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Intermittent Fasting with Moderate-Intensity Physical Exercise Increased Melatonin but Did Not Reduce Body Mass Index in Obese Rats

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Abstract: ***Background: Intermittent Fasting (IF) is a dietarymethod studied to positively affect circadian rhythm and metabolism by increasing melatonin hormone that may promote weight loss in obese individuals. Objective: This study aimed to measure IF with moderate-intensity exercise on increasing melatonin levels and reduce Body Mass Index (BMI). Methodology: An experimental study with pre and post-test control group design was conducted on 36 obese male Wistar rats aged 4-5 months. The subjects weredivided randomly into two groups, the study and control groups, with 18 subjects in each group. The study group underwent IF for 12 hours with moderate-intensity physical exercise, while the control group was given a standard diet with moderate-intensity physical exercise. After four weeks of intervention, melatonin levels assessedusing ELISA (enzyme-linked immunosorbent assay); meanwhile, BMI was measured before and after treatment. The comparative test was conducted to compare the results between groups. Results: The results showed that the mean pretest and post-test melatonin levels in the study group increased significantly (0.353±0.01vs.0.479±0.01; p<0.001). Melatonin levels in the study group at the end of the study also showed a significant increase compared to the control group (0.194±0.03 vs. 0.479±0.01; p<0.001). However, the mean pretest and post-test BMI in the study group did not show any significant differences (0.353±0.01 vs. 0.351±0.0; p=0.965). The BMI comparison between the study and control groups also did not show any significant differences (0.351±0.01 vs. 0.354±0.01; p=0.432). Conclusion: IF with moderate-intensity exercise increased melatonin levels but did not reduce BMI in obese male Wistar rats. The duration and diet regimes of IF need to be further evaluated.***

Keywords: Intermittent fasting, Obesity, Melatonin, Body mass index, Metabolism, Moderate physical activity

1. Introduction

The increasing global obesity incidence and prevalencehave resulted in various methods and dietary supplements claiming to help with weight loss. One of the well-known methods of dietary arrangements is Intermittent Fasting (IF). Time-limited feeding (TRF) is also known as IF, is a dietary regulation by consuming food for only a few hours per day. The general form of this TRF varies from fasting for 16 hours and eating only for 8 hours, fasting for 12 hours and eating for 12 hours, or eating only once and the rest fasting for 23 hours.[1] IF is hypothesized to affect metabolismvia effects on (a) circadian biology, (b) the gut microbiome, and (c) modified lifestyle behaviors, such as sleep time.[1,2] The association of IF with weight loss is made possible by an increase in melatonin hormone due to changes in circadian rhythm, lipolysis, and glycogenolysis during fasting.[2,3]

Studies in animals and humans claim that melatonin may enhance weight loss.[4]Melatonin, known as the sleep hormone, has a role in helping the body's circulation and metabolism regulation.[5]In addition to fostering circadian rhythms and their role as an antioxidant, previous research has shown that melatonin supplementation prevents obesity in experimental animals without affecting dietary intake and locomotor activity changes. This suggests that melatonin can act as a thermogenic agent.[5,6] Research conducted by Vazquez et al. (2018) suggests melatonin's role in increasing brown fat deposits (Brown Adipose Tissue), which functions as a thermogenic agent in a study based on mice, underlines its role as prevention of weight gain.[6]Olesçuck et al. (2019) also support the linkage of melatonin with a brown fat deposit, which functions as a thermoregulator in increasing energy metabolism.[5]

During fasting, the melatonin effect will cause insulin resistance and affect the liver's metabolism, such as glycogenolysis and gluconeogenesis, in fat tissue (lipolysis) and the pancreas (glucagon secretion). Meanwhile, melatonin's effect during the activity will increase insulin sensitivity, impacting glycogen synthesis and glycolysis in the liver, lipogenesis, adiponectin production in fat tissue, and insulin secretion.[7]In addition, exercise has an essential effect on the sleep cycle and modulates the circadian clock. Suprachiasmatic Nucleus (SCN) as a coordination center for circadian function increases during exercise, thus promote melatonin's secretion.[8]

Previous studies have not discussed changes in the body mass index (BMI) due to increased melatonin induced by IF in experimental animals. This study was initiated to increase the effectiveness of IF (12 hours of fasting followed by 12 hours of eating for four weeks) that moderate-intensity physical exercise increases melatonin levels and decreased body mass index in obese male Wistar rats.

2. Methodology

The experimental study used a randomized pre-posttest control group design on active male Wistar rats (*Rattus norvegicus*) aged 4-5 months. The determination of the sample size of 36 Wistar rats as research subjects followed the calculation using the Pocock formula. Samples that met the requirements were taken randomly and divided into two groups: the control group and the study group so that there were 18 rats per group.The study was carried out in the Integrated Laboratory of Biomedical Sciences, Faculty of Medicine, Udayana University. The study protocol was

approved by the institutional ethics committee with ethical clearance number: 56/UN14.2.9/PT.01.04/2020

Wistar rats were adapted to the environment for one week.In the next four weeks, the two groups of mice were given a high-fat and high-carbohydrate diet with composition of55% carbohydrate,35% fat and 10% protein to meet the obesity criteria according to Lee's index (> 0.3). The control group was underwentfour weeks moderate-intensity physical exercise with a standard diet consisting of 20-25% protein, 5% fat; starch 45-50%; crude fiber 5%; ash 4-5%, vitamin A 4.000 IU/kg; vitamin D 1.000 IU/kg; alpha-tocopherol 30g/kg; linoleic acid 3 g/kg; thiamine 4 mg/kg; riboflavin 3 mg/kg; vitamin B-12 50 ug/kg; biotin and choline 1.000 mg/kg. Meanwhile, the study group underwent fasting for 12 hours, and was given a standard diet in the next 12 hours along with moderate-intensity physical exercise for four weeks. The moderate-intensity physical exercise was swimming in a bucket with a duration of 20 minutes every day for four weeks. BMI measurements were carried out before the intervention started, and after the intervention was completed using the Tanita scale. Measurement of melatonin hormone levels using the ELISA technique in both groups was carried out at the end of the intervention.

A pairwise comparative test was used to compare the pretest and post-test data, the dependent t-test was performed on normally distributed data, and the Wilcoxon test was performed on skewed data. Comparison test between study groups and control groups was employed independent t-test on normally distributed data and Mann Whitney test on skewed data.

3. Results

The results of the comparative test analysis are shown in Table 1. The comparative analysis results on the mean pretest melatonin levels and BMI showed the uniformity of the research subjects' characteristics with a p value> 0.05.

Table 1: Comparative Analysis Between Groups

Variable	Group	*Pretest*	*Posttest*	*p***
		Mean±SD	Mean±SD	
Melatonin Levels (ng/L)	Control	0.237±0.04	0.194±0.03	0.009
	Study	0.253±0.04	0.479±0.07	<0.001
	*p**	0.342	<0.001	
BMI (Lee Index)	Control	0.353±0.01	0.354±0.01	0.432
	Study	0.353±0.01	0.351±0.01	0.965
	*p**	0.453	0.419	

*p * was analyzed with Mann-Whitney*
*p ** analyzed with Wilcoxon pair rank*

The results showed that the mean melatonin levels in the post-test study group were higher than the control group. Before and after the test, the mean melatonin levels in the study group also showed a significant difference (p-value <0.001). The post-test comparative analysis results on the mean BMI showed no difference between the study and control groups. The paired group test the mean BMI before and after the test did not show a significant difference (p-value = 0.965).

4. Discussion

This study suspected that IF could increase metabolism and hormone melatonin levels, which has a vital role in losing weight in obese people. Measurement of melatonin levels after IF and moderate-intensity physical activity showed that the mean melatonin levels in the study group was higher than the control group. This is in line with a study put forward by Longo (2016) where IF can optimize the activity of neurotransmitters and neuron networks to optimize brain function and peripheral energy metabolism to increase melatonin levels. The level and intensity of free radical damage are reduced during IF causes less inflammation and oxidative stress.[9–12] Thereby helping prevent damage to a person's genetic mechanisms.[9] This may explain why IF its effects can ward off cancer and degenerative diseases, leading to a longer life (Longevity).[9,13]Exercise is part of physical activity, recommended as part of weight loss therapy because it helps weight loss, abdominal fat, improves fitness, increases the work of several hormones, including melatonin, and helps maintain weight after a weight loss program.[14,15]

At the end of the study, the descriptive analysis of the mean in the study group showed a mean difference of 1.5 times higher than the control group. The comparability test between the two groups showed significant results with p-value = 0.001. These results indicate that IF with moderate-intensity physical exercise increases melatonin levels significantly in the study group compared to the control. This was also supported by the pre and post-test comparisons in the study group, which showed a significant increase (p <0.001). This study's results are in line with research conducted by Froy (2009) on mice with intermittent fasting settings, which have a significant effect on improving circadian rhythm. Although he did not measure melatonin levels directly in his studies, melatonin secretion from suprachiasmatic nuclear stimulation varied in response to circadian stimulation. Sleep disorders and circulatory rhythm due to obesity can affect the decrease in melatonin levels in the body.[16]

Another study conducted by Bubenik et al. (2011) stated that chronic food intake restriction maintains peak nighttime melatonin secretion, lost mainly in old age. In mice that were restricted in food intake, they had two times higher nighttime melatonin levels than ad libitum mice. Besides, the number of adrenergic receptors in the pineal gland involved in melatonin secretion was twice as high in food-restricted mice than in the control group. The mechanism of increased melatonin on dietary restriction is thought to be due to the secretion of this hormone in the digestive tract. The digestive tract is the body's primary source of melatonin, secreted from hormone-producing enterochromaffin cells from the intestinal mucosa.[17,18] Due to its large size, this organ is estimated to contain 400x more melatonin than the pineal gland. Fasting or dietary restriction increases melatonin secretion due to conditions similar to resting or sleeping conditions in mice, although this mechanism still needs to be studied further because contradictory findings are still found in other studies.[19]

The descriptive analysis of the mean BMI in the study group showed that the mean results were similarwith the control group. The comparability test between the two groups showed insignificant results with p-value = 0.419. These results indicate that IF with moderate-intensity physical exercise does not significantly affect BMI in the study group compared to the control group. The comparison between pre and post-test also did not show a significant difference (p = 0.965). These results are slightly different from the research conducted by Wilson et al. (2018), which states the significance of reducing fat and weight accumulation in experimental mice that do IF with training.[20] The study also stated that exercise carried out by experimental animals did not affect the significance of IF on metabolic improvement. Although it did not significantly reduce BMI, a study conducted by Liu et al. (2019) mentioned improving lipid and glucose metabolism, including reducing fat mass by inducing fat tissue remodeling in mice undergoing IF, even though there was no significant change in body weight.[3,21]

This study indicates that IF significantly increased melatonin levels, which may be in line with the provision of melatonin supplementation in Favero's study. Favero et al. (2015), in a study involving melatonin supplementation in mice for eight weeks, showed the involvement of melatonin in adipose tissue metabolism. Although not significant weight loss, melatonin supplementation was associated with partial improvement of the pathogenesis of obesity. Melatonin involves activation of central receptors, which results in changes in metabolic rate through sympathetic nervous activity and increases lipolysis and plasticity of adipose tissue.[4,22,23]Activation of adipose tissue receptors can affect energy storage by modulating metabolism or adipocyte proliferation. The findings suggest that adipose tissue is a peripheral target of melatonin for overall metabolic regulation.[22]

Also, IF studies on weight loss in obese subjects still provide mixed results. According to research conducted by Klempel et al. (2012), significant weight loss in adult female subjects who are overweight and obese by undergoing IF is still recommended to undergo calorie regulation and restriction, which is strictly observed by nutritionists. In his study, subjects received 900 kcal at the time of not fasting for eight weeks. The results showed a significant reduction in body weight and a decrease in metabolic syndrome indicators.[24] This may explain the study results, which did not show a significant difference in BMI because calorie restriction was not applied in the treatment group.Research by Smyers et al. (2020) gave different results in experimental animals undergoing long-term IF. In this study, IF was carried out for 14 weeks and was repeated in the following year. The results showed that the reduction in body weight and fat mass occurred in the treatment group even though they did not do significant physical activity and followed a standard diet.[25]The intervention duration is also recommended to continue to be evaluated until the weight loss results follow what is expected in the research hypothesis.

5. Conclusion

In this study, IF with moderate-intensity exercise increased the hormone melatonin levels but did not significantly reduce body mass index in obese male Wistar rats compared to controls. The results showed the effect of IF on improving the circulation and metabolism of experimental animals, as evidenced by the increase in melatonin levels in the study group compared to the control group. Melatonin is expected to reduce the effects of oxidative stress that often occurs in obese people. Oxidative stress can shorten telomere, which speeds up the aging process of cells.

However, this study showed no significant difference in the BMI of experimental animals, which prompted the need for further research regarding the effects of IF on the weight loss process through a more optimum IF duration, attention to nutrition or diet gave when not fasting and considering the optimum duration of physical activity. This study is expected to add to the research evidence on the potential of IF on melatonin enhancement.

6. Conflict of Interest

All authors declared that there is no conflict of interest regarding this publication.

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