.1 ± 1.22 (SC), 10.59 ± 1.47 (acute exercise), and 7.39 ± 1.4 (trained exercise). In tissue homogenate, GR activity was 37.25 ± 3.42 (SC), 24.95 ± 4.5 (acute exercise), and 18.45 ± 3.22 (trained exercise).

The reduced GSH (nmol/mg protein) in rat heart cytosol of SC rat was found to be 0.73 ± 0.08, which decreased to 0.66 ± 0.05 after acute exercise as shown in Fig. 6A. The GSSG (nmol/mg protein) in heart cytosol of SC rat was 1.14 ± 0.29, which increased to 1.55 ± 0.22 after acute exercise (Fig. 6B). Trained exercise increased GSH concentration to 0.95 ± 0.02 (mean ± SE) (n = 4). However, trained exercise decreased GSSG value to 0.54 ± 0.22 (n = 4).

The MDA (nmol/mg protein) in mitochondria of SC, acute exercise, and trained exercise were 0.16 ± 0.01, 0.17 ± 0.01, and 0.12 ± 0.01, respectively (Fig. 7). MDA levels decreased to 75% of SC in mitochondria of rat heart after trained exercise.

The mitochondria/cytosol ratio for SOD, CAT, and GSH-Px in rat heart after acute exercise was compared with that of trained exercise as shown in Table 3. This ratio for all antioxidant enzymes in acute exercise was much higher than in trained exercise, indicating the greater oxidative stress due to acute exercise.

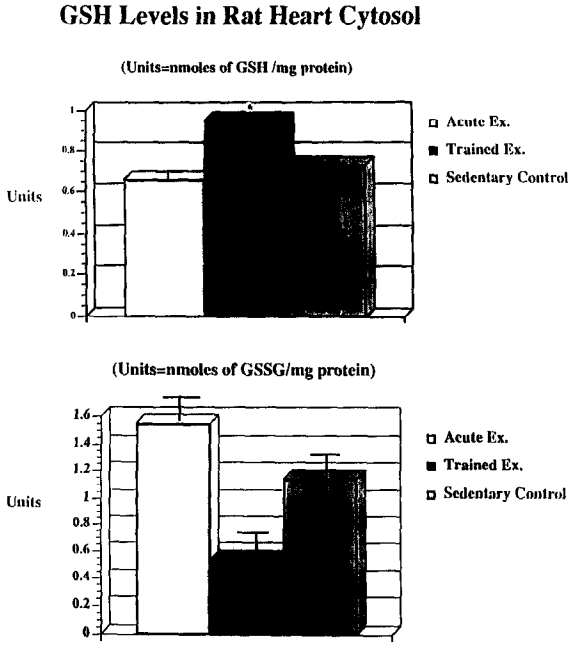

FIG. 6. (A) Glutathione levels in heart cytosol of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 5) rats. Values are means ± SEM. *$p < 0.05$ compared to the sedentary control group. (B) Oxidized glutathione levels in heart cytosol of acute exercise (n = 4), trained exercise (n = 4), and sedentary control (n = 5) rats. Values are means ± SEM. *$p < 0.05$ compared to the sedentary control group.

DISCUSSION

Mitochondria utilizes most of the oxygen (95%) and is reduced to water during biological oxidation via a two-electron transfer through cytochrome carriers and other well-known oxidation reduction systems. However, about 2–5% of the oxygen is reduced through univalent pathways, which leads to the production of highly reactive oxygen species (ROS). These ROS consist of superoxide anion (O_2^-), H_2O_2,

TABLE 3

RAT HEART MITOCHONDRIAL/CYTOSOL RATIO FOR ANTIOXIDANT ENZYME ACTIVITIES BASED ON AS PERCENT OF SEDENTARY CONTROL

	SOD	CAT	GSH-Px
Acute exercise (100% V_{O_2} max)	1.92	2.67	1.71
Trained Exercise (7.5 weeks)	1.26	1.59	1.32

OH, and singlet oxygen (19,42). The ROS cause damage to mitochondrial membranes and cytoplasmic structures through peroxidation of phospholipids, proteins, and nucleotides. Unsaturated free fatty acids, a constituent of phospholipids, are easily peroxidized. The product of peroxidation is MDA, which is measured conveniently. This peroxidation reaction can cause irreversible cell death by the loss of homeostasis and the specific activity of cellular organelle. However, this initiation of peroxidation can be reversed by the stimulation of AOE and by maintaining an adequate concentration of an intracellular antioxidant GSH.

This investigation clearly demonstrates that exercise, acute or trained, stimulates AOE in the heart mitochondria, thus providing the benefit to the heart.

In RBCs, SOD activity increased considerably after acute exercise and after trained exercise. CAT activity decreased after acute exercise and increased after trained exercise. The GSH-Px increased after acute and trained exercise. The discrepancy is unexplained other than the possibility of the presence of different isozymes of CAT in RBCs, which behaves differently than its counterpart in heart subcellular fractions. In RBCs, a different CAT isoenzyme is present. Overall increases in SOD, CAT, and GSH-Px in RBCs are more after trained exercise than acute exercise.

Antioxidant enzymes activity increased significantly in the mitochondria of the heart after acute and trained exercise. MnSOD activity was considerably more after acute exercise than after trained exercise. Similarly, CAT activity in mitochondria increased significantly after acute exercise and trained exercise, respectively. GSH-Px activity increased after acute exercise as well as trained exercise. Exercise, either acute or trained, produced oxidative stress on the heart tissue and its organelles. There are biochemical mechanisms to cope with this stress, which involve the induction of AOE. It is possible that the similar degree of oxidative stress is produced in acute vs. trained exercise. The difference is that the tissues from trained animals are better able to cope with the stress.

The ratio of mitochondria/cytosol for AOE after exercise training is less than after an acute single bout of exercise. This suggests that the mitochondria of the heart is under more oxidative stress after acute exercise than after trained exercise and are more susceptible to injury by O_2^-. Superoxide anions are excessively generated in mitochondria after acute exercise, which influences the increase in the activity of MnSOD in mitochondria. MnSOD is utilized to dismutate O_2^- to H_2O_2. Thus, the excess of H_2O_2 produced enhances the activity of CAT, thereby increasing the ratio of mitochondria vs. cytosol for CAT. This enzyme catalyzes H_2O_2 to H_2O and singlet oxygen. Similarly, GSH-Px activity is also stimulated in mitochondria of acutely exercised rats, giving higher ratio of mitochondria/cytosol for this group. GSH-Px catalyzed H_2O_2 or lipid peroxide to water or lipid utilizing GSH as a substrate, which is converted to GSSG.

GSSG levels were found to be almost twofold higher than GSH level in the cytosolic fraction of the heart after acute exercise. On the contrary, after trained exercise, GSSG levels were about half that of GSH level in heart cytosol. GSH serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of mitochondria, cell membrane, and cell system. It functions as a substrate for GSH-Px and scavenges free radicals, oxyradicals, and singlet oxygen produced during exercise. AE produces these radicals, which convert GSH to GSSG, thereby increasing the levels of GSSG. Our results are comparable with those of Lew et al. (29), who reported that GSSG levels increased significantly in other tissues such as muscle, liver, and plasma after exhaustive exercise. There was a simultaneous decrease in GSH levels in liver and muscle but an increase in plasma. However, Ji et al. (22) reported contradictory results, indicating an increase in GSH in muscle after acute (exhaustive) exercise. Our results show a significant increase in GSH after trained exercise but not after acute exercise. GR enzyme activity that converts GSSG to GSH increased during trained exercise. GSH is synthesized primarily in the liver, and then it is transported to different organs of the body via blood (23,24). Other mechanisms for an increase in GSH in heart tissue due to trained exercise might be involved. Exercise increases the blood flow to cardiac muscle, which in turn increases the delivery of GSH to this organ. This enhanced intracellular transport of GSH seems to be essential in maintaining the redox state and to cope with the oxidative stress during trained exercise. However, the same phenomenon does not seem to occur with acute exercise. The GSH level is almost half that of GSSG level after strenuous single exercise. It appears that transport barriers for GSH, if any, open up with trained exercise. The primary benefit of prolonged exercise may be due to increase in the density of myocardial capillaries, which increases blood flow and increases delivery of GSH to the myocardium. Another possible mechanism for the increase in GSH during trained exercise could be due to hormonal effects. Trained exercise triggers a hormonal response that can influence the efflux of GSH from liver to blood. Ji et al. (22) have shown that exercise training increased the GSH in blood, which might be due to glucagon and cyclic AMP generating hormones.

Oxygen uptake increases 10-fold during acute exhaustive exercise. This increased oxygen, which contributes to an increase in aerobic metabolism, primarily occuring in mitochondria, can increase ROS production causing damage to the tissues and mitochondria (11,12). The induction of AOE activities due to exercise would prevent this tissue damage. It is not yet known whether the induction of the AOE takes place after intracellular concentration of free radicals or ROS increase above normal (critical) levels.

Our results compared the effects of acute exercise and trained exercise on the status of AOE (SOD, CAT, and GSH-Px), GR, and antioxidant (GSH) in subcellular fractions of the heart. MDA, a by-product of lipid peroxidation, is a marker of oxidative stress. Trained exercise decreased MDA level to 75% of SC value in heart mitochondria, indicating a possible beneficial effect of trained exercise. Our studies show a significant increase in MnSOD, CAT, and GSH-Px activity in heart mitochondria. This increase in AOE activity leads to scavenging of excess free radicals or oxyradicals and thus may contributes to a decrease in MDA value. Trained exercise induces AOE activity in mitochondria, indicating that this organelle may be more susceptible to oxidative stress. Although other investigators have reported that trained exercise did not seem to significantly alter AOE activity in heart tissues (25), we found a significant increase in MnSOD, CAT, and GSH-Px activity in heart mitochondria, suggesting that mitochondria is more susceptible to oxidative stress. However, the mechanisms of increase in AOE activity are not known.

Mitochondria consume oxygen to produce adenosine triphosphate (ATP) by oxidative phosphorylation. Adenosine triphosphate is used as an energy source by the heart for contraction and other cellular functions (10). However, during acute exercise, this ATP consumption increases, which is then broken down to adenosine diphosphate, adenosine mono-

phosphate (AMP), and to adenosine. Adenosine may exert various regulative effects on the body's complex adaptation response to exercise (41).

Another possible mechanism of AOE induction due to trained exercise is due to gene regulation. Although very little is known about the molecular basis for regulation of these enzymes in mammalian tissues, the demonstration of the selective in vitro induction of AOE by different oxidant stresses (40) supports these observations. These authors showed that the addition of H_2O_2 caused a dose-dependent increase in CAT mRNA in cells, whereas the increases in the steady-state mRNA levels of MnSOD and GSH-Px were less striking. There is scant information on whether these AOE (SOD, CAT, and GSH-Px) show differential regulation in tissues in response to physical exercise to nullify the increased production of ROS during exercise. Our preliminary studies have shown that exercise training caused an increase of 130–150% of SC in mRNA corresponding to MnSOD, CuZnSOD, CAT, and GSH-Px in the hearts of old rats. It seems that an increase in the antioxidant enzyme activities during exercise training might be due to an increase in gene expression. Amsted et al. (3) have reported that the small deviations from the physiological activity ratio GSH-Px/SOD have a dramatic effect on the resistance of cells to oxidant-induced damage to the genome and cell killing. The fine balance between several components of the antioxidant defenses appears to be important for the cellular resistance to oxidative stress.

In conclusion, our results show that MnSOD, CAT, and GSH-Px activities in mitochondria increased with exercise. An increase in mitochondria/cytosol ratio of AOE after acute exercise indicated that acute exercise contributed to oxidative stress more than trained exercise. Trained exercise increased GSH levels in cytosol. Trained exercise decreased MDA levels in heart mitochondria, indicating the beneficial effect due to trained exercise.

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