

# High Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT) Against TNF- $\alpha$ and IL-6 levels In Rats

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**Abstract**—The aim of this study was to investigate the effect of exercise with High Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT) on inflammatory profile of proinflammatory cytokines TNF- $\alpha$  and IL-6 on the subject of obesity. This research is used 39 wistar rats of male-sex obesity. Intervention training is given for 6 weeks with frequency 4 times per week, HIIT intensity (90-100% of base line capability), MIT (60-80% of base line capability) TNF- $\alpha$  and IL-6 levels are obtained from blood testing with using ELISA test. Data analysis using Anova test. The results of this study showed that there were differences in levels of TNF- $\alpha$  HIIT with control ( $P = 0.003$ ), MIT with control ( $P = 0.001$ ) and no difference between HIIT group and MIT ( $P = 0.945$ ). There was a difference of IL-6 levels between HIIT and control ( $P = 0.000$ ), HIIT with MIT ( $P = 0.002$ ), no difference in MIT group with control ( $P = 0.747$ ). Exercise with the method of HIIT and MIT for 6 weeks with a frequency of 4 times per week can not create levels of TNF- $\alpha$  on the subject of obesity are lower as compared with sedentary groups, even the levels of TNF- $\alpha$  group of HIIT and MIT were significantly higher compared with the sedentary group. As for the levels of IL-6, HIIT is more effective in influencing the low IL-6 compared to MIT.

**Keywords**— HIIT, MIT, TNF- $\alpha$ , IL-6, Obesity

## I. INTRODUCTION

At this time the obesity became a very important health problem to be addressed, the increase in overweight and obesity are not immediately addressed will lead to occurrence of metabolic syndrome at someone [1]. Syndrome metabolic disorders a liquid metabolic marked with visceral obesity, Dislipidaemia (disorders of lipid profile), Hyperglycemia and Hypertension [2]. Meanwhile, the International diabetes federation (IDF) explained that the metabolic syndrome is a cluster of risk factors include obesity, diabetes, high cholesterol and hypertension [3]. If not handled properly, the metabolic syndrome will trigger the onset of physiological disorders such as cardiovascular disorders namely atherosclerosis (the hardening of blood vessel walls) [4].

Metabolic syndrome can increase the risk of coronary heart disease, stroke, cancer and renal insufficiency [5]. From a wide

range of physiological disorders, obesity or overweight contributed the biggest factor for the occurrence of metabolic syndrome [6]. Physiological disturbances caused by obesity are also inseparable from the role of the cytokine-like body biokhemis proinflamasi IL-6 (Interleukin 6) and TNF- $\alpha$  (Tumor Necrosis Factor-alpha) which can eventually lead to syndrome metabolic [7].

Individuals with overweight or obesity, significantly had the levels of IL-6 and TNF- $\alpha$  which is higher compared with individuals who have normal weight [8]. In individuals with overweight or obesity, undergo excess triglyceride accumulation in adiposit thus causing hiperthrophy (increase volume) adiposity [9]. Hiperthrophy adiposit pro inflammatory cytokines will improve, where Neutrophils, which are inflammatory responses against the initial response will creep into adipose tissue which then stimulates macrophage infiltration type M1 [10]. This type of M1 macrophages increases the production of IL-6 and TNF- $\alpha$  [11]. IL-6 and TNF- $\alpha$  contribute greatly to the onset of metabolic syndrome [12,13,14]. Because IL-6 and TNF- $\alpha$  has a great contribution towards the onset of metabolic syndrome, The International Diabetes Federation (IDF) insert of IL-6 and TNF- $\alpha$  as additional parameters to predict Diabetes Mellitus [3].

Metabolic syndrome itself can be addressed and prevented by exercise, people with metabolic syndrome decreased their risk factors through increased physical fitness after intervened with regular physical training [15]. Model exercise with Moderate Intensity Training (MIT) capable of lowering risk factors for metabolic syndrome with the reduction of body fat, increase insulin sensitivity, and lower levels of IL-6 and TNF- $\alpha$  [16,17]. The low body fat will prevent fat cell damage and hypoksia experience, so will reduce pro inflammatory cytokines IL-6 and TNF- $\alpha$  through increased secretion of adiponectin and anti inflammatory cytokines [18]. But on the other hand, modeling exercises with High Intensity Interval Training (HIIT) is also effective in improving lipid profiles, increasing the release of anti-inflammatory molecules, and increased insulin sensitivity [19,20]. This can occur because the exercise HIIT will improve muscle contractions, so that will maximize muscle mitochondria in enzymatic reactions

that will improve ambilan glucose in skeletal muscle and will give effect to increased adiponektin that affect the levels of *C-Reactive Protein* (CRP) is pro inflammatory cytokines IL-6 and TNF- $\alpha$  [21,22,23].

## II. MATERIALS AND METHOD

### A. Animals

This research uses thirty-nine rat *Rattus norvegicus* type white wistar strain of obese males, aged 2-3 months with weight 160 g > gained from animal house Tiput Jaya. The animals are placed in individual cages made from polyethylene and are stored in a room where the temperature is maintained at  $22 \pm 2^\circ \text{C}$ , humidity 50-55%, and controlled under the light-dark cycle of 12 hours. The rats were given a commercial rat feed and water ad libitum.

The rats were randomized into three groups. Control group ( $n = 13$ ) were not given any treatment/sedentary. HIIT Group ( $n = 13$ ) were given physical treatment of High Intensity Interval Training (HIIT). The MIT Group ( $n = 13$ ) were given physical treatment of Moderate Intensity Training. All procedures in this study was approved by the Ethics Committee of the University of Brawijaya, Malang (approval number: 900-KEP-UB).

### B. Exercise Training Protocol

Exercise program in this study using the method of exercise of the High Intensity Interval Training (HIIT) and Moderate Intensity Interval Training (MIT). Exercise program carried out for 6 weeks with a frequency of 4 times per week. The application of exercise program using treadmill running animals.

The total capacity of each test animals exercised first before doing the exercise program to search for veterinarian maximum ability a try. Determination of total capacity is done by way of an animal trying to put on a treadmill with an initial speed of 8 m/min, treadmill speed will be increased in the amount of 1 m/min every 2 minutes [24]. When animals try already touched shocker > 5 seconds, try stated has entered the threshold of exhaustion and the speed of the iniliah that will serve as the total capacity of each animal try [25].

HIIT done with intensity 90-100% of total capacity, the total duration of 60 minutes per week, the comparison interval workouts 1:1 (2 seconds on, 2 seconds off) [24]. MIT is done with intensity 50-60% of total capacity, the total duration of 120 minutes per week [24]. Load progress provided by the addition of speed treadmill 1 m/min per week.

### C. Immunoassays for Cytokines

Biomarkers of Inflammation TNF- $\alpha$  & IL-6 taken from blood serum. The taking of blood and the centrifuge is carried out according to standards by labolatories from Bioscience Laboratories Brawijaya Universitys. Blood serum will then be tested using Enzyme Linked Immunosorbent Assay (ELISA) to know the levels of TNF- $\alpha$  and IL-6. ELISA testing is carried out according to standards in laboratory of Molecular

Physiology, Faculty of medicine, University of Brawijaya, Malang.

### D. Statistical Analysis

Initial data processing using descriptive statistics showing the mean  $\pm$  standard deviation (SD). Test of normality test using the Shapiro-Wilk. Its homogeneity test using test Levene. The difference of influence of Moderate Intensity exercise Training model (MIT) and High Intensity Interval Training (HIIT) against the levels of TNF- $\alpha$  and IL-6 in blood was tested using Anova statistics. All analysis is tested by using SPSS V. 19.0 for windows with 0.01 significance level.

### E. Result

From the results of a blood test shows that the Group has done HIIT and MIT have levels of TNF- $\alpha$  which is higher compared to the control group. As for the levels of IL-6, a group of HIIT has the most low levels compared with the Group of MIT and control.

**Table 1. The levels of TNF- $\alpha$  and IL-6**

Group	Levels of TNF- $\alpha$ (pg/ml)	Levels of IL-6 (pg/ml)
HIIT	246.43 $\pm$ 68.5	2584 $\pm$ 697.1
MIT	258.16 $\pm$ 124.0	3551 $\pm$ 689.1
Control	116.07 $\pm$ 78.6	3741 $\pm$ 609.7

Mean  $\pm$  Standart Deviation

Test results showed that the different groups of HIIT and MIT have difference levels of TNF- $\alpha$  which is higher than in the control. The levels of TNF- $\alpha$  group of HIIT is significantly higher than in control group ( $P = 0.003$ ), the MIT group was significantly higher ( $P = 0.001$ ), whereas the Group of HIIT and MIT is not different ( $P = 0.945$ ).

**Table 2. The difference in the levels of TNF- $\alpha$  between groups**

Group	HIIT	MIT	control
HIIT	-	0.945	0.003*
MIT	-	-	0.001*

\* Significant with  $P < 0.01$

On the levels of IL-6, showed that the Group had levels of difference HIIT IL-6 are lower than on the MIT Group ( $P = 0.002$ ) and control ( $P = 0.000$ ). Whereas the levels of IL-6 Group of MIT with the control did not have a significant difference ( $P = 0.747$ ).

**Table 3. The difference in the levels of IL-6 between groups**

Group	HIIT	MIT	Control
HIIT	-	0.002*	0.000*
MIT	-	-	0.747

\* Significant with  $P < 0.01$

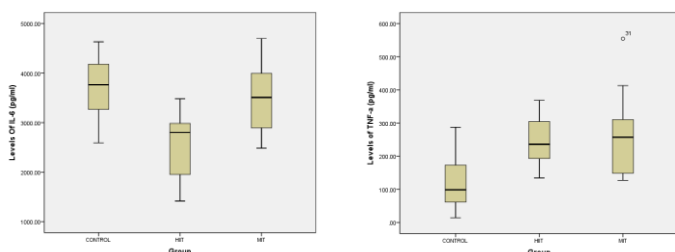


Fig. 1. Average levels of IL-6 and TNF- $\alpha$  in each group

### III. DISCUSSION

The levels of TNF- $\alpha$  on HIIT workout and MIT are higher than in the control group or the sedentary. In contrast to these findings, the levels of TNF- $\alpha$  and IL-6 in individuals who are obese, detected decreased after doing exercises on a regular basis [26]. Exercises with MIT were able to reduce the levels of TNF- $\alpha$  which is accompanied by a decrease in fat mass and body weight [27]. Exercises with HIIT is also capable in the reduction of the levels of TNF- $\alpha$  [19].

In the results of these researchers still have not been able to claim more high levels of TNF- $\alpha$  group of HIIT and MIT is dangerous and bad for the body effect conferring. Research of Neto et al. found similar results with this research, namely increased TNF- $\alpha$  in the exercise group, but the increase was not accompanied by an increase in HOMA-IR and insulin concentration changes that indicate that insulin sensitivity is not affected by the concentrations of TNF- $\alpha$  are different [28]. This is in contrast with the other opinions which stated that when the cells directly exposed by the high TNF- $\alpha$ , this will inhibit the signal insulin with Insulin Receptor Substrate proteins affect [29].

The findings in this study supports previous literature, that the development in the methods of the same exercise for 6 weeks in rats of obesity occurring increased levels of TNF- $\alpha$  from the sedentary group, an increase in TNF- $\alpha$  in the exercise groups can promote adipocytolysis and produce free fatty acids to be used by the muscles [30]. The increased TNF- $\alpha$  gene expression may play a role in lipid and glucose metabolism in adipose tissue through the effects of autokrin and in skeletal muscle through the effects parakrinnya [31]. The increased TNF- $\alpha$  via the sport can contribute to the activation of satellite cells that trigger an increase in muscle fibers and establishment of regeneration of new induction through the expression of calcineurin [32]. Another issue to consider is that the role of TNF- $\alpha$  dependent on receptors in the cell membranes of the recipient, there are two types of receptors (TNFR1 and 2) and the pro-inflammatory characteristics commonly associated with protein (mainly in adipose tissue) is bonded with the TNFR 1 (Neto et al., 2015:578). At this time also have been identified that TNF- $\alpha$  is able to induce an increase in lipolysis in adipose [33].

While on the measurement of IL-6 indicates that there is a difference between a group of HIIT with MIT and the control group, but there was no difference in the MIT group with control. These results support the findings stated that HIIT can be associated with a reduction in the expression of

inflammatory cytokines pro such as IL-6 and an increase in cytokines bitter taste, with greater efficiency when compared to moderate sports on reduction of the risk factors of metabolic diseases [19]. HIIT is a effective method of exercise to reduce body fat percentage which is an important factor in reducing the concentration of IL-6 [34]. The levels of IL-6 on the MIT group higher than HIIT on the study also supports the research results of Silva et al. which found that IL-6 on a group of moderate aerobic exercise or heavier than the other exercise groups [35]. The high levels of IL-6 on the MIT group could possibly be mediated from the still high on magrofag adipose infiltration.

More low levels of IL-6 from HIIT group at MIT and predictable control of mechanisms that enhance the HIIT training is the oxidation of fatty acids with increasing levels of adiponektin that circulate in skeletal muscle, which ultimately reduces the percentage of body fat [36]. HIIT done for six weeks was able to reduce magrofag on adipose infiltration [30]. Tereduksinya magrofag infiltration can be caused due to a decrease in expression of MCP-1 which also contribute to the tereduksinya gene transcript proinflamasi cytokine IL-6 [37].

### IV. CONCLUSION

Exercise with the method of HIIT and MIT for 6 weeks with a frequency of 4 times per week can not create levels of TNF- $\alpha$  on the subject of obesity are lower as compared with sedentary groups, even the levels of TNF- $\alpha$  group of HIIT and MIT were significantly higher compared with the sedentary group. As for the levels of IL-6, HIIT is more effective in influencing the low IL-6 compared to MIT.

There is no difference in the effects of exercise HIIT and influence within the MIT levels TNF- $\alpha$ , but no difference in the effects of exercise HIIT with MIT in influencing levels of IL-6, HIIT is more effective in lowering the levels of IL-6 compared to MIT.

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