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Original Article

The Comparative Study of Physical Exercise Towards Brain Natriuretic Peptide (BNP) and Malondialdehyde (MDA) as Oxidative Stress Markers in (*Rattus novergicus)* Wistar Strain Rats

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Abstract

Background: Physical exercise has an effect on biological functions, namely a positive effect in terms of cell repair and a negative effect, namely inhibiting or damaging cells. Strenuous physical exercise can increase muscle injury and oxidative stress in individuals who are not conditioned or are not used to physical exercise. In addition, oxidative stress also plays a role in chronic fatigue syndrome. This occurs because the speed of energy demand exceeds the speed and ability of the oxygen transport system to supply oxygen to the mitochondria. The heart muscle is a muscle that has a major role in physical exercise. Oxidative stress plays an important role in the pathogenesis of myocardial infarction. **Objectives:** The purpose of this research is to determine the difference between aerobic and anaerobic exercise in the formation of free radicals. The formation of free radicals can be predicted by measuring levels of malondialdehyde (MDA) and Brain Natriuetic Peptide (BNP). **Methods:** The design used in this study was an experimental study in vivo with a pretest-posttest design with a comparison group. The subjects of this study were 28 male Wistar rats, Rattus novergicus, which were divided into 6 treatment groups and 1 comparison group. The data is taken by doing treadmill physical exercise with a speed of 20m / minute for aerobics and 35m / minute for anaerobics. Different tests of average MDA and BNP levels were used using independent t-tests, ANOVA, and post hoc**. Result:** The results showed a decrease in MDA and BNP levels in the aerobic treatment group and an increase in anaerobic compared to the comparison group. The analysis showed that there was a significant difference between the aerobic group for 1 day, 3 times a week, and 7 days, respectively, with p 0.05. **Conclusion:** It can be concluded that aerobic and anaerobic physical exercise performed 1 day, 3 times a week, and 7 consecutive days can increase the antioxidant defence system so that oxidative stress decreases, which is indicated by a decrease in MDA and BNP levels.

Keywords: *Brain Natriuetic Peptide, Malondialdehyde, Physical Exercise*

Introduction

The incidence of sudden death is still high among athletes just before competition. A recent estimate of incidence ranged from 1 in 40,000 to 1 in 80,000 athletes per year. While there is controversy about the potential connection between intense endurance exercise and an increased risk of some cardiac conditions, However, for a small number of individuals who harbor cardiac conditions, exercise can sometimes be associated with the risk of sudden death (Wasfy, 2016).
Athletes usually do physical training with more intensity and more frequently**.** When doing physical exercise, the body needs a very large supply of oxygen, so this increase will trigger the emergence of free radicals in the body (Cowie *et al.,* 2003).

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If physical exercise is done with frequent frequency, there will be a buildup of free radicals in the body. According to the review by Weeks *et al*. (2016), an estimated 20–60% of athletes suffer from the stress caused by excessive exercise and inadequate recovery. At high intensities of physical exercise, free radicals can form due to the release of electrons from the spiration chain. Free radicals that are formed can cause interference at the molecular or cellular level by taking electrons from these molecules and cellular. In addition to free radicals formed during high-intensity physical exercise and light canal this increases the production of Brain Natriuretic Peptide (BNP) and Malondialdehide (MDA) in the blood (Aristoteles *et al.,* 2021)

BNP is a type-Bhormone that is stimulated when the heart ventricles are stretched and under pressure or respond to excessive volume and pressure changes. This changes because the oxygen supply could not meet the required level in order to maintain the heart's activity during high-intensity aerobic and anaerobic physical activity. On the other hand, MDA is produced when the body's antioxidants are unable to fight free radicals. Finally, electron-giving compounds or all compounds reduce the negative effects of oxidants, including enzymes and protein-binding proteins (Nur Wahyu *et al.,* 2023).

From above discussion a comparative study is needed between the effects of physical exercise of light intensity and high intensity on the levels of BNP and MDA in the blood as an indicator of oxidative stress. The aim of this study is to add information and scientific knowledge, especially in the field of biomedicine. Regarding the comparison of BNP and MDA levels in the blood of Wistar rats given the burden of physical exercise of light crossing with high-intensity physical exercise.

Material and Methods

This type of research is experimental laboratory research. The research was carried out in the animal house of the Faculty of Medicine, Sriwijaya, Indonesia. The research study has been approved by the research ethics committee of sultan Agung Islamic University, Semarang, No. 792/A.1/FIK-SA/XI/2022. The examination of BNP and MDA levels was carried out in the laboratory of the Department of Biochemistry and Molecular Biology of the Faculty of Medicine, Sriwijaya University. The population of this research subject is male Wistar strain rats obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. Inclusion Criteria Rats (Rattus norvegicus) male Wistar strain, 6–8 weeks old, weight 90–140 grams and Mice can participate in conducting research, and the exclusion criteria are that they are in sick condition, rats are lazy, or they cannot properly follow anaerobic physical activity. Data were collected by measuring the levels of BNP and MDA as parameters of oxidative stress. Analysis of the study *Paired t-test, unpaired t-test, and Anova and Post Hoc Test.* Materil and tool are: essay buffer concentration 20x (50 ml), peptide-biotin, standard peptides, TMB substrate solution, streptavidin-horseradish-peroxidase (SA-HRP), positive control, 2N HCL, spectrophotometer (biorad), stopwatch, microtube and micropipette, 96 well microtiter plates, microtiter plate reader, Acetat plate sealer, plate shaker, microtiter washer plate, multi-channel pipette, and EDTA Lavender Vacutaner tubes, Homogenate heart muscle of the Rattus noverticus wistar strain was used as a reagent for MDA and Aquades examinations. Procedures of study: rats were divided into 7 treatment groups; measurement of BNP content parameters; and Measurement of Oxidative Stress Parameters (MDA).

Results

Table 1. Average Rat Body Weight

Group	Early weight loss	P value
Comparison	98.75±10.30	0.179
Aerobic		
1 day	130.0±31.88	
3 times a week	111.2±13.15	
7 days	100.0±8.165	
Anaerobic		
1 day	108.7±11.81	
3 times a week	117.5±11.90	
7 days	92.50±25.98	

Homogenity of variance levene's test p>0.05

Characteristics of body weight (gr) of experimental rats (Rattus *novergicus*) based on treatment group (comparison group = P1, aerobic treatment group 1 day = P2, aerobic treatment group 3x a week = P3, aerobic treatment group 7 consecutive days = P4, treatment group anaerobic 1 day = P5, anaerobic treatment group 3x a week = P6, anaerobic treatment group 7 days in a row = P7), then the average body weight of rats was tested with Lavene's test and the results showed the average body weight of rats was relatively the same (homogeneous).

Table 2. Test the normality of research data on the level of Malondialdehyde

Group	p	1 day (nmol/gr)	p	3 times a week (nmol/gr)		7 days (nmol/gr)	p
Comparison 5.07±5.38	0.447						
Aerobic		1.92±0.78	0.369	1.57±0.44	0.332	0.80±0.21	0.577
Anaerobic		2.35 ±1.99	0.183	1.25±0.47	0.420	1.02±0.40	0.796

Normality Test with shapiro wilk α = 0.05

From the table above, MDA level measurement results in each experimental group (comparison group = P1, aerobics 1 day group = P2, aerobic group 3 times a week = P3, aerobics 7 days consecutive group = P4, anaerobic one day group = P5, 3 times an aerobic group = P6, and anaerobic groups 7 days in a row =P7) are listed in table 2 The normality test is then performed with Shapiro Wilk (p > 0.05), and the results show normal distribution data.

Table 3. Normality Test Data for BNP Level Research Data

Group	P	1 day (nmol/gr)	P	3 times a week (nmol/gr)	P	7 days (nmol/gr)	P
Comparison 4.42±2.90	0.859						
Aerobic		6.00±2.61	0.983	1.18±2.08	0.237	4.55±2.77	0.409
Anaerobic		6.93±1.04	0.350	7.88±2.50	0.029	9.44±5.59	0.569

Normality Test with shapiro wilk α = 0.05

The results of BNP level measurements in each experimental group (comparison group = P1, aerobic treatment group 1 day = P2, aerobic treatment group 3x a week = P3, aerobic treatment group for 7 days = P4, anaerobic treatment group 1 day = P5, treatment group anaerobic 3x a week = P6, and the anaerobic treatment group for 7 days = P7) are listed in table 3 and normality tests are performed with Shapiro wilk data (p≥0.05) BNP measurements in each experimental group. The results show that most of the data is normally distributed.

Table 4. Average Levels of MDA In The Aerobic Group

Group	No treatment	1 day (nmol/gr)	P	3 times a week (nmol/gr)	P	7 days (nmol/gr)	P
Comparison	5.07±5.38						
Aerobic		1.92 ±0.78	0.291	1.57±0.44	0.243	0.80±0.21	0.210

Meaningful difference (P<0.05)Independence t-test

The results of MDA level measurements in the aerobic group (P2, P3, P4) are listed in table 4, compared with the MDA level values of the comparison group (P1) by the unpaired t-test (p <0.05).

The results show that there was a decrease in MDA levels in the P2, P3, and P4 aerobic treatment groups compared to the comparison group.

Table 5. Average Level of MDA In The Anaerobic Group

Group	No Treatment	1 day (nmol/gr)	P	3 times a week (nmol/gr)	P	7 days (nmol/gr)	P
Comparison	5.07±5.38						
Anerobic		2.35 ±1.99	0.379	1.25±0.47	0.207	1.02±0.40	0.230

Meaningful Difference (p<0.05)Independence t-test

The measurement results of MDA levels in the anaerobic group (P5, P6, P7) compared to the MDA levels of the comparison group (P1) are listed in table 5, and then, by unpaired t-test (p<0.05), the results show that there is a decrease in MDA levels in the anaerobic treatment group compared with the comparison group.

Table 6. Average BNP Level of Aerobic Treatment Group

Group	No Treatment	1 day (pg/ml)	P	3 times a week (pg/ml)	P	7 days (pg/ml)	P
Comparison	4.42±29.06						
Aerobic		6.00±2.61	0.447	1.18±2.08	0.006	4.55±2.77	0.948

Meaningful Difference (p<0.05) Independence t-test

The results of BNP level measurements in the treatment group (P2, P3, P4) and the comparison group (P1) are listed in table 6. Furthermore, the mean BNP measurement results were calculated in each of the experimental groups, and the results showed an increase in BNP levels in the treatment group (P2) compared to the comparison group, while the other was a decrease in BNP levels in the treatment group (P3).

Table 7. Average BNP Levels in Anaerobic Groups (P4, P5, P6)

Group	No Treatment	1 day (pg/ml)	P	3 times a week (pg/ml)	P	7 days (pg/ml)	P
Comparison	4.42±29.06						
Anaerobic		6.93±10.47	0.392	7.88±25.08	0.029	9.44±55.98	0.162

Meaningful Difference (p<0.05) Independence t-test

The results of measurements of BNP levels in the treatment groups P5, P6, and P7 and the comparison group (P1) are in table 7. Furthermore, the mean BNP measurement results in each anaerobic treatment group were calculated, and the results showed that there was a significant increase in BNP levels in groups P5, P6, and P7 compared to the comparison group according to the frequency of exercises.

Table 8. MDA Level Conformity Test Between Comparative, Aerobic and Anaerobic Groups with Post-Hoc

Group	Comparison	1 day Aerobic	3 days Aerobic	7 days Aerobic	1 day Anaerobic	3 days Anaerobic	7 days Anaerobic
Comparison		0.057	**0.036**	**0.012**	0.096	**0.023**	**0.017**
1 day aerobic	0.057		0.825	0.480	0.788	0.670	0.571
3 days aerobic	**0.036**	0.825		0.625	0.625	0.837	0.729
7 days aerobic	**0.012**	0.480	0.625		0.333	0.776	0.887
1 day anaerobic	0.096	0.788	0.625	0.333		0.490	0.406
3 days anaerobic	**0.023**	0.670	0.837	0.776	0.490		. 0.887
7 days anaerobic	**0.017**	0.571	0.729	0.887	0.406	0.887	

Post hoc p<0.05

The results of MDA level measurements in all groups (P1, P2, P3, P4, P5, P6, and P7) are listed in the table above. Compared to using the post hoc test (p<0.05), the results show there are significant differences in groups P3, P4, P6, and P7 with the comparison group (P1).

Table 9 Comparison of BNP Levels between Comparative, Aerobic and Anaerobic Groups

Group	Comparison	1 day Aerobic	3 days Aerobic	7 days Aerobic	1 day Anaerobic	3 days Anaerobic	7 days Anaerobic
Comparison		0.451	**0.002**	0.947	0.237	**0.042**	**0.024**
1 day aerobic	0.451		**0.010**	0.490	0.658	**0.008**	0.111
3 days aerobic	**0.002**	**0.010**		**0.002**	**0.026**	**0.000**	0.253
7 days aerobic	0.947	0.490	**0.002**		0.263	**0.037**	**0.028**
1 day anaerobic	0.237	0.658	**0.026**	0.263		**0.003**	0.238
3 days anaerobic	**0.042**	**0.008**	**0.000**	**0.037**	**0.003**		**0.000**
7 days anaerobic	**0.024**	0.111	0.253	**0.028**	0.238	**0.000**	

Post hoc p<0.05

The table above shows which group is more meaningful than the three groups, namely the comparison, the aerobic treatment group, and the anaerobic treatment group, the conformity test with post-hoc.

Discussion

In this study, it was found that there was no significant difference in MDA levels in the aerobic physical exercise treatment group 1 day, 3 times a week, and 7 consecutive days with the comparison group (p> 0.05). According to the researchers, this shows that aerobic physical exercise done in 1 day will

cause greater oxidative stress than if the physical activity is carried out every day or 3x a week (Zhou *et al.*, 2022). In physical activity carried out 3x a week for 7 consecutive days, MDA levels in the network do not increase too much compared to if the activity is only done once. This is because at a frequency of 3x a week, there is a resting phase, and at a frequency of 7 consecutive days, an adaptation response arises (Rafaqat & Rafaqat, 2023). The Anova test results in the group 1 day, 3x a week, and 7 consecutive days between the aerobic group obtained a significant difference with a value of p = 0.04. This shows that aerobic physical activity carried out everyday results in the response of the heart muscle adaptation to the burden that is given, so that MDA levels in the network decrease (Aktaş *et al.,* 2024).

This is in line with research conducted by Sagnella (2000) that uses experimental study methods, but the number of samples is different, stating that the effect of physical exercise can occur instantaneously, called the acute response, and the long-term effect of regular and programmed exercise is called adaptation. The more often physical exercise is carried out, it will lead to heart muscle adaptation to the exercise (Ebrahimian, 2018).

The results of this study are also in line with research conducted by Niessner *et al.* (2003) with the same form of treatment and experimental methodology, but the number of different samples states that in mice who do physical activity using a treadmill, MDA levels of heart muscle are lower. Likewise, research conducted by Stein and Levin (1998) found that MDA levels in cardiac muscle and skeletal muscle were not significantly different compared to the control group. Conversely, in rats that performed physical activity in the form of exhaustive swimming, there was an increase in MDA levels.

MDA level measurement results in the anaerobic group found that there was no significant difference in the group that did anaerobic physical exercise 1 day, 3x a week, and 7 consecutive days compared to the comparison group (p> 0.05). Similarly, the average aerobic group MDA levels in the anaerobic group decreased with the duration of treatment. According to the researchers, the adaptation response and frequency of physical exercise play a significant role in reducing MDA levels in the heart muscle. This is in line with research conducted by Rahima *et al., (*2015) with the same methodology but different forms of treatment that, with an increasing 62 intensity of physical exercise carried out and programmed, will cause long-term effects called adaptation. Physical exercise will increase the antioxidant defence system and minimize the release of free radicals.

In this study, it was found that, there were significant differences in the aerobic group 3 times a week compared to the comparison group (p> 0.05). According to researchers, the presence of fluctuations in BNP levels is due to the fact that the frequency of physical exercise given to each group is different, so that the response of adaptation caused by the heart muscle is also different (El Assar *et al.,* 2022). The results of the study for the 1-day aerobics group are in line with the results of research conducted by Bordbar *et al.,* (2012), which states that, in the group that did aerobic physical exercise using a treadmill with a speed of 15 m/s for 45 minutes for 8 weeks, there was an increase in plasma BNP levels (p = 0.002) after doing aerobic exercise for 1 day compared to the value of BNP levels before doing aerobic physical exercise.

The results of measurements of BNP levels in the anaerobic group found that there were significant differences between the three groups that did anaerobic physical exercise and the control group (p<0.05). According to researchers, the increase in the average BNP level is due to physical exercise, given that the heart responds more and more to excessive burden. Other research conducted by Purnomo (2011) also shows that exhausting physical exercise can cause ischemia, which is a reduced state or loss of oxygen supply. Anova test results showed that there were significant differences in BNP levels in the three anaerobic groups (p<0.05) (Rahima *et al.,* 2022). According to researchers, this shows that anaerobic physical exercise with a different frequency of exercise will still affect the level of BNP.

MDA level measurement results in the aerobic and anaerobic groups showed that there were no significant differences between the 1 day, 3 times a week, and 7 consecutive days groups in the aerobic and anaerobic groups. This is in line with research conducted by Thompson *et al., (2007),* who found that the effects of physical exercise can occur instantaneously, called the acute response, and have long-term effects due to regular and programmed exercise, called adaptation.

From the results of this study, it was found that, there were significant differences in BNP levels only in the aerobic physical exercise group 3 times a week and anaerobic 3 times a week (p<0,05). The results of this study are in line with research conducted by Bordbar *et al.,* (2012), which explains that

the treatment group did endurance or aerobic exercise using a treadmill at 15 m/s for 45 minutes and the anaerobic treatment group used heavy exercise equipment for 30 minutes.

Conclusion

There is an increase in BNP levels before and after light physical exercise. Brain Natriuretic Peptide (BNP) is produced by heart muscle cells, especially ventricles, in response to excessive volume and pressure. Because the source of expenditure is specific to the heart, the BNP circulating in the blood can describe the level of stress burden on the heart. On the other hand, there is a decrease in MDA levels in accordance with the length of treatment days before and after light physical exercise. The influence of physical exercise can occur instantaneously, called acute response, and long-term, due to regular and programmatic exercise, called adaptation. The more often physical exercise is done, the greater the response to the adaptation of the heart muscle to the exercise. Light physical exercise is beneficial if done regularly and during the recovery phase so that the heart muscle is not burdened with aerobic physical activity. With the rest and recovery phase, there is time to restore muscle strength, energy, and nutrients for the heart so as to maintain the levels of brain natriuetic peptide (BNP) and malondialdehyde (MDA) in the body. The frequency of exercises like this is recommended to gain body fitness. This study has limitations that can be taken into consideration by the next researcher in order to get better research results. These limitations include the fact that the scope used in the study only includes several parameters related to physical exercise and oxidative stress, so that the research results cannot be generalized to broader parameters. The results of this research can be a reference, especially in biomedical science, which shows that physical activity carried out regularly can prevent oxidative stress on the heart muscle. Therefore, to maintain the health of the heart muscle, it is recommended that physical activity is not done every day, but there must be a resting period so that the heart muscle can recover optimally. Anaerobic physical exercise can result in loading on the heart muscle. Therefore, especially for athletes who often do continuous physical exercise, it is recommended to do exercise with light and moderate intensity so that it does not cause excessive load on the heart, which can trigger oxidative stress, which can have an impact on sudden death. Further research needs to be done to see the histopathological picture of the heart muscle given aerobic and anaerobic physical exercises so that we can know the picture that occurs in the heart muscle due to loading on aerobic and anaerobic physical exercises.

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Conflict of Interest

Nil.

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