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# *Impact of Maximum Physical Exercise on Male Mice (Mus musculus L.) Without Supplementation*

*E Yuniarti[1], Des M[1], N Izzati [2], V Laila Nuchair[2]*

[1]Lecturer of Biology Department, Faculty of Mathematics and Natural Sciences,
Universitas Negeri Padang
Jl. Prof. Dr. Hamka Air Tawar Padang - 25131, Indonesia

[2]Student of Bachelor Program of Biology Science,
Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang
Jl. Prof. Dr. Hamka Air Tawar Padang - 25131, Indonesia



**Abstract – Maximum physical exercise is the cause of an increase in free radicals. The imbalance between free radicals and antioxidants in the body is known as oxidative stress that causes erythrocyte damage after physical exercise that causes Sports Anemia. This study uses a type of experimental research. Held in May-June 2020 at the Animal Physiology Laboratory FMIPA UNP. The treatments taken are (K1) not treated and (K2) given the maximum physical exercise treatment in the form of a maximum swimming for 2 weeks. Data were analyzed by paired comparison test with a significance level of 5%. The data were processed using SPSS 23. The results of this study indicate that maximum physical exercise can reduce blood components in mice, namely a decrease in hemoglobin (K1) from 15.05 g/dL to 13.52 g/dL and (K2) from 13.22 g/dL to 8.35 g/dL, decrease in hematocrit (K1) 43.28% to 41.10% and (K2) 42.28% to 28.24%, decrease erythrocytes (K1) 11.88 106/mm$^3$ to 10.71 106/mm$^3$ and (K2) 8.82 106/ mm$^3$ to 6.38 106/mm$^3$ and the increase in leukocytes (K1) 4.83106/mm$^3$ to 5.25 106/ mm$^3$ and (K2) 5.18 106/mm$^3$ to 7.51106/mm$^3$. The conclusion of the research is the effect of maximal physical exercise on male mice without supplementation can significantly reduce hemoglobin, hematocrit and erythrocytes but not significantly reduce leukocyte levels.**

**Keywords –** ***Erythrocyte, Hemoglobin, Hematocrit, Leukocytes, Maximum Physical Exercise***

## I. INTRODUCTION

Sports can improve the degree of health and physical fitness level of a person [1] and to achieve and maintain physical fitness is to do physical exercise in the form of regular and measured physical exercise. [2] Benefits Physical exercise can also improve heart, lung, bone mass, muscle strength, and more. [3] Swimming as a sport that can train and develop overall body muscles, and swimming is also an excellent sport to improve and maintain one's physical fitness, because when swimming all members of the body move. Swimming movements can make the muscles of the body stretch, so that the body's muscles become elastic. Swimming is also useful for building strength and endurance. [4]

Physical exercise can have a positive effect, it can improving routine physical exercise with mild to moderate intensity can increase endurance and body fitness. In addition, physical exercise can have a negative effect, namely inhibiting or damaging if physical exercise is carried out until fatigue is called maximum physical exercise. [5]

**Corresponding Author:** E Yuniarti

Physical exercise that can affect Hb levels is moderate to severe physical exercise. [6] The impact of excessive physical exercise is an imbalance between physical training and recovery time. Excessive physical training can have a bad effect on the condition of homeostasis in the body, which ultimately also affects the work system of organs. [7] [8]

As a form of the body's adaptation to moderate-heavy intensity activities carried out, there can be changes in blood plasma volume where the blood plasma volume will decrease and will make the levels of hemoglobin in the blood appear to increase, besides that when doing moderate-heavy intensity activities, the body needs oxygen more, to compensate for oxygen demand, the body will do erythropoiesis which will also make the hemoglobin level increase. [9]

Physical exercise with maximum intensity in individuals who are not conditioned or not accustomed to doing physical exercise will result in oxidative damage and muscle injury. [10] Additionally, maximal physical exercise can trigger an imbalance between free radical production and the body's antioxidant defense system, known as oxidative stress. [5]

Oxidative stress is one of the factors responsible for erythrocyte damage during and after maximum physical exercise. From the research that has been done that erythrocyte damage occurs during and after maximum physical exercise can cause anemia, which is often called Sports Anemia. [11] During maximal physical exercise there is an increase in the amount of oxygen, increased activity of skeletal muscles, fatigue and decreased physical abilities. At the time of physical exercise the maximum need for oxygen increases. Oxygen, although very much needed, is also toxic. This will trigger an increase in Reactive Oxygen Species (ROS) and free radical products. [12] In this condition, free radicals will cause lipid peroxidation of cell membranes and damage the organization of cell membranes. Lipid peroxidation of cell membranes makes it easy for erythrocyte cells to undergo hemolysis, which is the occurrence of lysis of the erythrocyte membrane which causes oxidation of hemoglobin and ultimately causes hemoglobin levels to decrease. [13] A decrease in hemoglobin level will also be followed by a decrease in the hematocrit value. From the results of Simanullang's study [13] it was found that there was a decrease in hemoglobin levels and in line with a decrease in hematocrit in the treatment after maximum physical exercise in the form of swimming. According to Andrian in Tortoraet al., [14] [15] the addition of oxidative stress can result in an increase in the number of leukocytes exceeding 10,000 cells. Increased leukocyte count is a protective response to stress such as microbial infection, heavy activity, anesthesia and surgery. Stress causes an increase in a person's vulnerability to infection, so stress decreases health status. [16] The immune system is a system that is very sensitive to stress, both triggered physiologically and psychologically. [17] The results of the study by Senturk et al., [18] showed that after doing maximum physical exercise can cause changes in hematocrit, erythrocyte and leukocyte values.

Therefore, the purpose of this study was to determine the impact of maximal exercise in the form of maximum swimming on hemoglobin levels, hematocrit values, the number of erythrocytes and the number of leukocytes in male mice (*Mus musculus* L.) without supplementation.

## II. Research Method

This study uses an experimental research type with a treatment amounting to 2 each of 6 replications. Held in May-July 2020 in the Laboratory of Animal Physiology, Department of Biology FMIPA UNP

### *2.1. Tools and Materials*

The tools used are 1000 mL beaker glass, cube-shaped swimming containers (p = 32 xl = 16 xt = 24 cm), tweezers, stopwatch, Sahli haemometer, suction pipettes, stirring rods, drop pipettes, statics, surgical scissors, pipettes microhematocrit, hematocrit centrifuge, rat bottle, thoma pipette, haemositometer and microscope.

The materials used in this research are male mice weighing 30-35 grams with age of 8-10 weeks, pellets, tap water drinks, 0.1 N HCl solution, hayem solution, Turk solution, distilled water, 70% alcohol, xylol , tissue and cotton.

### *2.2. Research procedure*

#### 2.2.1. Test Animal Preparation

Mice were acclimatized for 7 days at room temperature before being treated. Mice are fed in the form of pellets and bottled water. Mice were then divided into 2 groups namely K1; group without treatment, K2; treatment group. Each group consisted of 6 mice which were then given a treatment in the form of maximum swimming.

#### 2.2.2. Maximum Physical Exercise Treatment in Mice

The procedure of maximum physical exercise treatment follows the procedure carried out by Ciulla et al., [19]. Meanwhile, the mouse swimming container follows Simanullang, [13] which is in the shape of a cube. Mice were taken from the K2 treatment group randomly for maximum physical exercise by means of mice forced to swim in a

container that had no escape with a size of 32 (p) x 16 (l) x 24 (t) cm filled with water with a depth of 8 cm. After the mice are put into a container a stopwatch is used to calculate the time needed for maximum physical exercise.

In an effort to get out of the mouse container swimming, diving and climbing the walls of the container with all their strength and if the mice appear to be resting to take power then the mice are submerged by using tweezers. When mice stop all their movements, except movements to survive (keep the head still on the surface of the water), this is considered to have done maximum physical exercise. [19] The treatment of swimming exercises is done with a maximum frequency of exercise 3 times per week for 28 days with a length of swimming 30 minutes.

### 2.2.3. Measurement of Hemoglobin Levels in Blood of Mice

Measurement of hemoglobin levels was carried out using the Sahli method, that is, a 0.1 N HCl solution was put in a sahli tube up to the boundary mark 2. Take one male mice and clean the tip of the tail with alcohol. Then cut about 2 mm from the tip of the tail with surgical scissors. Suck blood samples from the tails of mice that have been cut using a pipette to reach the line limit of 20 $mm^3$ (0.22 cc). Insert the blood sample into a tube that has been filled with HCl solution and wait for 3 minutes or until it turns blackish brown. Add the distilled drops drop by stirring using a stir bar until the color is the same as the standard color of the hemoglobinometer. Read the results of the hemoglobin level in the unit g / dL stated on the hemoglobin tube.

### 2.2.4. Measurement of Erythrocytes in Blood of Mice

The tail of the male mice then the tail end of the male mice is cleaned with alcohol, then cut about 2 mm from the tip of the tail using surgical scissors. Let the blood come out after a few seconds. Insert blood through the tip of the red microhematocrit pipette to ¾ the volume of the tube. The tip of the pipette is blocked with wax which is useful so that when blood is centrifuged it does not come out. Microhematocrit pipettes containing blood were centrifuged at a speed of 6,000 rpm for 10 minutes. Reading the results by observing the layers formed by comparing the volume of blood cells with all blood components. The hematocrit value is expressed in percent and is determined using the formula:

$$\frac{h_1}{h_2} \times 100\%$$

Information:

$h_1$: High red blood cell deposits

$h_2$: Height of all blood components

### 2.2.5. Measurement of Erythrocytes in Blood of Mice

The tail of the mice is smeared with warm water for about two minutes, then smeared with alcohol. The tip of the mouse tail is cut using surgical scissors. Blood from the tail of mice is released slowly by pressing the base of the tail, then sorted to the tip of the tail. Blood is sucked using a thoma pipette up to 0.5. The dilution of hayem diluent is sucked with a thoma pipette up to 101. A dilution of 1/200 occurs. Both ends of the pipette are closed using the thumb and middle finger, shaking is done by flipping through the pipette. 1-2 drops of liquid in the thoma pipette are removed and discarded, then in the next drop the tip of the thoma pipette is affixed to one of the contents of the cubicle which has been given a glass cover and tissue paper and the other side. The liquid in the thoma pipette will flow to meet the cubicle and then the cubicle will be placed under a microscope.

Erythrocytes that are in the 5 cubicle R count. The calculation starts from the left in zigzag. To avoid inaccurate calculations, erythrocytes in the left and upper border of a small chamber are counted as erythrocytes in the small chamber.

The actual number of erythrocytes can be determined by looking for factors to get the number of erythrocytes per µL of blood, which is 5 x 10 x 200 = 10,000. So to get the amount of erythrocytes ie:

$$\boxed{\text{N x } 10.000.}$$

Ket: N = the number of erythrocytes from 5 cubicles,
200 = dilution factor,
10 = depth of object,
5 = number of samples.

### 2.2.6. Leukocyte Measurement in Blood of Mice

Mice are removed from the cage by means of half of the tail removed, then the mice are put in a mouse bottle and left the tail hanging out. The tail of the mice is smeared with warm water for about two minutes, then smeared with alcohol. The tip of the tail is then cut using surgical scissors.

Blood from the tail of mice is released slowly by pressing the base of the tail, then sorted to the tip of the tail. Blood is sucked using a leukocyte thoma pipette to 0.5. The turk thinner solution is sucked with a thoma pipette to 11. There is a 1/20 dilution. Both ends of the pipette are closed using the thumb and middle finger, shaking is done by back and

forth. 1-2 drops of liquid in the thoma pipette are removed and discarded, then in the next drop the tip of the thoma pipette is affixed to one side of the counting booth which already contains a glass cover and tissue paper on the other side. The liquid in the thoma pipette will flow to meet the cubicle and then the cubicle will be placed in a microscope.

Leukocytes that are in 4 cubicles W are counted. The calculation starts from the left in zigzag. To avoid inaccurate calculations, leukocytes in the left and upper border of a booth W are counted as leukocytes in the count booth.

The number of leukocytes 103 / mm3 in the blood can be calculated using the formula:

$$(N/0,4) \times 20 \text{ or } N \times 50$$

Ket: N = the number of leukocytes in 4 counting chambers,
0,4 = cubicle volume count,
20 = dilution.

### *2.3. Data analysis*

The data obtained were analyzed by Paired Comparation T-test with a significance level of 5%. Data processed using SPSS 23.

## III. Results And Discussion

### *3.1. Results*

Based on observations of the blood component of male mice before being given treatment after (posttest) given the maximum physical exercise treatment can be seen in the following chart:

After the paired Comparation T-test in Table 1, Sig. (2-tailed) <0.05 means that the blood components of mice are hemoglobin, hematocrit, erythrocytes and leukocytes before given maximum physical exercise significantly different from the blood component of mice after being given maximal physical exercise. So it can be seen that the maximum physical exercise influences the blood component of male mice. As an explanation the data will be as presented in Figure 1.

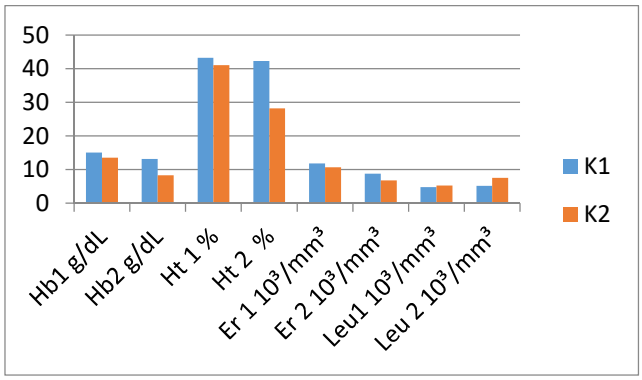


Figure 1. Comparison graph The average blood component of male mice before (pretest) and after (posttest) maximum physical exercise.

Tabel 1. Paired Comparation T-test results of male blood component (*Mus musculus* L.) before (preetest) and after (posttest) were treated with maximum physical exercise.

| Blood component | Treatment | Median | | Sig. (2-tailed) |
|---|---|---|---|---|
| | | Pretest | Posttest | |
| Hemoglobin | K1 | 15.05 g/dL | 13.52 g/dL | 0.000 |
| | K2 | 13.22 g/dL | 8.35 g/dL | |
| Hematocrit | K1 | 43.28% | 41.10% | 0.001 |
| | K2 | 42.28% | 28.24% | |
| Erythrocytes | K1 | 11.88 $10^6$/mm$^3$ | 10.71 $10^6$/mm$^3$ | 0.000 |
| | K2 | 8.82 $10^6$/mm$^3$ | 6.38 $10^6$/mm$^3$ | |

| | | | | |
|---|---|---|---|---|
| Leukocytes | K1 | 4.83 $10^3$/mm$^3$ | 5.25 $10^3$/mm$^3$ | 0.001 |
| | K2 | 5.18 $10^3$/mm$^3$ | 7.51 $10^3$/mm$^3$ | |

*Information: sig. (2-tailed)<Sig. 5% show significant differences.*

### *3.2. Discussion*

In this study, mice in the K2 group were treated with maximum physical exercise in the form of swimming 3 times/week for 28 days with ± 30 minutes every 1x swimming except in the K1 group. Physical exercise can increase the formation of free radicals and lipid peroxidation. Severe physical exercise in individuals who are not accustomed to doing physical exercise will result in oxidative damage and muscle injury. In conditions of oxidative stress, free radicals will cause lipid peroxidation of cell membranes and damage the organization of cell membranes. This cell membrane is very important for receptor function and enzyme function, so that the occurrence of cell membrane lipid peroxidation by free radicals can result in total cellular loss. Lipid peroxidation of cell membranes makes it easier for erythrocyte cells to undergo hemolysis, namely the lysis of erythrocyte membranes which causes hemoglobin to be liberated. and ultimately causes a decrease in hemoglobin levels [10], triggering the destruction of erythrocytes and will cause a decrease in the number of erythrocytes that cause anemia.

According to Hariyono [20], normal levels of hemoglobin in male mice range from 13-16 g/dL, if less than these levels, the mice are said to be anemia. From the observations, group 2 (K2) can reduce hemoglobin levels by 4.87 g/dL. This decrease in hemoglobin levels is due to the inability of endogenous antioxidants to neutralize free radicals in the body due to maximum physical exercise. According to the results of Simanullang's study [13], it also showed that there was a decrease in hemoglobin levels in mice treated with maximum physical exercise, ie swimming for 2 weeks by 2.0 g/dL. This decrease in hemoglobin level is caused by an increase in the number of erythrocyte cells damaged by maximum physical exercise. [21] In addition, if the formation of hemoglobin in the bone marrow decreases, the level of hemoglobin in cells can also decrease to below normal values. [22]

Physical exercise done with maximum intensity can cause anemia. According to Bonilla et al., [23] anemia can be defined as the amount and quality of circulating erythrocytes below the normal level for an individual determined according to their level of hemoglobin (Hb) and the number of erythrocytes. Along with hemoglobin levels, hematocrit values are usually associated with the degree of anemia suffered. Therefore, a decrease in hemoglobin level will also be followed by a decrease in the hematocrit value.

Based on the data obtained, the mean hematocrit value of male mice in group 1 (K1) that were not treated was 43.28% (pretest) and 41.10% (posttest) despite a decrease of 2.18% the hematocrit value of male mice was still in normal condition. This is consistent with what was said by Kusumawati [24], in normal mice hematocrit values range from 33.1%-49.9%. While the hematocrit value of male mice in group 2 (K2) who were trained in swimming were 42.28% (pretest) and 28.24% (post test). This shows that maximum physical exercise in the form of swimming can reduce the hematocrit value by 14.04%.

Another blood component is a decrease in the number of erythrocytes due to maximal physical exercise. According Kusumawati [4] the number of red blood cells in normal mice is 6.86-11.7 x $10^6$/mm$^3$, if it is less than that number then the mice are said to be anemia. In this study, the average number of mice erythocytes in group 2 (K2) post test 6.38 x106/mm$^3$, compared with the average number of erythrocytes pretest which was 8.82 x $10^6$/mm$^3$, so the maximum physical exercise was proven to reduce the number of mice erythrocytes by 2.44 x$10^6$/mm$^3$in 28 days.

In addition, the mean number of leukocytes in male mice in group 1 (K1) that were not given maximum physical exercise treatment were 4.83 x $10^3$/mm$^3$ (pre test) and 5.25 x $10^3$/mm$^3$ (post test), meanwhile the average number of male leukocytes in the group 2 (K2) who were given maximum physical training were 5.18 x $10^3$/mm$^3$ (pretest) and 7.51 x $10^3$/mm$^3$ (post test). According to Tharll [25], in normal circumstances the peripheral blood of mice contains leukocytes with a number ranging from 2.61-10.05 x $10^3$/mm$^3$ so that the average increase in the number of leukocytes in the K1 group even though it rises is still in normal conditions, but mice that are given maximum physical exercise treatment in the form swimming has the average number of leukocytes increased in the post test compared to the pretest. This shows that maximum physical exercise can increase the number of leukocytes in male mice.

An increase and decrease in the number of leukocytes can occur due to physiological or pathological influences. An increase in the number of leukocytes in the blood is called leukosity. Leukosity that occurs due to physiological factors can be caused by muscle activity, fear stimulation and

emotional disturbances, whereas disorders of pathological influence can be caused by apotological processes in response to disease attacks. [26] This allows the average number of leukocytes in male mice to increase after maximum physical exercise. The results of the Pharma Nutura team (2004) [27] showed that heavy physical activity can increase the production of free radicals. Physical exercise that is not in accordance with the principles of exercise causes free radicals that will bind to hemoglobin which causes tissue injury. [29]

According to Cooper [28] (in Harahap, [27]) in normal circumstances, free radicals are formed very slowly, 5% of oxygen consumption will form free radicals and then neutralized by antioxidants in the body. But if the rate of free radical formation greatly increases beyond 5% because it is triggered by strenuous and exhausting physical activity, the amount of free radicals will exceed the capacity of the antioxidant defense system capacity. This imbalance can cause oxidative stress and stimulate the activity of leukocyte cells.

Excessive physical exercise can have a bad effect on the homeostatic condition of the body, which ultimately affects the work system of the body's organs. [30] Regular physical exercise is needed to have beneficial health effects, such as reducing various cardiovascular diseases, disorders of metabolic syndrome and osteoporosis. [31]

## IV. Conclusions And Suggestions

### *4.1. Conclusions*

The results of this study indicate that maximum physical exercise can have an impact on blood components in mice, namely a decrease in hemoglobin, a decrease in hematocrit, a decrease in erythrocytes and an increase in leukocytes.

### *4.2. Suggestions*

Further research can be done to overcome anemia in maximal physical exercise.

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