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# Exercise decrease the expression of MCP-1 in perivascular adipose tissue (PVAT) in obese mice

*by* HotimahHotimah

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## Exercise decrease the expression of MCP-1 in perivascular adipose tissue (PVAT) in obese mice



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Hotimah Masdan Salim<sup>1\*</sup>, Aisyah<sup>2,3</sup>, Michio Shimabukuro<sup>4</sup>

### ABSTRACT

**Introduction:** Exercise has been believed to prevent metabolic syndrome (Mets) from occurring. Accumulation of inflammation factors such as monocytes chemoattractant protein-1 (MCP1) in adipose tissue is a factor in the development of MetS. However, the inflammatory mediators that play a role in the accumulation of macrophages are still unclear, so this study aims to determine the expression of MCP-1 in perivascular adipose tissue (PVAT).

**Methods:** Eight-week-old male wild-type mice, were divided into five groups. One control group was given a standard diet and four groups were given a high-fat diet (HFD) for 2 weeks. Exercise treatment was given to the HFD group by dividing HFD with exercise once a week, HFD with exercise three times a week, and HFD with exercise five times a week. As for the exercise, speed is given 10-18m/minute for 30 minutes. and this exercise is carried out for two weeks. Gene expression of MCP-1 in PVAT tissues was analyzed using quantitative chain reaction (PCR).

**Results:** The HFD group experienced an increase in body weight ( $P < 0.01$ ) and adipose tissue mass ( $P < 0.01$ ) compared to the control group. Furthermore, exercise decreased body weight ( $P < 0.05$ ) and adipose tissue in the HFD-Exc1 group ( $P < 0.01$ ). Furthermore, exercise tends to decrease the inflammatory mediator in macrophages MCP-1 in PVAT compared to the HFD group.

**Conclusion:** These results suggested that exercise partially reduces adiposity, tended to decreases MCP-1 in HFD-induced obesity.

**Keywords:** HFD, Exercise, PVAT, MCP-1.

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### INTRODUCTION

Obesity that occurs in humans is a risk factor for causing the inflammatory process and associated "metabolic syndrome", which is a collection of pathological conditions such as hypertension, insulin resistance, hyperglycemia, and dyslipidemia.<sup>1,2</sup> This metabolic syndrome can cause disruption of cytokines and chemokines, thereby increasing the release of inflammatory mediators in the tissues in the body.<sup>3</sup> Recent studies related to obesity have shown that excessive uptake of metabolic tissues such as liver, bone, muscle, and adipose tissue (AT) causes chronic inflammation associated with obesity and insulin resistance.<sup>4</sup>

In animal and human studies, it is explained that an important component in cells that causes inflammation is a collection of macrophages that enter and accumulate in visceral adipose tissue.<sup>5</sup> Insulin resistance in humans correlates with the accumulation of Adipose tissue

macrophage (ATM) numbers, which will lead to activation of inflammatory mediators such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and induced nitric oxide synthase.<sup>6,7</sup> Monocyte chemoattractant protein-1 (MCP-1 or CCL2) is a mediator associated with the accumulation of macrophages in the tissue. Previous studies in animals and humans have shown that there is a strong bond that causes insulin resistance by increasing levels of MCP-1 in adipose tissue.<sup>8</sup>

Exercise is a powerful treatment strategy for treating obesity and diabetes that has the added benefit of preventing the development of metabolic syndrome.<sup>9</sup> Exercise on a treadmill reduces inflammatory mediators, improves lipid profiles, reduces insulin resistance, and promotes weight loss in animal models.<sup>10</sup> Increased body weight is a major risk factor for obesity, so monitoring body weight to be stable can be used as a preventive measure in preventing obesity

inflammation.

Previous research has shown that weight loss can prevent the accumulation of macrophages into fat tissue by reducing oxidative stress.<sup>11</sup> These findings have also been corroborated by other studies that have shown that dietary composition and dietary restriction can reduce oxidative stress. In addition, exercise, which is one method of losing weight, is effective in reducing oxidative stress if you do regular exercise for a long period.<sup>12</sup>

However, the role of physical exercise on MCP-1 levels in adipose tissue is still not fully explained. Therefore, this study aims to determine the effect of physical exercise on MCP-1 expression in PVAT.

### METHODS

#### Animal

In this investigation, 25 male Wild type C57BL/6 mice aged 8 weeks were employed. Eight mice were given a regular diet after the adjustment period, and

the remaining twenty mice were given a high-fat diet (HFD) for two weeks before their samples were collected for the intervention. From twenty mice with a high-fat diet, we divided into four different groups: with HFD without exercise (HFD-Non-Ex), with HFD and exercise once a week (HFD-Ex1), HFD and exercise three times a week (HFD-Ex2), and HFD and exercise five times a week (HFD-Ex3) for two weeks.

#### Exercise training protocol

The exercise training adjustment was given with a speed of 10-18m/min for 30 minutes for one day, three days, and five days a week for two weeks.

#### Extraction of total RNA

Total RNA was extracted from the extraction in PVAT using a homogenizer added with TRIS reagent with a ratio of 100 mg of tissue plus 1 ml of TRIS. The entire RNA extraction process uses Wizard® Genomic DNA Purification (Promega). After extraction, the total RNA concentration was calculated using a UV spectrophotometer with an absorbance of 260 nm. Then the examination was carried out using the Select Bio-Product (USA) PCR tool to examine the expression of MCP-1. Primer sequences were as follows: Forward 5'-ACTGAAGCCAGCTCTCTC TTCTC-3' and Reverse 5'-TTCCTTCTT GGGTCAGCACAGAC-3'

#### Statistical analysis

Data processing was performed using SPSS V22.0 (SPSS Chicago) software, which was presented as mean ± SEM. To determine the expression of the MCP-1 gene, the analysis was carried out using the two-way ANOVA with the post-hoc Tukey test, where the P-value <0.05 indicated significance.

## RESULTS

### Parameters of high-fat diet-induced obese mice

To evaluate the effects of the administration of a high-fat diet, body weight was measured. The body weight in the control group (Ctrl) with normal chow showed lower than the high-fat diet (HFD) group significantly ( $P < 0.001$ ). In the last week of the exercise, the high-fat diet (HFD) group showed a significant increase in weight ( $p < 0.001$ ) compared to the control group (Ctrl) group (table.1). The HFD group showed a higher body weight than HFD + Exc1, HFD + Exc1, and HFD + Exc-3 (table.1). Food intake is a factor in increasing body weight. However, this study showed that there is no significant difference in of food consumption of food in all groups (table 1).

### The effects of exercise on Perivascular Adipose Tissue (PVAT) weight

Bodyweight was increased in the high-fat-diet group without intervention compared with the control group significantly ( $P < 0.001$ ). The bodyweight of intervention with exercise was decreased compared with the HFD group significantly. This in line with the decrease in adipose tissue mass, which in this study there was a difference in PVAT mass in the HFD group and the HFD + Exc1 group. However, there were no significant differences in the other exercise treatment groups (figure 1)

### The expression of MCP-1 in PVAT after exercise training

To examine the effect of high-fat diet on the accumulation of macrophages in adipose tissue, the expression of MCP-1 was measured in PVAT. The PCR result showed that the expression of MCP-1 in the exercise group tended to be downregulated compared with the HFD

group (figure 2).

## DISCUSSION

In this study, regular training of exercise in mice fed HFD showed decreased MCP-1 expression in adipose tissue. The reduction of macrophages in MCP-1 did not correlate with the decrease in adipose tissue mass. However, previous studies have shown that MCP-1 expression in adipose tissue determines the degree of obesity.<sup>13,14</sup>

Previous study was found that a HFD increased MCP-1 expression in PVAT, accompanied by an increase in body weight. However, regular physical exercise can reduce MCP-1 expression in perivascular adipose tissue.<sup>2</sup> This study also explains that the increased macrophage accumulation is due to the important role of MCP-1 in mobilizing macrophages into obese adipose tissue by decreasing MCP-1 in PVAT.<sup>15</sup>

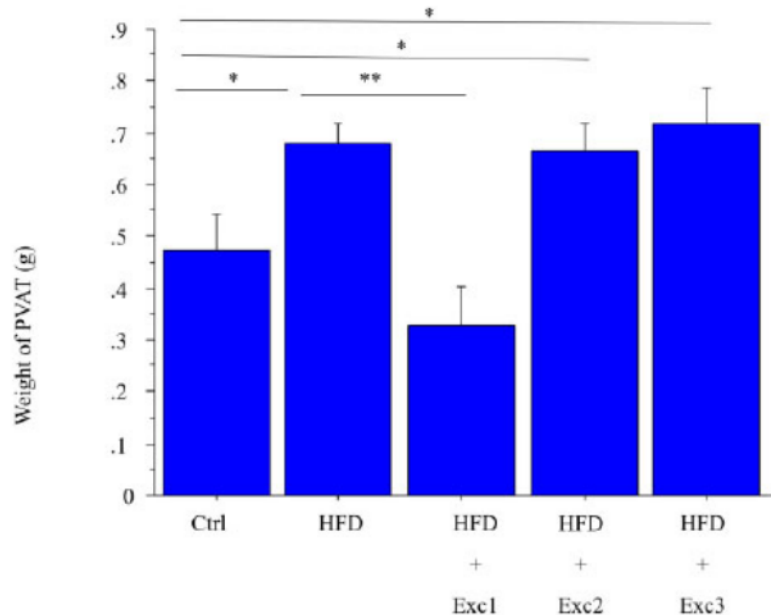
It is now well known that the secretion of several cytokines derived from adipose tissue is associated with obesity. This inflammatory process is a form of chronic subclinical process in obese individuals. This inflammation is also associated with the pathology of insulin resistance and causes metabolic syndrome and cardiovascular disease. It has not been doubted that weight loss affects the inflammatory phenotype in obesity<sup>15</sup>, previous studies explained in knockout mice in a role of obesity-induced the expression of mediator inflammation including MCP-1. Furthermore, the MCP-1 has mechanism to improve homeostasis in HFD and induce obese, by reducing liver fat, decreasing glucose and mass of liver in animal model.<sup>16</sup>

Physical exercise is an effort to reduce obesity. The previous study has shown that exercise training in the administration

**Table 1. Parameters of Body weight and Food Intake.**

Parameters	Ctrl	HFD	HFD+Exc1	HFD+Exc2	HFD+Exc3
Body weight pre-intervention (g)	30.52±1.003	31.50±1.33	31.92±1.84	32.28±0.819	31.14±1.56
Body weight post-intervention (g)	33±1.22	39.7±1.153*	33.87±1.03†	36.46±1.442**.	33.82±1.07†
Food intake (g)	65.2±20.63	48.2±23.71	54.4±30.705	57.4±15.42	51.8±21.04

Ctrl; control, HFD; high-fat diet, HFD-Ex1; exercise once a week, HFD-Exc2; HFD-Exc3; exercise five times a week. All values are mean ± SEM. \*,  $P < 0,05$ , \*\*,  $P < 0,01$  compared with control group. †;  $P < 0,05$ , ††;  $P < 0,01$  compared with HFD group.



**Figure 1.** High-fat-diet effects on perivascular adipose tissue (PVAT) Exercise training significantly reduced PVAT weight in group Exc-1 compared to the HFD group. However, physical exercise in groups Exc-1 and Exc-3 had no effect on decreasing PVAT compared to the HFD group. Ctrl; control, HFD; high-fat diet, HFD-Exc1; exercise once a week, HFD-Exc2; two times a week HFD-Exc3; exercise five times a week. All values are mean  $\pm$  SEM. \*,  $P < 0.05$ , compared with control group, †,  $P < 0.05$ , ††,  $P < 0.001$  compared with HFD group.

of a high-fat diet did not affect to induce the body to weigh loss and fat mass.<sup>17</sup> In contrast, exercise training for a long and short period improved glucose tolerance and aerobic capacity in obese mice. These improvements are related to the body weight and the expression of HOMA-IR in obese mice.<sup>18-20</sup> In this study was a short physical exercise that was given for two weeks. The results of this study also indicated that short-term exercise was affected the weight loss with the quantity of exercise once and five times a week. Furthermore, weight loss was not in line with decreasing total visceral fat tissue. This result is in line with previous studies gained similar weight and demonstrated similar total and visceral adiposity in mice with and without exercise fed a high-fat diet.<sup>19</sup> Therefore, our results suggested that exercise down-regulated the expression of MCP-1 in the perivascular adipose tissue, without a decrease in the weight of the adipose tissue in the body. The experiment

was conducted without isolating macrophages from adipose tissue, which was a disadvantage of this work.

## CONCLUSION

This study supported the participation of indirect effects in mice with a high-fat diet and indicated that short-term exercise training had positive benefits. Furthermore, exercise training to prevent the development of obesity by down-regulated MCP-1 expression in adipose tissue. Further research is needed to determine the effect of other factors on Exercise decrease the expression of MCP-1 in perivascular adipose tissue (PVAT) in obese mice.

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## AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

## FUNDING

The authors are responsible for all of the study funding without a grant or any external funding source.

## CONFLICT OF INTEREST

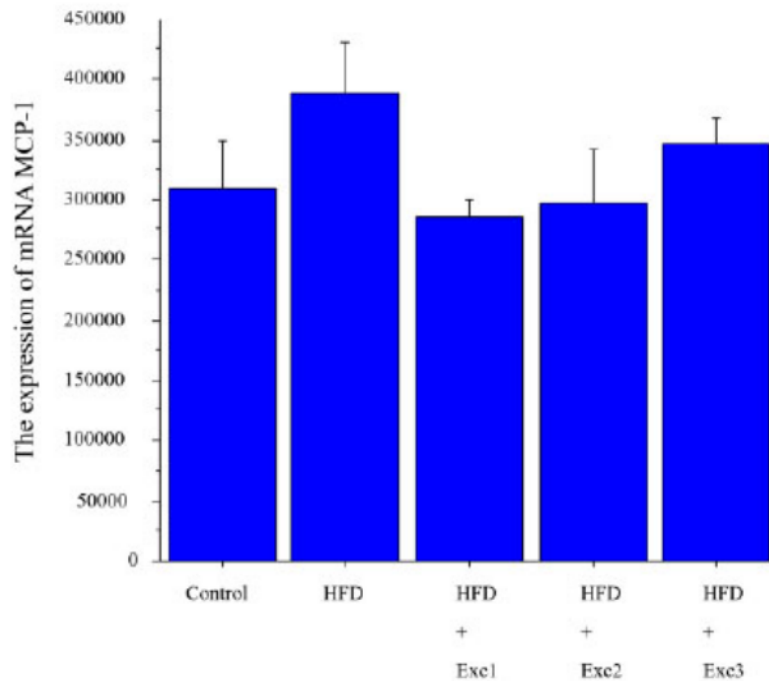
There is no conflict of interest for this manuscript.

## ETHICAL CONSIDERATION

Animal studies were approved by Health Research Ethics Committee, Medical School of Universitas Hang Tuah (approval number: E/012/UHT.KEPK.03/II/2020).

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**Figure 2.** The effect of exercise and HFD on mRNA expression in PVAT. Exercise training was decreased the expression of MCP-1 in PVAT, but there is no significant differences between groups. Ctrl; control, HFD; high-fat diet, HFD-Ex1; exercise once a week, HFD-Exc2; HFD-Exc3; exercise five times a week. All values are mean  $\pm$  SEM.

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