Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants

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Liu, Jiankang, Helen C. Yeo, Eva Övervik-Douki, Tory Hagen, Stephanie J. Doniger, Daniel W. Chu, George A. Brooks, and Bruce N. Ames. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 89: 21–28, 2000.—The responses to oxidative stress induced by chronic exercise (8-wk treadmill running) or acute exercise (treadmill running to exhaustion) were investigated in the brain, liver, heart, kidney, and muscles of rats. Various biomarkers of oxidative stress were measured, namely, lipid peroxidation [malondialdehyde (MDA)], protein oxidation (protein carbonyl levels and glutamine synthetase activity), oxidative DNA damage (8-hydroxy-2′-deoxyguanosine), and endogenous antioxidants (ascorbic acid, α-tocopherol, glutathione, ubiquinone, ubiquinol, and cysteine). The predominant changes are in MDA, ascorbic acid, glutathione, cysteine, and cystine. The mitochondrial fraction of brain and liver showed oxidative changes as assayed by MDA similar to those of the tissue homogenate. Our results show that the responses of the brain to oxidative stress by acute or chronic exercise are quite different from those in the liver, heart, fast muscle, and slow muscle; oxidative stress by acute or chronic exercise elicits different responses depending on the organ tissue type and its endogenous antioxidant levels.

chronic exercise; acute exercise; lipid peroxidation; protein oxidation; mitochondria; antioxidants

THE CONVERSION OF OXYGEN DURING normal metabolism to the by-products hydrogen peroxide, superoxide, and hydroxyl radical occurs by successive electron additions to oxygen. Because exercise increases whole body and tissue rates of oxygen consumption, it has been hypothesized that exercise may cause oxidative stress and tissue damage (1, 11–13). There have been many reports showing that exercise causes oxidative stress, e.g., the direct detection of free radical generation in rat muscle and liver (5, 33); increases in oxidative damage biomarkers such as protein carbonyls and thiobarbituric acid reactive substances (27, 28); effects on mitochondrial function (24, 39); and decreases in levels of antioxidants and antioxidant enzymes in the heart (25, 34), blood (10, 15, 38), lung (26), liver (2, 10, 15), brain (35), and muscles (3, 9, 10, 15, 23, 28, 29). However, there are also reports showing that exercise fails to result in a functionally significant level of oxidative stress in the heart (30). Thus far, the evidence for tissue oxidative stress and damage due to exercise remains incomplete because of the complexity of the exercise models. We still lack a comprehensive picture regarding the relationships between oxidative stress and exercise in the brain, liver, heart, and muscles. The relation between changes in endogenous antioxidants and oxidative stress remains to be clarified, although their correlation has been frequently hypothesized (10–12). In addition, in previous studies, lipid peroxidation, which is the most common index of oxidative stress, has been estimated by the nonspecific thiobarbituric acid assay, which has been shown to overestimate lipid peroxidation when compared with a more specific gas chromatography-mass spectrometry (GC-MS) assay (19, 40).

Although there have been many studies on oxidative stress caused by exercise that utilized oxidative biomarkers in various tissues, especially muscle, many questions still remain unanswered, such as 1) does exercise cause oxidative stress in organs, such as brain and kidney, other than liver, heart, and muscle?; 2) does chronic or acute exercise show different effects on oxidative stress in various organs?; and 3) what is the relationship between exercise-induced oxidative stress and endogenous antioxidants? We hypothesize that exercise may have important effects on organs such as the brain as well as on the muscle and heart by both chronic and acute exercise and that endogenous antioxidants may play an important role in the adaptation to exercise-induced oxidative stress in these organs. In addition, state-of-the-art biochemical techniques, such as the more specific GC-MS assay for lipid peroxidation, have been used in this work to eliminate artifacts. In the present study, rats were subjected to both acute and chronic exercise. Oxidative stress was assayed by examining lipid peroxidation, oxidative DNA damage, and protein oxidation. Assays were done on the brain,
liver, heart, kidney, the soleus (a predominantly slow-twitch muscle, hereafter called “slow muscle”), and the rectus femoris (a predominantly fast-twitch muscle, hereafter called “fast muscle”). Oxidative stress in mitochondria of brain and liver was also examined. Antioxidant levels of ascorbic acid, α-tocopherol, ubiquinone, ubiquinol, GSH, GSSG, cysteine, and cystine levels were examined by HPLC. Results indicate tissue heterogeneity in oxidative stress-strain relationships during stress.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (10 rats/group; 30 rats were used) were 6 wk old at the start of the experiments. Female rats are known to maintain body weight better than male rats (4). The animals were kept two to four per cage and had free access to water and normal chow. In order for the exercise to take place during their most active period, the animals were subjected to a reversed 12-h light cycle.

Chronic exercise and acute exercise. The animals in the chronic exercise groups were habituated by treadmill exercise over a 2-wk period during which the duration and speed of exercise increased to 2 h at 1.6 km/h. For 2 wk thereafter, animals were exercised at this level for 8 wk, 5 days/wk. Animals were rested for 48 h before death (4, 5, 35).

Animals in the acute exercise groups were also conditioned to the treadmill over a 2-wk period but only for 10 min at 0.8 km/h for 3 days/wk. Immediately before death, these animals were made to run on the treadmill at 1.6 km/h until exhaustion, which is defined as the animal touching the electrified grid at the rear of the treadmill five times in 2 min. The running time varied between 20 and 110 min. Age-matched control animals were kept sedentary in their cages throughout the study (4, 5, 35).

All animals were fasted for 12 h before death. They were anesthetized with CO₂, decapitated, and exsanguinated. The brain, liver, heart, kidney, and vastus lateralis muscles from both hind legs (subdivided into fast and slow muscle groups) were sampled. These are the muscles most widely studied, although these muscles may not be representative of the whole body. The tissues were removed from all animals in the same order: liver, kidney, heart, brain, and leg muscles. The tissues were immediately covered by ice-cold buffer and kept on ice and then immediately transported to a cold room where they were processed to small preparations and, in some cases, further to mitochondria. All the tissue preparations were frozen on dry ice and then transferred to a −80°C freezer. The mitochondria were isolated on the same day of death. The experiment was designed so that it would be possible to kill one group of animals per day. For each rat, the tissues were all removed in <10 min. Because some of the metabolites examined might be expected to change fairly rapidly once exercise is stopped, each animal was killed immediately after the cessation of exercise. Thus the delay between exercise cessation and death has been minimized.

Separation of mitochondria. The mitochondria of brain and liver were separated by the differential centrifugation method, with adaptations for each type of tissue. The fresh tissues were weighed and immediately placed in ice-cold buffer (10 mM Tris, 250 mM sucrose, pH 7.4). The mitochondria were characterized by electron microscopy, suspended in buffer, and stored at −80°C until analyzed.

Measurement of lipid peroxidation. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) by using GC-MS (19, 40).

Measurement of 8-hydroxy-2′-deoxyguanosine in nuclear DNA. Nuclear DNA of the brain, liver, kidney, heart, and muscles were isolated, and the level of 8-hydroxy-2′-deoxyguanosine (8oxo8dG) was analyzed by HPLC with electrochemical detection (31).

Protein oxidation determination. Protein oxidation was assayed spectrometrically by measuring protein carbonyls with 2,4-dinitrophenyl hydrazine (14).

Assay of glutamine synthetase activity. Glutamine synthetase activity was determined spectrophotometrically (32).

Assay of antioxidants. The levels of endogenous antioxidants, including ascorbic acid, α-tocopherol, glutathione (both GSH and GSSG), ubiquinone, ubiquinol, and cysteine (and cystine), were assayed by HPLC (20).

Protein measurement. Protein concentrations were measured with the bicinchoninic acid method by using the Pierce BCA protein assay reagent kit (Rockford, IL) with a 96-well microtitteration plate. Bovine serum albumin (fraction V) was used as the standard.

Statistical analysis. All results are from 8–10 animals. Means and SE were calculated, and multiple comparisons were performed by using one-way ANOVA with Bonferroni’s post hoc tests.

RESULTS

Acute exercise induced significant increases in MDA content in liver (Fig. 1). Chronic exercise induced a decrease in the MDA content in the brain, whereas chronic exercise induced significant MDA increases in the heart and fast and slow muscles (Fig. 1).

Acute exercise did not induce any significant increases in protein carbonyl levels in any organs (Fig. 2). Chronic exercise induced a small decrease in protein carbonyl levels in the liver and a similar increase in the slow muscle (Fig. 2).

Both acute and chronic exercise caused a decrease in glutamine synthetase activity in the liver, with the effect of acute exercise being greater than that of chronic exercise (Fig. 3). It is noteworthy that acute exercise and chronic exercise produced opposite effects on the glutamine synthetase activity in the fast and slow muscles. In the fast muscle, chronic exercise induced some increase, whereas acute exercise induced some decrease, and vice versa for slow muscle. As a result, there was a significant difference between the acutely exercised and the chronically exercised rats.

For kidney contents, mean values of MDA, protein carbonyl, or glutamine synthetase activity did not change as a result of either form of exercise (data not shown).

Similar to the results obtained with the tissue homogenates, only chronic exercise induced a significant decrease in MDA in the brain mitochondria (Fig. 4A), and only acute exercise induced a significant increase in MDA in the liver mitochondria (Fig. 4A). The changes in glutamine synthetase activity in liver mitochondria were the same as in the tissue homogenates: both acute and chronic exercise induced a decrease. The effect of acute exercise was greater than that of chronic exercise (Fig. 4B). There were no significant differences among the three groups in the brain mitochondria as the result of exercise. There were no significant changes in protein carbonyl levels...
in either the brain or the liver mitochondria (data not shown).

No significant changes were found in the levels of oxo8dG in the nuclear DNA of brain, liver, heart, kidney, and fast and slow muscles for both trained and acute exercises (data not shown).

The changes in tissue antioxidant levels induced by chronic and acute exercise are shown in Table 1. Chronic exercise induced a significant increase in ascorbic acid in the brain, a decrease in cysteine and cystine in the liver, a decrease in ascorbic acid and α-tocopherol in the fast muscle, and a decrease in ascorbic acid and an increase in ubiquinone in the slow muscle.

Acute exercise induced a significant decrease in ubiquinone in the brain, a decrease in cysteine and cystine in the liver, an increase in ascorbic acid and GSH in the heart, and a decrease in ascorbic acid in the slow muscle.

Patterns of changes in tissue antioxidant levels are summarized in Table 2.

DISCUSSION

We studied oxidative stress biomarkers and endogenous antioxidants in the brain, liver, heart, kidney, and muscles of rats subjected to acute or chronic exercise. The oxidative damage biomarkers included lipid peroxidation, protein oxidation, and DNA damage, and the endogenous antioxidants included ascorbic acid, α-tocopherol, GSH and GSSG, ubiquinone, ubiquinol, cysteine, and cystine. Our results showed that brain is also responsive to both acute and chronic exercise, although the responses of oxidative stress to acute and chronic exercise in the brain are quite different from those in the liver, heart, fast muscle, and slow muscle. The antioxidant status also showed quite different changes in direction and magnitude in different organs.

Fig. 1. Chronic and acute exercise-induced changes in malondialdehyde (MDA) in the brain, liver, heart, fast (twitch) muscle, and slow (twitch) muscle. Values are means ± SE of 8–10 animals. Significance was determined with one-way ANOVA and, when $P$ was <0.05, is shown as connected groups.

Fig. 2. Chronic and acute exercise-induced changes in protein carbonyls in the brain, liver, heart, fast muscle, and slow muscle. Values are means ± SE of 8–10 animals. Significance was determined with one-way ANOVA and, when $P$ was <0.05, is shown as connected groups.
and by different types of exercise. Changes due to exercise are subtle, suggesting that there are active stress-strain relationships between oxidant formation and scavenging during exercise.

It is well known that different forms of exercise result in different levels of oxidative stress. We have chosen treadmill running, which is a standard form of aerobic exercise used for rodent experiments. Treadmill running was chosen over swimming because swimming causes other forms of stress and aerobic responses are highly variable. Running involves a polymeric (eccentric) muscle component, and acutely exercised nontrained rats, therefore, suffered two stresses, oxidative and polymeric, the latter of which imposed a degree of muscle trauma.

No significant changes were found in levels of oxo8dG in the nuclear DNA in brain, liver, heart, kidney, and fast and slow muscles as a result of either chronic or acute exercise. This suggests either that exercise does not induce significant oxidant stress to cause DNA damage or that the activated repair system is sufficiently robust to prevent DNA damage from oxidative stress during exercise. Our results are consistent with a study in humans, which showed that acute exhaustive exercise does not cause changes in urinary excretion of oxo8dG and that oxo8dG is not accumulated by consecutive exercise, although it is sustained as long as the exercise is repeated (21, 37).

The HPLC-electrochemical detection method for measurement of oxidative DNA damage was improved in its sensitivity and precision after this work was completed (8).

As shown in Fig. 1, acute exercise induced a significant increase in MDA only in the liver. However, chronic exercise induced a decrease in the brain but an increase in heart and fast and slow muscles. An increased level of lipid peroxidation is the evidence most frequently cited in support of the involvement of oxidative stress in tissues (6, 7, 17). Davies et al.

Fig. 3. Chronic and acute exercise-induced changes in glutamine synthetase activity in the brain, liver, heart, fast muscle, and slow muscle. Values are means ± SE of 8–10 animals. Significance was determined with one-way ANOVA and, when $P$ was <0.05, is shown as connected groups.

Fig. 4. Chronic and acute exercise-induced changes in MDA (A) and glutamine synthetase activity (B) in the mitochondria of brain and liver. Values are means ± SE of 8–10 animals. Significance was determined with one-way ANOVA and, when $P$ was <0.05, is shown as connected groups.
Table 1. Antioxidants in rat tissues in the brain, liver, heart, kidney, fast muscle, and slow muscle as the result of chronic and acute exercise

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chronic</th>
<th>Acute</th>
<th>Control</th>
<th>Chronic</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascorbic acid</strong></td>
<td>510 ± 34.8</td>
<td>690 ± 37.9</td>
<td>530 ± 18.9</td>
<td>169.1 ± 21.8</td>
<td>171.0 ± 9.5</td>
<td>171.1 ± 9.8</td>
</tr>
<tr>
<td><strong>α-Tocopherol</strong></td>
<td>52.0 ± 4.3</td>
<td>51.8 ± 3.0</td>
<td>52.8 ± 5.7</td>
<td>42.2 ± 2.4</td>
<td>39.7 ± 2.6</td>
<td>33.0 ± 3.8†</td>
</tr>
<tr>
<td><strong>Total ubiquinol</strong></td>
<td>89.2 ± 14.4</td>
<td>109.2 ± 14.3</td>
<td>54.6 ± 6.7</td>
<td>247.7 ± 28.3</td>
<td>290.2 ± 30.3</td>
<td>203.3 ± 11.3</td>
</tr>
<tr>
<td><strong>Ubiquinol</strong></td>
<td>36.2 ± 13.6</td>
<td>43.9 ± 15.9</td>
<td>8.4 ± 3.4↑</td>
<td>46.1 ± 22.5</td>
<td>109.4 ± 29.6</td>
<td>13.2 ± 7.5</td>
</tr>
<tr>
<td><strong>Ubiquinone</strong></td>
<td>54.7 ± 3.5</td>
<td>60.9 ± 3.8</td>
<td>46.1 ± 4.8‡</td>
<td>200.2 ± 16.3</td>
<td>170.7 ± 9.9</td>
<td>190.1 ± 13.1</td>
</tr>
<tr>
<td><strong>Ubiquinol/ubiquinone</strong></td>
<td>0.66</td>
<td>0.72</td>
<td>0.18</td>
<td>0.23</td>
<td>0.64</td>
<td>0.07</td>
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<tr>
<td><strong>GSH</strong></td>
<td>2.84 ± 0.5</td>
<td>3.74 ± 0.4</td>
<td>1.83 ± 0.6</td>
<td>5.11 ± 0.47</td>
<td>5.53 ± 0.54</td>
<td>3.34 ± 0.28§</td>
</tr>
<tr>
<td><strong>GSSG</strong></td>
<td>0.029 ± 0.015</td>
<td>0.050 ± 0.032</td>
<td>0.020 ± 0.008</td>
<td>GSSG</td>
<td>0.308 ± 0.10</td>
<td>0.603 ± 0.12</td>
</tr>
<tr>
<td><strong>GSH/GSSG</strong></td>
<td>97.6</td>
<td>75.6</td>
<td>93.3</td>
<td>GSH/GSSG</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td><strong>Cysteine</strong></td>
<td>68.1 ± 7.6</td>
<td>99.3 ± 9.3*</td>
<td>63.9 ± 9.1</td>
<td>Cysteine</td>
<td>167.1 ± 14.3</td>
<td>181.6 ± 24.0</td>
</tr>
<tr>
<td><strong>Cystine</strong></td>
<td>118.3 ± 14.3</td>
<td>145.1 ± 13.3</td>
<td>94.7 ± 12.5</td>
<td>Cystine</td>
<td>611.4 ± 36.4</td>
<td>627.0 ± 28.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM in pmol/mg protein for α-tocopherol, ubiquinol, ubiquinone, and total ubiquinol; in nmol/mg protein for GSH, GSSG, cysteine, and cystine; and in nmol/100 mg wet tissue for ascorbic acid; n = 10 animals. Ubiquinol/ubiquinone, ratio of ubiquinol to ubiquinone; GSH/GSSG, ratio of GSH to GSSG; ND, not detectable; N/A, not applicable. Statistical difference was calculated by ANOVA. Significant * increase or † decrease, P < 0.05; Significant ‡ increase or § decrease, P < 0.01.

(5) have shown that exhaustive exercise induces a two- to threefold increase in free radical concentrations in the muscle and liver, accompanied by a significant increase in thiobarbituric acid-reactive substances. Therefore, chronic exercise decreases the MDA level in the brain, indicating a possible beneficial effect of chronic exercise. Because acute exercise increases the MDA level in the liver, it

Table 2. Summary of changes in tissue antioxidant levels after 8 wk of chronic and acute exercise

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Fast Muscle</th>
<th>Slow Muscle</th>
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<tr>
<td></td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td><strong>Ascorbic acid</strong></td>
<td>↑↑</td>
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<td>↑</td>
<td>↑</td>
<td>↓↓</td>
<td>↓↓</td>
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<tr>
<td><strong>α-Tocopherol</strong></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td><strong>Total ubiquinol</strong></td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
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<tr>
<td><strong>Ubiquinol</strong></td>
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<tr>
<td><strong>Ubiquinone</strong></td>
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<td><strong>GSH</strong></td>
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<td><strong>GSSG</strong></td>
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<tr>
<td><strong>Cysteine</strong></td>
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<tr>
<td><strong>Cystine</strong></td>
<td>↓</td>
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<td>↓</td>
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</tbody>
</table>

C, chronic; A, acute. Significant ↑↑ increase or ↓↓ decrease compared with sedentary controls, P < 0.05; significant ↑↑ increase or ↓↓ decrease, P < 0.01.
supports the hypothesis of the involvement of oxidative stress in tissues by acute exercise.

Somani et al. (34) showed that, in rat heart, acute exercise results in a larger increase in antioxidant enzyme activities than does chronic exercise. This difference was proposed to be a result of a compensatory mechanism to cope with the enhanced production of superoxide and oxyradicals during exhaustive exercise. The heart is an aerobic organ and has one of the highest mass-specific oxygen consumption rates in the body; therefore, the heart copes with high rates of oxidant formation and stress. Heart tissue has four times less superoxide dismutase (SOD) activity than the liver, and the catalase activity is also extremely low (33). Reflective of this stress is the accumulation of MDA in hearts of chronically exercised animals. A single bout of acute exercise may not actually cause significant oxidative stress to heart tissue because of the buffering actions of antioxidant systems, but the daily imposition of lengthy exercise bouts appears to leave traces of accumulated oxidative stress. This may also happen in muscles, as demonstrated by our MDA results from both fast and slow muscles. Our results also support the idea (11) that chronic exercise has dual effects: chronic exercise results in oxidant formation and oxidative stress and, perhaps as a consequence, induces antioxidant enzymes and antioxidant synthesis that minimize the effects of oxidants.

Oxidative damage to proteins is accompanied by an increase in the number of carbonyl residues, which can be assayed by the stable hydrazone derivatives formed with 2,4-dinitrophenyl hydrazine (14). The protein carbonyl assay has provided useful information about protein damage during aging and ischemia-reperfusion in the brains of gerbils (36). In the present experiment, we showed that chronic exercise significantly decreased protein carbonyls in the liver, suggesting that chronic exercise may enhance the antioxidant defense against oxidative damage to proteins. The increase in the slow muscle is not clear, possibly due to recruitment patterns or some unknown fiber-type-specific mechanism. MDA, a measure of oxidative damage to lipids, showed a positive correlation with protein carbonyls in the slow muscle in chronic exercise and in the brain and liver in acute exercise. This suggests that oxidants produced during exercise attack both lipids and protein.

Glutamine synthetase, a key enzyme in cellular nitrogen regulation, facilitates the uptake of excitatory neurotransmitter glutamate and is among the enzymes that are rapidly induced by stress hormone glucocorticoids. Decreased uptake of glutamate due to decreased glutamine synthetase activity could result in neurotoxic effects of abnormally prolonged N-methyl-D-aspartate receptor activation and generation of oxidants (32). Therefore, an increase indicates a stress response. Our results showed that MDA was positively correlated with glutamine synthetase activity in the fast muscle in chronic exercise and acute exercise. However, exercise resulted in a large increase in glutamine synthetase in slow muscle, reflecting the role of glutamine as a gluconeogenic regulator during stress (22). The evidence is also consistent with the hypothesis that increased glucocorticoids during stress may cause the generation of oxidants (18). The demonstration of significantly decreased glutamine synthetase activity in the liver by both acute and chronic exercise supports the hypothesis of an accumulation of oxidized glutamine synthetase protein. This loss is best accounted for by the known vulnerability of glutamine synthetase to oxidation, as was seen in aging studies (32). However, chronically exercised animals exhibit a higher glutamine synthetase activity than those acutely exercised in fast muscle, whereas the opposite is seen in slow muscle. Again, this may be due to recruitment patterns or to some unknown fiber-type-specific mechanism.

Davies et al. (5) showed that exhaustive exercise decreases mitochondrial respiratory control, causing an increase in free radicals and thiobarbituric acid-reactive substances and suggesting that endurance training induces free radical damage. The rate of free radical or oxidant generation in biological tissue is closely related to oxygen consumption: under physiological conditions, the majority of oxidants are produced in the mitochondria. Thus it seems likely that mitochondria, in addition to being the sources of oxidant production, also should be the targets of oxidants. Exercise, by increasing the oxygen consumption rate, may result in oxidative stress in mitochondria. This results in an increased production of oxidants, which could be detrimental to tissue (9, 12). The oxidants cause damage to mitochondrial membranes and cytoplasmic structures through the peroxidation of phospholipids, proteins, and nucleotides. We showed that acute exercise increases the MDA level accompanied by a decrease in glutamine synthetase activity in the liver mitochondria, as was seen in the tissue homogenate. Chronic exercise decreases the MDA level in brain mitochondria, indicating a possible beneficial effect of chronic exercise on brain functioning. The increase in antioxidant ascorbic acid and cysteine may contribute to a decrease in brain mitochondrial lipid peroxidation. It is also possible that the increased oxidative stress may cause mitochondrial genesis during stress.

Oxidative damage can cause irreversible cell damage through the loss of homeostasis functioning and the loss of mitochondria. However, the initiation of oxidative damage can be reversed by the stimulation of antioxidant enzymes, by maintaining an adequate concentration of intracellular antioxidants, and by repair systems. The increase in the levels of antioxidants leads to the scavenging of excess free radicals and thereby may contribute to a decrease in oxidative damage, whereas a decrease in the levels of antioxidants should lead to an increase in oxidative damage.

The brain utilizes 20% of the total oxygen consumed by the whole body at rest. The oxygen consumption rate increases 10- to 15-fold during exercise; however, the brain oxygen consumption is known to be constant during exercise. Thus it is unlikely that exercise poses an oxidative stress to brain. The lack of oxidative stress to brain during exercise is noteworthy because the brain could be susceptible to lipid peroxidation damage.
due to the high concentration of polyunsaturated fatty acids and lower levels of antioxidant enzymes (SOD, catalase, GSH-peroxidase) and GSH (7, 16). Chronic exercise caused an increase in the levels of antioxidants and antioxidant enzymes in the brain, which would help to protect the brain from oxidative damage. Consistent with our results, Somani et al. (35) studied the SOD, GSH-peroxidase, and ratio of GSSG to GSH in the brains of chronically exercised rats. They found that chronic exercise induced an increase in SOD in brain stem and in corpus striatum. The GSH-to-GSSG ratio significantly increased in the cerebral cortex and in the brain stem. A result of exercise training is to cope with oxidative stress: our results showing MDA decreases and ascorbic acid increases during chronic exercise provide further support to this notion.

Somani et al. (34) also showed that exhaustive exercise decreases the GSH concentration in liver and muscle and chronic exercise increases the GSH concentration in plasma and tissue. This may be due to the fact shown in our experiments that acute exercise causes a significant decrease in cysteine and cystine in the liver. Cystine is the rate-limiting precursor for glutathione synthesis. The decrease in GSH and cystine in the liver as a result of acute exercise may account for the increased MDA and protein carbonyls. We have shown that both MDA and protein carbonyl are negatively correlated with GSH and cysteine or cystine in acute exercise.

The differences among organs may be dependent on several factors, such as oxygen consumption, susceptibility to oxidants and to antioxidant enzyme activation, antioxidant levels, and other repair systems. Muscle and heart appear to respond to oxidative stress quite differently than other organs, such as brain and liver, possibly due to the difference in mitochondrial biogenesis and the occurrence of oxidant-induced degeneration.

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