THE EFFECT OF AEROBIC EXERCISE ON THE ERYTHROCYTE OSMOTIC FRAGILITY OF THE WISTAR WHITE RATS

Sugiharto
Department of Sport Sciences
Malang State University School of Education Sciences

ABSTRACT

Exercise is known to increase the production of free radicals and reduce anti free radicals capability, thus, damaging the tissues. This research was conducted in order to understand the effects of aerobic exercise towards the erythrocyte osmotic fragility. The design of this research was posttest only control group, involving white male rats; the Wistar strain Rattus norvegicus as samples. The aerobic exercise was conducted on the tested animals by running on a treadmill three times a week for eight weeks. The data were analysed with t test. As derived from the results of this research, aerobic exercises produced effect on erythrocyte osmotic fragility ($p < 0.05$). This showed that the aerobic exercises had a potential upon the erythrocyte osmotic fragility. Therefore, to avoid such condition, exercise should be done according to accurate dose and monitoring of the dietary aspect, especially the free anti-radical elements, such as vitamin C and E- containing food, is necessary.

Keywords: *erythrocyte osmotic fragility, aerobic exercise*

Correspondence: Sugiharto, Department of Sport Sciences, Malang State University
School of Education Sciences, Jl. Surabaya 6, Malang 65145, phone 62-341-551312, fax. 62-341-551921

INTRODUCTION

Exercise is related to the issues of health development and physical fitness as an effort to enhance the quality of human resource. However, according to Bouchard (1990), the quality of human resource does not only depend on the physical strength, but also on health. Sports and exercises concerned in physical strength to gain achievement have inconveniences because they do not only possess benefits, but also risks (Rost 1993). Risks of damaged tissues could occur when maximal or sub maximal exercises are performed (Gervino 1993). This is because exercises have potential to increase free radicals and decrease the free anti-radical protections of the body (Balkhrishnan 1998). Exercising, either to achieve excellence, or when it is performed irregularly, will cause significant setback on the health condition.

Basically, exercise is a stressor that is anticipated as a stimulator where in the end it would be adapted by the body (Lamb 1984). However, when sports are concerned mainly to increase physical strength, it may cause homeostatic disturbance on the body (Ji 1998), where there is an increase of pathological incidents, damaged tissues (Balakhrisnan 1998), and damaged cell membranes that is caused by toxic molecules such as MDA (Malondialdehyde) (Halliwel 1999). Such disturbances are due to the free radicals, especially free oxygen radicals, produced by the exercise (Sjodin 1990). Several authors reported that exercises could increase intracellular enzymes in the serum (Stansbie 1991), glutamate oxaloacetate transaminase enzyme up to 182% after doing a 90 km cross country race (Statland 1982), creatine kinase enzyme ten times compared to that before exercise (Galun 1985), and lactate dehydrogenase enzyme as much as 37 after padie-ball for an hour (Stansbie 1991). Free radicals can also cause erythrocytes destruction. Exercise does not only initiate mechanical trauma on the erythrocytes due to muscles contraction. It can also cause increasing body temperature, fluid loss, hemoconcentration and oxidation stress that lead to erythrocytes haemolysis during exercise or when the regeneration process take place (Entiirk 2001).

Increased free radicals and damaged tissues in exercise are due to increasing need of energy during the activity. Meanwhile, the intracellular energy supply is very limited. Hence, the continuous energy production occurs through the oxidation process, Krebs cycle and electron transport. Oxygen is used to produce energy through the oxidation, Krebs cycle and electron transport. Therefore, the oxygen requirements increase drastically (Sjodin 1990). The increasing need of energy while exercising, especially the aerobic type, usually intensifies the oxygen requirements, resulting in an increase of 10-15 times while exercising (Sen 1995). Even during maximal exercise, the oxygen need increases in the tissues up to 20 times compared to normal (Ji 1999). Brites (1999) had reported that

exercise is related to increasing oxygen consumption, especially in active tissues and also to increasing free radicals productions. Exercise has a potentiality to cause ischemic and, for that reason, the adenylate kinase system becomes very active. The active adenylate kinase converts two mol of ADP to ATP and AMP. The accumulated AMP causes hypoxanthine build-up in skeletal muscles and plasma. During resting, hypoxanthine will be transformed back to AMP. However, hypoxanthine tends to be converted to uric acid by xanthine oxidase. Xanthine oxidase is an enzyme that forms super oxide free radicals (Flaherty 1991).

In fact, exercise is a stressor that helps the body to adapt to the increasing functional capacities towards the body system (Lamb 1984), and also enhances the protection system of the free anti-radicals. Based on that fact, this research was aimed to reveal the effects of aerobic exercise that was conducted for eight weeks towards the erythrocytes osmotic fragility on the Wistar rats. Therefore, new information about the benefits of healthy exercises could be obtained from this research to observe the body adaptation system through free radicals concepts on regular and organized exercises in an accurate dose.

MATERIALS AND METHODS

This experimental research was done on healthy, male Wistar strain *Rattus norvegicus* rats, aged three months, and weighed 150 – 200 grams. The design of this research was posttest only control group design, consisting of two groups, which were the aerobic exercise and control groups, and the groupings were done randomly. The aerobic exercise was conducted on experimental animals by running on a treadmill three times a week, one to ten sets, and four rounds in 1-10 minutes within eight weeks. The control group did not have to do the aerobic exercise. Both groups had the 521 food types and drinks from tap water. Blood samples were taken and erythrocytes osmotic fragility examination was done 24 hours after exercise. The erythrocytes were examined by using a photocolorimetric method. The osmotic fragility was tested on the principle of dispensing NaCl substance that was prepared with a few NaCl concentrations, and followed by a curve that linked the % haemolysis with NaCl concentration, whereas the blood samples were examined to test the fragility in the Prodia Laboratory Malang. To test the differences between the aerobic activity group and control group, an analysis using a t-test with significance level of 5 % was done.

RESULTS

The average total of erythrocytes osmotic fragility for exercise group was 0.1880 with standard deviation (SD) of 0.0110, whereas the average total of the fragility for the control group was 0.1760 with SD of 0.0089. Based on this test, there were erythrocytes osmotic fragility differences between the aerobic activity and the control at 1.89 ($p < 0.05$).

DISCUSSION

This research was an experimental study using posttest only control group design. The samples of this research were Wistar strain *Rattus norvegicus* white rats. A treadmill, built especially for the rats, was used in this research to perform the aerobic exercise. This treadmill had a convenient way to provide exercise workload for the rats, allowing them to keep on moving in the spinning treadmill and did not cause any stress. The force of the treadmill could be organized by setting the velocity and the timing of the spinning treadmill, until the intensity of the exercise could be adjusted to those settings. Meanwhile, the blood samples were taken for erythrocytes osmotic fragility test 24 hours after exercise. Entiirk (2001) and Yalcin (2000) had also mentioned in their reports that the fragility test is to be done 24 hours after exercise. In this research, the osmotic fragility was an indicator to damaged erythrocytes due to free radicals that were produced while exercising. The erythrocytes osmotic fragility is an examination that can be used to explicate the erythrocyte condition in maintaining its survival.

Based on the results, there were differences of erythrocytes osmotic fragility due to aerobic exercises ($P < 0.05$). This proves that performing aerobic exercises for three times a week within eight weeks had certain indications on erythrocytes osmotic fragility. Entiirk (2001) had stated that when the tested white rats were given swimming exercises; there were differences between the erythrocytes osmotic fragility and thiobarbituric acid reactive (TBARS) compared to control groups. This also happened in Jain's (1989) experiment when he was trying to correlate the increasing glucose, lipid peroxide (free radicals) with the erythrocytes osmotic fragility, and to find that there was a significant relation between lipid peroxide and erythrocytes osmotic fragility. According to Brites (1999), free radicals have some functions on erythrocytes osmotic fragility. This shows that exercise does have effects on the erythrocytes and increase haemolysis due to free radicals. Free radicals are known to have important roles on damaged tissues and disadvantages effects on erythrocytes. The emerged free

radicals in mitochondria are related to the changes of oxygen consuming, reperfusion-ischemic, and the depressed leucocytes that also contributed to stress oxidation (oxygen free radicals) during exercise (Entiirk 2001).

Basically, exercise is a body stressor that increases body functional capacity to create an adaptation response (Viru 1995), and anti free radicals adaptation system. However, the adaptation system depends on the intensity, duration, and types of muscle fibres that involve in exercise. The endurance of exercise for eight weeks did not show any significant changes in the anti free radicals enzymes. Even after nine weeks it did not show any changes of anti free radical superoxide dismutase enzymes (SOD) (Ji 1995). According to Kanter (1985), the changes of anti free radical enzymes occur significantly while exercising for at least 21 weeks. This occurrence depends mostly on the adaptation response of the body from the exercise workload. The response to body adaptation over the workload determines the quality of the body system. However, each body system has different tolerance on the adaptation process of the body over the workload (Lamb, 1984). The response to body adaptation over the exercise load was distinctively known as the general adaptation syndrome (GAS) that consisting of shock phase, adaptation phase and fatigue (Rushall, 1990). During exercise, there is an imbalance between free radicals and anti free radicals production, which have an impact on the damaged cells and tissues (Ji 1999). Sen (1995) stated that 1.8 times increase of lipid peroxidation occurred in human conducting exercise with a load 75 times from maximum oxygen given for 8 weeks. However, the body response over the exercise workload determines the ability to adapt on such load; the increasing inflammation response from the body that occur due to the strenuous exercise workload, activated leucocytes, increasing lactate acid and the increasing free radicals that deeply affected the damaged red cells. In the meantime, regular exercise causes response in the body adaptation to undergo changes towards the abilities to produce anti free radicals and shield the body from free radicals that emerge during exercise (Yalcin 2000). By exercising regularly, the anti free radicals enzyme is able to increase its ability and productions to protect the tissues from free radicals reactivity. This has been proven by a professional football player who trains regularly and acquires more anti-radicals in total plasma compared to those who never had trainings (Brites 1999).

From the results of analysis, exercise by running on a treadmill three times a week for eight weeks showed that the presence of erythrocytes osmotic fragility. The increasing fragility was an indicator of damaged erythrocytes caused by free radicals. The free radicals were generated while doing the exercises. Exercise has a great potential to produce free radicals, especially the oxygen free radicals (OFR) (Sjodin 1990). The oxygen free radicals obtained its name from oxygen, one of the molecules needed by the aerobic organisms, including human. Aerobic organisms need oxygen to produce chemical energy in the form of ATP (adenosine triphosphate) through the oxidative phosphorylation process that occurs in mitochondria (Halliwell 1999). During exercise or generating process, the needs for energy increase, thus building up the oxygen requirement. According to Ji (1999), the oxygen requirement increases up to 15 – 20 times more than normal during exercise. The increasing oxygen requirement during exercise is needed to form the energy (ATP) constantly because intracellular ATP supply is very limited. However, as the exercise goes on, the needs for ATP will remain high until the process to form ATP is needed through oxidation, Krebs cycle and electron transportation that require oxygen in the process (Sjodin 1990). The increasing oxygen consumption is related with the increasing reactive oxygen species production. The latter is a response to physiological and biochemical changes in exercise, and the decreasing level of anti free radicals and increasing lipid peroxide occur in all of the target cells, tissues and red cells (Brites, 1999). Ischemia occurs during exercise due to free radicals through the increasing prostaglandin synthesis from arachidonic acid.

The G_2 prostaglandin conversion to H_2 prostaglandin by prostaglandin hydroperoxide forms oxygen free radicals (Flahery 1991). The adenylate kinase activation occurs while ischemia occurs, thus converting two mol of ADP to ATP and AMP. The eliminated AMP will be accumulated in the skeletal muscles and causes hypoxanthine build-up. However, hypoxanthine tends to be converted to uric acid by xanthine oxidase. Xanthine oxidase is an enzyme that forms superoxide free radicals. Xanthine oxidase uses O_2 as an electron acceptor, and then the O_2 will be reduced to superoxide anion free radicals (Sjodin 1990).

The damaged erythrocytes, besides being impaired by free radicals that emerge during exercise, is also caused by erythrocyte pressure in the capillary while the active muscles contracted, body temperature increase, dehydration, and haemoconcentration that causes haemolysis (Entiirk 2001). The free radicals are known to have important roles in damaged tissues and have its disadvantages effects towards erythrocytes (Ji 1999). In addition, free radicals emerge from the mitochondria due to the increasing oxygen consumption for oxidation process to form energy while exercise and generating process. The intensified ischemic situation and activated

leucocytes are factors that contribute to the emerged free radicals while exercising. Sub maximal exercise was found to cause erythrocytes oxidation exposures and osmotic pressure to damaged red cells (Yalcin 2000).

CONCLUSIONS

It was concluded that performing aerobic exercise three times a week for eight weeks showed no significant differences in damaged erythrocyte by using erythrocytes osmotic fragility as an indicator. However, on average, there was an indication on the erythrocytes osmotic fragility indicator. This proved that the exercise done for eight weeks was not sufficient for the body to adapt to anti free radicals system. Subsequently, to avoid from reactivated free radicals that emerge during exercise, accurate, regular and measured amount of exercises should be monitored. The damaged tissues that are caused by the free radicals that emerge in the exercise needs certain attention and awareness by watching the diet consumptions, especially the anti free radicals nutrients, as an effort to reactivate free radicals and avoid more damaged tissues. This helps to give better meaning in increasing the effort to improve health.

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