



CONTINUOUS SWIMMING TRAINING IN MODERATE INTENSITY DECREASED FOLLICLE STIMULATING HORMONE LEVEL IN FEMALE MICE ON A HIGH-CALORIE DIET

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Abstract

Background: The increase of calorie intake can increase the risk of obesity and diabetes mellitus. One of the efforts to reduce the risk is doing physical exercise. Types of physical exercise that can affect the system of female reproduction are not clearly defined. **Purpose:** To analyze the effect of moderate intensity continuous physical exercise on FSH levels in female rats exposed to a high-calorie diet. **Methods:** Laboratory experimental was research with Randomized Post test only Control Group Design. Sample size was 27 rats (150-200, adults) in the estrus phase, then divided into 3 groups (negative control group, positive control group (high-calorie diet), and the Moderate Intensity Continuous Training group (MICT with high-calorie diet)). **Results:** The results showed that there was an significant effect of continuous physical exercise in moderate intensity on FSH level in female rats exposed to other group. The FSH level in the MICT group had the lowest level (16.78 ± 4.83 mIU/ml). **Conclusion:** There is an effect of MICT on FSH level decreases in female rats exposed to a high diet calories. This can be caused, that high-calorie diet can inhibit GnRH secretion and continuous moderate-intensity physical exercise changes the rat energy metabolism. **Keywords:** FSH, moderate intensity physical exercise, MICT (Swimming), high-calorie diet.

INTRODUCTION

A high-calorie diet is an increase in calorie intake in high amounts that exceeds the intake requirement. Increased consumption of high-carbohydrate foods and fat can put you at risk of obesity, obesity and can even induce diabetes mellitus type 2 (Alvarez-Curto & Milligan, 2016). Consumption of over-represented carbohydrates or fats in the modern diet compared to physiological needs and overconsumption of the three macronutrients is associated with weight gain and obesity (BA et al., 2004). Ovaries in obese women have higher circulating insulin levels compared to normal-weight women, the effects of insulin retention and

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impaired Glucose tolerance in obese patients will certainly affect blood sugar levels (Purwandari, 2014). Insulin resistance spurs the body to compensate with the pancreas increase insulin production resulting in hyperinsulinemia which then stimulates the ovaries to produce androgens in women who causes premature follicular atresia (Wahyuni et al., 2015). Follicle arrest and anovulation can cause secretions Abnormal gonadotropins such as FSH and LH may also be stimulated direct by hyperinsulinemia, intraovarian excess of androgens, direct effect of insulin or a combination of these factors (Akbar et al., 2020). Therefore, excess weight can affect subfertility in a woman and can affect reproductive organs, causing irregular menstruation, subfertility, Polycystic Ovary Syndrome (PCOS), uterine, endometrial, breast, and cervical cancer (Irene et al., 2019).

According to the Ministry of Health, obesity management can be done through increasing physical activity whose movement is continuous with low-intensity movement moderate so that there is an increase in energy expenditure and an increase in muscle mass (Kemenkes RI, 2019). Changes in physical activity have a positive effect on sexual health in the form of: more frequent sexual intercourse, better vaginal lubrication, and overall sexual function 5 years later (Wekker et al., 2018). Exercise with intensity moderate indicates a tendency for a higher number of follicles (Rahayu et al., 2021). The study also shows that moderate-intensity exercise has a positive effect on ovarian reserve due to the number of apoptotic follicles in the group undergoing less sports training. However, some studies have reported that exercise of various intensities had different effects on ovarian follicle. In addition to our study, moderate exercise may increase the responsiveness and sensitivity of the follicle to FSH and LH with a concomitant improvement in ovulatory status in young women (Ruder et al., 2008). Research conducted by (Kiranmayee et al., 2017) found that moderate-intensity physical activity was associated with higher levels of anti-Müllerian hormone and FSH in 162 women aged between 19 and 42 years. The purpose of this study was to determine the effect of continuous physical exercise on against Follicle Stimulating Hormone (FSH) in rats exposed to a high diet calories.



METHOD

The research design used is Randomized Posttest Only Control Group Design. The population in this study was female rats. Sample size calculated using the faderar formula, the results obtained for each control group and treatment in this study were more than equal to 9 tails with a total of 27 female mouse tails.

The research was carried out at the Experimental Animal Laboratory, Faculty of medicine Universitas Airlangga. Prior to conducting the study, female rats were studied, acclimatization or environmental adjustment was carried out for one week in the Experimental Animal Laboratory enclosure, Faculty of Medicine, Universitas Airlangga. Rats were randomly divided into three groups: control group negative (no high-calorie diet and not given swimming treatment), group positive control (high-calorie diet and not given swimming treatment) and MICT group (high calorie diet and given moderate intensity physical exercise in the form of swimming with an additional weight of 6% body weight). High calorie diet with Sonde of 40% Dextrose glucose solution as much as 0.013 ml/g BW female rats every morning for 4 weeks based on previous research by Herawati, L (2020) (Herawati et al., 2020). Swimming protocol adapted from (Lee et al., 2010; Lutfi et al., 2021) administered 5x/week for 4 weeks, the treatment is carried out gradually in the first week for 10 minutes swimming with a load of 6% of the rat's body weight, then in the second week 20 minutes of swimming with a load of 6% of the rat's body weight, and in the third week too fourth for 30 minutes with a load of 6% of the rat's body weight. The swimming treatment was carried out during the day to ensure that the mice are in the proper reproductive phase. In the same way, before starting the treatment, a vaginal swab was performed first. All rats that will be given treatment are in the estrus phase.

Surgery was performed after 4 weeks of treatment under anesthesia using 70% ether, and then an incision is made in the medial abdomen to take blood samples from the hearts of mice. Next done checking blood FSH levels using the ELISA (Enzyme Linked) method (Immune-Sorbent Assay).

Processing and analyzing data using descriptive statistical tests, test normality with Shapiro Wilk, homogeneity test using Levene's test and one-way nova analysis difference test because the data is normally distributed with the help

of IBM SPSS ver.25 computer program. After a significant difference was found, it was confirmed using the smallest post-hoc significance difference or test Mann-Whitney. The significance level used is 5%. This animal study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Indonesia (202/EC/KEPK/FKUA/2021).

RESULT AND DISCUSSION

Descriptive statistics

The following is a descriptive statistical result of FSH levels in female rats.

Table 1 The Average of Body Weight Difference

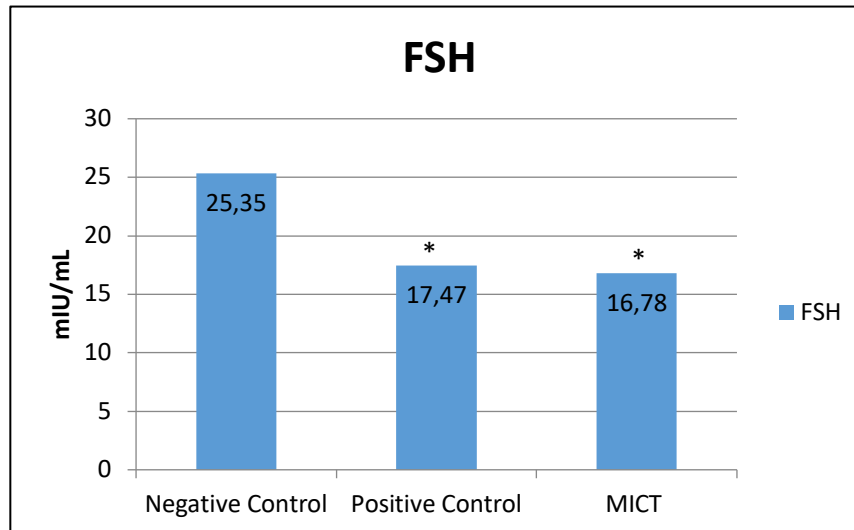
Group	N	Mean Weight Difference \pm SD
Negative Control	9	2.56 \pm 7.923
Positive Control	9	0.22 \pm 9.795
MICT	9	4.89 \pm 10.422

FSH level

Table 2 The Average Serum FSH Level

Group	Mean FSH level (mIU/mL) \pm SD	Sig.
Negative Control	25.35 \pm 7.65	0,007
Positive Control	17.47 \pm 4.01	
MICT	16.78 \pm 4.83	

Based on table 2 it is found that the p value is 0.007 ($p > 0.05$) which means that there is a significant difference in FSH levels in female rats between the negative control group, the positive control group, and the MICT. Followed by a post hoc test to determine the significance differences between each group.



*significantly different from the negative group ($p<0.05$)

Figure 1. Comparison of FSH level each group

From Figure 1, it is found that the negative control group is different significantly with the positive control group and MICT.

FSH is a hormone secreted by the anterior pituitary which functions in the growth of ovarian follicles until the antrum develops, induces the secretion of estrogen and progesterone from the ovaries by activating aromatase and P450 enzymes, induce granulosa cell proliferation and expression of LH receptors and direct negative feedback on GnRH secretion (BKKBN, 2013).

The MICT group showed that the lowest mean FSH level was compared to the negative control group and the positive control group. From these data it can be concluded that the mice given the treatment tended to have low FSH levels. These results are in line with studies that were performed by Wijayanti (2020) which states that the average decline of Serum FSH levels in rats given high-intensity physical exercise is getting heavier (Wijayanti, 2020). The decrease in FSH can occur due to doing physical exercise. Doing physical exercise can release high energy. Exercising can also inhibit the reproductive axis through GnRH, thereby reducing secretion of LH and FSH (Rahmawati, 2014). In a study conducted by SN Seyed S, et al (2016), it was stated that in the group of rats with swimming treatment, have lower levels of estradiol and FSH (Rohmawati, 2021). Physical activity can affect neurohumoral modulation through energy metabolism pathways. Reserve low energy, is thought to cause various disturbances of the HPO axis, especially in

female athletes (Loucks & Thuma, 2003). In line with research conducted by Li N, et.al (2021) found that FSH levels decreased after rats were given swimming treatment with Masashi swimming protocol (120 minutes per session, 6 day/week, for 15 days) (Li et al., 2021).

However, there are several studies that are not in line with this study. Research conducted by Kelley, et.al (2011) showed that there was no difference in FSH levels between groups, where this study was conducted on animal subjects, namely, horses were divided into 2 groups (Kelley et al., 2011). The control group was without physical exercise and the treatment group was given physical exercise treatment. Blood sampling carried out every day during the first cycle to the end. Other research was using 26-day-old rats which were divided into 2 groups. Namely, the control group was given the treatment of physical exercise running on a rotating wheel voluntarily (not set by time) while the treatment group was given physical exercise running on a swivel wheel given an extended duration of time. Blood samples were taken when the mice were 7 weeks old, blood samples taken every 5-6 minutes after treatment during the metestrus cycle. Result showed that there was no significant difference in FSH levels between groups these (Manning & Bronson, 1991). This difference in results may occur due to different types of physical exercise used and the time of blood collection.

The results of this study showed FSH levels in each group had a difference of 0.007 ($p < 0.05$) between the negative control groups, positive control group and MICT group (table 2). But also need to know that the difference in body weight was the highest in the MICT group (table 1). This matter can occur because a high-calorie diet is an increase in calorie intake in the body amounts that exceed the intake requirement. A high-carbohydrate diet increases muscle and liver glycogen stores (Zajac et al., 2014). Excessive consumption of carbohydrates or fats is widely represented in the modern diet compared to the physiological needs, and overconsumption of all three macronutrients is associated with weight gain, body weight and obesity (BA et al., 2004). Obesity is a key component of the metabolic syndrome, has a significant impact on female fertility, mainly because of changes in the function of the HPO axis (Cena, 2020). The HPO axis is a regulated system that strictly controls female reproduction. The occurrence of dysfunction in the



HPO axis result in impaired ovulation (Mikhael et al., 2019). In line with research conducted by Zhang et al., 2021 high-calorie diet significantly lowers serum levels luteinizing hormone (LH) and testosterone (T), inhibits the expression of FSH and LH in pituitary, increases cytochrome P450 enzymes and LH receptor expression in the ovaries, and cause impaired glucose tolerance (Zhang et al., 2021).

There are factors that may be the cause of weight gain in the MICT group as in previous studies which stated that exercise moderate-intensity aerobic activity increases OFC (orbitofrontal cortex) activity associated with high-calorie foods and stimulates appetite in individuals dependent on methamphetamine (Wang et al., 2019). In addition, the weight gain causes mice to become obese, is one of the factors that causes mice to become more sensitive (Coleman, 1978). Even the physical exercise given can be the wrong factor that triggers changes in the metabolism of the rat's body. In line with research, it was stated that rats are animals that easily experience stress thus affecting all aspects of metabolism, one of which is energy regulation including diet, energy expenditure and body composition (27). Stress in mice used for research can be triggered by compulsion to swim, routine handling, noise or social isolation due to being placed cages and other research equipment (Ellacott et al., 2010).

However, there are inconsistent studies. Acute moderate intensity exercise temporarily suppresses appetite and increases PYY and GLP-1 in hours after exercise without stimulating compensatory changes in appetite in underweight or overweight/obese individuals (Douglas et al., 2017). Sports for 2 weeks, regardless of intensity, does not change appetite or taste hormones hungry after eating, despite a slight reduction in food intake and body weight (Heiston et al., 2019). This difference in results may occur due to different types of physical exercise used and different research subjects.

CONCLUSION

It can be concluded that there is an effect of MICT (swimming) on FSH level in rats females exposed to a high-calorie diet. This can be caused, that a high-calories diet can inhibit GnRH secretion and moderate intensity exercise causes the changes in the metabolism of the rats.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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