Effect of Submaximal Exercise on Trained and Untrained Intensity on Estrogen Levels in Female White Rats (*Rattus Norvegicus*) Wistar Strains

Cinthya Fitri Paramitha¹, Mita² Herdiyantini³*, Eric Mayo Dagradi³, Annisa Ullya Rasyida⁴

Abstract

Background: Trained and continuous physical activity can increase estrogen levels in women, while irregular activity can increase excessive Estrogen levels. Submaximal exercise is a recommended exercise to improve a woman's physiological function. The purpose of this experiment was to determine the effect of submaximal exercise on trained and untrained intensity on Estrogen levels in female white rats (*Rattus norvegicus*) Wistar strains. Methods: This research was an experimental study using a post-test-only control group design using 30 female Wistar rats which were divided into 5 groups: control group not treated (K1), experimental group of animals with exercise intensity without intervention (K2), group of experimental animals with untrained intensity without intervention (K3), group of experimental animals with exercise intensity with intervention (K4), and experimental group of animals with untrained intensity with intervention (K5). The intervention was swimming at moderate intensity with a duration of 85% of the maximum time to swim and a load of 8% of the rat’s body weight. After the treatment period, all groups were terminated to take intracardiac blood measured using ELISA. Results: The data results were analyzed using statistical tests. The results of this study found that the trained group had lower Estrogen levels compared to the untrained group, but higher than the control group. The Kruskal-Wallis test show a significance value (p = 0.157), p > 0.05. Conclusion: In this study, there was no effect of submaximal exercise between trained and untrained intensity on Estrogen levels in Wistar strain female white rat.

Keywords: Estrogen, submaximal exercise, trained intensity, untrained intensity

Original Research Article

INTRODUCTION

Nowadays, social media is very easy to access things that people want, such as accessing fashion. Most of the fashion in the advertising world shows many female figures who have slim bodies. This makes people want a beautiful body like their idols so they strive for various ways, one of which is by...
exercising. To get the results that are as expected, we need to know what exercise is right in order to have a healthy and ideal body.

Physical exercise is one of the forms of improving the quality of the human being based on his physique by carrying out competitive activities that involve the strict exertion of physical exertion or the use of relatively complex physical skills motivated by personal pleasure and external benefits (Millah et al., 2018). Meanwhile, according to (Parwata, 2015) exercise is an effort that is carried out continuously and systematically to improve functional capabilities. Trained people have higher metabolic qualities and fat oxidation proportions than untrained ones. Regular physical activity can increase muscle mass and reduce body fat mass (Guyton & Hall, 2014).

Submaximal physical exercise is a physical exercise that has a close to high intensity around (80-90 % maximum heart rate) that can reduce the use of muscle glycogen for energy and increase the use of fatty acids as energy. This happens because the body adapts to the stressful stresses that arise during exercise (Lesmana & Broto, 2019). Physical exercise is classified into two types, aerobic and anaerobic (Radtke et al., 2017). Aerobic exercise using submaximal intensity can induce metabolism and improve so that this exercise is reported to be effective for maintaining body fitness (Utamayasa et al., 2022). According to Utamayasa et al (2022), women who do submaximal exercises or moderate exercises can improve body fitness with aerobic training and can provide long-term health. Exercises that are done in a trained and continuous manner will have a good effect on the body’s organs to achieve optimal fitness compared to untrained exercises.

Estrogen is a female primary genital hormone and is one of the important hormones to support female function, and has a role in the menstrual cycle. reproduction, as well as a role in bone mineral density (Pratama et al., 2011; Rietjens et al., 2017).

According to the Ministry of Health 2020, osteoporosis in women aged 50-80 years is around 23% and in women aged 70-80 years is around 53%. Osteoporosis itself is defined as a systemic bone disease characterized by a decrease in bone strength, so that bones break easily (Fitriani, 2018). One of the factors of osteoporosis is the presence of Estrogen deficiency. Estrogen has a very important role in bone metabolism, affecting the activity of osteoblast and osteoclast cells, including maintaining the working balance of the two cells (Humaryanto, 2017). One of the factors that affect Estrogen levels is exercise. Regular exercise with submaximal exercise zones is known to increase estrogen hormone levels.

Therefore, we will conduct a study to see the influence between submaximal exercise on trained and untrained intensity on Estrogen levels that can be caused by both exercise methods.

**MATERIALS AND METHODS**

This study used the Post-test only Randomized Control Group Design method using 30 female rats of 2-month-old Wistar Strains with a body weight of 180-200 grams and nullipara divided into 5 groups: control group not treated (K1), a group of experimental animals treated with trained intensity without intervention (K2), group of experimental animals treated with untrained intensity without intervention (K3), the experimental animal group that was given the intervention intensity treatment (K4), and the experimental animal group that was given the untrained intensity treatment with the intervention (K5) which was carried out at the Biochemistry Laboratory of the Faculty of Medicine, Hang Tuah University, Surabaya. Adaptation of the environment is carried out for 13 days with the same cage conditions.

After adaptation, each group of mice performed swimming exercises for 2 weeks. The exercise performed was to perform swimming with moderate intensity with a duration of 85% of the maximum time the rat was able to swim and a load of 8% of the rat’s body weight (Hita, 2020; Prasetya et al., 2018). Groups 2 and 4 were groups with trained intensity where each rat performed exercises for 2 weeks every day with a predetermined exercise method. Groups 3 and 5 were untrained intensity groups where each rat performed exercise once a week for 2 weeks, while group 1, or the rat control group no exercise was performed.
After the exercise, all groups will go through a recovery process for 1 week. Then, groups 4 and 5 were treated again at the same time. After the treatment period, all groups are terminated for intracardial blood draws. Measurement of Estrogen levels is carried out using ELISA. The data results will be analyzed using statistical tests.

**RESULTS**

**Estrogen Level Examination Results**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>1295.40180</td>
</tr>
<tr>
<td>K2</td>
<td>1366.84360</td>
</tr>
<tr>
<td>K3</td>
<td>1437.06340</td>
</tr>
<tr>
<td>K4</td>
<td>1233.62640</td>
</tr>
<tr>
<td>K5</td>
<td>1337.00620</td>
</tr>
</tbody>
</table>

K1: Control Group; K2: Trained Intensity without Intervention Group; K3: Untrained Intensity without Intervention Group; K4: Trained Intensity with Intervention Group, K5: Untrained Intensity with Intervention Group

The average Estrogen level in experimental animals after descriptive testing and results were obtained that the untrained group had a higher average compared to the trained group, while the control group had the lowest average compared to the two groups.

**Estrogen Level Normality Test Results**

<table>
<thead>
<tr>
<th>Group</th>
<th>Shapiro-Wilk Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.979</td>
<td>0.931</td>
</tr>
<tr>
<td>K2</td>
<td>0.940</td>
<td>0.666</td>
</tr>
<tr>
<td>K3</td>
<td>0.827</td>
<td>0.133</td>
</tr>
<tr>
<td>K4</td>
<td>0.948</td>
<td>0.720</td>
</tr>
<tr>
<td>K5</td>
<td>0.797</td>
<td>0.076</td>
</tr>
</tbody>
</table>

The results of the normality test obtained a significance value of p > 0.05, thus indicating that the distribution of data from the five groups was normally distributed.

**Results of Homogeneity Tests of Experimental Animal Group Variants**

After conducting a normality test and normally distributed data, proceed to perform a variant homogeneity test. The homogeneity test used is levene’s test.

<table>
<thead>
<tr>
<th>Data</th>
<th>Levene Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>4.380</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Levene test results of Estrogen levels showed a significance value of 0.011, meaning that estrogen levels were not homogeneous (p < 0.05).
Kruskal-Wallis Test Results

Kruskal-Wallis non-parametric statistical tests were conducted to prove the hypothesis in this study.

Table 4. Kruskal-Wallis Test Results

<table>
<thead>
<tr>
<th></th>
<th>Estrogen Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kruskal-Wallis</td>
<td>6.624</td>
</tr>
<tr>
<td>Df</td>
<td>4</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>0.157</td>
</tr>
</tbody>
</table>

The result of the data has a significance value of 0.157. This showed a $p > 0.05$, thus there was no effect of submaximal exercise on trained and untrained intensity on Estrogen levels in female rats (*rattus norvegicus*) of the wistar strain.

**DISCUSSION**

In the results of studies that have been carried out, it was found that the trained group had lower Estrogen levels compared to the untrained group, but higher than the control group. These results, showed that the trained intensity group was able to increase Estrogen levels and the untrained group was able to increase excess Estrogen levels.

Women who exercise irregularly will have excess estrogen than women who are used to doing regular and intensive exercise (Daiyah et al., 2021). Regular physical activity is also able to increase estrogen levels in women but is still within normal levels compared to those who do not do physical activity irregularly (Nyoman Kanca, 2015). Women will experience stages of reproductive development in the form of menarche, menstruation, pregnancy, climacterium, to menopause. Sport or physical exercise can affect such physiological functions. Women who go through the early menarche stage are much longer experiencing menopause in the future, while the slower the menarche stage, the faster menopause will occur, so the productive period will be shorter. Trained submaximal physical exercise is recommended to be performed on women because it can support female physiological function (Swasta, 2012).

The intensity of this exercise can lead to an increase in the activity of these enzymes. Exercise can also influence the occurrence of weight gain which is also related to the production of Estrogen. People with obesity produce far more Estrogen than those with normal weight. This increase in Estrogen levels can be converted into ineffective Estrogen due to the risk of metabolic diseases, such as diabetes mellitus, hypertension, and coronary heart disease (Bebasari, 2017; Moraska et al., 2000).

The role of oxidative stress in the pathophysiology of diseases caused by free radicals is widely recognized, as it leads to tissue damage or necrosis (Yuslianti, 2018). ROS (Reactive Oxygen Species) production is dependent on both enzymatic and non-enzymatic reactions during the biological processes of living organisms. The primary source of ROS production is mitochondria, which can occur during both physiological and pathological conditions (Pizzino et al., 2017). The formation of free radicals can be caused not only by higher intensity but also by a long duration of time. However, if we do high-intensity activities for a short duration, the increase in free radicals will not occur (Rahmawati, 2014). The accumulation of free radicals can harm estrogen-producing cells due to the lipid peroxidation process. This damage can result in a decrease in estrogen levels (Rahmawati, 2014; Wardani et al., 2012). An imbalance of free radicals, especially reactive oxygen species (ROS), can lead to the formation of oxidative stress. A decrease in estrogen levels can cause oxidative stress (Permatasari et al., 2017). When ROS production increases, physiological adaptations occur to deal with it, namely by forming endogenous antioxidants (Sylviana et al., 2017). Oxidative stress can lead to a reduction in estrogen levels and play a role in the development of various diseases (Sugiritama & Adiputra, 2019).

The differences in these studies can be caused by differences in the type of exercise, duration of exercise, mice used, and the condition of the mice (ovariectomy and normal). In study (Daghigh et al., 2014)
2018) there was a significant improvement after exercise treatment by swimming in ovariectomy white rats compared to those who were not treated. These different results are likely due to the duration of exercise given, samples of rats that are not ovariectomy, the intensity of exercise, and the duration of treatment. In study (Daghigh et al., 2018) the duration of exercise given was about 60 minutes per day for 8 weeks. In this study, the duration of exercise was only 41 seconds per mouse per day for 2 weeks for trained intensity and 41 seconds per rat 1 time a week for 2 weeks. The sampling time in the study (Daghigh et al., 2018) was taken after the last 24 hours of swimming time. In this study, samples were taken after 1 week of rest and 15 after intervention. The duration of exercise treatment in the study (Daghigh et al., 2018) 8 weeks, while in this study it was 2 weeks with the same frequency, namely every day per week for the trained. To conduct training in trained and untrained mice in this study was trained (7x / week) for 2 weeks and untrained (1x / week) for 2 weeks, while in research showed that trained (7x / week) for 2 weeks and untrained did not conduct training before intervention. The length of recovery time in this study was 1 week. The study was about 1-6 weeks (Carpentieri et al., 2016).

The results in this study found that the group of mice that were not treated had an average of 1295.40180 pg/ml, the group of experimental animals given trained intensity treatment without intervention had an average of 1366.84360 pg/ml, the group of experimental animals given untrained intensity treatment without intervention had an average of 1437.06340 pg/ml, the group of experimental animals treated with trained intensity treatment with intervention had an average of 1233.62640 pg/ml, and the group of experimental animals treated with untrained intensity with interventions had an average of 1337.00620 pg/ml. From these figures, it can be seen that mice with untrained intensity with interventions had the highest average amount of Estrogen (Daiyah et al., 2021).

CONCLUSION

There was no submaximal exercise effect on the intensity of trained and untrained estrogen levels in female white rats (rattus norvegicus) of the wistar strain.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this study

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