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The Effect of Moderate Intensity Aerobic Training on MDA Level, Osmotic Fragility, and Erythrocyte Amount

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***Abstract*— This study aimed to analyze the amount of oxidative stress level reduction, the increase in membrane endurance and the amount of erythrocyte cells due to moderate intensity aerobic exercise. This research was a quasi-experimental study with quantitative approach, with a randomized group pretest and posttest design. The population in this study was students majoring in PKO especially in Faculty of Sport and Science of Universitas Negeri Malang, while the sampling technique used was purposive random sampling, and the sample size was 20 people. The independent variable in this study was moderate intensity aerobic exercise. The exercise was done 3 times a week, for 8 weeks period and 30 minutes long. While, the dependent variables were: (1) MDA blood plasma level, as an oxidative stress degree level, 2) erythrocytes osmotic fragility, as an indicator of erythrocyte membrane cell resistance, and (3) the amount of erythrocyte cells. The dependent variable data were collected by examining venous blood and the data analysis used the T test technique by implementing α 0.05. The analysis of the data showed that all components of the dependent variable obtained p <0.05. The conclusion of this study was that moderate intensity aerobic exercise significantly decreased the degree of oxidative stress, increased membrane endurance and increased the amount of erythrocyte cells.**

Keywords*— *exercise, moderate intensity, MDA, osmotic fragility, erythrocytes.

I. INTRODUCTION

The exercise response and adaptation to erythrocytes system have becoming a hot topic in sports medicine studies in the last few decades, and most of the previous studies focused on 'Sports anemia'. [1]. Research conducted to determine the adaptation of exercise to erythrocyte system varied widely. Factors that influence the adaptation of exercise to erythrocyte system cover: differences in exercise programs, types of cells measured, research subjects and measurement methods. Differences in training programs lead to different results since this is closely related to the intensity and the duration of training as well as the length of the training program. [2].

Exercise stimulates erythropoiesis and increases hemoglobin level and red blood cell mass, which increases oxygen transport capacity as well. Different results based on research on 13 female athletes who were given aerobic exercise for 8 weeks resulted in a hemoglobin level to drop [3]. Thus, a high intensity interval training did not increase the level of hemoglobin, erythrocytes and hematocrit [4].

Aerobic can also result in the free radicals formation. When doing aerobic exercise, oxygen consumption will increase up to 20 times, even oxygen consumption in the muscles can increase up to 100 times. Aerobic exercise can result in a lack of oxygen in the tissues. In addition, it can also cause temporary hypoxia in the inactive tissues of organs such as the kidneys, liver and intestines. Aerobic exercise with submaximal intensity (from pulse 80-85% of the maximum pulse), will make the muscle tissues experience hypoxia, because as the muscular system contracts intensively, it will squeeze intramuscular blood vessels in the active muscle tissues and this results in the decrease of blood flow to the active muscle tissues. When aerobic exercise submaximal intensity is complete, the blood quickly returns to other organs that lack of blood flow, causing reperfusion which can cause a number of free radicals to participate in circulation.

Excessive production of free radicals in the body will trigger a condition called oxidative stress. Malondialdehyde (MDA) is the final result of fat peroxidation which is used as a biological sign of fat peroxidation and can reflect the degree of oxidative stress. Whereas the superoxide dismutase enzyme (SOD) is a major endogenous antioxidant enzyme that plays an essential role directly in defensing cells from free radicals interference, and indirectly maintains toxic oxygen balance [5]. Erythrocytes are cells which are vital for their function, but erythrocyte is also one of the body's cells that is very vulnerable towards the free radicals' effect. Moreover, the response of physical activity can increase oxidative stress, yet the adaptation of regular physical exercise can reduce oxidative stress [6].

Osmotic fragility of erythrocytes is one way of examination to describe the resistance of erythrocyte cell membranes in maintaining the survival of the damaged erythrocyte cells. Physical activity carried out for 8 weeks has not been sufficient to provide the body's adaptation to erythrocytes osmotic fragility [7]. Thus, moderate intensity exercise can improve aggregation, increase osmotic fragility and reduce erythrocyte cell damage [8].

These differences in assumptions became the basis for reassessing the effect of adaptation of moderate intensity aerobic exercise on reducing the degree of oxidative stress, increasing endurance of erythrocyte membranes and increasing the amount of erythrocyte cells.

II. METHOD

This research was a quasi-experimental study, with a randomized group research design by using pre-test and post-test design. Variables in this study consisted of: A). The independent variables which included: (1) The treatment variable which was the moderate intensity aerobic exercise, with a frequency of 3 times per week for 8 weeks period and 30 minutes duration. (2). The control variables, namely: (a) Males, (b) 18-20 year-old subjects, and (c) Normal Body Mass Index. (d) Non-smokers. (3)The manipulated variables, namely (a) Out-of-treatment activities, and (b). Nutritional intake. B). The dependent variables consisted of: (1). The number of erythrocyte cells, (2). Erythrocyte osmotic fragility as an indicator of erythrocyte membrane resistance, and (3). MDA blood plasma level as an indicator of the degree of oxidative stress. Blood samples were taken before the treatment and at the end of the treatment. Moreover, they were also taken 24 hours after the physical activity.

The population of this study was 92 students of PKO from Sports and Science Department at UM in 2015/2016. The research sample was taken by using Purposive Randomized Sampling. The total of research samples were 20 male students. Data were analyzed by t-test technique. The technical analysis requirements for the t-test covered data normality test (Shapiro-Wilk test), and a homogeneity test for variants in groups (Levene's technique). The data were analyzed by using SPSS Version 23 and the test was assessed by using α 0.05.

III. RESULT & DISCUSSION

TABLE 1. RESULTS OF THE DESCRIPTION OF THE MEAN AND THE SD OF THE DEPENDENT VARIABLES

VARIABEL	PRETEST	POSTTEST
MDA (NG/ML)	1000,80 ± 81,66	865,75 ± 18,97
FO (%)	0,46 ± 0,02	0,43 ± 0,02
ERYTHROCYTES (MIL/UL)	5,4580 ± 0,223	5,6230 ± 0,142

Table 1 showed the results of the average and standard deviation analysis of the pretest and posttest data. It could be concluded from the average of the posttest data that MDA blood plasma level experienced a deflation, the osmotic erythrocyte fragility (FO) also decreased while the amount of erythrocyte cells increased.

TABLE 2. RESULTS OF T-TEST ANALYSIS OF DEPENDENT VARIABLES

VARIABLES	SIG	NOTES
PAIR MDA PRE-POST	0,000	SIGNIFICANT
PAIR FO PRE-POST	0,029	SIGNIFICANT
PAIR ERYTHROCYTES PRE-POST	0,021	SIGNIFICANT

Based on Table 2, the results of the t test revealed that all components of the dependent variable both on the pretest and posttest mean were significantly different ($p < 0.05$). This meant that due to moderate intensity aerobic exercise there was a significant difference between the mean values of the pretest and posttest on the whole components of the dependent variable.

TABLE 3. RESULTS OF ANALYSIS OF THE EFFECTIVENESS OF MODERATE INTENSITY AEROBIC EXERCISE ON DEPENDENT VARIABLES

VARIABLES	DELTA	NOTES
MDA (NG/ML)	-135,00±85,2	↓13,49%
FO (%)	-0,03±0,03	↓6,52%
ERYTHROCYTES (MIL/UL)	0,1650± 0,057	↑3,02%

This research used quasi-experimental research with randomized group pretest and posttest design. The sampling technique used purposive randomized sampling with a large sample of 20 people. The sample criteria used included: male sex, age between 18-20 years, normal body mass index and non-smokers. It was expected that the more variables controlled used the more changes in the dependent variable would happen due to more independent variables involved. The independent variables used in this study were moderate intensity aerobic exercise, with a frequency of 3 times a week done for 8 weeks, and duration of 30 minutes. It was expected that with this treatment, the adaptation would occur due to the exercise. The dependent variables in this study were: (1) MDA blood plasma level as an indicator of the degree of oxidative stress, (2) erythrocyte osmotic fragility as an indicator of erythrocyte cell membrane resistance, and (3) the amount of erythrocyte cells. The dependent variable was measured through venous blood sampling. Whereas, venous blood samples were taken before and after the exercise program and that venous blood samples were taken 24 hours after physical activity. The venous blood samples taken after 24 hours of physical activity were expected to be the result of exercise adaptation, not the result of the response from the last exercise. Above all, by taking the blood samples 24 hours after the physical activity, it was also expected that the existing blood system in our body has recovered [9].

Exercise is a modulator of biological functions that can have a wide impact on both positive (increasing and improving) and negative (inhibiting and damaging) effects. Exercise is a vital thing in life as adaptation to exercise can maintain and improve the health of the body, and will be able to improve the physical performance of the body and can also prevent premature aging [10]. Aerobic exercise will help increasing the body's metabolism, especially in the musculoskeletal muscles system. The increase of metabolism during aerobic exercise aims to increase energy production (ATP) to meet the energy needs to do the activity. This process is followed by an increase of O2. To meet the increasing needs of O2 and the release of CO2 as well as the heat requires the integrated work of various systems in our body, especially the cardiovascular and respiratory systems. Changes in the cardiorespiratory system will increase blood transport to the muscles, while adequate circulation to other tissues must be maintained [11].

To get the maximum benefit from the exercise, all exercises must be carried out with regard to the exercise dose with the principle of FIT or frequency, intensity, and tempo. Frequency is how many times exercise done in a week in order to give an exercise impact. The ideal frequency of exercise is 3-5 times a week. Exercise which is less than 3 times a week does not show adaptation to exercise, whereas exercise which is done more than 5 times a week does not provide an opportunity for the body to recover. Next, intensity has something to do with the the given training load. Aerobic exercise is done with light to moderate intensity. An exercise can sufficiently increase the ability of the heart if the intensity of the exercise is between 70-85% of the maximum pulse rate (DNM). Meanwhile, tempo is the duration or length of training given. Research showed that the tempo of the exercise between 20-30 minutes is enough to provide a skill improvement as much as 35% if done with a frequency of 3 times a week within one and a half months.

Aerobic exercise carried out with the good and right principles will guarantee a good biological change and adaptation to the body. Changes that occur due to good and right exercise to the body, among others: biochemical changes, stroke volume increase, cardiac output volume increase in a minute, blood volume and hemoglobin increase. The effects of exercise at cellular level include: increasing the amount and diameter of mitochondria, increasing the variety of oxidative enzymes used in the Kreb's cycle and electron transfer mechanisms [12]. Exercise can be done with different duration and intensity. The duration of the exercise is the length of the exercise in one training session. Meanwhile, the intensity of the exercise is primarily the workload training. The intensity of the exercise can be stated in absolute and relative terms. In an absolute term, the intensity of the exercise can be assessed from the expenditure of energy used in kcal or joules per minute.

While relatively, the intensity of exercise can be assessed by calculating the percentage of the pulse when exercising out of the maximum pulse rate (% DNM) or calculating the percentage of the oxygen exertion from the total maximum oxygen consumption (VO2 max) in ml/kg unit per minute. Physiologically, exercise gives physical stress to the body which can produce an adaptation response. The recommended physical exercise is as long as the body able to adapt to the excessive body load (the principle of overload). Practicing at a rather high intensity level can induce specific adaptations which allow the body to function more efficiently.

The major function of erythrocyte cells is transporting O2 to cells and tissues which then returning CO2 from cells and tissues to the lungs. Erythrocytes are flexible and biconcave, this is useful for passing capillaries or microcirculations as many as Ø 3,5µ, as well as keeping hemoglobin in a reduced state, and also for maintaining osmotic balance even though there is a high concentration of protein in cells [13].

The oxygen condition of cells and tissues is the basis for the formation of erythrocytes. The functional ability of cells to transport oxygen to cells and tissues in relation to tissue oxygen requirements regulates the speed of erythrocytes formation. Any situation causes the reduction of oxygen transported to the tissue will increase the speed of erythrocytes production. The theory of oxygen lacking (hypoxia) is also supported by the results of studies that described individuals who live in the highlands with low oxygen pressure conditions, will experience hypoxic conditions. This persistent hypoxic condition will trigger a significant increase in hemoglobin level.

Adaptation to exercise training is also known to trigger an increase antioxidant production, such as catalase (CAT), superoxide dismutase (SOD), and Gluthathion Sulfur Hydroxyl (GSH) [14]. Antioxidant defense is very necessary for cells, because cells will continue to form free radicals reactive oxygen species (ROS) during respiration and inflammatory conditions [15]. Excessive production of free radicals in the body will trigger a condition called oxidative stress. MDA is a final result of fat peroxidation which is used as a biological sign of fat peroxidation and can be used to assess the degree of oxidative stress. While the SOD enzyme is the main endogenous antioxidant enzyme that has an substantial role to directly protect cells from free radicals interference, and indirectly maintain toxic oxygen balance. Moreover, physical activity triggers oxidative stress responses in untrained people compared to trained people, and there is no significant difference between sexes in the level of oxidative stress during physical activity [16]. It proves that aerobic exercise is one thing that can trigger the formation of free radicals, but if we do the exercises properly and correctly, our bodies will experience adaptation to help us reducing the impact of free radicals.

Erythrocytes are vital cells for our body's function, yet erythrocytes are also one of the body's cells that are irresistance towards the effects of free radicals. The oxidants formed in erythrocytes are superoxide (O.2-), hydrogen peroxide (H2O2), and peroxyl radicals (ROOD.). The source of free radicals caused by exercise derives from (1) the increase of auto oxidation process of hemoglobin (Hb) to methemoglobin, (2) the increase of electron transport system in the mitochondria, (3) the accumulation of lactic acid, (4) the increase of xanthine oxidase (XO), and (5) the increase of cathecolamine production.While doing exercise, our body

Experiences hypoxia. The condition of hypoxia is a major factor in our body which forms the erythropoietin hormone. The erythropoetin hormone will trigger the marrow bone to produce more erythrocyte cells. The increase production and the amount of erythrocyte cells caused by aerobic exercise will increase hemoglobin levels in the blood. Finally, this increase in hemoglobin levels will also increase the maximum oxygen capacity even though other hematological parameters do not change much.

Discussion of MDA Level Results

MDA blood plasma level is lipid peroxidation in the body due to free radicals, this could indicate the level of oxidative stress that occurs [17]. Moderate intensity exercise and high intensity were known to result in mass production of free radicals. The escalation of free radicals caused damage to cells such as muscles and heart. [18]. Exercise with high intensity and a long period of time increased the formation of reactive oxygen and nitrogen species (RONS), this caused an imbalance between oxidants and antioxidants. What is more, this occurs in anaerobic and aerobic exercise. High free radicals which are not offset by an increase of antioxidants will cause stress on cells called as oxidative stress [19]. Exercises carried out with the right exercise principles and proper dosage, will also cause the formation of high free radicals formed in the body. But it seems that the body's adaptation to exercise would be able to form antioxidants so that they can ward off free radicals formed.

Exercise adaptation with low intensity (40 to 60% of maximal pulse (DNM)) to moderate intensity (69-75% DNM) would increase cellular antioxidant defense. High intensity exercise (75-90% DNM) would lead to oxidative stress, due to the antioxidants formed were unable to deal with excessive amount of free radicals [20]. Response to exhausting exercise resulted in an increase in oxidative stress and disturbs the structure of erythrocytes in the inactive group, while in the trained group there was no function impairment. Exercise adaptation resulted in an increase in SOD enzyme activity and reduced the degree of oxidative stress [21] Furthermore, aerobic exercise induced an increase in the antioxidant enzymes activity (superoxide dismutase, Catalase and glutathione peroxidase), as well as a decrease in MDA levels. High intensity interval training for 8 weeks resulted in an increase of aerobic capacity and decrease in lipid peroxidation [22] Aerobic exercise for 12 weeks increased the CAT and the SOD levels as an indicator of an increase in antioxidant status, as well as a decrease in MDA levels as an indicator of decreased oxidative stress [23]. The effect of sprint exercise twice a week done for 12 weeks on mice showed a decrease in MDA levels [24].

This proved that high-intensity sprint exercises could reduce the degree of oxidative stress. Aerobic exercise could also reduce TBARS (81%) and increase the activity of antioxidant enzymes such as Cu, Zn-SOD [25]. While, exercise adaptation resulted in the adaptation of oxidative stress to muscle cells [26]. From the above studies, it was known that the effect of exercise adaptation resulted in a decrease in oxidative stress with the decrease of blood plasma MDA levels indicator and a reduction in oxidative stress on muscle cells. Thus, it could be said that the training response would result in an increase of free radicals in our body cells, but if we did training with the right principles and proper exercise then our body would experience adaptation to exercise, so that our bodies would produce more endogenous antioxidants, and eventually would be able to ward off the negative effects of these free radicals.

From this study, the researcher revealed that there was significant difference towards the average score of pretest and posttest of blood plasma MDA levels ($p < 0.05$), and the results at the posttest showed a decrease. This showed that the adaptation of moderate intensity aerobic exercise was able to reduce the degree of oxidative stress. According to Table 3, a moderate intensity aerobic exercise could reduce the degree of oxidative stress by 13.49%.

Discussion of Erythrocyte Osmotic Fragility Results

The process of hematopoiesis occurred in the marrow bone. While, retyculocytes which were the premature forms of erythrocytes, would grow and form erythrocytes that were 8 µm in diameter, in the form of a biconcave disc with a cell aged 120 days [27]. Erythrocytes are a major component of blood after leukocytes, platelets and plasma. Erythrocyte membranes are permeable to water molecules (H2O) [28]. This is because of the presence of AQP1 protein transport. Erythrocytes that are put into hypertonic solution will experience cell shrinkage because more water is leaving the cell than entering it. And if erythrocytes are in a hypotonic environment, then osmosis will occur from the outside into cells which will cause cells to bulge. If the plasma membrane cannot withstand a high intracellular pressure due to the achievement of critical volume, the cell will rupture and hemoglobin will be released [29].

Erythrocytes osmotic fragility test assessed the process of erythrocytes lysis due to osmotic stress. The level of osmotic fragility of erythrocytes was also influenced by the ratio of cell surface area to cell volume. The acceleration of osmotic fragility could also be affected by free radicals. Free radicals also became one of the causes of erythrocytes damage. Free radicals took a role in the erythrocytes osmotic fragility. During the physical activity there was mechanical trauma to erythrocytes caused by muscle contraction. Besides, during physical activity there was an increase in body temperature, lack of body fluids, hemoconcentration and oxidative stress which caused a erythrocyte himolysis during exercise or during the recovery period. The physical activity load carried out regularly provided the body's adaptation to changes in the body capability of building anti-free radical production and the ability to counteract free radicals caused by physical activity.

Cycling interval training done in 5 days per week in around 30 minutes with an average intensity of 60% VO2Max for 5 weeks could significantly improve aggregation, osmotic fragility and reduce erythrocyte cells damage. After 5 times per week exercise done for 5 weeks, showed an increase in VO2 max and a decrease in osmotic fragility of erythrocytes which meant there was an increase also in endurance of the erythrocyte membranes. Eventually the adaptation of aerobic exercise led to a decrease in the degree of oxidative stress, this was closely correlated with an increase in endurance of cell membranes.

It was also found that the moderate intensity aerobic exercise gave an impact on osmotic fragility (FO) and it was as a marker of erythrocyte membranes endurance. Moreover, the results of pretest and posttest on osmotic fragility showed significant differences ($p < 0.05$). This revealed that there was a significant difference between the average pretest results and the results in posttest on osmotic erythrocyte fragility variable. We could draw a conclusion that there was also a significant effect of moderate intensity aerobic exercise on the increase of erythrocyte cell membranes endurance. In this study, the average posttest erythrocyte osmotic fragility decreased, it meant that in the erythrocytes osmotic fragility test with the fluid concentration (hypotonic) subtraction, it was still unable to rupture since the osmotic fragility test assessed the occurence of erythrocyte lysis due to osmotic stress. This also meant that if erythrocytes were in a hypotonic environment, osmosis would occur from the outside into cells which would cause the cells to bulge. If the plasma membranes could not withstand a high intracellular pressure due to the achievement of critical volume, the cell would rupture. In this study based on Table 3, osmotic fragility of erythrocytes decreased by 6.52%. It could be concluded that moderate intensity aerobic exercise was effective in increasing the resistance of erythrocyte membranes by 6.52%.

Discussion of Erythrocyte Cells Amount Results

Physiological adaptation due to exercise requires sufficient exercise intensity to stimulate the threshold value of aerobic excitement. Aerobic exercise will result in an increased metabolism, especially in the musculoskeletal system. This enhancement of metabolism is useful for increasing ATP production, so that the energy needed for activities can be met. Furthermore, this will certainly be followed by an increase in the need for O2, then to meet the needs of O2 and the release of CO2 and heat, requires the integrated work of various cardiovascular and respiratory mechanisms. Changes in the cardiorespiratory system during aerobic exercise will increase blood flow to the muscles, while adequate circulation to other tissues must be maintained [30].

As a result of aerobic exercise that continued with moderate intensity would cause an oxygen lacking (hypoxia) state in the body. The condition of hypoxia at the cellular level would then trigger an increase in the production of hypoxia induced factor-1 (HIF-1) transcription which played an essential role in the response of cells and tissues to low oxygen conditions. Increased production of HIF-1 in the kidneys and liver would generate the transcription of erythropoietin genes so that the hormone erythropoietin then be released into the bloodstream. Erythropoeitin hormone is a hormone in the circulatory system which goes through the hematopoietic system (red marrow) and sticks to its receptors in stem cells, this bond will trigger the maturation of these stem cells into erythroid precursor cells which will undergo a maturation process through a series of reactions with cytokines such as stem cell factor, interleukin-3, interleukin-11, granulocyte-macrophage colony stimulating factor and thromopoietin. This erythropoetin hormones acceleration would eventually trigger an increase in erythrocyte cells production. Exercise stimulated erythropoiesis and increased HB levels and red cell mass, which at the same time also lifted up the oxygen transport. The underlying mechanisms, especially in the marrow bone, included stimulating erythropoiesis by hyperplasia of the hematopoietic marrow bone, uplifting the hematopoietic microenvironment induced by exercise, and accelerating the erythropoiesis by utilizing hormones erythropoetin and cytokines.

Aerobic and anerobic exercises had an effect on accelerating the amount of erythrocyte cells [31]. There was a significant effect of interval training on an increase in erythrocyte cells, and an increase in VO2 max [32]. The high intensity interval training method could also boost the VO2 max but did not increase the value of hemoglobin, erythrocytes, and hematocrit.

The results of this study were projected on table 2 above. From the t test, the average value of pretest and posttest of erythrocytes amount obtained $p = 0.021$ ($p < 0.05$). This meant that there was a significant difference between the pretest and posttest mean scores on the amount of erythrocyte cells variable. So it could be concluded that moderate intensity aerobic exercise with frequency of 3 times per week and done for 8 weeks could significantly increase the amount of erythrocyte cells. The increase amount of erythrocyte cells in this study was as much as 3.02%.

IV. CONCLUSION

Moderate intensity aerobic exercise significantly reduced oxidative stress level, increased endurance of erythrocyte membranes, and increased the amount of erythrocytes.

V. SUGGESTION

As an effort to maintain health and fitness, it is recommended to do a moderate intensity aerobic exercise 3 times a week and in 30 minutes. It can reduce the degree of oxidative stress, increase the resistance of the erythrocyte membrane and increase the amount of erythrocytes. This good erythrocyte system condition is closely related to maintaining health and fitness of our bodies, especially the cardiorespiratory system.

REFERENCES

[1] Hu, M., & Lin, W. (2012). Effects of exercise training on red blood cell production: implications for anemia. Acta haematologica, 127 (3), 156-164.

[2] Yunus, M. (2017). Effect of Interval Exercise on Increasing Erythrocyte Cells and VO2Max. Motion: Journal of Research of Physical Education, 8 (1), 79-89.

[3] Moosavizademonir. (2011). Effect of One Period of Training on Hemoglobin, Hematocrit & RBC of Athlete Girls. Annals of Biological Research (6): 642-44.

[4] Putra, K. P., Al Ardha, M. A., Kinasih, A., & Aji, R. S. Correlation of changes in the value of VO2max, erythrocytes, hemoglobin and hematocrit after high intensity interval training. Journal of Sports, 5 (2), 161-170.

[5] Wresdiyati, T., Mamba, K., Adnyane, I., & Aisyah, U. S. (2002). The effect of stress conditions on the intracellular antioxidant copper, zinc oxidase dismutase in the rat kidney: an immunohistochemical study. Hayati, 9 (3), 85-88.

[6] Candrawati, S. (2015). Effect of Physical Activity on Oxidative Stress. MANDALA of Health, 6 (1), 454-461.

[7] Sugiharto. (2005). Erythrocyte Osmotic Fragility in Aerobic Physical Activity. Sports Science and Technology Journal. Vol 7, No. 3, September 2005.

[8] Chou, S. L., Huang, Y. C., Fu, T. C., Hsu, C. C., & Wang, J. S. (2016). Cycling Exercise Training Alleviates Hypoxia-Impaired Erythrocyte Rheology. Medicine & Science in Sports & Exercise, 48 (1), 57-65.

[9] Şentürk, U. K., Gündüz, F., Kuru, O., Actekin, M. R., Kipmen, D., Yalçin, O., & Başkurt, O. K. (2001). Exercise-induced oxidative stress Affects erythrocytes in sedentary rats but not exercise-trained rats. Journal of applied physiology, 91 (5), 1999-2004.

[10] Adiputra, N. (2008). Sports Health Textbook of the Faculty of Medicine, Udayana University, Denpasar.

[11] Barrett, K. E., Barman, S. M., Boitano, S., & Brooks, H. (2010). Ganong's review of medical physiology. 23. USA: McGraw Hill.

[12] Warburton, D. E., Nicol, C. W., & Bredin, S. S. (2006). Health benefits of physical activity: the evidence. Canadian medical association journal, 174 (6), 801-809.

[13] Arber, D. A., Glader, B., List, A. F., Means, R. T., Paraskevas, F., & Rodgers, G. M. (2013). Wintrobe's clinical hematology. Lippincott Williams & Wilkins.

[14] Marius-Daniel, R. A. D. U., Stelian, S. C. H. I. O. P. U., & Dragomir, C. (2010). The effect of acute physical exercise on the antioxidant status of the skeletal and cardiac muscle in the Wistar rat. Romanian Biotechnol Lett, 15 (3), 56-61.

[15] Leeuwenburgh, C., & Heinecke, J. W. (2001). Oxidative stress and antioxidants in exercise. Current medicinal chemistry, 8 (7), 829-838.

[16] Stanković, M., & Radovanović, D. (2012). Oxidative stress and physical activity. Sport Logia, 8 (1), 1-11.

[17] Souza, C. F., & Fernandes, L. C. (2006). Production of reactive oxygen species during the aerobic and anaerobic exercise. Brazilian Journal of Kinanthropometry and Human Performance, 8 (2), 102-109.

[18] Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiological reviews, 88 (4), 1243-1276.

[19] Powers SK, Nelson WB, Hudson MB. (2011). Exercise induced oxidative stress in humans: cause and consequences. Journal of Free radical Biology & Medicine 51; 942-950.

[20] Lamina, S., Ezema, C. I., Theresa, A. I., & Anthonia, E. U. (2013). Effects of free radicals and antioxidants on exercise performance. Oxidants and Antioxidants in Medical Science, 2 (2), 83-91.

[21] Andriichuk, A., Tkachenko, H., Kurhaluk, N., & Tkachova, I. (2013). Oxidative stress biomarkers and erythrocyte hemolysis in trained ukrainian warm blood horses during training sessions. Біологія тварин, (15, № 4), 9-23

[22] Sandeep, H. S and Hassan, M. A (2013). "Effect of High Intensity Interval Training on Malondialdehyde and Aerobic Capacity of Male Physical Education Students". International Journal of Current Advanced Research Research Vol.2, Issue, .57 - 59,

[23] Ajabi, F.J, Mohammad R Z, Asghar, (2013). "Influence of aerobic training on red cell antioxidants defense, plasma malondialdehyde capacity in multiple sclerosis patients". International Research Journal of Applied and Basic Sciences Vol, 4 (7): 1757-1761

[24] Cunningham, P., Geary, M., Harper, R., Pendleton, A., & Stover, S. (2005). High intensity sprint training reduces lipid peroxidation in fast-twitch skeletal muscle. Journal of Exercise Physiology Online, 8 (6).

[25] Lambertucci, R. H., Levada-Pires, A.C., Rossoni, L.V., Curi, R., & Pithon-Curi, T. C. (2007). Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. Mechanisms of aging and development, 128 (3), 267-275.

[26] Steinbacher, P., & Eckl, P. (2015). Impact of oxidative stress on exercising skeletal muscle. Biomolecules, 5 (2), 356-377.

[27] Pasini, E. M., Kirkegaard, M., Mortensen, P., Lutz, H. U., Thomas, A. W., & Mann, M. (2006). In-depth analysis of the membrane and cytosolic proteome of red blood cells. Blood, 10