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# High intensity interval training (HIIT) VS moderate intensity training (MIT) for interleukin-6 (IL-6) in obesity

# Entrenamiento en intervalos de alta intensidad (HIIT) vs. entrenamiento de intensidad moderada (MIT) para la interleucina-6 (IL-6) en la obesidad

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**Abstract**

This study aims to determine the effect of High Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT) exercises seen from the biomolecular parameters of Interleukin-6 (IL-6). Male Rattus Norvegicus mice used aged 2 - 3 months with obesity status were trained 4 times a week for 6 weeks. HIIT uses an intensity of 90 - 100% of the total maximum speed/baseline with an interval of 1: 1 (2 minutes active: 2 minutes rest) for 15 minutes/exercise. MIT uses an intensity of 50-60% of the total maximum speed/baseline for 30 minutes/exercise. IL-6 levels were tested using Elisa. There was a significant difference (P = 0.000) in the three groups at the end of the study. The results of body weight between the HIIT and control groups were (P = 0.000), while the MIT and control groups were (0.002). The results of IL-6 levels in the HIIT group and the control group were significantly different with a P value of 0.000, while in the HIIT group and the MIT group the significance value was P = 0.002. The conclusion of this study shows that IL-6 levels in the HIIT group were lower than those in the MIT group and the control group. Although HIIT is effective in losing weight, it still needs to be monitored and strictly controlled, considering that HIIT is a high-intensity activity to avoid injury.

**Keywords:** exercise, HIIT, MIT, interleukin-6 (IL-6), obesity

**Resumen**

Este estudio tiene como objetivo determinar el efecto de los ejercicios de entrenamiento en intervalos de alta intensidad (HIIT) y entrenamiento de intensidad moderada (MIT) vistos a partir de los parámetros biomoleculares de la interleucina-6 (IL-6). Se entrenaron ratones macho Rattus Norvegicus de 2 a 3 meses de edad con estado de obesidad 4 veces por semana durante 6 semanas. El HIIT utiliza una intensidad del 90 - 100% de la velocidad máxima total/línea de base con un intervalo de 1:1 (2 minutos activo : 2 minutos de descanso) durante 15 minutos/ejercicio. El MIT utiliza una intensidad del 50 - 60% de la velocidad máxima total/línea de base durante 30 minutos/ejercicio. Los niveles de IL-6 se probaron utilizando Elisa. Hubo una diferencia significativa (P = 0,000) en los tres grupos al final del estudio. Los resultados del peso corporal entre los grupos HIIT y control fueron (P = 0,000), mientras que los grupos MIT y control fueron (0,002). Los resultados de los niveles de IL-6 en el grupo HIIT y el grupo control fueron significativamente diferentes, con un valor de p de 0,000, mientras que en los grupos HIIT y MIT el valor de significancia fue de p = 0,002. La conclusión de este estudio muestra que los niveles de IL-6 en el grupo HIIT fueron inferiores a los del grupo MIT y el grupo control. Si bien el HIIT es eficaz para perder peso, aún requiere un seguimiento y un control estricto, considerando que es una actividad de alta intensidad para evitar lesiones.

**Palabras Clave:** ejercicio, HIIT, MIT, interleucina-6 (IL-6), obesidad

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## Introduction

Obesity is a problem that currently needs special attention to be addressed. Obese adults with a body mass index (BMI) > 30Kg/m2 were estimated at 10.8% of men (266 million men) and 14.9% of women (375 million women) worldwide in 2014. This figure has more than doubled compared to the 1975 figures of 3.2% in men and 6.4% in women. If this condition continues, it can be predicted that by 2025 it is possible that obesity will occur in men by 18% and women by 21% ((NCD-RisC, 2016). A person's weight condition can be used as a parameter to see the characteristics of energy metabolism, hormonal status and as an indicator of a person's physiological condition (Syamsudin et al., 2021). Obesity as seen from the increase in BMI is a factor that can increase risk factors for degerative diseases such as diabetes, cardiovascular disease, and musculoskeletal disorders that can reduce the quality and life expectancy of the community (Lin & Li, 2021).

Obesity can occur due to a person's lack of physical activity, Physical activity that individuals do each week has a correlation with individual weight (Shalgumbayeva et al., 2023). The obesity epidemic is the result of the interaction of various aspects such as environmental factors, genetic and human behavior (Omer, 2020). Psychological stress can be a trigger in increasing the risk of obesity (Cuevas et al., 2019). Chronic psychosocial stress can increase glucocorticoids that contribute to emotions and increased food consumption (Masood & Moorthy, 2023).

In obesity, adipose tissue plays a crucial role. The more accumulation of excess fat in adipose tissue, it can change the size of adipose tissue to become larger (hypertrophy) and increase its number (hyperplasia) (Longo et al., 2019). Obesity is a condition characterized by an increase in inflammatory conditions, especially the consequences will be higher if stored in Visceral Adipose Tissue (VAT) (Guerreiro et al., 2022). Inflammation in obesity is mediated by excess adipose tissue storage which results in increased macrophage infiltration associated with apoptosis and can increase the recruitment of neutrophil, CD4+ and CD8+ T sets which can cause insulin resistance in adipose tissue (Fico et al., 2023).

Obesity is one of the features of metabolic syndrome that is closely associated with chronic inflammation (Stępień et al., 2014). Excess macronutrients in adipose tissue stimulate the tissue to release inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), and reduce the production of adiponectin, which is a trigger for pro-

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inflammatory conditions and oxidative stress. Increased levels of interleukin 6 stimulate the liver to synthesize and secrete C-reactive protein (Ellulu et al., 2017). Interleukin 6 is a member of pro inflammation cytokines that can induce the expression of proteins that can affect acute inflammation (Peter et al., 2020). High levels of circulating IL-6 can describe the intensity of chronic inflammation in obesity, which can lead to the development of atherosclerosis and coronary heart disease (Zimowska et al., 2024). IL-6 can destabilize the insulin receptor substrate protein 1 (IRS1), leading to insulin resistance (Radványi & Röszer, 2024).

Exercise is one way to overcome obesity problems by maximizing energy balance and also modulating immune cells, adipokines and inflammatory cytokines (Krüger et al., 2014). Exercise can reduce the mass of visceral adipose tissue (Nordby et al., 2012). High Intensity Interval Training (HIIT) exercise model using high intensity can reduce visceral fat (Zhang et al., 2021). IL-6 levels decreased after doing HIIT exercise (Rhibi et al., 2022). This decrease can occur because weight loss can reduce cytokines derived from adipose tissue (Phillips & Grayson, 2020). Other results show that exercise with moderate intensity training can also reduce IL-6 levels (Hayes et al., 2021). Exercise with moderate intensity performed with a frequency of 3 times a week can reduce IL-6 levels (Reis et al., 2024).

Researchers decided to use animal models because the training, particularly HIIT, involves an intensity of up to 100%. There are concerns that administering it to humans could cause injury.

## Methodology

*Subjects*

This study used 39 male white rats of the obese strain Rattus norvegicus aged 2 - 3 months and weight > 160 grams. Wistars were housed in individual cages with room temperature of 22˚C, humidity of 50 - 55%, and controlled on a 12-hour light-dark cycle. Wistars were divided into three groups. The control group (n = 13) was untreated/sedentary. HIIT group (n = 13) was given exercise with High Intensity Interval Training (HIIT) model. The MIT group (n = 13) was treated with Moderate Intensity Exercise. The ethical procedures in this study were approved by the Ethics Committee of Brawijaya University, Malang (approval number: 900-KEP-UB).

*Exercise Training Protocol*

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The training program uses HIIT and MIT methods. HIIT uses an intensity of 90 - 100% of the total maximum speed/base line with a 1 : 1 interval (2 minutes on : 2 minutes off) for a duration of 15 minutes per workout. MIT uses an intensity of 50 - 60% of the total maximum speed/base line with a duration of 30 minutes per exercise. The increase in training load was given by increasing the treadmill speed by 1 m/min in one week. Exercise was carried out for 6 weeks with a frequency of 4x per week. Implementation of HIIT and MIT exercise programs using a special wistar treadmill.

We used the special wistar treadmill because we can setting up the speed for every groups. For example, the MIT group, we can set up the speed start from 8m/minutes and increase the speed 1m/minutes every 2 minutes. In other hand, the treadmill have a shocker to give a sign when the wistar exhausted.

*IL-6 and Whole Blood Measurement*

IL-6 was taken from blood serum. Blood was taken in accordance with the standards of the Biosciences Laboratory of Brawijaya University. Blood serum will then be tested using the Enzyme Linked Immunosorbent Assay (ELISA) test to determine IL-6 levels.

*Statistical Analysis*

Initial data processing used descriptive statistics to describe the average results of each variable. Normality test using Shapiro-Wilk. Levene test is used to determine data homogeneity. Furthermore, to determine differences in IL-6 from Moderate Intensity Training (MIT) and High intensity Interval Training (HIIT) were tested using Anova.

## Results

The results of the weight measurements taken every week can be seen in table 1, showing that in the last week the body weight of the three groups had a significant difference (P=0.000). A more detailed analysis showed that there was a difference between the HIIT group and the control (P=0.000) and the MIT group and the control (0.002).

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**Table 1**.
Average Body weight per Week (grams)

| | Group | HIIT | MIT | Control |
|---|---|---|---|---|
| The Average body weight per week (Grams) | Week 0 | 200±20.4 | 206±22.7 | 215±21.7 |
| | Week 1 | 229±24.7 | 236±24.6 | 243±24.8 |
| | Week 2 | 238±24.2 | 247±23.5 | 251±26.4 |
| | Week 3 | 242±25.1 | 249±22.7 | 261±27.3 |
| | Week 4 | 246±22.7 | 253±22.0 | 274±27.8 |
| | Week 5 | 257±23.3 | 268±22.5 | 284±28.3 |
| | Week 6 | 249±23.7 | 258±19.6 | 291±24.8 |

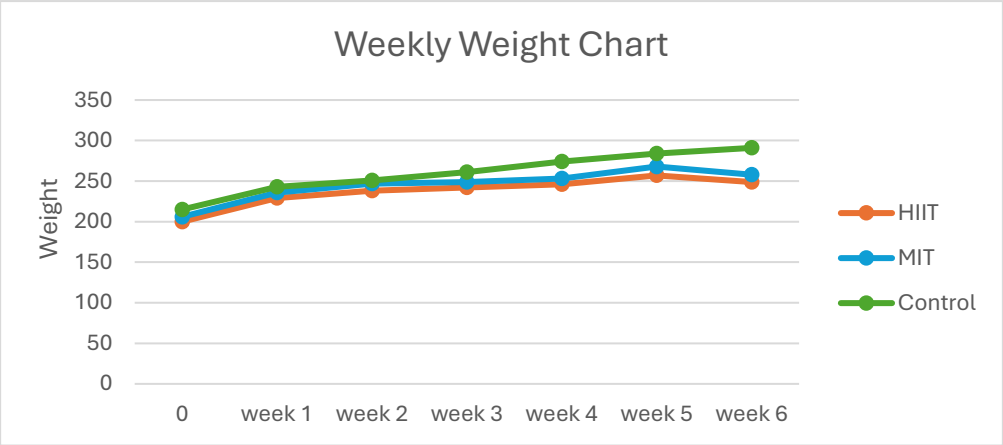


**Figure 1**. Weekly Weight Chart

From table 1 and figure 1 it can be explained that the HIIT group with an initial body weight of 200 ± 20.4 grams experienced an increase in body weight from week one to week five reaching a body weight of 257 ± 23.3 grams, but there was a decrease in body weight after week six to 249 ± 23.7 grams. The MIT group with an initial body weight of 206±21.7 grams also experienced an increase in body weight from week one to week five to reach a body weight of 268±22.5 grams, but also experienced a decrease in body weight after week six to 258±19.6 grams. Meanwhile, the control group with an initial body weight of 215 ± 21.7 grams experienced an increase in body weight from week one to week six with a body weight of 291 ± 24.8 and did not experience any weight loss during the study.

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**Table 2**.
Blood Test Results

| Variabel | HIIT | MIT | Kontrol |
|---|---|---|---|
| Eritrosit | 8.12±0.49 | 8.03±0.51 | 7.59±0.47 |
| Leukosit | 6.98±1.97 | 5.83±1.45 | 6.16±2.60 |
| Platelet | 824.83±94.02 | 916.67±142.92 | 857.43±64.21 |
| IL-6 | 2584.33±697.1* | 3551±689.1 | 3741.77±827.2 |

*IL-6 levels of HIIT VS Control were significantly different with P=0.000, HIIT VS MIT were different with a significance level of P=0.002.

Table 2 shows that HIIT has an average IL-6 level of 2584 ± 697.1 pg/ml which is lower than the MIT and control groups. The MIT group itself had IL-6 levels of 3551 ± 689.1 pg / ml higher than the HIIT group but lower than the control group. The control group had the highest average IL-6 level of 3741±609.7 pg/ml.

The results of the ANOVA test of differences between the three groups namely HIIT, MIT and Control showed the results of P = o.ooo which means there is a significant difference between the three groups. The Tukey test results showed that there was a significant difference in IL-6 levels between the HIIT group with control (P = 0.003) and the HIIT group with MIT (P = 0.002), while IL-6 levels in the MIT group with control had no significant difference (P = 0.747).

## Discussion

The HIIT group exhibited a lower mean IL-6 level (2584 ± 697.1 pg/ml) compared to the MIT group (3551 ± 689.1 pg/ml) and the control group (3741 ± 609.7 pg/ml). Statistical analysis revealed significant differences between the HIIT group and both the MIT and control groups, whereas no significant difference was found between the MIT and control groups.

These results support the findings of another study which stated that HIIT may be associated with reduced expression of pro-inflammatory cytokines such as IL-6 and increased anti-inflammatory cytokines, with greater efficiency when compared to moderate exercise on reducing risk factors for metabolic diseases (Steckling et al., 2015). HIIT is a good exercise method to reduce body fat percentage which is an important factor in reducing IL-6 concentration (Maillard et al., 2019). IL-6 levels in the MIT group which were higher than HIIT in this study also support the results of other studies which found that IL-6 in aerobic or moderate exercise groups was higher than other exercise groups (Silva et al., 2016). Higher IL-

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6 levels in the MIT group may be mediated by the high infiltration of magrophages in adipose tissue.

The lower IL-6 levels of the HIIT group than MIT and control were predicted from the mechanism that HIIT training increases fatty acid oxidation by increasing circulating adiponectin levels in skeletal muscle, which ultimately reduces body fat percentage (Guo et al., 2024). Similar to this study, HIIT can improve metabolic (Glucose), Hormonal (Cortisol) and decrease Inflammatory markers IL-6 (Wang et al., 2022). The observed reduction in macrophage infiltration may be attributed to the downregulation of MCP-1 expression, which in turn contributes to the decreased transcription of the pro-inflammatory cytokine IL-6 gene. (Linden et al., 2014).

Physical exercise is directly involved in the prevention of obesity and fat accumulation mediated by IL-6 (Li et al., 2021). This supports the findings in this study which showed that there was a trend of weight loss at week six in the HIIT and MIT exercise groups. This weight loss was also accompanied by lower IL-6 levels in the HIIT and MIT groups. Other studies have shown that HIIT is more effective in weight loss because HIIT can reduce visceral fat (Maillard et al., 2018). This could be due to the possibility of greater energy expenditure after exercise. After exercise there is an increase in the lipolytic ability of visceral adipose tissue which is higher in HIIT, besides the expression of β3-AR plays a key role in mediating the process (Liu et al., 2022). Visceral fat has a positive correlation with IL-6, high visceral fat will affect high levels of IL-6 but if lower visceral fat can also affect lower levels of IL-6 (Nam et al., 2015). While other studies suggest that fat loss in HIIT can be caused by increased fat oxidation which can affect IL-6 regulation (Soeria & Boenyamin, 2019).

However, in another study, although IL-6 is considered a pro-inflammatory cytokine, other evidence suggests that IL-6 has a more complex role in the regulation of fat metabolism and oxidation (Pal & Whitham, 2014). IL-6 may be involved in the mobility and oxidation of visceral fat tissue, so an increase in IL-6 can reduce visceral adipose tissue (Hoover et al., 2021). In terms of exercise that has an effect on weight loss can also be mediated by IL-6 which during exercise IL-6 stimulates lipolysis released from skeletal muscle (Lehrskov et al., 2019). Other results stated that HIIT and MIT training can be used as an alternative to overcome obesity (Hadiono et al., 2023).

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## Conclusion

HIIT and MIT exercise models turned out to be equally effective in weight loss, but in terms of biomolecular IL-6, HIIT had lower IL-6 levels than MIT and control. An important limitation of this study is that it was conducted in an animal model, which limits the direct generalization of the results to the human population. So, the HIIT and MIT exercises can be used as an alternative in overcoming obesity problems. Therefore, it is recommended that future research consider human clinical studies and expand the evaluation to other inflammatory and metabolic biomarkers to better understand the mechanisms involved.

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