The Combination of High Calorie Diet and Moderate Interval Training Decreases Follicle Stimulating Hormone (FSH) Level In Rats

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Abstract

Background: Obesity can cause leptin and insulin resistance which will affect the production of GnRH and FSH. One of risks factor of obesity is high calorie intake chronicaly. Exercise plays a role in the regulation of blood calorie and body weight. However, the effect of exercise on subjects with a high-calorie diet on FSH is unknown. This study aims to determine the effect of a high calorie diet with moderate intensity interval training (MIIT) on FSH level in female rats. **Method:** Sample was 27 female rats (Rattus norvegicus) wistar strain were divided into 3 groups. The negative control group (standard diet), the positive control group (high calorie diet), and the MIIT group (high calorie diet and moderate-intensity exercise with intervals). Measurement of FSH using the ELISA method after treatment for 4 weeks. **Result:** Statistical analysis showed that there was a significant difference in FSH level with a value (p = 0.001). The mean FSH was 25.32 mIU/ml ± 7.89 in the negative control group, 19.71 mIU/ml ± 6.55 in the positive control group, and 12.12 mIU/ml ± 3.64 in the MIIT group. The decrease FSH in the positive control and MIIT group due to a high calorie diet caused insulin and leptin resistance which affected GnRH. **Conclusion:** Moderate interval training decreases the FSH level may due to the level of physical stress.

Keywords: FSH;Exercise;High calorie diet;Healthy lifestyle

Introduction

Being overweight or obese is defined as abnormal or excessive fat accumulation that can interfere with health [1]. The prevalence of obesity in Indonesia continues to increase, from 10.5% (2007) to 14.8% (2013) and then 21.8% (2018). Likewise, the prevalence of overweight increased from 8.6%(2007) to 11.5% (2013) and then 13.6%(2018)[2]. The prevalence of overweight and obesity in women is greater than in men [3].

Overweight and obesity are associated with significant menstrual cycle irregularities and reduced pregnancy rates, increased gonadotropin requirements and increased miscarriage incidence [4]. Female reproductive function is affected by obesity due to high level of leptin and insulin. Leptin and insulin resistance affect GnRH to decrease FSH, which results in low estrogen in the follicle. Hyperinsulin affects theca cells and LH production in the long term resulting in hyperandrogen [5].

Environmental changes such as stress, intense exercise, and extreme weight changes can cause disturbances in the pulsation of these hormones [6]. Physical activity with strenuous intensity is associated with the incidence of amenorrhea, oligomenorrhea, luteal phase deficiency, and anovulation. The hypothalamic-pituitary-ovarian axis mechanism is impaired [7]. The effect of moderate-intensity physical exercise at intervals on reproductive hormone level is not widely known. Therefore, the aim of this study was to analyze the effect of moderate-intensity physical exercise at intervals on FSH level in people with hyperglycemia.

Method

This research using experimental animals has received approval from the Ethics Committee of the Faculty of Medicine, Airlangga University, Indonesia (169 / EC / KEPK / FKUA / 2021), carried out in the laboratory of the biochemical test animal unit, Faculty of Medicine, Airlangga University, Surabaya. The time of the research was carried out in September-October 2021. The type of research carried out this time was a laboratory experimental study using a Randomized Posttest Only Control Group Design.

Animal Test

In this study, 27 female rats (Rattus norvegicus) wistar strain with a body weight of 150-200 grams were in good health. Rats were housed under standard laboratory conditions with cages in the form of a plastic tub measuring 50x40x20 cm with a wire cover at the top. Inside the cage there is a place for food and drink bottles, and at the bottom of the tub rice husk is given to absorb rat droppings which are regularly replaced. Rats were randomly assigned to three treatment groups, namely a negative control group (with a standard diet), a positive control group (a high-calorie diet), and the MIIT group (a high-calorie diet and moderate-intensity exercise at intervals). To ensure that the rats were aligned in the reproductive phase, a vaginal swab was performed.

High calorie diet

Every day rats were given standard feed during the day as much as 133 grams and every day given additional sonde of 40% calorie dextrose solution as much as 0.013 ml/g BW every morning [8]. (Herawati, 2020) in the positive control group and MIIT. The negative control group was given a sonde in the form of plain water. Sonde done 5 times a week for 4 weeks

Swimming

Moderate Intensity Interval Training which is done in the form of swimming with an additional load of 6% body weight which is carried out at intervals. The swimming duration is increased gradually. The additional load was placed in the middle position of the rat's tail [9].

The swimming protocol was adapted from Foss et al., 1998; Herawati, 2004; Putri et al., 2018, [10,11,12]. using a swimming pool with a water depth of 50 cm. Swimming is done 5 times a week for 4 weeks with different swimming times. Swimming time in MIIT uses a 2:1 ratio of swimming to rest. In the first week, swimming was carried out for 5 minutes with a 2.5 minute rest for 2 repetitions, before that, swimming was carried out without weights for 3 minutes. In the second week, swimming was carried out for 5 minutes with a 2.5 minute rest for 4 repetitions, before that, swimming was carried out without weights for 1 minute. In the third and fourth weeks, swimming was carried out for 5 minutes with a 2.5 minute rest for

6 repetitions, before that, swimming was carried out without weights for 1 minute.
At the end of the treatment, surgery was carried out at the Experimental Animal Laboratory, Faculty of Medicine, Airlangga University, Surabaya. Rats were anesthetized using 70% ether placed in a closed container and samples were taken by taking blood from the heart. Blood was put into a gel clot activator tube and then centrifuged and then checked for blood FSH level using the ELISA (Enzyme Linked Immune-Sorbent Assay) method.

FSH (Follicle Stimulating Hormone)
FSH level was measured using the ELISA (Enzyme Linked Immune-Sorbent Assay) method using rat serum. The use of the FSH rat reagent from elabscience with a sensitivity of 1.88 ng/mL.

The data were analyzed using the SPSS application. The analysis begins with a descriptive statistical test. Then continued with the normality test of the data using the Shapiro Wilk test with the results showing normal distribution, followed by the homogeneity test using the Levene test with the results that all data had homogeneous variance (p> 0.05). then proceed with the different test using one way ANOVA. If there is a significant difference, then the statistical test is continued with the Post Hoc test.

Results

Subject Characteristics

Table 1. The Average of Body Weight Difference

Group	Mean	SD
Negative Control	1.22	8.36
Positive Control	0.44	9.76
MIIT	-6.11	14.26

No significant difference (P = 0.322) among group

FSH level

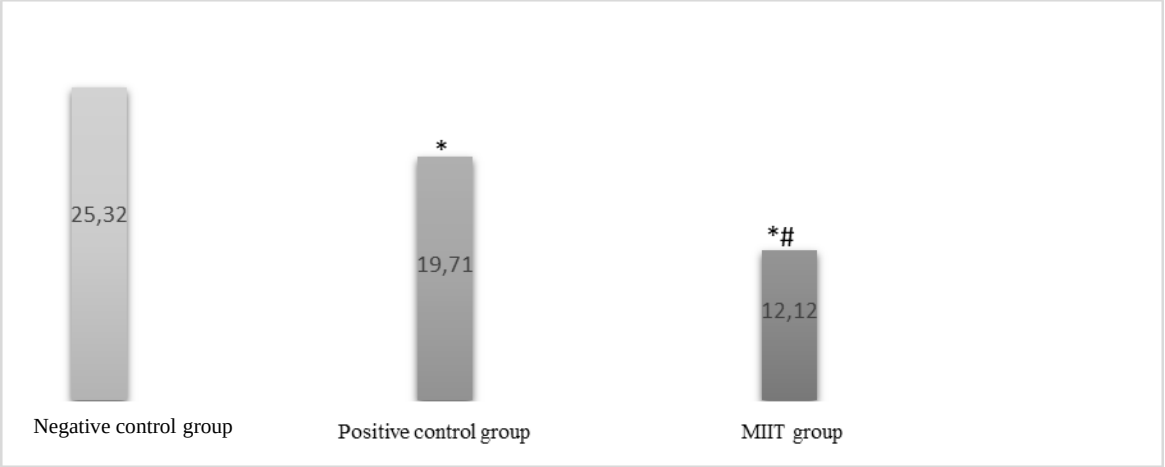
Table 2. The Average Serum FSH Level

Group	N	Mean (mIU/ml)	SD
Negative Control	9	25.32	7.89
Positive Control	9	19.71	6.55
MIIT	9	12.12	3.64

The data on FSH serum level of female rats are data that are normally distributed and are homogeneous data so that the different test is carried out using the One-way Anova test. The results showed that the p value was 0.001 (p <0.05). There was a significant difference in serum FSH level in female rats between the negative control group, positive control group, and the MIIT group. Furthermore, it is necessary to do

a post hoc test to determine the significance of the differences between each group. In the post hoc test, the measurement of serum FSH level in female rats used the LSD (Least Significance Different) test.

Fig 1. Comparison of FSH level each group



* Significantly different with the negative control group
\# Significantly different with the positive control group

The results of the further test between the negative control group and the positive control group showed that the p value was 0.070 ($p>0.05$), which means that there was no significant difference between the negative control group and the positive control group. The results of the follow-up test between the positive control group and the MIIT group showed that the p-value was 0.017 ($p<0.05$), which means that there was a significant difference between the positive control group and the MIIT group. Furthermore, for further test results between the MIIT group and the negative control group, the p-value is 0.000 ($p<0.05$) which means that there is a significant difference between the MIIT group and the negative control group.

Discussion

In this study, there was no significant difference in body weight in each group. There was a change in body weight between before and after the study which was very varied in each group. Rats are animals that easily experience stress when given treatment, so that it can affect all aspects of metabolism, one of which is energy regulation including food intake, energy expenditure and body composition [13].

Varied changes in body weight indicate that changes in body weight are not directly related to changes in FSH level. In this study, it was found that there was no significant difference between the negative control group and the positive control group. Although there was no significant difference in FSH level in the positive control group, the group that was given an additional high-calorie diet showed a tendency to decrease FSH level compared to the negative control group, without a high-calorie diet. Someone who has

an increase in blood sugar or hyperglycemia will have excess weight and one of the causes is excessive sugar consumption. When blood sugar increases, there is an increase in the hormone insulin to control blood sugar level. The hormone insulin increases, there is stimulation of GnRH-producing neurons, thereby increasing GnRH secretion in producing more LH and lower FSH secretion [14].

Obesity increases androgen production and reduces sex hormone binding globulin (SHBG) level, which will cause androgen effects to increase in the body [15]. Similar results were shown with this study, women with a high BMI were in line with a higher incidence of hyperandrogens [16]. The aromatase system will be disturbed by high level of androgens in the granulosa cells, triggering early follicular atresia which can lead to infertility [17].

In obesity, the female reproductive function is affected. Leptin, an anorexigenic peptide hormone secreted by adipose tissue and circulating in plasma as free or protein-bound adipokines, is produced in greater quantities [18]. Leptin enters the blood circulation and then reaches the brain and functions to regulate eating habits, energy balance, and reduce the production of neuropeptide Y (NPY) from the hypothalamus which plays a role in stimulating appetite. There is a reciprocal relationship between food intake, level of leptin, hypothalamus (NPY) and insulin [19]. The abundance of leptin level in obese people is thought to be due to increased expression of the leptin gene and adipose tissue. Leptin resistance occurs in obese people. High level of leptin in the blood is not followed by suppression of the level of NPY produced in the brain [20]. The state of high leptin level is associated with hyperinsulinemia that occurs as a compensatory reaction to insulin insensitivity caused by insulin resistance. High insulin level will stimulate the production of free androgens. The high state of androgens results in negative feedback on the production of the FSH hormone produced by the hypothalamus [21].

Regular exercise is an important component of supporting long-term weight loss. Changes in body weight occur as a result of compensatory mechanisms by the body [22]. Different results were shown with this study, a study on 162 women between the ages of 19 and 42 and found that moderate-intensity physical activity was associated with higher level of anti-Müllerian hormones and FSH. Physical activity can influence neurohumoral modulation of metabolic pathways involved in energy metabolism and reproduction. Moderate exercise can increase the response and sensitivity of follicles to FSH and LH with improved ovulatory status in young women [23].

The results of this study were that FSH level was the lowest in the MIIT group, which was given high calorie diet and swimming. This study is in line with research conducted on female students at a university in Nigeria, the group who did moderate to vigorous physical exercise showed a decrease in the level of the hormones estrogen, FSH, and LH [24]. This can be caused in addition to exercise that uses repetitions many times as well as giving a high-calorie diet. In general, the reproductive axis is inhibited due to exercise through direct or indirect inhibition of GnRH by corticotropin-releasing hormones, endorphins, and glucocorticoids, there by reducing the secretion of luteinizing hormone (LH) and FSH, as well as gonadal hormonal secretion. and make sex steroid target tissues resistant to this hormone [25].

The different results in this study may be due to stress in rats, namely moderate-intensity exercise but

added to a high-calorie diet. Stress activates the hypothalamic-pituitary-adrenal axis which causes an increase in glucocoticoid secretion and simultaneously inhibits gonadotropin secretion [26]. Adaptation to stress consists of early autonomic activation and release of catecholamines from the sympathetic nervous system, followed by slower and prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis and increased secretion of CRH, arginine vasopressin, ACTH, and glucocorticoids [27].

Stress is closely related to an increase in cortisol level, cortisol acts centrally to suppress the pulsatile secretion of GnRH [26]. An increase in plasma cortisol concentration initiates a negative estrogen feedback, which then suppresses the frequency or amplitude of GnRH, reduces FSH secretion, ultimately inhibiting ovulation and triggering the formation of small ovarian cysts [28]. Cortisol also has an inhibitory effect on GnRH secretion in the form of a decrease in FSH production [29]. However, this study has limitations, namely that there is no examination of cortisol level to determine stress level in research subjects. It would be better to check for cortisol also, so that the results are more accurate.

Conclusion

Based on the result of the analysis and discussion that have been discussed previously, the combination of high calorie diet and moderate interval training decreases FSH level. It maybe a result of the higher level of stress in physical activity. However, it needs further study to uncover the underlying mechanism and to figure out the best physical exercise to influence FSH level.

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