

ORIGINAL ARTICLE

**Cholesterol Levels After Maximum Physical Activity in White Rats
(*Rattus Norvegicus*)**

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Abstract: The effect of physical activity on biological functions can be either positive or negative influences. Severe physical activity can increase oxygen consumption by 100-200 times compared to resting conditions. Increased use of oxygen, especially by muscle contracting, causes an increase in the number of free radicals. This study aims to determine the effect of maximum physical activity on cholesterol levels in white rats. The research design was used an experimental study with a post-test and control group design. Subjects were male white rats (*Rattus Norvegicus*), 20 of them were 3-4 months old, body weight was 180-200 grams. Subjects were divided into 2 groups randomly, each group consisted of 10 white rats, namely the control group: not given maximum physical activity; group treated: given maximum physical activity in the form of swimming until almost submerged. The results showed a significant increase in cholesterol levels ($p=0.035$; $p<0.05$) in the treatment group, namely the group that was given maximum physical activity in the form of swimming to almost drowning, compared to the control group, namely the group that did not swim. The study concluded that there was an effect of maximum physical activity on blood cholesterol levels in white rats (*Rattus Norvegicus*).

Keywords: maximum physical activity, cholesterol, free radicals

INTRODUCTION

Physical activity is a body movement produced by skeletal muscles that require energy expenditure. Physical activity results from skeletal muscle contraction. Every human being every day always performs the physical activity. The benefits of physical activity when carried out healthily and enjoyably, with light to moderate intensity, will improve health and fitness.¹

Physical activity has a positive effect if it is carried out with light to moderate intensity, the goal is to improve fitness, health, and the

dignity of human life. However, physical activity can also have a negative effect, namely inhibiting or disrupting physiological processes in the body.² The effect of physical activity on biological functions can be in the form of a positive influence that improves or a negative effect, namely inhibiting or damaging. These various physical activities are very prone to cause stress if done excessively, such as strenuous physical activity.^{3,4}

In certain circumstances, strenuous physical activity can have a negative effect,

namely inhibiting or disrupting physiological processes in the body. Strenuous physical activity can increase oxygen consumption 100–200 times compared to resting conditions. Increased use of oxygen, especially by contracting muscles, causes the leakage of electrons from the mitochondria which will become ROS (Reactive Oxygen Species). In that situation, 2-5% of oxygen will be oxidized to free radicals. So that when doing physical activity with high intensity, there will be an increase in the number of free radicals.⁵

When the production of free radicals exceeds the cellular defense antioxidants, there will be oxidative stress.⁶ In conditions of oxidative stress, an increase in free radicals causes an increase in lipid peroxidation and damages cell membranes.⁷ The buildup of oxidative stress can cause damage to the activation of stress-sensitive signaling pathways such as cardiovascular disease, insulin resistance, and metabolic syndrome.⁸

Oxidative stress can cause cell damage and is the basis of pathogenesis for cardiovascular disease processes, pulmonary disease, autoimmune diseases, malignancies, metabolic disorders, and aging. The effect of physical activity on oxidative stress is divided into acute response and chronic response. Acutely, physical activity can increase the formation of free radicals thereby increasing oxidative stress in the body. Meanwhile, chronically, regular physical activity can increase endogenous antioxidant capacity, thereby reducing oxidative stress in the body.⁹

Cholesterol is a fatty substance circulating in the blood, yellowish in color and waxy, which is produced by the liver and is needed by the body. Cholesterol has an important meaning because it is a major

element in plasma lipoproteins and plasma membranes and a precursor to a large number of steroid compounds.¹⁰ The results of previous studies stated that the relationship between physical activity and cholesterol levels was significant.^{11,12}

METHODS

The research method used was experimental with post-test design and control group design. This research was conducted at the Pharmacology Laboratory of the USU Faculty of Pharmacy and has received ethical approval from the USU FMIPA Animal Ethics Commission No.00290 / KEPH-FMIPA / 2020. The subjects of the study were 20 male white rats (*Rattus Norvegicus*), aged 3–4 months, weighing 180–200 grams, obtained from the Pharmacology Laboratory of the USU Faculty of Pharmacy. Furthermore, the experimental animals were randomly divided into 2 groups, each group consisting of 10 white rats, namely the control group: not given maximum physical activity; treatment group: given maximum physical activity in the form of swimming until almost drowning.

Treatment of maximum physical activity in the sample was adjusted to the O'Toole method, in which the rats were soaked for approximately 45 minutes based on the maximum swimming time of the rats. Maximum physical activity is the maximum activity in the form of swimming as hard as possible until almost drowning or there are signs of fatigue in the form of drowning almost everything except the nose, weakening of movement, decreased muscle strength, decreased reaction time, and frequency of movement, and decreased reflexes.

Cholesterol level is the result of cholesterol measurement from laboratory tests using the direct CHOD-PAP test method, the cholesterol level unit is mg/dl.

The equipment that will be used in this research is a stopwatch to calculate the time or length of time that mice can swim until almost drowning, a spectrophotometer for cholesterol examination, a tub designed with a length of 10 cm and a diameter of 25 cm, where only one mouse can swim.

Before treatment, all rats were adapted and kept in groups (4 rats per cage) in experimental animal cages made of plastic material (30x20x10 cm) covered with fine gauze. Food in the form of pellets and drinks (tap water) in excess (*ad libitum*). The base of the cage is covered with rice husks 0.5–1 cm thick and replaced every day. The maintenance room light is controlled exactly 12 hours bright and 12 hours dark, while the room temperature and humidity are left in the natural range.

One by one, the rats were given maximum physical activity treatment in the form of swimming as hard as possible until they almost drowned or there were signs of fatigue in the form of drowning almost all of the body except the nose and weakening movement of the limbs. The swimming duration ranges from 40-45 minutes. The rats were given a stimulus (their head was pressed into the water) to keep swimming as hard as possible until they almost drowned so that the maximum physical activity of the rats was achieved. Furthermore, the mice were sedated using ketamine to draw blood immediately intracardial. Then do a cholesterol level check.

The distribution of each data obtained is determined by the normality test (Kolmogorov-Smirnov test). If the data is normally distributed, an unpaired t-test will

be carried out with $\alpha = 0.05$, whereas if the data is not normally distributed, it will be followed by a non-parametric test. The data obtained were then processed using statistical procedures.

RESULTS

We analyzed data on the results of cholesterol checks using the independent t-test. To be able to perform the t-test, it must meet several requirements. Among them are that the samples are normally distributed and come from a homogeneous population. Testing the normality of cholesterol data using the Kolmogorov-Smirnov test can be seen in the table below.

Table 1. Normality test result

Variable	Treatment group	p
Cholesterol level	P1	0.123
	P2	0.139

Description: P1 Control group
P2 Treatment group

The results of the Kolmogorov-Smirnov normality test showed that cholesterol levels showed that it normally distributed the data ($p > 0.05$) (table 1), so the next test was the parametric test, namely the independent t-test.

Table 2. Cholesterol level

Cholesterol (mg/dl)	P1	P2	P value
Mean	74.90	7.781	0.035
SD	84.8	11.351	

Description: P1 control group
P2 treatment group
SD standard deviation

The results showed that there was a significant increase in cholesterol levels ($p = 0.035$; $p < 0.05$) in the treatment group, namely the group that was given maximum

physical activity like swimming to fatigue and almost drowning, compared to the control group, namely the group that did not swim (table 2).

DISCUSSION

Based on the results of the study, we found that the maximum physical activity as swimming to fatigue caused an increase in cholesterol levels compared to the control group, namely the group that was not given swimming treatment. This increase in cholesterol levels proves that during maximum physical activity it forms excessive free radicals. The number of cholesterol levels increased due to the stress experienced by rats, namely an imbalance between the number of free radicals and the body's antioxidants. In normal circumstances, the body's antioxidant system can suppress the activity of Reactive Oxygen Species (ROS) in the body.^{13,14}

Reactive Oxygen Species (ROS) is an oxidant that is very reactive and has different activities. The negative impact of these compounds arises because of their activity so that they can damage cell components that are very important for maintaining cell integrity. Each ROS formed can start a chain reaction which continues until the ROS remove by the other ROS or its antioxidant system. This excessive free radical formation can cause oxidative stress.¹⁵ Oxidative stress can damage cell lipid membranes through a series of lipid peroxidation reactions, causing increased cell membrane permeability and impaired mitochondrial function.¹⁶

Lipid peroxidation is the oxidative damage to lipid biomolecules due to ROS reactivity. Lipid peroxidation can cause changes in lipid profile levels. At the maximum physical activity, we obtain an

increase in cholesterol levels due to this process.¹⁷

According to the American Heart Association, one of the main or direct risk factors for increasing cholesterol levels in the body are genetic factors, free radicals, and intake of foods high in saturated fat. The results of this study support by other studies which suggest that high-intensity, acute resistance exercise can lead to changes in lipid profiles.¹⁸

Cholesterol is the most sterol found in the body, its form can be as free cholesterol or bound to fatty acids as cholesterol esters. Cholesterol in the blood and lymph is seen as a cholesterol ester while that in the blood cells of the muscle, liver, and other tissues is free. Free fatty acids that are not oxidized and will re-esterify into triglycerides in the adipose, liver, and intramuscular tissue, if there is more re-esterification than lipolytic, there is an increase in the concentration of free fatty acids in plasma which can cause various lipid-related diseases such as hypercholesterolemia.¹⁹

CONCLUSION

The conclusion from the results is that there is an effect of maximum physical activity on blood cholesterol levels in white rats (*Rattus Norvegicus*).

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