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Effect of acute and regular exercise on antioxidative enzymes, tissue damage markers and membran lipid peroxidation of erythrocytes in sedentary students

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Abstract: 15 healthy sedentary men, 19-25 years old an did not have any programmed physical activity, were studied. The subjects were asked to run submaximal 15-20 min every day for 5 weeks. Erythrocyte lipid peroxidation, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and glucose and uric acid levels were determined in blood samples which were taken before and immediately after acute exercise and after the end of 5-week training program. Malonaldialdehyde (MDA), sing of lipid peroxidation, creatin phosphokinase (CK), CK-MB, lactate dehydrigenase (LDH) concentrations increased ($p<0.0001$, $p<0.01$, $p<0.05$, $p<0.05$, respectively) and GSH-Px and SOD activities decreased significantly ($p<0.0001$ and $p<0.05$, respectively) after acute exercise. Although MDA level after the 5 week training program was lower than the MDA level after acute exercise period, it was still higher than sedentary period ($p<0.01$). GSH-Px activity after the 5-week training program was significantly higher than this of sedentary period ($p<0.0001$). SOD activity after 5-week training program was also higher than this of sedentary period but it was not statistically significant ($p>0.05$). We also found that uric acid and glucose levels increased immediately after acute exercise ($p<0.05$), but there was no significant differences between uric acid and glucose levels of sedentary and training period. It is concluded that acute exercise causes oxidative stress in sedentary men. Thus irregular exercise, "weekend physical activities", may be harmful in contradiction with the common concept. On the other hand, regular exercise may prevent this deleterious condition by decreasing lipid peroxidation, augmenting antioxidant system and decrease the exercise-induced muscle damage.

Key Words: Sedentary - exercise - lipid peroxidation - antioxidants- damage

Introduction

Reactive oxygen species are the natural products of aerobic organisms. It has been known that oxygen consumption and metabolic activity are elevated due to increased muscle contraction during physical activity and exercise (1, 2, 3, 4). Elevated oxygen consumption during increased metabolic activity increases the electron leakage from the mitochondrial transport system and causes an increase in oxidative stress, lipid peroxidation and generation of free radicals such as; superoxide, hydrogen peroxide and the hydroxyl radical (5, 6).

Recently, much attention has been paid to the role of lipid peroxidation and antioxidant system in exercise and physical training. Many studies (1, 2) have reported that acute submaximal exercise increases exercise-induced lipid peroxidation. Regular physical training, on the other hand, causes an increase in the antioxidant system and a reduction in lipid peroxidation (5, 7, 8). It has been suggested that (9, 10) individuals who exercise regularly, placing a constant oxidative stress on the muscles and other cells, have an augmented antioxidant defence system to reduce exercise-induced oxidative risk. However, it is very difficult to reduce te exercise-induced oxidative threat in didividuals who do not exercise regularly.

Enzymatic and non enzymatic antioxidant functions regulate free radical reactions by scavenging, repairing, quenching and chain breaking reactions. Under normal physiological conditions an organism protects cellular homeostasis by an antioxidant network which is responsible for the coordination and integration of the various cellular defence systems that are not well understood (11). The elevated generation of free radicals in the cell causes poly unsaturated fatty acid (PUFA)

peroxidation in membranes. This affects the membrane permeability and ion balance of the cell. It this way all cellular components, especially membrane phospholipids can be damaged (11, 12). Because erythrocytes, vital for metabolism and circulation, do not have a nucleus or mitochondria, they are very sensitive to oxidative stress (1, 2).

The antioxidant defence system is also very important for sudden oxidative stress that occurs upon exercise. Up until now most studies have examined the mechanisms of the antioxidant defence system (1, 5, 6). Although acute exercise may induce oxidative stress in untrained individuals, after exercising regularly, the antioxidant system can develop to reduce oxidative stress (13). In this report, we have investigated the effect of acute exercise and regular physical trdaining on lipid peroxidation and antioxidant defence mechanisms in sedentary males.

Materials and Methods

Fiftheen healthy male subjects who did not any programmed physical activity for at least 6 months beforeexperiment were studied. Physical characteristics of individuals are shown in Table I. Individuals did not take any vitamin, antioxidant or drug during the study period. They were also stoopped any physical activity for at least one week before the study. They were asked to run submaximal 20 min very day for five weeks. Erythrocyte malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), serum creatin kinase (CK), CK-MB, lactate dehydrogenase (LDH), uric acid and glucose levels wereeasured in blood samples which werecollected three times; before (sedentary period) and immediately after an acute exercise and after the end of the 5-week exercise program.

Table 1. Mean characteristics of subjects.

Number and sex	15 men
Age (years)	21 (19-25)
Height (cm)	173.86 (168-180)
Weight (kg)	68.86 (61-80)
BMI	22.77 (20.66-26.12)
Running state before (minute/day)	-
Training program (minute/day)	18 (15-20)
Cigarette smoking and alcohol taking	-

Determination of MDA and Enzyme Activities

The eryhrocyte MDA level was measured using a thiobarbituric acid reactive substance (TBARS) method. Briefly, phosphate buffer (pH 7.4), butylated hydroxytoluene (BHT) and 30% thrichloroacetic acid (TBA) were added into the erythrocyte packs. After incubating two hours at -20°C, the mixture was centrifuged (2000 g) for 15 min at 4°C. The supernatant was collected and 0.1 mol EDTA and 1% thiobarbituric acid (TBA) were added. Tubes with teflon-lined screw caps were incubated at 100°C in water bath for 15 min and cooled to room temperature. The mixture was examined in a spectrophomoter (Novaspec II Pharmacia-Biotech) with excitation-emmision wavelengths of 532 and 600 nm. Results were expressed as nM/10^{10} erythrocyte.

Erythrocyte GSH-Px and SOD activities were determined in an otoanalyser (Technicon RA-XT 9219104) using commercial kits (Randox-Ransod Lot No 8136 C and Randox-Ransel Lot No 7843 C). Serum uric acid and glucose levels were also measured in the otoanalyser using commercial kits (Biotrol).

Statistical analysis

Data were expressed as mean±standard deviation (SD) and analyzed using nonparametric paired *t*-test

Table 2. The data of sedentary, acute exercise and training program. Data is expressed as mean±standart deviation (SD).

	sedentary	acute period	after taining
MDA (nM/10 10 eryt)	1.281±0.084	2.023±0.140*	1.482±0.077**
SOD activity (U/grHb)	1965±267	1417.6±95.6***	20.80±148
GSH-Px activity (U/grHb)	72.84±1.88	65.82±1.63*	79.87±1.83*
Uric acid (mg/dL)	3.900±0.246	4.475±0.266*	4.242±0.302
Glucose (mg/dL)	89.92±2.71	108.50±4.02*	88.67±1.34
CK (U/l)	138.5±25	161.4±27***	150.4±25.6
CK-MB (U/l)	20.75±2.15	25.17±2.16***	19.33±2.44
LDH (U/l)	170.8±11.6	188.58±9.37***	187.2±10.8***

*p<0.0001 **p<0.01 ***p<0.05

(Wilcoxon). Pearson' correlation method was used to test the relationship among means.

Results

Changes in erythrocyte MDA, SOD, GSH-Px, serum CK CK-MB, LHD, uric acid and glucose levels are presented in Table II. We found that MDA, serum tissue damage markers, uric acid and glucose levels increased (respectively $p<0.0001$, $p<0.01$, $p<0.05$), SOD and GSH-Px activities decreased ($p<0.001$, $p<0.05$) immediately after acute exercise. However, at the end of 5 week period. MDA level was lower ($p<0.05$) than acute period but it was still higher than the MDA level of sedentary period. CK-CK-MB and LDH levels returned to sedentary. On the other hand, GSH-Px level was highest at the end of the 5-week period. There was no significant difference between SOD, glucose and uric acid levels sedentary period and those of after training. Therewas positive correlation between erythrocyte MDA level and serum glucose concentration after acute exercise ($r=0.534$ $p<0.05$).

Discussion

The present study indicated that acute submaximal exercise increases eryhthrocyte membrane lipid peroxidation. We found that MDA level of after training was lower than this of acute exercise and higher than the MDA level of sedentary period. This indicated an occurrence of an antioxidant defense system with a regular training that reduced the MDA level and the damage caused by free radicals. We though that if we let the subjects to continue theri physical training for morehan five weeks, MDA level could have returned to sedentary period level. Our result was consistent with the results of followings. Yagi (14) suggested that MDA level decreases regularly during nine month exercise period. Lovlin et al (15) showed that plasma and erythrocyte MDA level increases after acute exercise. Roberton et al. (13) demonstrated that MDA level in sedentaries is higher than moderate and elite athletes after acute exercise.

During any exercise form there is an increase in the requirement of oxygen to speed metabolism (1). There is a correlation between free radical production of organisms and metabolic rate, tissue blood circulation and oxygen consumption (3). It is known that during exercise VO_2 is elevated 10- to 200- fold above resting level (16). This elevation in oxygen consumption has been shown to be indicator of oxidative stress and reactive oxygen species (17). The decrease in antioxidant enzyme activities after acute exercise might be due to their use against the free radicals and their inhibition by free radical species (18). It is known that hydrogen peroxide can inhibit SOD activity by reducing Cu^{+2} to Cu^{+1}'in SOD (8). Increased rate of reduced Cu^{+1} can act promoter of hydroxyl by Haber-Weis reaction (19, 20).

Studies have shown that glutathione concentration in eryhrocytes may be altered after exercise. We found that GSH-Px level decreased after acute exercise and increased after 5-week regular training program. Our result was consistent with the result of Duthie et al. (9) who reported that erythrocyte antioxidant status (GSH) reduces immediately after an exercise and erythrocytes become susceptible to lipid peroxidation. Kanter et al. (13) reported that the protective antioxidant capacity of blood is enhanced in endurance runners and the improvement in the blood antioxidant potential may be related to the physical activity.

In the present study we alsoinvestigated serum glucose and uric acid concentrations as an antioxidant scavencges hydroxyl radical (11). These parameters increased immediately after acute exercise and returned to sedentary levels at the end of training program. Gohil et al. (22) also observed that after acute exercise glucose level was elevated. This increase is related pentoz shophate shunt which increases glucose oxidation induced NADPH requirement (22). Glutathione reductase catalyses the reaction of GSSG to GSH and needs NADPH for it's activity (11). The major source of NADPH is the pentoz phosphate shunt provided by direct oxidation of glucose (12, 22). For this reason the NADPH requirement during acute exercise is supplied by glucose releasing glycogen sources (11).

Thiol group, especially albumin, seruloplasmin and uric acid, oxidaction is elevated in organism exposed to oxidative stress due to increased reactions of oxidation and reduction (12, 23, 24). After acute exercise only uric acid changes was noted suggesting that this change may be related to purine metabolism (13). Uric acid as an antioxidant plays a role on preventing ascorbic acid oxidation, scavenging peroxyl, hydroxyl, superoxyde radicals and binding transition metals for preventing the radical reproduction reactions (11). In this study acute exercise uric acid concentration was found significantly higher than that of sedentary and training program. After acute submaximal exercise, decreased antioxidant enzyme concentrations of erythrocytes was probably due to an increase in the usage and destruction of them in

intracellular defense sytstem.

In this study, acute physical activity caused oxidative sterss, lipid peroxidation and inefficiency of antioxidant defense system. Exercise program 15-20 min/day for five weeks, appeared to reduce lipid peroxidation and caused changes in lipid parameters and augment antioxidant defense system. From these result we concluded that regular exercise makes individuals stronger against oxidative stress and provides a healthy life.

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