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Full Length Research Paper

Oxidative stress and antioxidant status in sportsmen two hours after strenuous exercise and in sedentary control subjects

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Regular exercise has well documented health benefits. However, exercise can also induce imbalance between oxidant stress and antioxidant status. This study was designed to investigate the serum lipid profile and non-enzymatic antioxidants markers (serum uric acid and albumin) as well as lipid hydroperoxide (a marker of oxidative stress) in 39 sportsmen after 2 h of strenuous training exercise and also in 24 sedentary age-matched males who served as controls subjects. Total cholesterol, LDL- and HDL-cholesterol were higher in the sportsmen but the difference was only significant in total and LDL-cholesterol ($p<0.05$). Triacylgylcerol was significantly lower ($p<0.05$) in the sportsmen. In addition, serum uric acid level was higher in the sportsmen ($p<0.05$), but the albumin values were not significantly different. The lipid hydroperoxide was significantly higher in the sportsmen ($p<0.05$) suggesting higher oxidative stress. It is possible that the higher uric acid, HDL-cholesterol and comparable albumin levels could ameliorate oxidative stress in the sportsmen. Since exercise remains a key aspect of a healthy life, a better knowledge on how to balance oxidative stress and antioxidant status during exercise would help to promote good health.

Key words: Sportsmen, antioxidant status, serum lipids, uric acid, albumin, lipid hydroperoxide.

INTRODUCTION

Exercise remains a key aspect of a healthy lifestyle. However, strenuous physical exercise results in an enhanced uptake of oxygen leading to increased metabolism, which can increase the production of reactive oxygen species (ROS) (Kelle et al., 1998). These radicals trigger chain reactions in the fatty acids of phospholipids present in the lipid bilayer, leading to membrane lipid peroxidation and the loss of membrane bilayer organization, which is necessary for membrane-bound enzyme and receptor function (Berdanier, 1988; Evans, 2001). Generally, aerobic organisms produce reactive oxygen species during normal respiration and inflammatory conditions. But exercise can create an imbalance between oxidant and antioxidant levels, a situation know as oxidative stress (Leeuwenburgh and Heinecke, 2001).

Without the intervention of the cell's antioxidant defense mechanisms, free radical-mediated lipid peroxidation can lead to the loss of the integrity of cell membrane and tissue damage (Maxwell, 1995; Clarkson and Thompson, 2000). The efficiency of the antioxidant defense system depends on the balanced diet. Multiple enzymatic and non-enzymatic antioxidant defense sys-tems are present in cells to protect the membranes and other cell organelles from the damaging effects of free radical reactions (Evans, 2001). Thiol groups especially albumin and uric acid are increased in individuals exposed to oxidative stress (Grootveld and Halliwell, 1987). Uric acid is an antioxidant that prevents ascorbic acid oxidation; it scavenges peroxyl, hydroperoxyl superoxide radicals by binding transition metals, thereby preventing free radical reproduction (Aruoma, 1994). Recent evidence indicates that albumin may provide antioxidant protection by functioning as a serum peroxidase in the presence of reduced glutathione, which is an intracellular antioxidant

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Table 1. Non-enzymatic serum antioxidants and lipid hydroperoxide levels in participants.

Parameter	Sportsmen after exercise	Sedentary controls	p-value
Uric acid (mg/dl)	5.5.0±1.44* (39) √	4.50±2.37 (24)	<0.05
Albumin (g/dl)	3.61±0.99 (19)	3.39±0.89 (24)	ns
Lipid hydroperoxide (µmol/l)	7.58±0.81 (20)	5.91±1.22 (16)	<0.01

*Mean±SD; √ number of samples; ns =not significant.

Table 2. Serum lipid levels in sportsmen and sedentary controls**.**

Lipid	Sportsmen (19) √	Sedentary controls (24)	p-value
Total cholesterol	121.30±28.29*	91.53±26.37	<0.001
Triacylglycerol Mg/dl	51.70±19.66	64.50±27.64	ns
HDL-cholesterol	35.70±17.23	30.29±12.03	ns
LDL-cholesterol	146.67±39.69	108.93±33.14	<0.05

*Mean±SD; √ number of samples; ns =not significant.

(Aruoma, 1994). This study was designed to compare oxidative stress (serum lipid hydroperoxide level) and antioxidant status (serum uric acid and albumin levels) in regular sportsmen after two and a half hours of strenuous training exercise with that of sedentary age-matched control subjects not involved in any regular physical exercise.

MATERIALS AND METHODS

Subjects

This study was carried out in Delta State of Nigeria between April and May, 2004. A total of 63 male volunteers aged between 18-27 years took part in this study. Questionnaires were administered to determine the level of activity and dietary pattern. The participants were apparently healthy and not on any special medication or anabolics. Thirty-nine of them were regular sportsmen (footballers and joggers) while the remaining 24 sedentary males who served, as control subjects were not involved in any regular physical exercise.

Sample collection

Fasting venous blood sample (5 ml) was collected from the sedentary controls and sportsmen immediately after the sportsmen had been involved in 2 h of strenuous rigorous training exercise. The samples were centrifuged at 1500 g for 10 min at room temperature. Plasma was then collected and stored frozen at -20°C until analyzed.

Analysis

The serum samples were analyzed for total cholesterol (Richmond, 1973), and triacylglycerol (Wybenga and Inkpen, 1974). The HDL-cholesterol in the supernatant was determined after the very low-density lipoprotein and LDL as well as chylomicrons in the plasma had been precipitated by addition of phosphotungstic acid in the presence of magnesium ions. LDL-cholesterol was estimated using formula (Friedewald et al., 1972). Serum albumin, uric acid and lipid hydroperoxide were also determined (Doumas et al., 1971; Caraway, 1963; Buege and Aust, 1978) respectively. Due to non-availability of reagents not all the analysis was carried out on all the samples.

Statistical analysis

Data are presented as mean ±1SD. The means were compared using Student's't' test. Differences are considered significant at $p<0.05$. Associations between parameters were determined using Pearson's correlation coefficient. Associations were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The non-enzymatic plasma antioxidants and lipid hydroperoxide levels are presented in Table 1. The uric acid and lipid hydroperoxide levels were significantly lower in the sedentary controls. There was no significant difference in the serum albumin levels (3.61±0.99 and 3.39±0.89 mg/dl in sportsmen and control subjects, respectively). Table 2 shows the plasma lipid levels of participants. The total cholesterol and LDL-cholesterol levels were all significantly higher in the sportsmen after the two hours strenuous exercise.

This study was designed to compare oxidative stress (serum lipid hydroperoxide level) and antioxidant status (serum uric acid and albumin levels) in regular sportsmen after two and a half hours of strenuous training exercise with that of sedentary age-matched control subjects not involved in any regular physical exercise. The results show that strenuous exercise induced oxidative stress as shown by the significantly higher level ($p<0.01$) of serum lipid hydroperoxide in the sportsmen. Lipid hydroperoxide an index of membrane lipid peroxidation is a marker of oxidative stress A similar observation was also reported in a study where malondialdehyde (MDA) was used as a marker of oxidative stress during exercise (Kelle et al., 1998; Kelle et al., 1999). Strenuous physical exercise is reported to reduce antioxidant level and increase peroxidation, (Brites et al., 1999) this has been confirmed by the significantly higher levels of serum lipid hydroperoxide level in the sportsmen at the end of two hours rigours training exercise. Regular physical exercise has well-documented health benefits, and is reported to prolong life span in experimental animals (McCarter, 2000; Leeuwenburgh and Heinecke, 2001).

Robertson et al. (1991) examined the antioxidant status of highly trained runners, low-moderately trained runners and also in sedentary individuals and found that antioxidant capacity was enhanced in the highly trained runners. Uric acid and albumin were used as makers of antioxidant status in this study. Uric acid has been identified as one of the biological important antioxidant for training athletes or exercising persons. Urate scavenges organic and inorganic oxygen radicals and can bind iron and copper ions and stops or slows down the catalysis of free radical reactions (Halliwell, 1994). Uric acid level increased after exercise in humans (Arouma, 1994; Aslan et al., 1998), and also in experimental animals after exercise compared to sedentary controls (Kelle et al., 1999). These observations have been confirmed in this study where serum the uric acid level in the sportsmen after strenuous exercise was significantly higher ($p<0.05$) compared to controls. Albumin is another serum antioxidant and also the most abundant protein in serum. Recent evidence indicates that albumin may provide antioxidant protection by functioning as a serum peroxidase in the presence of reduced glutathione, which is an intracellular antioxidant. (Arouma, 1994) Albumin is a superb binder of metals such as copper and iron ions and one of its chief roles is to bind and transport fatty acids in the plasma (Arouma, 1994; Nelson and Cox, 2005). In this study the serum albumin level was not significantly different in the two groups. Low serum albumin may also reflect a serum antioxidant deficit that contributes to risk of oxidative stress (Halliwell, 1997).

Serum lipid profile was also investigated in this study. The total plasma cholesterol level in the sportsmen at the end of 2 h strenuous exercise was significantly higher than in the sedentary controls, ($p<0.001$). A similar result has been reported in experimental animals after exercise (Kelle et al., 1998). This supports earlier observations that plasma cholesterol may protect erythrocytes against peroxidant stress (Duthie et al., 1990; Kelle et al., 1999; Bereza et al., 1985). Most of the cell cholesterol is located in the plasma membrane (Lange et al., 2004). It may be that cholesterol moderates membrane stability without greatly compromising fluidity because cholesterol contains both rigid and flexible structural elements (Nelson and Cox, 2005). Cholesterol depletion increases stiffness of membrane by altering the properties of the sub-membrane F-actin and or its attachment to the membrane (Fitzroy et al., 2004) and thus compromising the integrity of the cell. In this study, serum total cholesterol, LDL-cholesterol and HDL-cholesterol levels were also found to be higher in the sportsmen compared to the sedentary controls although the difference in HDL-cholesterol level was not significant. HDL-cholesterol plays an antiaterogenic role because it transports excess cholesterol from peripheral tissues to the liver for excretion (Brites et al., 1999) and also protects LDL-cholesterol from oxidative damage by metals ions. It is possible that the higher serum LDL-cholesterol observed in the sportsmen in this study could be attributed to their low HDL-cholesterol level. High concentration of HDL-cholesterol is associated with low risk of atherosclerosis and is reported to be high in individuals who exercise regularly (Fruchart and Packard, 1997), though our results do not support the latter observation.

Serum triacylglycerol (TAG) was also measured in this study. TAG represents the largest fuel reserve in the body. While most endogenous TAGs are stored in the adipose tissue, some are stored in the skeletal muscle and also found in the plasma (Coyle, 2000). Hydrolysis of TAG from adipose tissue, muscle and plasma would release fatty acids to skeletal muscle mitochondria for β-oxidation (Nelson and Cox, 2005) to provide part of the energy needed for the exercise. However, only a small fraction of the total energy production is derived from plasma TAGs during exercise because the conversion to free fatty acid is slow (Terjung et al., 1983). This could explain the comparable levels of serum TAG in the sportsmen and controls in this study (51.70±19.66 and 64.50±27.64 mg/dl, respectively). Although regular exercise has well documented benefits and has been confirmed in this study we could not ascertain the effect of exercise in the sedentary controls because acute exercise in untrained individuals is reported to increase exercise-induced lipid peroxidation and oxidative stress (Powers et al., 1999) while regular exercise causes an increase in the antioxidant system and a relatively less increase in lipid peroxidation (Leeuwenburgh and Heinecke, 2001). Individuals who exercise regularly place a constant oxidative stress on the muscles but other cells augment antioxidant defense system thereby reducing exercise-induced oxidative stress (Leeuwenburgh and Heinecke, 2001). The direct effect of acute exercise in untrained individuals could be done in experimental animals to further confirm the advantages of regular exercise.

Therefore while there was an increased production of indices of oxidative stress in exercising individuals compared to sedentary controls, our results suggest that they also showed slightly better antioxidant status as indicated by the higher level of serum uric acid and comparable serum albumin concentrations.

It is possible that oxidative stress incurred during exercise was not sufficient to cause significant free radical-mediated peroxidation of fatty acids in the cell membrane because of higher level of serum uric acid and comparable serum albumin level. Higher cholesterol protects against erythrocyte peroxidant stress to some extent. It is becoming clear that a person's antioxidant status and therefore health and well being are improved by physical exercise as well as by a nutritious diet (Lesgards et al., 2002). Thus high intake of antioxidants either through diet or supplements could be important for very active people. However, since normal oxidative metabolism depends on the production of free radicals it is possible that too much free radical quenching could occur by high doses of antioxidants.

Conclusion

We conclude from our findings that high strenuous exercise could induce oxidative stress. However, such physical activity could also contribute to an enhanced antioxidant defence potential and possibly ameliorate oxidative stress in sportsmen.

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