Research Paper Biochemistry

Oxidative stress and antioxidant status of endurance athletes

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ABSTRACT

Status of lipid peroxidation and activity levels of Catalase (EC 1.11.1.6) & Superoxide dismutase (SOD, EC 1.15.1.1) in male athletes and sedentary population was investigated. Both the groups' i.e. sedentary (S group) and athlete (A group) consist of thirty subjects each. Group A includes endurance athletes of 20KM Walk involved in regular physical activity while the group S includes sedentary subjects those who are not involved in regular physical activity. The blood serum was used for activity levels of enzymes and other parameter. The serum catalse, superoxide dismutase & malondialdehyde were significantly (P< 0.05) high in group A (athletes) as compare to group S (sedentary). Due to regular physical activity both oxidative stress and antioxidant capacity increased in athletes as compared with sedentary controls.

Keywords : Oxidative stress, Antioxidant status, Malondialdehyde, Catalase, Superoxide- dismutase.

INTRODUCTION

Cells continuously produce free radicals and ROS as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids. Exercise can cause an imbalance between ROS and antioxidants, which is referred to as oxidative stress (Urso & Clarkson, 2003). While regular exercise training is associated with numerous health benefits, it can be viewed as an intense physical stressor leading to increased oxidative cellular damage, likely due to enhanced production of ROS (Bloomer et al, 2005). The high production of reactive oxygen species may be responsible for a series of physiological and biochemical changes that occur during exercise (Alessio, 1993). It has been reported that strenuous physical exercise produces a decrease in antioxidant levels and an increase in the markers of lipid peroxidation in target tissues and blood (Davies et al, 1982; Vasankari et al, 1995). A proliferation of these free radicals can cause a decrease in the function of affected cells and can result in a decreased ability of muscles to maintain work. Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase (EC 1.11.1.6) is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al, 1996). Superoxide dismutase (SOD, EC 1.15.1.1) is a class of enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation. Lipid peroxidation is used as an indicator of oxidative stress in cells and tissues (Yagi, 1998; Armstrong & Brown, 1994). Lipid peroxides, derived from polyunsaturated fatty acids are unstable and decompose to form a complex series of compounds such as malondialdehyde. The aim of present study is to know the status of lipid peroxidation and antioxidant status of Indian endurance athletes in comparison to age and sex matched sedentary counterparts.

Methodology

Two groups comprising 30 male endurance athletes, (average age ± SD, 23.18 ± 1.83 years) and 30 male sedentary controls (average age ± SD, 24.55 ± 1.8 years) were studied. The endurance athletes belong to 20 Km Walk events of athletics. On the other hand the control subjects were sedentary do not involve in regular physical exercise, but are healthy. Persons with any type of illness and smokers were excluded from the study. A prior written consent was taken from each participant that they are voluntarily ready for this research work. Five (5) ml of venous blood was withdrawn from each subject after an overnight fasting of 12 hours. Malondialdehyde, activities of catalase and superoxide dismutase in serum was determined by a colorimetric method employing Cayman's assay kits. The results obtained by all the parameters were analyzed by t-test to find out the difference between the two groups.

Cayman's Catalase Assay Kit utilizes the per-oxidative function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color. The assay temperature is 25°C and catalase activity is measured at 540 nm. When a series of 45 catalase measurement were performed on the same day, the intra-assay coefficient of variation was 3.8%. When a series of 45 catalase measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 9.9% (Johansson and Borg, 1988).

Cayman's Superoxide Dismutase (SOD) Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The assay temperature is 25°C. SOD activity is measured at 530-540 nm. When a series of 60 SOD standard measurements were performed on the same day, the intra- assay coefficient of variation was 3.2%. When a series of 60 SOD standard measurements were performed on five different days under the same experimental conditions, the inter- assay coefficient of variation was 3.7% (Marklund, 1980).

Cayman's TBARS (Thiobarbituric acid reactive substances) Assay Kit provides a simple, reproducible, and standardized tool for assaying lipid peroxidation in serum. The MDA-TBA adducts formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions is measured at 530-540 nm. The assay is performed at room temperature. When a series of ten human plasma/serum samples were assayed on the same day, the intra-assay coefficient of variation was 5.5%. When a series of eight human plasma/serum samples were assayed on seven different days under the same experimental conditions, the inter-assay coefficient of variation was 5.9% (Ohkawa, et. al., 1979; Draper, et. al., 1993).

Results

Table 1 Mean SD and t value of malondialdehyde and antioxidants of both the groups

BIOCHEMICAL PARAMETERS	Group S (N=30)	Group A (N=30)	t-Value
	Mean±SD	Mean±SD	
MALONDIALDEHYDE (nmole/ml)	15.83±6.37	46.86±8.96	15.71*
CATALASE (nmole/min./ml)	9.30±2.95	22.97±5.55	**12.10***
SUPEROXIDE DISMUTASE(U/ml)	0.09±0.05	0.21±0.18	**3.63***

*Significant at 5% Level (2.00)

As shown in table no.1 there is significant difference found in the level of malondialdehyde between sedentary (15.83±6.37) and athlete group (46.86±8.96) at 5% level of significance (15.71) .The level of catalase between sedentary (9.30±2.95) and athlete group (22.97±5.55) has shown significant difference at 5% level of significance (12.10). The level of superoxide dismutase between sedentary (0.09±0.05) and athlete group (0.21±0.18) has shown significant difference at 5% level of significance (3.63).

Discussion

The results of the present study show that the athlete group have significantly high values of catalase, superoxide dismutase and amount of lipid peroxidation product i.e. malondialdehyde. That means there is a significant increase in lipid peroxidation and antioxidant status of athletes, which is supported by the various studies showing that long-term habitual exercise always creates oxidative stress while developing the antioxidant system (Powers & Lennon, 1999; Ji, 1995). Powers & Lennon (1999) proposed that the exercise causes an increase in the production of reactive oxygen species. Studies suggest that regular exercise can increase the antioxidant capacity (Kahraman, et al, 2003; Alessio, 1993; Close et al, 2006; Medved, et al, 2004). In the present study the subjects were athletes, they had been exercising regularly for several years and took part in competitive sport. We are of the opinion that this may be a factor in the increased enzymatic antioxidant defense in these athletes as compare to sedentary group. Athletes belong to long endurance activity, therefore during physical activity fat metabolism is dominated and this might be a reason for increased lipid peroxidation in athletes. Several studies have shown that lipid peroxidation produced by exercise is higher in relation to the control group (Cooper et al, 2002; Groussard et al, 2003). However; another study has found no difference in lipid peroxidation (Ookawara et al, 2003).

CONCLUSION

Indian endurance athletes have increased level of malondialdehyde and antioxidant enzyme markers as compare to sedentary counterparts. Increase in lipid peroxidation and antioxidant status of Indian athletes may be due to long-term regular and endurance type of physical activity.

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