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Changes in the Histological Appearance of White Fat in Young Rats as a Result of Different Exercise Intensities

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Abstract

Sedentary lifestyle can cause the accumulation of energy reserves in the form of lipids in white adipose cells. Lifestyle modification by means of exercise is done to reduce fat reserves in white adipose tissue (WAT). This study aims to determine the effect of exercise in various intensities on the histological changes of white adipose cells. Research method exploratory with Federer formula to determine the number of samples carried out. Using 28 Wistar rats located at the Central Laboratory of Padjadjran University, Sumedang from May to June 2022. The data taken were the average diameter ± standard error (SEM) of the inguinal white adipose cells of the experimental rats. Data analysis using Microsoft Excel 2016 and SPSS v.28 software. Of the 28 samples studied, only 25 samples met the inclusion criteria. Group K is 0.15% ($p = 0.024$) greater than P2, and 0.25% (p 0.001) is greater than P3. The P1 group had a value of 0.17% ($p = 0.025$) greater than P3. Moderate and vigorous exercise can be used to reduce the size of white adipose cells. While low-intensity exercise is considered ineffective. Further research can be carried out to assess changes in white adipose cells based on exercise intensity from other sides besides their morphology.

Keywords: Histological, White Adipose Tissue, Exercise

INTRODUCTION

A sedentary lifestyle is characterized by a lack of moderate to strenuous physical activity. This harmful lifestyle can result in obesity and other metabolic disorders. In 2020, 23% of adults and 81% of adolescents will have a sedentary lifestyle, according to the WHO. This lifestyle, along with a high-fat diet, can disrupt the balance between energy intake and energy expenditure, leading to obesity. Obese individuals have excess fat tissue and adipose cell hypertrophy, particularly in white adipose tissue (WAT). (6) It is anticipated that restoring adipose cell size because of lifestyle modification involving increased physical activity as a treatment for obesity will restore adipose cell size.

Exercise is widely recognized as a subcategory of physical activity that can be regulated; exercise and a regulated diet can prevent obesity in individuals. (7, 8) In previous studies examining the effects of exercise at varying intensities, moderate and heavy intensity exercise significantly reduced body fat percentage, total body fat mass, and WAT area. Morphologically, exercise can decrease lipid reserves in adipose cells, particularly in adipose cells. Changes in WAT include increased expression of mitochondrial biogenesis genes in subcutaneous WAT (scWAT) and visceral WAT (vWAT), increased mitochondrial activity, adipocyte morphology, altered secretion of adipokines, and conversion of some white adipose cells in scWAT to beige adipose cells. Other studies (7,14) have shown that WAT transplants improve the metabolic rate of exercising individuals. It is hypothesized that exercise can influence the histological appearance of white fat cells, particularly moderate and vigorous exercise. Changes in the histological appearance of white fat cells caused by different exercise intensities have not been studied. The purpose of this study was to identify histologically significant changes in white adipose cells in response to different exercise intensities.

METHOD

28 Wistar rats were subjected to four treatments using an exploratory experimental design (Group K, control; Group P1, light intensity exercise; Group P2, moderate intensity exercise; and Group P3, heavy intensity exercise) (16–18). Using the Federer formula (n6) to determine the number of samples From May to June 2022, the research was conducted in the C2 and C4 laboratories of the Faculty of Medicine Building, as well as the Central Laboratory of Padjadjran University, Sumedang. This study used samples from previous studies and obtained ethical approval for experimental animals from the Padjadjaran University Research Ethics Commission, Bandung, no. 676/UN6.KEP/EC/2018.

Experimental Animals

Male white rats of the Wistar strain (*Rattus norvegicus*) (28 rats), approximately 8 weeks old, weighing between 200 and 220 grams, not sick or injured, and free of parasites, were used to determine the sample's utility. According to Dishman's score, rats are excluded if they have anatomical abnormalities or if they refuse to run on the treadmill.(16,19) Rats were housed in standard cages, fed rodent pellets, and provided with water ad libitum. There were 12 -hour cycles of light and darkness in the cage, and the temperature was maintained between 22 and 24 degrees Celsius. After two weeks of acclimatization, the experimental animals will be separated into four groups: one control group and three groups with varying intensities. (16-18)

Habituation Protocol

The protocol was carried out with reference to previous experiments, and animals were habituated to prevent stress. On the first day of habituation, the rats (including the control group rats) were placed on a treadmill machine for 60 minutes. On the second day, the treadmill's speed increased to 2.0 meters per minute for twenty minutes. On day five, gradually increase the speed of the treadmill until it reaches 11 meters per minute. After the habituation process is complete, the Dishman score is used to determine the running capacity. (16,19)

Dishman score

The running capacity of the experimental animals will be evaluated using a five-point scale developed by Dishman et al. as follows: point 1, refusing to run; point 2, sporadic, run-stop, wrong direction; point 3, able to run, requires constant attention; point 4, able to run consistently and without stimulus, several times carried away by the treadmill; and point 5, able to run consistently on the front of the treadmill, without stimulus.(19) Rats with a Dishman score of five are eligible for participation in the experiment.(16)

Treadmill Exercise Protocol

After the habituation phase, the rats were placed on a treadmill at a speed determined by the treatment group (16–18). The control group did not receive physical activity, the light exercise treatment group was given a speed of 10 m/min, the moderate exercise treatment group was given a speed of 20 m/min, and the heavy exercise treatment group was given a speed of 30 m/min for 30 minutes per day, given 2 rest days per week, and lasts for 8 weeks. (17,18) On the final day of competition, the rats were euthanized by inhaling isoflurane at a concentration of at least 5% for one minute, until they ceased breathing. (17,18) Then, samples of WAT were collected for histological analysis.

Sampling

The part used in the study was a micrograph of WAT stained with hematoxylin and eosin (H&E). WAT will be extracted from the same location on each experimental rat, namely the inguinal region, placed on a glass slide, and stained with H&E.

Data processing

Using the ZEISS Z2 Microscope and the ZEN software application, the preparations were observed and photographed. One field of view was analyzed by measuring the diameter (m) of white adipose cells. In one preparation, five images were captured, one image containing cells with the most spherical shape was chosen to eliminate bias, and all adipose cells within the image were measured. Following measurement, the average cell diameter was calculated for each treatment group.

As quantitative data, the mean (SEM) of the samples in each treatment group was used in this study's data analysis, followed by a one-way ANOVA (Analysis of Variance) after the homogeneity test. The follow-up test for one-way ANOVA uses the LSD (Least Significant Difference) test. The LSD test is used to determine significant differences between treatment groups, with p 0.05 indicating that the data contains significant differences. This study's data were processed and analyzed utilizing Microsoft Excel 2016 and SPSS v.28.

RESULTS

From a total of 28 experimental rat samples, rats in groups P1 (n = 1) and P2 (n = 2) that did not meet the inclusion and exclusion criteria could not be included in the results. After collecting data on the histological appearance of white adipose tissue (WAT) in experimental rats from groups K, P1, P2, and P3, it was determined that the average diameter of white adipose cells varied between treatment groups. Group K had the largest average diameter of white adipose cells compared to groups P1, P2, and P3, which was 244.24 m. Group P1 has an average diameter of 219.78 m, which is larger than group P2's average diameter of 206.53 m and group P3's average diameter of 182.44 m (Figure 1). The LSD follow-up test was then administered to determine whether there were any significant differences between treatment groups. The average diameter of white adipose cells was significantly different between the K group and the P2 group between treatment groups. The average diameter of white adipose cells was significantly different between the K group and the P2 group (p = 0.024), with the K group being 0.15 percent* larger in diameter than the P2 group. Group K differed significantly from group P3, p 0.001, with a value of 0.25 percentage points higher than group P3. A significant effect was also observed when comparing group P1 to group P3, p = 0.025, 0.17%* greater.

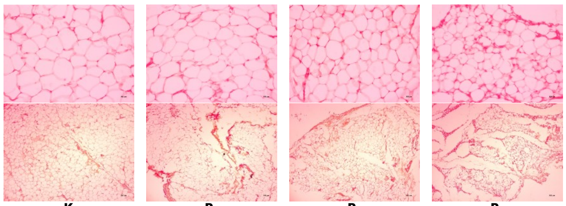

Figure 1. Histological images of differences in white adipose cells according to exercise intensity, histological images of WAT stained with H&E at 40x10 magnification (top) and 10x10 magnification (bottom).

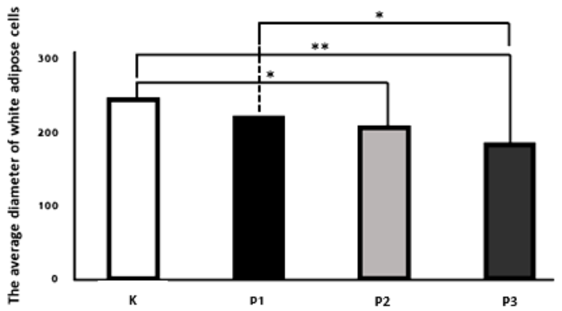

Figure 2. Example of image format for significant differences between treatment groups; data are presented as mean standard error of the mean (n=7) at the 95% confidence level; significant differences between groups are indicated by: K: control; P1: low intensity exercise; P2: moderate intensity exercise; P3: high intensity exercise

DISCUSSION

Compared to controls, moderate and heavy-intensity exercise have a significant effect on white adipose cells. Heavy-intensity exercise also has a significant effect compared to light intensity, as evidenced by the average diameter of white adipose cells decreasing with increasing exercise intensity.

This study collected scWAT from the inguinal region because the specimens ob-

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served were WAT, and scWAT contained significantly more cells than vWAT. VWAT is metabolically more hazardous than scWAT due to its proximity to the portal vein, which facilitates the entry of free fatty acids (FFA) into the liver via the portal vein and causes various metabolic complications related to the liver. (7,9,20,21) vWAT also indicates that scWAT can no longer store excess lipids. (7,9,20) Several systematic reviews and meta-analyses have demonstrated that moderate and vigorous intensity exercise have a positive effect on reducing body fat percentage (%) and total body fat mass (kg) when compared to light intensity exercise. The findings of previous studies are consistent with the findings of this study, namely a positive effect on reducing lipid content in the body, particularly vWAT. In this study, the beneficial effect of reducing lipid content in the body took the form of a histological comparison of visceral adipose cell size, which varies significantly with moderate and vigorous exercise intensity.

Because exercise can reduce lipid levels in the body, it has been utilized as a treatment for obesity for a long time. The energy reserves in adipose cells, in the form of triacylglycerols, are hydrolyzed back into FFA and enter lipid metabolism to be used as muscle-driving energy when a person exercises. (13,22) Thus, regular sports-related physical activity can reduce the amount of triacylglycerol stored within adipose cells. Adipose cells are useful for storing energy in the form of lipids, particularly WAT, which contains a single large unilocular lipid drop. (23) If the amount of lipid reserves in adipose cells decreases, the number of adipose cells will decrease dramatically, particularly in WAT, where the majority of adipose cells are used to store lipid reserves. size. This is also supported by a comparison of the histological characteristics of vWAT, which reveals that white adipose cells in group K have the largest average diameter compared to those in groups P1, P2, and P3 (Figure 1).

On a metabolic level, exercise of extreme intensity has a significant anti-inflammatory effect. (24) In the study by Balducci et al. (2010), sedentary, light-intensity, high-intensity aerobic, and mixed programs of resistance and high-intensity aerobic exercise were studied over a period of 12 months to determine their effect on high-sensitivity C-reactive protein (hs-CRP) and other inflammatory markers in type 2 diabetes and metabolic syndrome patients. Studies have shown that high-intensity exercise reduces hs-CRP, IL-1b, IL-6, TNF-a, IFN-g, leptin, and resistin while increasing IL-4, IL-10, and adiponectin, particularly in mixed resistance and aerobic programs. (24,25) C-reactive protein (CRP) is a protein known to be produced by the liver during inflammation; however, it can also be produced by adipose cells. It is frequently used as a marker to determine the risk of type 2 diabetes, insulin resistance, endothelial dysfunction, and cardiovascular disease (CVD), with disease risk calculations in the next 10 years in the hs-CRP range of 10 to 20%. (24,26,27)

In another study reviewed from the standpoint of energy use, trials of energy restriction accompanied by moderate or vigorous exercise with matched energy expenditure yielded comparable outcomes for body weight, fat mass, and biological markers. This demonstrates that the benefits of exercise with equal energy expenditure and energy restriction are independent of the intensity of the chosen sport. In the author's study, moderate and vigorous-intensity exercise yielded significant results with the same time and volume of exercise.

In 2020, the World Health Organization (WHO) recommends 150–300 minutes of moderate-intensity aerobic exercise or 75–150 minutes of vigorous-intensity aerobic exercise for adults with chronic diseases such as hypertension and type 2 diabetes mellitus. By calculating the maximum heart rate per minute (bpm) according to age and matching it with the percent target heart rate according to exercise intensity, with a light exercise intensity value (40–54%), a moderate exercise intensity value (55–69%), and a severe exercise intensity value (70–89%), the CDC in 2022 will provide an alternative method for measuring exercise intensity that can be performed by the general population. (28,29) Moderate intensity exercise includes yoga, ballet dancing, golf, walking 5–7 km/hour, or cycling 8–14 km/hour, whereas vigorous intensity exercise includes walking above 8 km/hour, running, cycling above 14 km/hour, cycling uphill, football, basketball, karate, etc. (30)

This study's design has limitations, including the possibility of human error when making sample pieces for preparations that can damage the sample and measurement errors that can occur when measuring white adipose cells due to a lack of precision. To overcome this limitation, visual field and leisurely measurements of white adipose cells were performed. In addition, the use of experimental animals, which may produce results distinct from those of humans, is a potential limitation of this study. To address the limitations of this study, this section of the discussion compares it to other studies involving human populations and research samples.

CONCLUSION

It can be concluded from this discussion that the histological appearance of white adipose cells can be used to determine significant differences between individuals who do not exercise and those who exercise with light, moderate, and intense intensity. However, low-intensity exercise is ineffective compared to non-exercising individuals in producing significant differences. To determine the effect of differences in exercise intensity on adipose cells other than their morphology, such as the characteristics and identification of adipose cells and the measurement of target fat protein, additional research is required.

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