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THE EFFECT OF MODERATE SWIMMING EXERCISE ON ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION LEVELS IN CHILDREN

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Abstract : Strenuous exercise is characterized by increased oxygen consumption and the disturbance between intracellular pro-oxidant and antioxidant homeostasis. Although there are several studies related to an increase in antioxidant enzyme activity in adults doing exercise, the effect of regular exercise on antioxidant enzymes and lipid peroxidation levels has not been examined in children. In our study, the effects of a four week regular swimming exercise on antioxidant enzymes (superoxide dismutase and glutathione peroxidase) activities in erythrocytes and plasma thiobarbituric acid reactive substances (TBARS) levels, an indicator of lipid peroxidation, were investigated in previously untrained healthy children.

We found that superoxide dismutase (SOD) activity was increased significantly following a four week swimming course (from 581.1 ± 146.2 to 791.1 ± 221.9 U/gHb, $P<0.01$). Conversly, plasma TBARS levels were decreased from 1.1 ± 0.4 to 0.9 ± 0.3 nmol/ml ($P<0.05$). Glutathione peroxidase (GPx) activity appeared to increase following swimming course, albeit not statistically significant (from 45.5 ± 16.5 to 50.3 ± 14.8 U/gHb). According to these findings, regular swimming exercise has beneficial effects on antioxidant defence in healthy children.

Key words : children, swimming exercise, lipid peroxidation, antioxidant enzymes

INTRODUCTION

A major internal threat to cellular homeostasis of aerobic organisms arises from reactive oxygen intermediates and the by products generated from oxygen metabolism. Ironically, these reactive oxygen species (ROS) are derived from normal physiological and metabolic processes that are essential to cell (1). During this process, most oxygen molecules are reduced to water, but a fraction of oxygen (2–5%) is univalently reduced to various intermediates representing the electron reductants of oxygen one, superoxide ($O_2^{\cdot-}$), two, hydrogen peroxide

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(H_2O_2); and three, hydroxyl radical (OH·) (2). Hydroxyl radical reacts at great speed with almost every molecule found in living cells, including DNA, membrane lipids and carbohydrates. This reaction results in lipid peroxidation. These lipid peroxides readily decompose to liberate highly reactive carbonyle fragments such as malondialdehyde. Gutteridge demonstrated that malondialdehyde (MDA) was the major species responsible for thiobarbituric acid reactive substances (TBARS) (3). Aerobic organisms would not survive without protective mechanisms counteracting the detrimental effects of ROS. Thus, higher organisms have developed effective antioxidant systems during the course of evalution (1, 2). The enzyme superoxide dismutase (SOD), present in red blood cells, catalysis the conversion of the O_2^- to H_2O_2 and O_2. The H_2O_2 is then decomposed by the glutathione peroxidase (GPx) or by catalase (CAT), both also present in the red blood cells (4). These antioxidant defense systems preserve homeostasis for normal cell function at rest and perhaps during mild oxidative stress. When ROS production is excessive, or the antioxidant defence is severely compromised due to nutritional deficiency or biochemical inhibition, extensive cell and tissue damage may occur, leading to various pathogenic conditions and/or aging (5).

Strenuous exercise is characterized by the disturbance between intracellular pro-oxidant and antioxidant homeostasis. An increase in energy demand during physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues. The rate of oxygen uptake by the body during exercise may increase by 10–to–15 fold. Oxygen flux in the active peripheral skeletal muscle tissue may, however, increase by ~ 100-fold with an ~ 30 fold increase in blood flow and 3-fold increase in arteriovenous oxygen difference (6). Although there are several studies related to an increase in antioxidant enzyme activity in adults doing exercise (5, 7, 8), the effect of regular exercise on antioxidant enzymes and lipid peroxidation levels has not been examined in children.

In present study, the effects of a four week swimming exercise on lipid peroxidation levels and antioxidant enzymes such as superoxide dismutase and glutathione peroxidase have been studied in healthy children.

METHODS

Twelve previously untrained healthy children aged between 6–11 years (8.3 ± 1.7) were enrolled the study. The experimental procedures were explained to the children and their family and voluntary informed consent was obtained. Blood samples were collected before the initiation of the swimming course and 24 hours after the termination of the course in order to minimize the residual effect of the last exercise. Heparinized venous whole blood was used for erythrocyte antioxidant enzymes (superoxide dismutase and glutathione peroxidase) activities and plasma thiobarbituric acid reactive substances (TBARS) levels as an indicator of lipid peroxidation. The swimming exercise program exercise program was consisted of a base education of swimming twice daily

(between 10–11 A.M. and 3–4 P.M.) and a technical swimming education (between 11–12 A.M. and 4–5 P.M.) for 4 weeks.

Determination of SOD activity

Erythrocyte SOD was determined with a Randox test combination (Randox, Crumlin, U.K.). Xanthine and xanthine oxidase were used to generate superoxide radicals reacting with 2-(4-iodophenyl) 3-(4-nitrophenol)-5 phenyl tetrazolium chloride (INT) to form a red formazan dye. Concentration substrates were 0.075 µmol for xanthine and 0.037 mmol for INT. Superoxide dismutase inhibits this reaction by converting the superoxide radical to oxygen. A SOD unit inhibites the rate of reduction of INT by 50% in a complex system with xanthine and xanthine oxidase. Because of the small linearity range of the test, the sample was diluted so that the percentage of inhibition fell between 30% and 60%. A standard curve was prepared, using the kit standard, and the value for the diluted sample was read from this curve. SOD activity was measured at 505 nm on a Shimadzu UV–1201v spectrometer on hemolysates of washed erythrocytes obtained by centrifugation of whole blood. Results were expressed in SOD U/g hemoglobin.

Determination of GPx activity

GPx was also determined with a Randox test combination. (Randox, Crumlin, UK). GPk catalyses the oxidaton of glutathione (at concentration 5 mmol) by cumene hydroperoxide according to the method of Paglia et al (9). In the presence of glutathione reductase (at concentration $> 0.75 \times 10^3$ U) and 0.35 mmol NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to NAD^+. The decrease in absorbance at 340 nm was measured at 37°C. The assay was performed on a hemolysate of washed erythrocytes obtained from the mixing of 0.05 ml whole blood with 1 ml cold diluting agent and 1 ml Drabkin reagent. The GPx unit was defined as the enzyme activity necessary to convert 1 mmol of NADPH to NADP in 1 minute. The results were expressed in GPx U/g hemoglobin.

Determination of TBARS

In a modified Yagi method, (10) 0.05 ml of blood was sampled with a pipette for determination of blood cells and placed in 1.0 ml of normal saline in a centrifuge tube. After gently shaking, the tube was spun at 3000 rpm for 10 minute and 0.5 ml of the supernatant was transferred to another centrifuge tube. The 4.0 ml of N/12 H_2SO_4 was added to this solution and the mixture was shaken gently. Then 0.5 ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 minutes, the mixture was centrifuged at 3000 rpm for 10 min. After the supernatant was discarded, the sediment was mixed with 2.0 ml of N/12 H_2SO_4 and 0.3 ml of 10% phosphotungstic acid and the mixture was centrifuged. The sediment was suspended in 4.0 ml of distilled water and 1.0 ml of thiobarbituric acid (TBA) reagent was added. The reaction mixture was heated for 60 min at 100°C in a water bath. After cooling with tap water, 5.0 ml of n-butanol was added and the mixture was shaken vigorously, then centrifuged at 3000 rpm for 15 min. Finally, the n-butanol layer was taken for spectophotometric measurement at 532 nm. A standard curve was prepared

using the MDA standard (1, 1, 3, 3-tetraethoxypropane) and the value for the plasma was read from this curve. The results were expressed as nmol/ml.

Statistical analysis

Results before and after the swimming exercise were compared with Wilcoxon signed rank test.

RESULTS

In this study, we found a statistically significant increase in SOD activity following a four week swimming course (from 581.1 ± 146.2 to 791.1 ± 221.9 U/gHb respectively, P<0.01). GPx activity appeared to increase following swimming course, albeit not statistically significant (from 45.5 ± 16.5 to 50.3 ± 14.8 U/gHb, P>0.05). In this research, there was a statistically significant decrease in plasma MDA levels following the swimming course (from 1.1 ± 0.4 to 0.9 ± 0.3 nmol/ml, P<0.05).

DISCUSSION

Exercise and the subsequent production of reactive species may overwhelm the ability of the endogenous and exogenous antioxidant defences leading to increased lipid peroxidation (11). In the past decade, evidence has accumulated showing that unaccustomed and strenuous exercise may manifest and imbalance between ROS and antioxidant defences, resulting in an oxidatively stressful environment in the body (12). We found that SOD activity was increased significantly following a four-week swimming course in children. Conversly, plasma TBARS levels were decreased. GPx activity appeared to increase following swimming course, but not statistically significant. The effect of regular exercise on antioxidant enzymes and lipid peroxidation levels has not been examined in children. It is known that an increase in antioxidant enzyme activity occurs during training in adults (5, 7, 8). However, a controversy still exists as to which enzyme and under what condition an enzyme can be activated. (7, 8, 13, 14). Available data suggest that each of the antioxidant systems may have a different response to acute and chronic exercise depending upon their biochemical and molecular mechanism of regulation (15). Ohno et al did not find any significant changes in antioxidant enzymes after 30 minutes of training (4). Although plasma Mn-SOD level was higher compared to sedanters for runners, it was not found any significant difference in Cu-Zn SOD level (16). Mena et al researched antioxidant enzymes in 3 groups of control, amateur and professional group. At rest, SOD, GPx and CAT was found to be significantly higher than control (17).

Although the effect of acute exercise in animals has uniformly resulted in an increase in tissue lipid peroxidation, the data on lipid peroxidation in humans in sparse and not at all in agreement (7). Kanter et al have shown that heavy running exercise caused a marked increase in plasma MDA levels in human (18). Sumidea et al (19) showed that an increment in MDA levels with maximum intensity exercise in bicycle ergometry for untrained persons. Vinikka et al did not observe any changes in MDA amounts with the same procedure (20). Jenkins et al also stated that MDA levels were decreased related to training adaptation (8).

There is some controversy as to whether high SOD activity is desirable, because the dismutation product, H_2O_2, has a higher diffuseability and a longer half-life, and may become a source of $OH^{\cdot}$ production (21). However, $O_2^{-\cdot}$ can generate H_2O_2 independent of SOD in the cell by attacking the ion-sulfur protein (4Fe-4S) cluster, thereby releasing Fe(II), which sets the stage for $OH^{\cdot}$ production via the Fenton reaction or via Haber-Weiss reaction (2). Radak et al show that down-regulation of SOD by high-altitude exposure is associated with an increased lipid peroxidation in skeletal muscle, whereas supplementation with an SOD derivative (SM-SOD) attenuated plasma lipid peroxidation in rats that were run to exhaustion (22). In our study, we found an increase in SOD activity with a subsequent decrease in lipid peroxidation levels confirming this suggestion.

In conclusion, these findings showing possible beneficial effects of a four week swimming exercise on antioxidant enzyme dismutase with a subsequent decrease in MDA in healthy children. Research studies on exercise in this aspect, can make a great contribution to a healthy long life.

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