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Effects of High Intensity Swimming on Heat Shock Protein 70, Superoxide Dismutase and Malondialdehyde of *Rattus norvegicus* Male Rats

Dody Taruna¹, Bambang Purwanto², Harianto Notopuro², Widjiati², Budi Utomo², Lilik Herawati², Reny I'tishom², Aryati^{2,*}

Dody Taruna¹, Bambang Purwanto², Harianto Notopuro², Widjiati², Budi Utomo², Lilik Herawati², Reny I'tishom², Aryati^{2,*}

¹Affiliated Doctoral Program of Medical Science, Medical Faculty, Airlangga University, Surabaya, Indonesia. Medical Faculty, Hang Tuah University, Surabaya, INDONESIA.

²Department of Doctoral Program, Medical Faculty, Airlangga University, Surabaya, INDONESIA.

Correspondence

Aryati

Department of Doctoral Program, Medical Faculty, Airlangga University, Surabaya, INDONESIA.

E-mail: aryati@fk.unair.ac.id

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ABSTRACT

Introduction: This study aims to analyze relationship between physical activity with high intensity swimming by proving the difference in the mean levels of HSP-70, SOD levels and MDA levels in male white rats (*Rattus norvegicus*) who perform vigorous-intensity physical activity compared to male white rat (*Rattus norvegicus*) who do not engage in strenuous physical activity. **Method:** This research is a laboratory experimental research. This research is a True Experimental Research type that uses a Post Test Only Control Group Design research design. The experimental unit consisted of 28 white rats (*Rattus norvegicus*). The study was started by dividing the rats into 2 groups. Group 0 who did not receive heavy physical activity treatment and group 1 who received heavy intensity physical activity treatment 3 times a week for 4 weeks. **Results:** The results of the Shapiro-Wilk test showed that the HSP-70 data were normally distributed ($p < 0.05$). SOD and MDA data for all groups were normally distributed ($p > 0.05$). The results of the Kruskal Wallis test showed that there was a significant difference in HSP-70 between groups ($p > 0.05$), the results of the Mann Whitney test showed that the HSP-70 of the K0 group was significantly different from the K1 group. The results of the analysis of variance with Brown-Forsythe showed that there was a significant difference in SOD between groups ($p < 0.05$). The results of the Games Howell test showed that the SOD of group K0 was significantly different from that of group K1. The results of the analysis of variance showed that there was a significant difference in MDA between groups ($p < 0.05$). The results of the LSD test showed that the MDA of the K0 group was significantly different from the K1 group. **Conclusion:** There is a difference in the mean levels of HSP-70 in white male rat (*Rattus norvegicus*) who perform strenuous physical activity, lower than male white rat (*Rattus norvegicus*) who do not engage in strenuous physical activity. There is a difference in the mean SOD levels in male white rat (*Rattus norvegicus*) which perform strenuous physical activity, lower than male white rat (*Rattus norvegicus*) who do not do strenuous physical activity. There is a difference in the mean MDA levels in male white rat (*Rattus norvegicus*) after doing strenuous physical activity, higher than male white rat (*Rattus norvegicus*) who do not engage in strenuous physical activity.

Key words: Strenuous physical activity, HSP-70, SOD, MDA.

INTRODUCTION

Currently, many people are already doing physical activities such as walking, swimming, cycling and making this physical activity a daily habit. Regular physical activity can help keep your physical condition healthy, energetic and independent walking with age. Physical activity plays an important role in preventing disease and stroke. The health benefits of regular physical activity have been demonstrated in many studies. Physical activity has benefits for all body systems, reducing stress and anxiety, increasing feelings of happiness, increasing self-confidence, increasing brain power, sharpening memory and increasing muscle and bone strength, preventing and reducing heart disease, obesity, blood sugar fluctuations, cardiovascular disease and obesity. cancer.¹ WHO (World Health Organization)² recommends exercise in the form of physical activity for 150-300 minutes of moderate intensity or 75-150 minutes of heavy intensity, or a combination of both per week. Due to the busyness of modern people, many people do sports only on weekends, and are nicknamed weekend warriors.³ Exercise in the form of physical activity on the weekends can have

a positive effect on health, according to research conducted by Haskell *et al.*⁴ which states that a person can do 30 minutes of moderate physical activity five days a week or 75 minutes of heavy-intensity physical activity one day a week.⁴ This statement is reinforced by research conducted by O, Donovan *et al.*⁵

Regular physical activity can improve quality of life, decrease damage caused by oxidative stress, repair tissue growth, remodeling, revascularization regeneration, and stem cell differentiation. Physical activity protects various organs of the body, such as the liver, kidneys and brain against the effects of oxidative stress. Physical activity has also been shown to protect male reproductive organs from oxidative damage caused by cytotoxic drugs, but the mechanism of this repair is not known.⁶ Physical activity with light and moderate intensity can have a positive effect on cardiovascular conditions, while physical activity with heavy intensity will have the opposite effect.⁷ During physical activity, the ability of skeletal muscles to meet increased energy demands is limited by the availability of oxygen in the tissues. In a state of physical activity, there is an insufficient supply of O₂ to the tissues compared to the tissue's

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need for oxygen (relative hypoxia).⁸ HSP-70 has anti-inflammatory effects, including vascular inflammation. Physical activity increases vascular levels or alter the phosphorylation status of various HSP-70 and these conditions are associated with an anti-inflammatory state. The increase in HSP-70 is due to physical activity and is maintained with repetitive physical activity (repetitive exercise/exercise training). Physical activity was associated with increased expression of HSPs, particularly HSP-70. Heat shock protein has an anti-inflammatory effect by inhibiting vascular mediators and inflammation (Noble, 2012). Physical activity can cause changes in the expression of antioxidant enzymes. The balance of oxidation-reduction in the body can express endogenous antioxidant enzymes, including: peroxiredoxins (Prx), thioredoxins (Trx), superoxide dismutases (SOD) and glutathione peroxidases (Gpx). This activity also limits the increase in oxidative stress. This group of enzymes can function in epigenetic regulation, such as DNA methylation, in response to the environment, inactivating reactive oxygen species (ROS) and maintaining them at physiological levels.

Oxygen is indispensable for life; however, in certain situations it has a deleterious effect due to the formation and activity of a number of chemical compounds, known as free radicals or reactive oxygen species (ROS). To neutralize ROS, cellular enzymatic and non-enzymatic mechanisms occur. Superoxide dismutase (SOD) is a family of enzymes that exist throughout the body that function to catalyze superoxide dismutation.⁹ Malondialdehyde (MDA) is an important marker reflecting low-grade systemic inflammation. Inflammation that occurs can be due to heating or tissue damage due to infection. MDA produced in various tissues is often involved in the pathogenesis of micro and macrovascular diseases.¹⁰ Relationship between physical activity vigorous-intensity swimming with HSP-70, SOD and MDA in male white rats (*Rattus norvegicus*) still very little is known, so this research is expected to be a guide for people who will be physically active with this type of sport.

MATERIALS AND METHODS

Research design

The research was conducted experimentally in the laboratory. This study was designed to fulfill the research objective which was to prove that strenuous physical activity could improve spermatogenesis in testicular tissue of male white rats (*Rattus norvegicus*) exposed to heat. This type of research was a laboratory experimental research. This research was a true experimental research type that uses a post-test-only control group design.

Experimental animal

The sample population in this study was Wistar rat, 15 weeks old male with a body weight of 170-200 grams with a healthy physical condition. From the population randomly took 28 samples and then ordinal pairing according to body weight was allocated into 2 groups. Then by simple random sampling, they were grouped into 2 different groups.

Analysis of HSP-70, SOD and MDA

Measurement of HSP-70 mouse blood was calculated using ELISA and optical density (OD value) 450 nm, Rat HSP-70/HSPA9 (Heat Shock Protein 70) ELISA KIT Kit Catalog: E-EL-R0479 Brand: Elabscience with ELISA measuring instrument Reader Thermo Fisher. Measurement of SOD (Superoxide dismutase) of rat blood was calculated using ELISA and optical density (OD value) 450 nm, Rat ELISA KIT SOD1 (Superoxide Dismutase 1, Soluble) ELISA Kit Catalog: E-EL-R1424 Brand: Elabscience with ELISA Reader measuring instrument ThermoFisher. Measurement of MDA (Malondialdehyde) of rat blood was calculated using ELISA and optical density (OD value)

450 nm, MDA (Malondialdehyde) ELISA KIT Kit Catalog : E-EL-0060 Brand : Elabscience with ELISA Reader Thermo Fisher measuring instrument. Physical activity of heavy intensity Physical activity of swimming with high intensity (Brito, 2015) with a modification, the rats were swam to stop if they started to sink / breath bubbles were seen. The research was conducted at the Embryology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya. Acclimatization of experimental animals for 7 days to water, food and air under laboratory conditions. The distribution of experimental animal groups was carried out randomly and consisted of 2 groups, namely:

K0: Group control (Without physical activity, a male white rat (*Rattus norvegicus*) blood was drawn for further examination).

K1: The treatment group (acclimatized for 1 week then given heavy intensity physical activity 3 times a week for 4 weeks. Then after 72 hours, white rats (*Rattus norvegicus*) were drawn blood.

Data analysis

Data analysis was carried out on HSP-70 levels, MDA levels, SOD. Levels each group by analyzing the mean (mean) and standard deviation, then tested for normality in all groups using the Kolmogorov-Smirnov. To determine the variance of homogeneity between groups, Levene's test was used. The significance level of used is 5%. The data were normally distributed and had homogeneous variations, followed by a statistical analysis of variance (ANOVA) test followed by a multiple comparison Tukey HSD test if $p < 0.05$. If the data distribution is not normal and or the data variance is not homogeneous, an analysis of variance (ANOVA) test is carried out followed by multiple comparisons to find out the differences between treatment groups using the Games-Howell test $p < 0.05$. The selection of statistical tests pays attention to the type of data from the dependent variable and the independent variable. On the data with interval/ratio dependent variable and with the independent variable as well as the interval ratio scale, path analysis will be carried out, namely HIF-1 levels, Testosterone levels, HSP-70 levels, IL-6 levels, TNF levels, MDA levels, SOD levels and spermatozoa quality. The mechanism is tested by regression analysis (Purnomo and Bramantoro, 2016).

RESULTS

Effect of physical activity on HSP-70, SOD and MDA levels

As result in Table 1 showed that HSP-70 and SOD levels on K0 group showed higher result than K1 group. Otherwise on K1 group had MDA level higher than K0 group. Moreover, Figure 1 exhibited levels of HSP-70 in the group that received heavy physical activity treatment (K1 group) were lower than not receive heavy physical activity treatment group (K0).

Furthermore, Figure 2 displayed SOD levels in heavy physical activity treatment (K1 group) were lower than not receive heavy physical activity treatment (K0 group).

Here in after, Figure 3 showed MDA levels on received heavy physical activity treatment group (K1 group) were lower than group that did not receive heavy physical activity treatment (K0 group).

Statistical analysis of HSP-70, SOD and MDA levels

As result in Table 2, the results of the Shapiro-Wilk test showed that the HSP-70 data were normally distributed ($p < 0.05$). SOD and MDA data for all groups were normally distributed ($p > 0.05$). Differences in data between groups where all groups were normally distributed were analyzed using analysis of variance, whereas if there were groups that were not normally distributed, the Kruskal Wallis test was used. The results of the Kruskal Wallis test showed that there was a significant

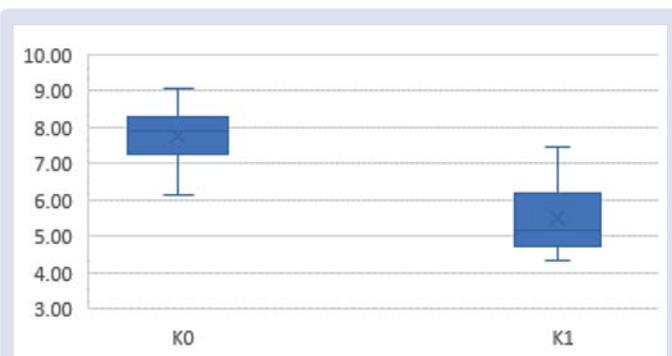


Figure 1: Overview of HSP-70 levels between treatment groups.

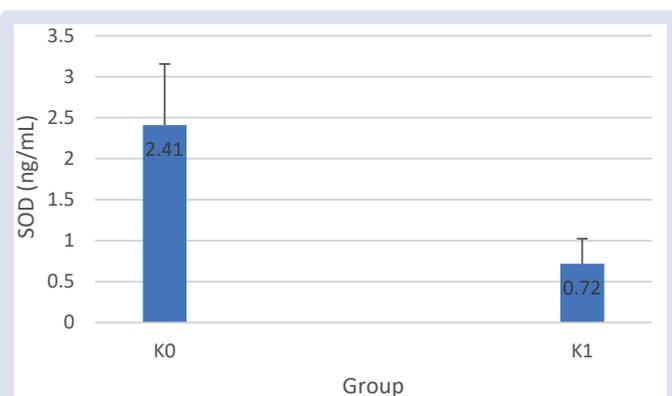


Figure 2: Overview of SOD levels between treatment groups.

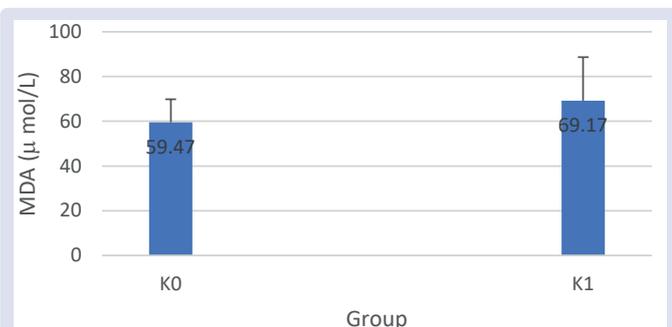


Figure 3: Overview of MDA levels between treatment groups.

Table 1: Mean and standard deviation of treatment groups.

No	Variable	Group			
		K0		K1	
		Average	SD	Average	SD
1.	HSP-70 level	7.77	0.84	5.528	0.960
2.	SOD level	2.41	0.74	0.721	0.305
3.	MDA level	59.47	10.29	69,174	19,498

Table 2: Test results for the normal distribution of data.

Variable	p value	
	K0	K1
HSP-70	0.662	0.348
SOD level	0.165	0.706
MDA level	0.883	0.122

Table 3: Differences in HSP-70 between groups.

Group	n	Median (min – max)	p Nilai value
K0	11	7.89 (6.13 – 9.05) a	< 0.001
K1	13	5.19 (4.35 – 7.47) b	

Table 4: Differences in SOD between groups.

Group	n	Average±Standard deviation	p Nilai value
K0	11	2.41±0.745 a	< 0.001
K1	13	0.72±0.305 b	

Table 5: Differences in MDA between groups.

Group	n	Average±Standard deviation	p Nilai value
K0	11	59.47±10,293 a	0.030
K1	13	69.17±19,498 ab	

difference in HSP-70 levels between groups ($p > 0.05$), the results of the Mann Whitney test showed that the HSP-70 levels of the K0 group was significantly different from the K1 group (Table 3).

Moreover, analysis of variance with Brown-Forsythe test showed that there was a significant difference in SOD levels between groups ($p < 0.05$). Games Howell's test results showed that the SOD levels of group K0 was significantly different from that of group K1 (Table 4).

The analysis of variance also showed significant difference in MDA levels between groups ($p < 0.05$). The results of the LSD test exhibited MDA levels of K0 group was significantly different from the K1 group (Table 5).

DISCUSSION

Effects of physical activity on HSP-70

HSP-70 is the most studied HSP. HSP-70 is a protein with a molecular mass of 66-78 kilodaltons. HSP-70 is encoded by multiple genes consisting of at least 11 genes (Lannau, 2008). HSP 70 consists of constitutive HSP-70 and inducible HSP-70 (Kiang, 1998; Parcalier, 2003). The most important and frequently studied group of HSPs is HSP-70. HSP-70 is a protein whose synthesis is stimulated by stress. HSP-70 has a gene that consists of a specific group of nucleotide markers (nucleotide block markers) called heat shock elements (HSE). The nucleotide group is CT-GAA-TTC-AG. HSP-70 consists of 13 isoforms. HSP-70 is divided into 2, namely constitutive HSP-70 and inducible HSP-70. Constitutive HSP-70 has high basal levels and is weakly induced by stress. Inducible HSP-70 is not synthesized under normal conditions and is only synthesized under stress conditions.¹¹⁻¹³ HSP-70 has a dimeric form, where each monomer contains 3 functional domains. The functional domains are ATPase N domain, substrate domain and C domain. Each domain has a different function. These three domains form a unique molecule called a triptych. The ATPase N domain is the domain that binds and hydrolyzes ATP. The function of this domain breaks down the molecular structure. The ATPase N domain consists of 4 subdomains which are divided into 2 lobes. Lobe I consists of sub-domain IA and sub-domain IB. Lobe II consists of subdomains IIA and IIB. These two lobes are linked *via* subdomains IA and IIA. The IB and IIB sub domains form a gap at the base. The bottom of this gap is the site for ATP binding. The IB and IIB sub domains approach each other when ATP is bound to the gap. Domain C consists of 5 spirals ($\alpha A-\alpha E$). This domain is a flexible domain. Domain C forms a structure that resembles a cap on the flexible part. This domain is used to open and close the substrate domain. The cap of this substrate domain will be occupied by hydrogen bonds and ionic bonds. Changes in the opening and closing of the C domain will cause a conformational change of the domain.¹¹

HSP-70 in eukaryotic cells interacts in the process of protein synthesis. HSP-70 plays a role in ensuring the formation of appropriate and correct proteins. This process begins when HSP-40 helps each hydrophobic portion of the protein being synthesized appear on the ribosome. It binds to the substrate domain of HSP-70. HSP-40 initiates ATP hydrolysis between the N domains. This process increases the protein affinity of the substrate domain and at the same time the flexible C domain closes the protein bound to the top. HSP-70 is bound to the hydrophobic area of the polypeptide chain and acts as a protective barrier. This HSP-70 prevents unwanted aggregation of polypeptide chains. The loss of HSP-70 will cause protein synthesis to malfunction. Hip protein is a protein that interacts with the ATPase domain HSP-70d with a molecular weight of 43 kDa. Hip will maintain the hydrophobic area bound to HSP-70 during polypeptide synthesis. The polypeptide chain that emerges from the ribosome will be ready to bend into a native tertiary structure. This process will cause the exchange of nucleotide exchange factor (NEF) from ADP domain N to ATP.¹¹⁻¹³ Nucleotide exchange factor consists of Bag-1 and HSPBP1. Bag-1 interacts with the gap between the IB and IIB subdomains on the N domain in HSP-70 and induces rotation of the IIB subdomain. The IIB subdomain will hold the ADP and rotate to free it. This mechanism causes Bag-1 to increase ADP release. Bag-1 is a factor of a protein that accelerates the dissociation of ADP from the N domain of HSP-70 (a dissociation activator).¹¹⁻¹³ Hip is a Bag-1 protein antagonist. Hip interacts with the ATPase domain of HSP-70. Hip is competent with Bag-1 in binding to the ATPase domain of HSP-70 and preventing Bag-1 from stimulating ADP nucleotide release. Inhibition of ADP release will cause immature substrates.¹¹⁻¹³

ATP hydrolysis can be enhanced by the interaction of HSP-70 with co-factors such as HSP-40. HSP-40 assists ATP hydrolysis by interacting with HSP-70 *via* the J domain. The protein folding process also involves HSP-60 which has a barrel-like shape. HSP-60 will perform polypeptide synthesis or protein denaturation to ensure protein folding. The protein folding process does not only occur in the cytoplasm but also in the endoplasmic reticulum. The process of protein synthesis in the endoplasmic reticulum is on the ribosomes. Ribosomes are transported into the endoplasmic reticulum *via* a translocon. The protein that first encounters the translocon is HSP-70. HSP-70 in the endoplasmic reticulum is referred to as BiP. BiP binds to areas of hydrophobic proteins that appear on the endoplasmic reticulum. This binding prevents the polypeptide chain from returning to the cytoplasm. This binding also aids in repeating the ATPase cycle, which will allow BiP to bind anywhere next to the hydrophobic areas of the endoplasmic reticulum. BiP will pull the polypeptide chain into the lumen of the reticulum like a ratchet. HSP-40 will attract BiP and regulate ATPase activity through a translocon located on the endoplasmic reticulum membrane.¹¹⁻¹³ Mitochondrial proteins are also bound to HSP-70. The cytoplasmic function of HSP-70 is to maintain the linear state of the protein chain. This allows the protein to penetrate through the translocon in the mitochondrial membrane. The initial process begins when a polypeptide chain appears in the mitochondrial matrix and HSP-70 which carries ATP energy will carry the protein chain into the mitochondrial matrix. The regulator of this cycle is regulated in the matrix by the nucleotide exchange factor. The translocon protein will accelerate the acceleration of the mitochondrial ATPase activity of HSP-70. The HSP-70 protein which functions to ensure protein folding causes HSP-70 to be referred to as chaperones while HSP-40 which helps HSP-70 is called co-chaperones.^{12,13}

Table 3, it can be seen that there are significant differences in the levels of HSP-70 between groups ($p > 0.05$), and then there are significant differences in levels of HSP-70 in the group that did not receive physical activity and warming treatment (group K0) with treatment group K1. This is in accordance with what was discussed by Krüger (2019) that

in fact physical activity is a physiological stress factor accompanied by various physiological changes that are known to increase the concentration of HSP-70. Oxidative stress, changes in temperature, pH, ion concentration, decreased concentration of calcium, intramuscular glycogen, impaired membrane integrity, and glucose deficiency during and after exercise provide conditions of instability and consequent homeostatic imbalance. Another condition is tissue hypoxia where lower than normal oxygen content and pressure in cells can trigger HSP expression.¹⁴ Consequently, an increase in HSP-70/90 was described in plasma and mononuclear cells and various organs and tissues such as muscle, liver, cardiac tissue and brain after acute exercise. In particular, the response of HSP-70 appears to be highly dependent on exercise mode. Exercise duration and volume, exercise type and intensity, subject's exercise status, and environmental factors such as heat have been shown to influence the degree of upregulation of HSP after exercise. Most studies use endurance exercise protocols and show that higher intensity appears to increase plasma and muscle production of HSP-70 more than moderate-intensity activity. However, Walsh *et al.* (2001) demonstrated that moderate-intensity resistance training also resulted in a significant increase in circulating concentrations of HSP-70 in humans. In another study, it was shown that prolonged exercise induces a more pronounced serum HSP-70 response than shorter or more intense exercise. A correlation was shown between pre-exercise HSP values and post-exercise HSP-70 increases in human muscle biopsies measured over 5-8 weeks of exercise. After a period of intense training, higher basal levels of HSP-70 were found, which may reflect HSP accumulation in leukocytes after several subsequent exercises. This finding is supported by the finding that HSP-70 is particularly elevated after a period of intense exercise with only a brief recovery period in muscle tissue.¹⁵

Progressive accumulation of HSP-70 was found in the skeletal muscles of trained rowers after 3 weeks of high-intensity strength training. During the next week of recovery, the value of HSP-70 decreased to the baseline value. Chronically elevated basal levels of HSP-70 were found in the heart tissue of trained mice. Interestingly, in these mice, acute treadmill exercise did not lead to further increases, suggesting some kind of preconditioning in cardiac tissue after training. In contrast, lower basal HSP-70 concentrations were demonstrated in peripheral blood mononuclear cells from trained subjects, which may also reflect adaptation to regular exercise. The accumulation of HSP-70 in muscle is suggested to act as a negative feedback regulator for inducible HSP-70 gene transcription. This may be an important mechanism for securing controlled regulation of stress proteins and for maintaining efficient recovery capacity. Therefore, endurance athletes may have—except in cardiac tissue—lower tissue HSP concentrations compared to untrained individuals, although increases in HSP are still observed in response to acute exercise. It has also been shown that heat stress during exercise, for example, during running in a hot environment, enhances HSP-70 concentrations in muscle, cardiac tissue and mononuclear cells, suggesting that external heat is an additional stress factor. Furthermore, the type of exercise affects the concentration of HSP. Although resistance and endurance training appear to induce similar expression of HSP-70.

Effects of physical activity on SOD

Oxygen is indispensable for life; however, in certain situations it has a deleterious effect due to the formation and activity of a number of chemical compounds, known as free radicals or reactive oxygen species (ROS). To neutralize ROS, cellular enzymatic and non-enzymatic mechanisms occur. Superoxide dismutase (SODs) is a family of enzymes that exist throughout the body that function to catalyze superoxide dismutation. The antioxidant capacity of mammalian organ systems can meet the needs of oxygen consumption and free radical production in the body. Oxidative tissues, such as skeletal muscle,

heart and kidneys, have the greatest antioxidant enzyme activity. Acute exercise, especially intense or prolonged exercise. The increase in the production of free radicals and reactive free radicals will be balanced by an antioxidant defense system under the control of signaling pathways in response to the increase in free radicals to maintain homeostasis, sports or physical training.⁹ Furthermore, from table 4, it can be seen that there is a significant difference in SOD levels between the treatment groups ($p < 0.05$), and significantly shows the SOD of the group that did not receive strenuous physical activity treatment (group K0) was significantly different from the treatment group K1. This is different from the research conducted by Yan (2020) which says that physical activity causes antioxidants to tend to increase, and pro-oxidant indicators tend to decrease. The reduction in oxidative stress in organ systems with exercise training is likely due to increased antioxidant defenses in tissues. Slow-twitch oxidative skeletal muscle fibers have a high oxidative capacity and have a higher antioxidant capacity than fast-twitch glycolytic fibers with lower oxidative potential. Most of the effects of exercise training in the antioxidant defense system are intracellular, including CuZnSOD, MnSOD and CAT, EcSOD is the only one that functions extracellularly.⁹

Exercise-induced expression of EcSOD appears to be specific for endurance (aerobic) exercise, not resistance training induced. Finally, genetically engineered mice with enhanced expression of EcSOD in skeletal muscle showed increased levels of EcSOD in blood and all peripheral tissues and organs, such as kidney, liver, heart, lung, and adipose tissue. These findings support that resistance training promotes EcSOD expression in skeletal muscle, the largest organ of the body, leading to enhanced extracellular antioxidant defenses in circulation and peripheral tissues as molecular transducers of the health and disease benefits of exercise.⁹ According to Ulvie (2012), the low levels of SOD in the group that received physical activity treatment could occur because the response of the antioxidant defense system to aerobic exercise depends on many factors. These factors are, duration of exercise, intensity of exercise, exposure to previous exercise, and age. The variability of the results was caused by differences in the use of the exercise model, the time of sampling, the status of the exercise subject, and the environment, such as height factors (Selman *et al.*, 2002). on the antioxidant enzyme activity of skeletal muscle, no significant changes were found. After the exercise was increased to twice a day for one week, there was a significant increase in antioxidant enzyme activity.¹⁶

Effects of physical activity on MDA

Malondialdehyde (MDA) is an important marker reflecting low-grade systemic inflammation. MDA produced in various tissues is often involved in the pathogenesis of micro and macrovascular diseases.¹⁰ Furthermore, from table 5, it can be seen that there is a significant difference in MDA levels between groups ($p < 0.05$), and it significantly shows that the MDA of the group that did not receive physical activity treatment (group K0) was significantly different from the group that received physical activity treatment. (K1 group). This is in accordance with research by Arslan (2014) which concluded that MDA as a marker of oxidative stress, showed a very significant relationship with diastolic hypertension in the research conducted. Lipid hydroperoxides decompose to form various products including malondialdehyde. Malondialdehyde is used as an indicator of oxidative damage to cells and tissues. There is growing evidence that increased oxidative stress and associated oxidative damage are mediators of vascular injury in cardiovascular pathology, including hypertension, and atherosclerosis. Exercise as a targeted therapy against reactive oxygen intermediates by reducing the generation of reactive oxygen species may be useful in minimizing vascular injury and end-organ damage in hypertension. The same thing was expressed by Bauzid *et al.* (2015) who concluded that MDA levels were higher during the recovery period in the HFG

(High level fitness group) compared to other groups. This study concluded that low or high levels of physical fitness help maintain better antioxidant defenses in older adults.¹⁷

CONCLUSION

There is a difference in the mean levels of HSP-70 in white rat (*Rattus norvegicus*) the man whoperform strenuous physical activity, lower than white rat (*Rattus norvegicus*) male who do not engage in strenuous physical activity. There is a difference in the mean SOD levels in white rat (*Rattus norvegicus*) male whichperform strenuous physical activity, lower than white rat (*Rattus norvegicus*) male who do not do strenuous physical activity. There is a difference in the mean MDA levels inwhite rat (*Rattus norvegicus*)male afterdoing strenuous physical activity, higher than white rat (*Rattus norvegicus*) male who do not engage in strenuous physical activity. This study was still has limitation such as variations in the intensity and type of physical activity which cannot be seen from this study.

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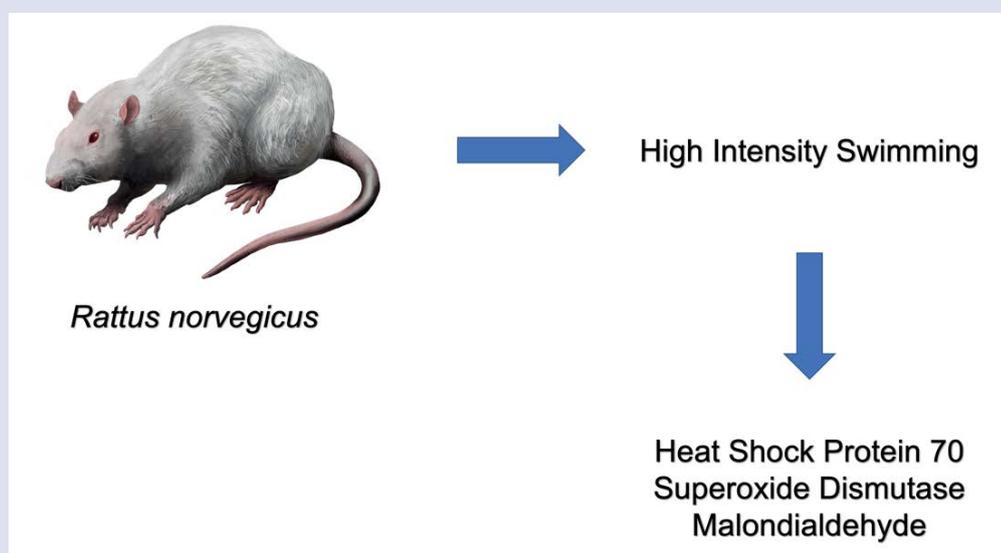
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GRAPHICAL ABSTRACT



ABOUT AUTHORS



Dody Taruna: He is a doctoral student from Faculty of Medicine, Universitas Airlangga. He is also a lecturer at Universitas Hang Tuah. Recently, his research topic about the effect of physical activity on male physiology and fertility.



Bambang Purwanto: He was graduated as medical doctor from Universitas Airlangga. Exercise physiology was interested field on his master and Ph.D program. Since 2017, he was attributed for Associate Profesor in Medical Physiology at Universitas Airlangga.



Harianto Notopuro: He is Professor at Faculty of medicine, Universitas Airlangga. His main topic research is related to biochemistry and biomolecular in human.



Widjiati: She is Professor at the Faculty of Veterinary Medicine, Universitas Airlangga. The research topic is currently being carried out is related to the reproduction of livestock.



Budi Utomo: He is senior lecturer in Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga. Experience with data management, epidemiology research and biostatistics. Strong background in research project management and data management.



Lilik Herawati: She is lecturer at Department of Physiology, Faculty of Medicine, Universitas Airlangga. His expertise is human physiology. Recently, she works in diabetes and obesity research.



Reny l'tishom: He is Associate Professor at Department of Biomedical Science, Faculty of Medicine, Universitas Airlangga. His publication subjects are male infertility, herbal Medicine and toxicology.



Aryati: She is Professor of Clinical Pathology at Faculty of Medicine, Universitas Airlangga. Her expertise is in Immunology and molecular biology.

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