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Different Effects of Acute and Chronic Strenuous Physical Exercise on Superoxide Dismutase (SOD), Malondialdehyde (MDA) Levels, and Sperm Quality of the Wistar Rats

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ABSTRACT

Background: Normal exercise can improve human physical abilities, but strenuous exercise can damage human cells. Strenuous exercise causes oxidative stress to the body. In order to determine the level of oxidative stress, it is important to check the levels of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in the body. SOD is the first line of defence in fighting against the oxidative stress, whereas MDA is the result of oxidative stress cell damage in the body. The sperm cell is the one that is affected by oxidative stress. This research aimed to investigate the differences in the effects of acute and chronic strenuous exercise on SOD production, MDA, and sperm quality.

Methods: The research was based on experimental design with post-test only control group design with Wistar rats. Eighteen male Wistar rats were randomly divided into 3 groups (n = 6). Group I: Normal control, Group II: Rats were treated to swim for about 25-40 minutes until they were drowning for 1 day (acute strenuous physical exercise), and Group III: Rats were treated to swim for about 25-40 minutes until they were drowning for almost every day for 2 weeks (chronic strenuous physical exercise). Examination of SOD and MDA levels was done using spectrophotometry, examination of sperm quality was done by looking at the morphology, motility, and sperm quantity through the light microscope at x1000 with haemocytometer.

Results: Chronic strenuous exercise significantly affects the decreasing SOD levels, increasing MDA levels, and decreasing sperm quality compared to the control group and acute strenuous exercise ($P < 0.05$).

Conclusion: According to the results of this study, the chronic strenuous exercise effects increase oxidative stress and sperm damage.

Keywords: Strenuous physical exercise, Oxidative stress, Superoxide dismutase, Malondialdehyde, Sperm quality, Sport physiology

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Introduction

Physical exercise is one of the basic human needs. By exercising regularly and periodically, people will gain a functional increase of various systems in the body (1). Physical exercise that is done repeatedly and on a regular basis is called training (2). In general, the physical exercise consists of two types of activities, anaerobic and aerobic activities (3). Both types are distinguished by the metabolic processes of the formation of energy when doing the physical exercise (4). Physical exercise can increase body temperature, blood acidity, and it can reduce the O_2 capacity in the body (5). An exercise is a form of physical stressors that have a variety of side effects, therefore, the physical exercise that is incompatible with the basic principles of exercise will adversely affect the function of the human system (6). One of the physical exercises that are not in accordance with the basic principles of training can be described as an excessive physical exercise or commonly known as "overtraining" (7).

Strenuous physical exercise or overtraining may cause health problems (8). This is because strenuous physical exercise will increase the production of free radicals in the body or commonly known as Reactive Oxygen Species (ROS) (9). Strenuous physical exercise needs more oxygen than the currently planned exercise (10). There are several choices of the formation of oxidants in the body during physical exercise such as oxidative phosphorylation, xanthine oxidase, autoxidation, and inflammatory responses (11). It causes an increase of more than 100% in ROS production when doing the physical exercise activity (12). The increasing levels of ROS in the body can be lethal (13).

Strenuous physical exercise is usually done by the athletes who are seriously preparing for a competition. Physical training programs for athletes are usually arranged for 5-6 times a week, sometimes they also have to do exercises in the morning and evening in the training center. In a long duration, strenuous physical exercise intensity may be harmful and may cause adverse effects on the body (14). One of the effects of strenuous physical exercise is the increasing oxidant compounds in the body, which is commonly known as oxidative stress (15). The release of free radicals will be involved in the process of fat oxidation in muscle cell membranes. It is called lipid peroxidation and it causes cells to be more prone to aging or other damage (16). Oxidative stress can lead to various chronic diseases, and also inflammation in the body and male infertility (17).

One of the biomarkers commonly used by researchers to measure the level of oxidative stress in the body is Malondialdehyde (MDA) (18). Malondialdehyde is the product of lipid peroxidation in aerobic metabolic processes. When the lipid peroxidation process takes place, superoxide dismutase (SOD) enzyme has a duty as the first line of defence against the excessive lipid peroxidation processes in the body (19). In this process, its activities depend on mineral metals such as manganese (Mn) and copper (Cu) so that they can work optimally (20). Excessive accumulation of free radical in the body results in accelerated apoptosis and damage to organs (19). One of the effects of oxidative stress is the occurrence of male infertility (21,22).

Thirty to eighty percent of male infertility cases are caused by oxidation stress (23). One of the causal factors for male infertility is the low antioxidant levels in the body. This is because the majority of infertile men are shown to have significantly more inflammation of the reproductive tract than fertile men (24). In inflammatory conditions, neutrophils are the dominant leukocytes contained in semen and have a high production capacity for ROS. Reactive oxygen species can damage the outer structure that can lead to decreased sperm motility, sperm count, and sperm morphology. It also affects DNA fragmentation. DNA fragmentation can occur due to exposure to ROS during the replacement of histones by protamine during the spermatogenesis process (25). So that ROS can lead to male infertility. It is very important to understand and determine the impact of strenuous physical exercise on the human body.

Materials and Methods

Research Design

This is an experimental study with a post-test group control design to determine the acute and chronic effects of strenuous physical exercise on the levels of superoxide dismutase (SOD), Malondialdehyde (MDA), and sperm quality.

Animal

In this experimental study, 18 male Wistar rats (aged 8 weeks with the weight of 200-250 g) were used. The animals were divided into three groups, 6 in each group. Before given the treatment, the rats were acclimated for 7 days without intervention at room temperature (25 ± 2°C). The rats were fed with standardized rat food with the nutritional content of 14% moisture, 18% protein, 8% fat, 6% fiber, and 8% ash. This research has gained the official permission from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang (Ethical number 216/VII/2020/Bioethics Commission). This research was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia.

Animal Grouping

Group I: Control group rats were only given standard foods and drinks without any strenuous physical exercise treatment.

Group II: The rats were fed a standardized foods and drinks. The rats were given strenuous physical exercise for one day. The physical exercise was that the rats were given swimming treatment for 25-40 minutes until they were almost drowned.

Group III: The rats were given standard foods and drinks. The rats were given strenuous physical exercise every day for two weeks. The physical exercise was the rats given the treatment to swim for 25-40 minutes until they were almost drowned.

Procedure for Giving Physical Exercise

The aquarium was filled with water level at 70-80 cm, the water temperature in the aquarium was 36°C, then, the rats were put into the aquarium, the rats swam for 25-40 minutes until they got tired with the signs that they were almost drowning. Swimming activities were conducted for 1 day for Group II (acute strenuous physical exercise) and 14 days or two weeks for Group III (chronic strenuous physical exercise).

Blood Sampling

In Group I, blood samples were taken on day 14. In Group II, the samples were taken after physical exercise on day 1, and in Group III, blood samples were taken after physical exercise on day 14. Blood samples were taken through the orbital sinus of the eye. Afterwards, the blood sample was put into the vacutainer tube, then, the vacutainer tube was inserted into a centrifuge at a speed of 3000 rpm for 30 minutes to get blood serum. The blood serum was then put into a micro-tube, and then, placed in a cooling machine with a temperature of -20°C.

Procedure for checking Superoxide Dismutase (SOD) levels

Superoxide dismutase levels were determined biochemically using the Ran-SOD kit at the Integrated Biomedical Laboratory of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia. The reagents in this kit consisted of a mixed substrate containing xanthine, phosphate buffer to dilute (standard or sample), xanthine oxidase, and standard solution to create standard curves. A total of 25 µL of plasma was used to measure blood SOD levels. Initially, 25 µL of the sample was put into the cuvette and 850 µL of the mixed substrate was added and mixed well. To inhibit SOD, 5 µL of 5 mM sodium cyanide was added to the mixture until well mixed. Afterwards, 125 µL of xanthine oxidase was added. The absorption was read at a wavelength of 505 nm using a spectrophotometer (Genesis). Superoxide dismutase (SOD) levels were determined using the equation obtained from the standard curve.

Procedure for checking Malondialdehyde (MDA) levels

Measurement of the concentration of the experimental sample was carried out in the same way as the standard solution, which was 1.0 mL of blood plasma reacted with 1.0 mL of 20% TCA and 1.0 mL of 1% TBA in 50% glacial acetic acid, then, incubated for 45 minutes at 95°C. The solution was centrifuged at 1000 rpm for 15 minutes. The supernatant was separated, and then, its absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 532.2 nm. The sample concentration was obtained by plotting the absorbance data of the sample into a standard curve.

Sperm Sampling

After collecting the blood samples, sperm samples were taken from the Wistar rats. Before taking rats' sperm cells, the rats were sacrificed by cervical dislocation. Then, surgery was performed on the rats by opening the abdominal wall so that the testes and epididymis were visible. Epididymal cauda was recorded in a petri dish containing 1 ml of 0.9% NaCl. Then cut with scissors the proximal part of the cauda epididymis, press the cauda epididymis slowly until the epididymal fluid secretion comes out suspended with 0.9% NaCl. Spermatozoa suspension that was successfully made could be used for morphological measurements. Examination of the amount of sperm, sperm morphology, and motility of spermatozoa was done using a light microscope at x1000 with a hemocytometer.

Statistical Analysis

All values were reported as mean ± standard deviation (SD). Statistical analysis was done using SPSS version 26 with one-way analysis of variance (ANOVA) and using the post-hoc test. Statistical significant level was considered at $P < 0.05$.

Results

Superoxide Dismutase (SOD) Levels

In this study, strenuous physical exercise could significantly increase SOD levels ($P < 0.05$). In the control group, the average SOD level was 83 U/ml, after being given strenuous exercise for 1 day, the average SOD level decreased to 73.3 U/ml. Meanwhile, after being given strenuous exercise for 2 weeks or 14 days, the SOD level dropped dramatically to 36.5 U/ml. Based on the data analysis, it can be concluded that giving strenuous physical exercise for 2 weeks has a significant effect on reducing levels of SOD in male Wistar rats. The significance of the differences in the mean SOD levels between the groups in the study is shown in Figure 1.

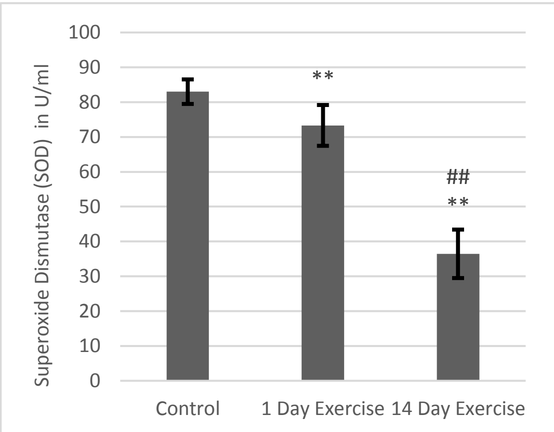

Figure 1. Effects of strenuous exercise on the average levels of superoxide dismutase (SOD).
Values are represented as Mean ± Standard Error of Mean (SEM) (n=6). ANOVA followed by the Post-hoc analysis by SPSS version 26. **P < 0.05, 1-day strenuous exercise and 14-day strenuous exercise compared to control. ##P < 0.05, 14-day strenuous exercise compared to 1-day strenuous exercise.

Malondialdehyde (MDA) Levels

In this research, strenuous physical exercise significantly reduced levels of Malondialdehyde (MDA) ($P < 0.05$). In the control group, the average level of MDA was 2.1 nmol/ml, after being given strenuous physical exercise for one day, the average level of MDA increased to 3.5 nmol/ml. Meanwhile, after being given strenuous physical exercise for two weeks or 14 days, the level of MDA increased dramatically to 8.5 nmol/ml. Based on the data analysis, it can be concluded that giving strenuous physical exercise for two weeks has a significant effect on the increasing levels of MDA in male Wistar rats. The significance of the differences in the mean levels of MDA between the groups in the study is shown in Figure 2.

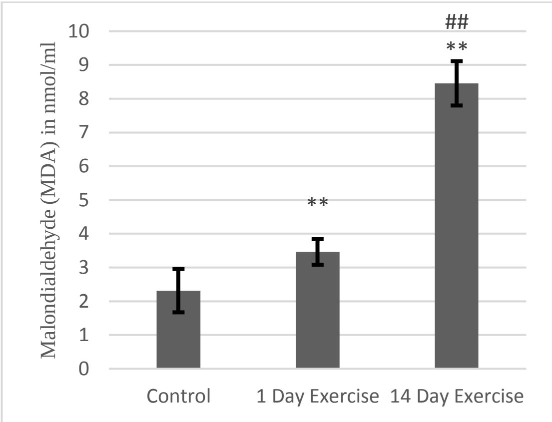

Figure 2. Effects of strenuous exercise on the average levels of Malondialdehyde (MDA).
Values are represented as Mean ± Standard Error of Mean (SEM) (n=6). ANOVA followed by the Post-hoc analysis by SPSS version 26. **P < 0.05, 1-day strenuous exercise and 14-day strenuous exercise compared to control. ##P < 0.05, 14-day strenuous exercise compared to 1-day strenuous exercise.

Sperm Count

In this research, strenuous physical exercise could significantly reduce the sperm count ($P < 0.05$). In the control group, the average sperm count was 30.7 million/ml. After one day of strenuous exercise, the mean of sperm count decreased to 25.3 million/ml. Meanwhile, after being given strenuous exercise for two weeks or 14 days, the number of spermatozoa decreased dramatically to 11.6 million/ml. Based on the data analysis, it can be concluded that giving strenuous physical exercise for two weeks has a significant effect on reducing the number of spermatozoa in male Wistar rats. The significance of the difference in the mean number of spermatozoa between the groups in the study is shown in Figure 3.

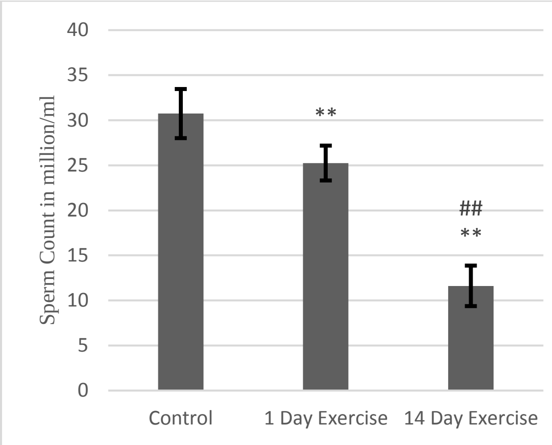

Figure 3. The effect of strenuous physical exercise on the average sperm count.
Values are represented as Mean ± Standard Error of Mean (SEM) (n=6). ANOVA followed by the Post-hoc analysis by SPSS version 26. **P < 0.05, 1-day strenuous exercise and 14-day strenuous exercise compared to control. ##P < 0.05, 14-day strenuous exercise compared to 1-day strenuous exercise

Sperm Motility

In this research, strenuous physical exercise significantly reduced spermatozoa motility (P < 0.05). In the control group, the mean of spermatozoa motility was 53.8%. After being given strenuous physical exercise for one day, the mean spermatozoa motility decreased to 42.9%. Meanwhile, after strenuous physical exercise for two weeks or 14 days, sperm motility decreased dramatically to 28.5%. Based on the data analysis, it can be concluded that giving strenuous physical exercise for 2 weeks has a significant effect on decreasing spermatozoa motility in male Wistar rats. The significance of the difference in the mean number of spermatozoa between the groups in the study is shown in Figure 4.

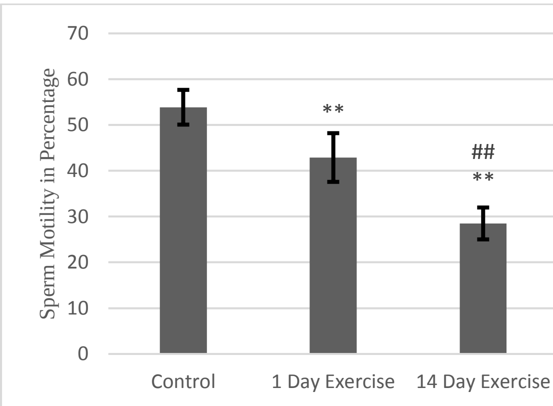

Figure 4. The effect of strenuous physical exercise on the average sperm motility.
Values are represented as Mean ± Standard Error of Mean (SEM) (n=6). ANOVA followed by the Post-hoc analysis by SPSS version 26. **P < 0.05, 1-day strenuous exercise and 14-day strenuous exercise compared to control. ##P < 0.05, 14-day strenuous exercise compared to 1-day strenuous exercise.

Sperm Morphology

In this research, strenuous physical exercise significantly decreased spermatozoa motility (P < 0.05). In the control group, the mean of spermatozoa motility was 84.9%. After being given strenuous physical exercise for one day, the mean spermatozoa motility decreased to 77%. Meanwhile, after being inforced to do strenous physical exercise for two weeks or 14 days, sperm motility decreased dramatically to 55.8%. Based on the data analysis, it can be concluded that strenous physical exercise for two weeks has a significant effect on decreasing spermatozoa motility in male Wistar rats. The significance of the difference in the mean number of spermatozoa between the groups in the study is shown in Figure 5.

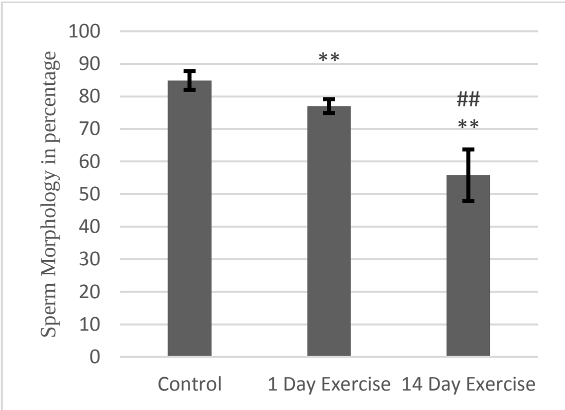

Figure 5. The effect of strenuous physical exercise on the average sperm morphology.
Values are represented as Mean ± Standard Error of Mean (SEM) (n=6). ANOVA followed by the Post-hoc analysis by SPSS version 26. **P < 0.05, 1-day strenuous exercise and 14-day strenuous exercise compared to control. ##P < 0.05, 14-day strenuous exercise compared to 1-day strenuous exercise.

Discussion

Physical exercise has a weighty effect on the condition of the human body. Physical exercise gives the effect of increasing immunity, physical fitness, and even it is able to prevent various aging-related diseases. Physical exercise is the movement of limbs that causes energy expenditure which is important for maintaining physical and mental health for maintaining the immune system in the body. Physical exercise is divided into several intensities including light, moderate, and heavy intensity. The intensity categories are distinguished by the attainment of a maximum pulse rate or Maximal Heart Rate (MHR) during the activity. Physical exercise is categorized in the category of light intensity if it reaches 50-63% MHR, in the category of moderate-intensity if it reaches 64-76% MHR, and in the category of heavy intensity if it reaches 77-93% MHR. However, physical exercise should be in accordance with the nature of the discipline of sport science that is measured and orderly.

Strenuous physical exercise usually occurs in an athlete who will compete in particular event of tournament. An athlete also performs strenuous physical exercise which can cause psychological disorders, physiological disorders, and decreased performance. Strenuous physical exercise can induce oxidative stress and although there is no evidence that this affects sporting performance in a short-term period, it may have long-term health consequences. Strenuous physical exercise will increase the oxygen consumption greater than the normal consumption in the bloodstream. Active muscles may increase the oxygen demand by 100% from a passive muscle condition (12). This increasing rate is due to the increased demand for ATP, while the intracellular supply of ATP is very limited, so that there is a continuous formation of ATP through oxidative processes, the Krebs cycle, and electron transport (26). Oxygen is needed in the process of ATP formation, oxygen consumption in the respiratory chain in mitochondria affects the increasing production of free radicals in the body. The production of reactive oxygen species (ROS) in mitochondria occurs to increase the activity of the electron transfer system through the oxidative phosphorylation process caused by increased metabolism and oxygen (27). Reactive oxygen species are converted into hydrogen peroxide (H_2O_2) and oxygen by superoxide dismutase (SOD) (28). Increased metabolic activity and oxygen consumption occurs when the body requires high energy requirements. This occurs during strenuous physical activity and the energy required is high enough, so the more energy is needed, the more free radicals will be produced (14).

During the exercise, there is an increase in sympathetic nerve activity, it may increase the metabolism of epinephrine and other catecholamines with O_2 to form free radicals (29). In long-term exercise, catecholamines in plasma increase, resulting in stimulation of beta-adrenergic receptors, an increase in oxidative metabolism, especially in the skeletal muscle and heart muscle (30). This metabolism in forming the energy from lipids increases lipolysis via the beta-oxidation pathway. This beta-oxidation pathway generates free radicals. Moreover, the autocorrelation of epinephrine to adrenochromes will form superoxide (O_2*) (19).

Superoxide dismutase is a primary antioxidant that works to prevent the formation of new free radicals because it can be changed into free radical molecules that diminish the negative impacts before they could react (31). Superoxide dismutase is one of the indicators used to determine the formation of radical compounds or intermediate reactions used to determine the formation of radical compounds or reactions between radical compounds and body molecules. Decreased SOD levels indicate the oxidative stress caused by an imbalance between antioxidants and free radicals (19,32).

Superoxide dismutase testing is a parameter used to determine the presence of antioxidant activity. The basic principle of measuring the activity of SOD is the reaction between xanthine and xanthine oxidase, which is used to produce superoxide radicals. Superoxide dismutase catalyzes the dismutation of superoxide radicals to more stable hydrogen peroxide. The results show that there is a significant difference between SOD activity in Group I against Group II and Group III. However, the results show that SOD activity tends to be lower in the group of rats that enforced to do strenuous physical exercise for 2 weeks (36.5 U/ml) compared to the group of rats that enforced to do the strenuous physical exercise for 1 day (73.3 U/ml) and the group that was not given any physical exercise (83.01 U/ml). This result is consistent with the results of a study by Yunarsa and Adiatmika that reported the average SOD activity in rats given excessive physical exercise (34.84 U/ml) compared to normal group (68.39 U/ml) (33).

In addition to measuring SOD levels, the measurement of oxidative stress conditions can also be done by measuring Malondialdehyde (MDA). Malondialdehyde is the final product of lipid oxidation which is toxic to cells and is a dialdehyde compound that has three carbon chains and it has a low molecular weight and can be produced by different mechanisms (16). Malondialdehyde is a white hygroscopic crystalline compound obtained from hydrolysis of tetraethoxypropane acid (34). Malondialdehyde levels have been widely used as an indicator of oxidative stress in unsaturated fats as well as an indicator of the presence of free radicals. Malondialdehyde levels are measured using the Thiobarbituric Acid Reactive

Substances (TBARS) test (18). Free radicals that were formed before will react with SOD and Cu^{2+} ions to produce H_2O_2. Furthermore, H_2O_2 reacts with Fe^{+2} and Cu^{+2} to form hydroxyl free radicals through the Fenton and Haber-Weiss reactions. The hydroxyl radical is a very reactive thing (16). Whereas the cell membrane consists of many important components such as phospholipids and glycolipids, both of them contain unsaturated fatty acids and cholesterol (35). These unsaturated fatty acids are very sensitive to hydroxyl radicals. This hydroxyl radical will form a chain reaction with one hydrogen atom from the cell membrane and form lipid peroxides. Based on the results of this research, there was a significant difference between the average MDA blood serum levels in the group of rats that enforced to do strenuous physical exercise for 2 weeks, and the group of rats that enforced to do physical exercise for one day, and the normal group. However, the results show that MDA activity tends to be higher in the group of rats that enforced to do strenuous physical exercise for 2 weeks (8.5 nMol/mL) compared to the group of rats that enforced to do the strenuous physical exercise for 1 day (3.2 nMol/mL) and the group that was not given any physical exercise (2.1 nMol/mL). The results of this research are consistent with the results of a research by Ilyas et al. (2017), that have reported an increase in the serum MDA levels were doing such vigorous exercises (36).

Reactive oxygen species levels that exceed the threshold for antioxidant defense in the reproductive tract oxidize protein, lipid, and DNA biomolecules, which cause spermatogenesis disorders. This increase in free radicals will damage the membranes of spermatogenic cells, thus, interfering with the transport of ions essential for the proliferation and growth of spermatogenic cells, damaging spermatozoa DNA, and increasing the apoptosis of spermatozoa (21). Increased apoptosis of spermatozoa can affect the morphology of spermatozoa. In this research, the group that was enforced to do strenuous physical exercise for 2 weeks had a morphological percentage of spermatozoa at 55.8%, the group enforced to do strenuous physical exercise for 1 day was at 77% and the normal group was at 84.9%. The increase in ROS production capacity is due to an increase in NADPH oxidase in the spermatozoa membrane (37). Increased NADPH is formed by the enzyme glucose-6-phosphate dehydrogenase in the immature cell cytoplasm. The formation of ROS will cause the damage to the mitochondrial membrane, and mitochondrial damage will also lead to increased production of ROS. Increased ROS also causes damage to Leydig cells and apoptosis in spermatozoa. Leydig cell damage may lead to decreased testosterone levels of intratesticular, which results in a decrease in the number of spermatozoa (38). In this research, the normal group had a sperm count of 30.7 million/ml, in the group that was enforced to do strenuous physical exercise for 1 day, the sperm count decreased to 25.3 million/ml, and in the group that was enforced to do strenuous physical exercise for 2 weeks, the sperm count dropped dramatically to 11.6 million/ml. ROS also damages mitochondrial DNA causing a decrease in ATP and resulting in decreased spermatozoa motility (38,39). In this research, the normal group had fairly high motility (53.8%), while in the group that was enforced to do physical exercise for 1 day, it was reported to be 42.9%, and in the group that was enforced to do physical exercise for 2 weeks, a very drastic decrease to 28.5% was reported.

Conclusion

Oxidative stress is a condition that occurs due to an imbalance between the production of free radicals in the body and the antioxidant defense system, which has a negative impact on the body. According to the results of this study, chronic strenuous physical exercise for 14 days or two weeks can reduce levels of superoxide dismutase, increase Malondialdehyde, and decrease sperm quality in male Wistar rats. Therefore, researchers suggested to reduce the negative effects of oxidative stress by providing antioxidants from outside the body.

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Conflict of Interests

The authors declare that there is no conflict of interests. The authors alone are responsible for the content and writing of the paper.

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