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EFFECT OF TRAINING ON LIPID PEROXIDATION, THIOL STATUS AND ANTIOXIDANT ENZYMES IN TISSUES OF RATS

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Abstract : The effect of training on lipid peroxidation, thiol-status and certain antioxidant enzymes of glutathione system was studied in blood and tissues such as liver and skeletal muscle of rats. Exercise training was given by subjecting the rats to treadmill running. Training for a period of 6 weeks resulted in increased lipid peroxidation ($P < 0.001$) as indicated by thiobarbituric acid test and conjugated diene measurement in liver and muscle. Thiol levels (total and non - protein) were reduced. However, glutathione level in blood was increased and blood lipid peroxides were unaltered as a result of training compared to sedentary controls. Adaptive increases in three antioxidant enzyme activities were observed. The study indicates that training induces adaptation in the glutathione system.

Key words : training lipid peroxidation antioxidant enzymes thiol status

INTRODUCTION

It is well known that exercise increases oxygen metabolism and following physical exercise, significant oxidative damage is detected in different tissues in animals (1). The metabolic leakage from mitochondria are suggested to be an important source of oxygen derived free radicals which mediate lipid peroxidation (2). Without the intervention of the cells' antioxidant defence mechanisms, free radical–mediated lipid peroxidation, can lead to a loss of integrity of cell membranes and tissue damage. Our studies and others concerning the effects of physical exercise have reported an increase in blood lipid peroxidation and alterations in circulating antioxidants mainly glutathione, vitamin E and vitamin C in humans (3, 4).

Glutathione, a major non-protein thiol in living organisms, plays a central role in coordinating the body's antioxidant defence processes. Reduced thiols have long been reported to be essential for recycling of other antioxidants like vitamin E and vitamin C (5). Perturbation of glutathione status of a biological system has been reported to lead to serious consequences (6). Administration of thiol compounds such as glutathione, cysteine and methionine are shown to protect against oxidative stress in humans and animals (7).

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Physical exercise, a mechanism that creates oxidative stress to cells, is accompanied by a decrease in glutathione and increase in oxidised glutathione contents in blood, liver and muscle of rats (8). This demonstrates increased glutathione oxidation in tissues resulting in a reduced export rate of glutathione from tissues to plasma. Glutathione peroxidases are cytosolic enzymes involved in this oxidation. The cells maintain the cellular level of glutathione predominantly in the reduced state by fast reduction of oxidised glutathione to reduced glutathione using the nicotinamide adenine dinucleotide phosphate (NADPH) - dependent glutathione reductase. NADPH is regenerated from $NADP^+$ by the pentose pathway enzyme, glucose - 6- phosphate dehydrogenase. The efficiency of the glutathione defense system depends on the endogenous synthesis of glutathione and its replenishment from its oxidised form in tissues.

Physical training has been shown to enhance microsomal cytochrome p-450 dependent drug metabolism in rat liver (9). This is important since cytochrome p-450 acts as a monooxygenase and oxidase liberating oxygen radicals. Endurance training increases oxidative potential in muscle enabling the muscle to utilize more oxygen. Training also induces many skeletal muscle mitochondrial enzymes thereby increasing the endurance capacity of trained animals and humans (10). There is a wealth of data on the effect of training on blood glutathione, thiol status and lipid peroxidation in tissues (8, 11, 12). However, data on the influence of training on the enzymes of glutathione defence system in liver and muscle are sparse. Because training involves chronic intermittent increased exposure to oxygen, it is of interest to know whether glutathione system will be able to adapt to training.

In this, we report the effect of 6 week exercise training on lipid peroxidation, thiol status in blood, liver and skeletal muscle of rats. We have also measured the changes in the activities of enzymes involved in the glutathione defence system due to training. The data are compared with those of untrained control animals.

METHODS

1, 1',3,3' tetramethoxypropane, 2-thiobarbituric acid (TBA) and 5,5' dithio (bis)nitrobenzoic acid were obtained from Sigma Chemical Company, MO, St Louis, U.S.A. 1-Chloro 2,4-dinitrobenzene, glutathione, oxidised glutathione and NADPH were purchased from s.d. fine chemicals, Bombay, India. Other chemicals and solvents were of analar grade and were purchased from CDH Laboratories, New Delhi, India.

Animals

Sixteen adult male rats (body weights 100-120 g) were purchased and maintained in Central Animal House, Raja Muthiah Institute of Health Sciences, Annamalai Nagar. The animals lived under normal cage conditions with free access to solid food pellets (Lipton India Ltd) and tap water. Temperature, humidity and light rhythm were kept constant. The rats were acclimatized to the rodent treadmill by walking at 2m/min for 5 minutes/day for a week after which the rats were divided into two groups of eight animals.

I) Sedentary controls : Treadmill walking was continued at the rate of 2m/min for 5 minutes for 5 days/week for a period of 6 weeks. This was done to ensure equivalent handling.

II) Exercise training : These animals were exercised on the treadmill for 5 days/week for 6 weeks utilising an incremental exercise programme as shown in Table I.

TABLE I : Exercise training protocol for group II rats. The rats were exercised for 5 days in a week for 6 weeks.

Week	*Inclination (% grade)*	*Belt speed (m/min)*	*Total time (min/day)*
0 – 1	0	6	10
1 – 2	0	6	30
3 – 4	5	18	30
4 – 6	5	27	30

In the first two weeks the treadmill belt speeds were 6 m/min and the angle of inclination wa O° grade. Duration of run was 10 min/day for the 1st week and 30 min/day during the second. The angle of inclination and the belt speed were increased during the next two weeks as shown in Table I to obtain progressive levels of exercise.

Exercise training was stopped 48 hrs prior to sacrifice in order to avoid the acute effect of physical exercise. The final mean body weights of trained animals did not differ significantly from controls (sedentary control - 162.0 ± 12.0 g; exercise training - 160.0 ± 14.0 g). Blood sample were collected in heparinised test tubes. Liver and muscle were dissected out and washed in physiological saline. Blood was centrifuged at 3,000 rpm to separate plasma and portions of liver and kidney were used for the preparation of homogenates.

Assays

The measurement of thiobarbituric acid reactive substances (TBARS) was done in plasma (13) as an index of plasma lipid peroxidation. Tissue lipid peroxidation was determined (14) by treating aliquots of tissue homogenate with a mixture containing equal volume of 0.35% TBA, 15% trichloroacetic acid and 0.025N hydrochloric acid. The contents were boiled at 90°C for 20 min in a water bath. 1,1,'3,'3 tetramethoxypropane was used as the standard. The pink colour formed was measured at 535 nm after centrifugation. The levels of TBARS was expressed as μmol/mg protein.

Conjugated dienes content was measured in tissue lipid extracts (15). Aliquots of lipid extract were evaporated to dryness and suspended in methanol. The absorbance were read at 215 and 233 nm with solvent blank. The diene content was determined by computing the ratio of absorbance at 233 and 215 nm.

Glutathione in blood was determined after deproteinisation by the method of Beutler and Kelley (16). The method is based on the measurement of a yellow colour when dithionitrobenzoic acid is added to compounds containing sulfhydryl groups.

Total and non-protein sulfhydryl groups in tissues were determined according to the method of Sedlack and Lindsay (17).

Succinate dehydrogenase (EC 1.3.99.1) was determined by the method of Egtabrook and Pullman (18) using phenazine methosulphate and 2,6 dichlorophenol indophenol. Glutathione peroxidase (EC. 1.11.1.9) was assayed by the method of Rotruck et al (19). Assay involved measurement of glutathione consumed after carrying out the reaction for a specified period of time. Glutathione transferase (EC 2.5.1.18) was assayed by the method of Habig et al (20) using 1-chloro, 2,4-dinitrobenzene as substrate and glutathione reductase (EC 1.6.4.2) by the method of Horn and Burns (21). Protein content was measured by the method of Lowry et al (22).

Results obtained are presented as means ± SD for eight animals. Statistical significance of difference in means was analysed by Student's 't' test.

RESULTS AND DISCUSSION

Increased formation of TBARS and diene conjugates were observed in liver and muscle of trained rats compared to control in the present study ($P<0.001$). However, the level of TBARS was found to be unaltered in plasma in trained rats (Table II). The activity of succinate dehydrogenase (mmol succinate oxidised/min/mg protein) an enzyme involved in energy metabolism, was also increased ($P<0.001$) in the trained group (10.2 ± 0.1) compared with sedentary controls (6.3 ± 0.3). This suggests that physical activity induces oxidative stress by increased oxygen metabolism and enhanced formation and leakage of oxygen radicals from mitochondrial electron transport chain. The production of free radicals in cells is also found to be dependent on the intensity and duration of exercise (23). Alessio and Goldfarb (11) obtained no significant difference in liver lipid peroxidation in trained and sedentary group of rats at rest. In their study training was given by 20 min treadmill running for a 10 week period which was longer than that of our study.

TABLE II : The content of TBARS and conjugated dienes in control and trained animals. Values are means ± SD. (n = 8).

	Sedentary control	*Exercise training*
TBARS		
Plasma (nmol/ml)	2.16 ± 0.42	2.0 ± 0.36
Tissues (nmol/mg protein)		
Liver	0.66 ± 0.13	1.0 ± 0.002***
Muscle	0.33 ± 0.03	0.76 ± 0.06***
Conjugated dienes		
(A 233/215 nm)		
Liver	0.19 ± 0.02	0.25 ± 0.03***
Muscle	0.20 ± 0.01	0.30 ± 0.03***

Significant at *** $P<0.001$.

The reduction in plasma lipid peroxidation can be attributed to the body's antioxidant capabilities. Chronic aerobic training could reduce lipid peroxidation by augmenting the antioxidant mechanism. We therefore undertook to study the thiol status in blood, liver and muscle. Blood glutathione levels were found to be increased and the levels of total and non-protein sulfhydryl group were lowered in liver and muscle after training (Table III). The non-protein sulfhydryl group level in liver is decreased to nearly 30% and that in muscle to 12% of

control. Prolonged treadmill running could alter the total sulfhydryl content and intracellular redox state and this is presumed to be due to oxidation of essential thiols (24).

TABLE III : Glutathione in blood, total and non-protein sulfhydryl groups (T-SH and NP-SH) in tissues of control and trained animals. Values are means ± SD. (n = 8).

	Sedentary control	*Exercise training*
Glutathione (mg/dl) in blood	20.68 ± 4.80	30.6 ± 3.6***
NP-SH (μmol/mg protein)		
Liver	5.65 ± 0.46	4.0 ± 0.36*
Muscle	3.78 ± 0.24	3.16 ± 0.36**
T-SH (μg/mg protein)		
Liver	14.62 ± 0.63	12.80 ± 0.26***
Muscle	10.72 ± 0.87	9.13 ± 0.50***

Significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The non-protein sulfhydryl group is predominantly contributed by glutathione, cysteine and some minor thiols. Liver plays a major role in glutathione homeostasis and is the main export organ for glutathione. It is likely that non-protein sulfhydryl group levels in plasma are kept fairly high while liver is depleted as a result of training. Plasma glutathione levels are increased in well trained humans with increased muscle mass (12) suggesting that training increases glutathione export from skeletal muscle into circulation. Marin et al (25) observed increase in plasma and muscle glutathione in dogs after training for a year.

The decline in thiol status in liver and muscle is accompanied by profound alterations in the enzymic protective capacity. The activies of glutathione peroxidase, glutathione transferase and glutathione reductase are significantly increased ($P<0.001$) in liver and muscle. Glutathione peroxidase activity was elevated in liver (25%) and muscle (22%) of the trained group which may be due to adaptive response to remove the hydrogen peroxide. Gohil et al (4) reported that blood glutathione decreased about 60% and oxidized glutathione increases about 100% in trained humans. Chronic training is reported to be an effective inducer of muscle mitochondrial selenium - dependent glutathione peroxidase in rats (26). Elevated glutathione peroxidase in muscle after chronic exercise training in rats has been observed (24). The utilization of more glutathione in the peroxidase reaction accounts for the reduction in non-protein sulfhydryl group content.

Glutathione transferase plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. Increased activity of glutathione transferase in liver (35%) occurs as an adaptive mechanism due to increased uptake from plasma. Products of lipid peroxidation released into plasma are taken up by the liver and probably by muscle for detoxification.

The increase in the activity of glutathione reductase in trained liver (20%) and muscle (43%) indicates that the enzyme is capable of adaptation to training to replenish glutathione. Elevated activity of glucose -6- phosphate deydrogenase in muscle has been reported in endurance trained rats which reflects possible

adaptation to increased demand of reducing equivalents in the cytosol (27).

TABLE IV : The activities of glutathione peroxidase, glutathione transferase and glutathione reductase in control and trained animals. Values are means ± SD (n = 8).

	Sedentary control	*Exercise training*
Glutathione peroxidase		
Liver	11.06 ± 2.31	13.78 ± 2.02*
Muscle	11.61 ± 1.33	14.90 ± 1.17***
Glutathione transferase		
Liver	6.33 ± 1.44	8.55 ± 1.21***
Muscle	3.82 ± 0.98	4.75 ± 0.73**
Glutathione reductase		
Liver	23.33 ± 3.43	28.82 ± 3.23***
Muscle	6.84 ± 1.14	9.84 ± 0.84***

Significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Glutathione peroxidase – μmol glutathione consumed/min/mg protein

Glutathione transferase – μmol choloro dinitrobenzene - glutathione conjugate formed/min/mg protein

Glutathione reductase – μmol of NADPH oxidised/hr/mg protein.

The findings of the present study show that in the resting state of normal sedentary animal, adequate protective mechanism operate to keep lipid peroxidation to minimum. A six week physical training causes a depletion in non-protein sulfhydryl groups in tissues such as liver and muscle accompained by an increase in lipid peroxidation. However, training provides possible adaptation by increasing the activities of glutathione peroxidase and transferase which are involved in the removal of toxic peroxides and glutathione reductase which is involved in the replenishment of glutathione. The level of glutathione in blood was increased and TBARS concentration in plasma was not influenced by training. During prolonged exercise glutathione is transported from liver and muscle to blood and other tissues as a source of antioxidant protection. It is also possible that enzymes involved in the glutathione synthesis are also stimulated in this condition. An increase in glutathione synthesis was observed in the muscle of trained animals (25). Other defensive components such as vitamin E, vitamin C and β carotene may also have contributed to the regulation of lipid peroxidation in plasma. Training has been shown to partially compensate age dependent decrease in plasma glutathione (12).

Increased comsumption of thiol compounds may prove beneficial under this condition to overcome the decline in thiol status in liver and muscle. Novelli et al (28) have reported that exogenous glutathione at a dosage of 500–1000 mg/day increased endurance to swim in experimental mice.

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