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EFFECTS OF MODERATE-INTENSITY EXERCISE TRAINING ON STRESS OXIDATIVE MARKER: MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE ACTIVITY IN ABDOMINAL AORTA OF JUVENILE RATS

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Abstract

To improve cardiovascular health, the WHO recommends 60 minutes of frequent moderate intensity physical activity in childhood. ACSM also recommends physical activity 30 minutes moderate activity or 30 minutes vigorous intensity, 3-4 times per week. However, limited data concerned in exercise starting from childhood effect to oxidative stress marker in vascular. Therefore the long-term effects of moderate intensity aerobic exercise training in early age on the cardiovascular, specifically on vascular stress oxidative marker needed to be studied. This study was conducted on male Wistar rats aged 3 weeks (60-70 grams), randomly allocated into 2 groups: 1) control group and 2) training group. Aerobic exercise training was conducted for 8 weeks on treadmill with age-dependent speeds. Training was intermittently 5 days each week for 20 minutes. Vascular oxidative stress marker was analyzed by measuring the level of malondialdehyde (MDA) and superoxide dismutase (SOD) activity on the abdominal aorta. Both the levels of MDA and SOD activity tended to increase in training group compared to the control group. The resuls of this study showed that long-term effects of moderate intensity aerobic exercise training in juvenile tended to increase the levels of MDA and specific SOD activity in the abdominal aorta tissues.

Keywords: Moderate Intensity Aerobic Exercise Training; Juvenile; Stress Oxidative Marker; MDA; SOD; Abdominal Aorta.

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1. Introduction

Cardiovascular diseases may have their origins in the early childhood [1,2]. All of risk factor of cardiovascular diseases are related with increased radical oxygen species (ROS) production in the vascular wall [3]. Sufficient ROS concentration play a number of regulatory function in normal condition, but increased ROS production can cause various cardiovascular diseases [3,4]. ROS play an important role in vascular homeostasis in physiological condition. In vascular wall, ROS can produced by NADPH oxidase, xanthine oxidase, uncoupling eNOS, mitochondrial respiration [3,5], myeloperoxidase, lypoxogenases, and transition metal [5]. Increased ROS in vascular wall can activate transcription of pro-inflammatory genes, endothelial cells, and also leukocyte cells, that leading to atherosclerosis [3].

Vascular wall also contain antioxidant defense system, such as SOD, Catalase, and Glutation peroxidase [3]. All of this antioxidant enzymes would reduce the ROS by change the ROS to non-radical molecule [3,5]. This antioxidant can reduce peroxynitrite formation which improve nitric oxide bioavailability [6]. So, antioxidant system can prevent cardiovascular diseases [4]. Aerobic exercise training is beneficial in the prevention as well as treatment for cardiovascular and metabolic diseases [7,8]. Regular aerobic exercise training can increase antioxidant mechanism to reduce ROS. It known cause increase SOD expression [9] and activity in vascular wall [10].

Lifestyle choices in childhood that continue to adulthood can affect long-term health [1]. The WHO stated that lack of physical activity is one mortality risk factor which can cause 6% mortality in the world [11]. Therefore, regular aerobic exercise training from childhood can be used to prevent cardiovascular diseases later in adulthood [1,2,12]. However, studies reviewed by Silva and Lima [13], showed that intense exercise causes oxidative stress in human and animals. Data on long term effects of exercise for juvenile to oxidative stress marker in vascular wall is still limited. However, WHO and some countries recommend 60 minutes of moderate intensity physical activity regularly for children to improve the cardiovascular health [14]. ACSM also recommend 30 minutes moderate intensity plus 30 minutes vigorous intensity for 3-4 times/weeks, almost every days will be better for children and youth [15].

The aim of this study was to evaluate the long-term effects of moderate intensity aerobic exercise training in early age on oxidative stress marker by measuring the level of malondialdehyde (MDA) and superoxide dismutase (SOD) specific activity from abdominal aorta tissue.

2. Materials and Methods

Ten juvenile male Wistar rats (3 week-old, 60-70 gram) were randomly divided into two groups, 1) 5 rats as control group and 2) 5 rats as training group. Before and during intervention, the rats were maintained properly according to the ethics of experimental animal usage. Rats were housed in an environmentally controlled room (temperature 23±1°C; lights on at 0600 h and off at 1800 h) in groups of five per cage with rat food and water ad libitum. This study was approved

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by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo Hospital. Aerobic exercise training protocol was adopted from Hsu [16] with some modification [17]. The protocol for training group was described in Fig.1.

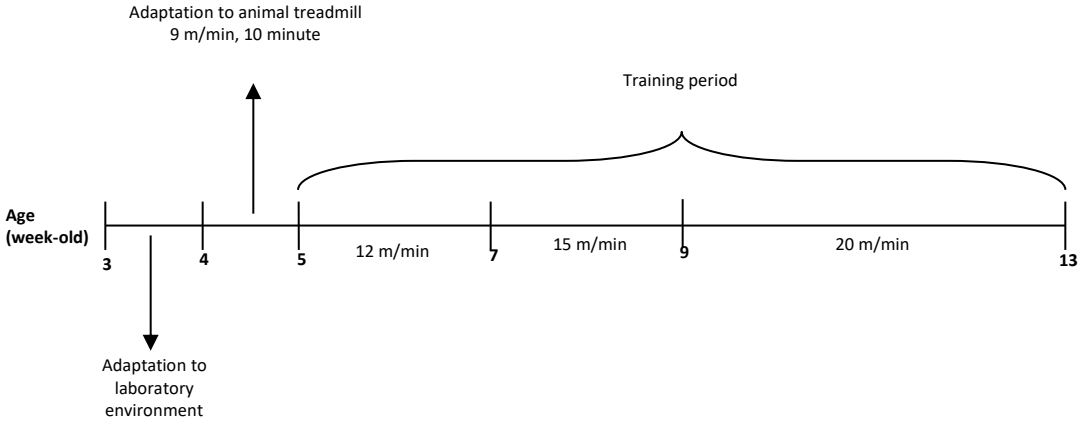

Figure 1: Training Protocol

In this period of adaptation to animal treadmill, the control group was placed on the animal treadmill without running for 10 minutes. At training period, the training group ran on the animal treadmill at an age-dependent speed described by Fig.1. Exercise was conducted 5 times a week Mondays to Fridays, with a 20-minute duration of running and a 90-second period of rest every 5 minutes running to avoid fatigue [17]. The control group was placed inside the animal treadmill without running for 20 minutes. To avoid the acute effects on exercise, the rats were decapitated by cervical dislocation on day-3 after treatment. Abdominal aorta was isolated, weighed and stored in the freezer at -80°C until used for homogenate. Then made into 10% abdominal aorta homogenate in a medium containing 10 mM Tris-HCL, 0.1 mM EDTA-2Na, 10 mM sucrose, 0.8% NaCl and pH 7.4. A supernatant was obtained from the tissue homogenate by centrifugation at 5000 rpm, 4°C, for 10 minutes. The supernatant was used for measuring the levels of MDA and SOD specific activity. MDA measurement was conducted to determine the level of lipid peroxide, the product of lipid peroxidation by radical oxygen species. This measurement used TBA method using thiobarbituric acid as chromogen to detect lipid peroxide. Formation of MDA-TBA adduct occurs by a nucleophilic attack involving carbon-5 of TBA and carbon-1 of MDA, followed by dehydration and similar reaction with a second molecule of TBA, producing a red pigment. The intensity of pink pigment formed from MDA-TBA condensation indicates the extent of lipid peroxidation. The level of MDA was measured by spectrophotometer using absorbance 530 nm. SOD activity in abdominal aorta tissue was measured using RANSOD kit by Randox Lab^{TM}, UK. This measurement used xanthine and xanthine oxidase to formed superoxide radical. SOD acivity measured by spectrophotometry method reflected by the amount of red formazan dye that showed the degree of inhibition of reaction between superoxide radical, with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. MDA and SOD values were given as means±SD. The data were analyzed using the SPSS version 16.0. All comparisons were performed using t-test independent and 1-sided p values of < 0.025 were considered statistically significant.

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3. Results

3.1. Malondialdehyde

The level of malondialdehyde can be seen in Fig. 2. The level of malondialdehyde in the training group (0.1395 ± 0.0179 nmol/mg of tissue) was higher than in the control group (0.1224 ± 0.0175 nmol/mg of tissue). Fig. 2 showed that malondialdehyde level of the training group tended to increase compared to the control group, but the difference was not significant (p = 0.084).

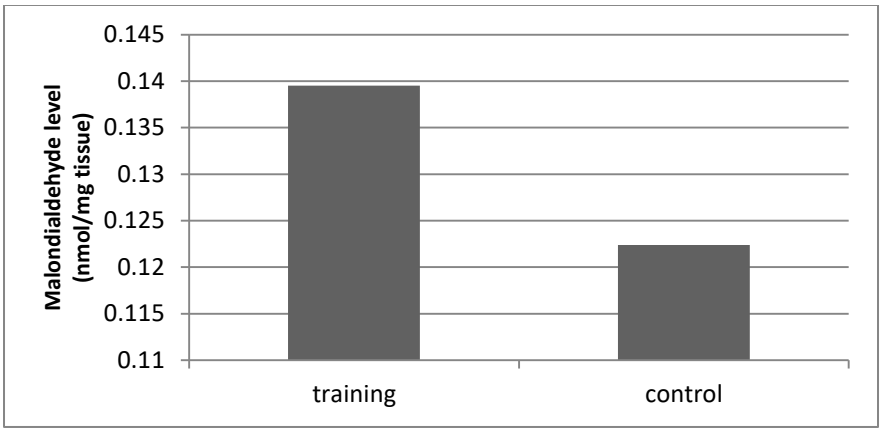

Figure 2: Comparison of Malondialdehyde (MDA) levels in the training group and control group

3.2. SOD Specific Activity

The level of SOD specific activity is presented in Fig. 3. The level of SOD specific activity in the training group (0.2806 ± 0.0882 U/mg of protein) was higher than in the control group (0.2185 ± 0.0621 U/mg of protein). Fig. 3 showed that SOD specific activity of the training group tended to increase compared to the control group, but the difference was not significant (p = 0.117).

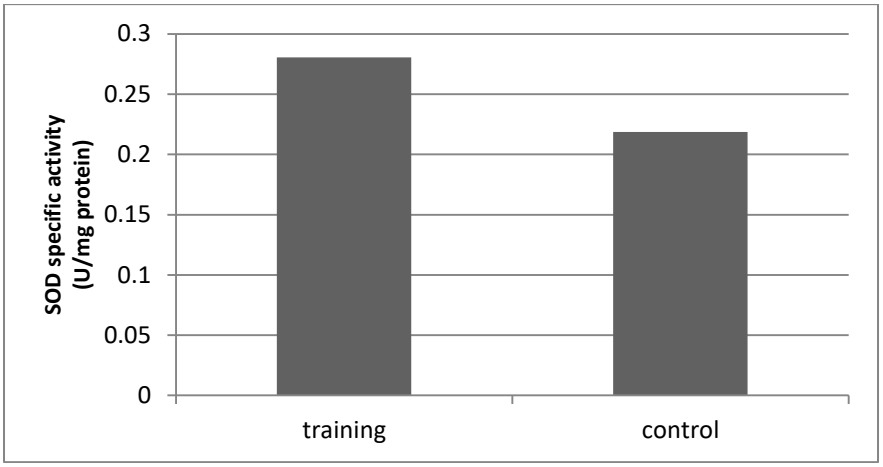

Figure 3: Comparison of superoxide dismutase (SOD) levels in the training group And control group

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4. Discussions

Wistar rats were used for this study because of their more active nature than other strains. Therefore it was expected the rats can follow the exercise protocol. At the beginning of the training program, the rats were aged 5 weeks, which was approximately equivalent to 10-12.5 years in human and therefore sexually immature [12,18].

The speed of the treadmill in this study classified as moderate intensity [16], similar to the WHO recommendation for children's physical activity [14]. The 8 weeks aerobic training for the rats was categorized as long-term aerobic training that was sufficient as chronic effect adaptation [16,19].

Pathological studies have shown that the abdominal aorta is first site of lipid deposit in large vessels [2]. Therefore, the abdominal aorta was used in this study to evaluate vascular oxidative stress marker.

This study showed that the level of malondialdehyde in the training group was higher than in the control group, although the difference was not significant. This meant that moderate intensity aerobic exercise training from early age tended to increase radical oxygen species in the abdominal aorta tissue. Real-life exercise program in healthy children (8-10 years old) also showed that it can increase systemic oxidative stress measured in the urinary concentration of 8-isoprostaglandin $F_{2\alpha}$ [20]. Sahin [21] research with 16 weeks swimming training also showed that there was significant increased in erythrocyte TBARs level in juvenile swimming training compared to control. In this study, intermittent moderate intensity training at a frequency of 5 days per week, with duration of 20 minutes on the juvenile rats showed increased radical oxygen species in abdominal aorta tissue. It also showed that might be the exercise dose was still too strenuous for juvenile rats. Bello et al result study reviewed by Schneider and Oliveira [22], showed that no increase on TBAr level in the hearts of 3-week old rats that training at low-intensity for 4 weeks. Therefore, children involved in training or interested in developing as an athlete must have sufficient time to rest between training for recovery. Beside that, children should begin the training with low-intensity and gradually increased until it reaches moderate-intensity.

Although aerobic exercise training on juvenile rats caused an increase in malondialdehyde level, an increase in SOD specific activity was also present. This showed that there was an effort by the body to resolve the MDA level in the abdominal aorta tissue. In a study conducted by de Moraes showed that exercise training increase SOD expression in the aorta tissue [9].This condition (increase MDA level and SOD activity) showed unperfect antioxidant mechanism to reduce ROS in juvenile training group. It is showed difference response of exercise in adult rat. It perhaps related to factors that influence SOD activity in early age. To know more detail impact of exercise in juvenile rat to oxidative stress marker in vascular tissue, it suggest to measure oxidative stress marker from vascular tissue, every week.

Exercise is physical stressor that disturbs the homeostatic balance of the body, which can increase ROS [23]. If the stressor is given regularly and continuously with a specified time and interval, the body will adapt to this stressor and change the stressor to become a stimulator [23].

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From this study, adaptation in juvenile rat looks like unperfect. It had difference response from adult rat. Increased in SOD activity still could not reduce ROS, that could seen from MDA level that higher in training group compare to control. Although MDA level in training group was higher than control, it could not said this condition as an oxidative stress condition because SOD activity increase with exercise training. Oxidative stress occurred if increased formation of oxidant is accompanied by a loss of antioxidant or accumulation of oxidized forms of the antioxidant [5]. Also remain unknown if it had clinical impact or not. Therefore, it needs more research about clinical impact from exercise in juvenile to vascular function.

5. Conclusions

There were limitations to this study, since the data obtained were on the quantity of biomarkers in oxidative stress marker and not directly on vascular function, which could be measured by vascular reactivity or blood pressure of the rats. Data for impact exercise in vascular also could evaluated by immunology marker and histological analysis. The conclusions of this study is the long-term effect of moderate intensity aerobic exercise training in juveniles can increase the level of MDA and also the SOD specific activity.

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Competing Interests

No financial interests to declare in this study.

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