ORIGINAL ARTICLE

Ghrelin and Serotonin as Indicators of Obesity due to The Influence of Circadia on Wistar Rats

*Retno Indrawati[1], Anis Irmawati[1], Indeswati Diyatri[1], Ninuk Hariyani[2], Mukhammad Fauzi[3]

[1] Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia
[2] Department of Public Health, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia
[3] Undergraduate Student, Faculty of Dental Medicne, Universitas Airlangga, Surabaya 60132, Indonesia

ABSTRACT

Introduction: One of the main factors supporting obesity is the disruption in the performance of the circadian rhythm which results in a decrease in the quality and quantity of sleep, which triggers stress which is regulated by the hormone serotonin in the body. This increase in serotonin is related to factors that trigger the risk of obesity, besides that melatonin and leptin in the body can decrease, thereby increasing ghrelin which stimulates appetite. The aim of this study was to prove that ghrelin and serotonin can be used as indicators of obesity in Wistar rats as experimental animals. **Materials and Methods:** This study involved 3 groups with each group consisting of 6 samples. Group 1 was the normal group (12 hours light, 12 hours dark), group 2 was the dark group (24 hours dark) and group 3 was the light group (24 hours light. Each group was treated with circadian and modified feed until obesity was found and then the blood measured using the Rat ELISA Kit. **Results:** The results of the correlation test of body weight of Wistar rats with ghrelin and serotonin show that there is a strong relationship between body weight and ghrelin with p-value = 0.006 ($p< 0.05$), $r= 0.609$. The correlation between body weight and serotonin was moderate with p-value = 0.023 ($p< 0.05$), $r= 0.517$. **Conclusion:** Ghrelin and Serotonin can be used as indicators of obesity in Wistar rats.

Keywords: Obesity, Circadian rhythm, Ghrelin, Serotonin

Corresponding Author:
Retno Indrawati, DDS, MDS
Email: retnoindrawati@fkg.unair.ac.id
Tel: +62315030255

INTRODUCTION

Obesity is a pathological condition in which excess fat is deposited in subcutaneous tissue, as well as a low-level chronic inflammatory condition in white adipose tissue. Obesity is classified as a chronic disease due to excessive food consumption, according to WHO (1). Obesity cases in the world increase threefold betwen 1975 and 2016. In 2016, more than 650 million people were obese (1). The percentage of obesity is about 13% of the world's adult population, which is 11% in men and 15% in women. Indonesians who are obese are not less than 12% and are expected to continue to increase every year (2). In addition, people who are obese have a 7 to 11 times higher risk of diabetes. In addition, if the person has diabetes, the risk of heart disease increases up to 8-fold. Obesity is a very serious health problem, not only for individuals but has become a global health problem.

The disruption of circadian rhythms, which act as regulators of biological functions and behaviors, is one of the risk factors that contribute to obesity (3). This messed-up circadian rhythm causes a drop in sleep quality and quantity, triggering the onset of stress, which is controlled by the hormone serotonin in the body (4). The increase in serotonin is linked to obesity risk factors, on the other hand, melatonin and leptin levels in the body can drop, causing ghrelin levels to rise, stimulating appetite (5–7).

Current technological developments have caused 62% of the earth's population to be exposed to artificial light at night (8). Increased exposure to light at night is known to lead to increased metabolic disorders, impaired reproductive function and neuroendocrine (8). Changes in the duration of light or treatment of certain photoperiodes are also a triggering factor for stress response (9).

This study was conducted to determine the effect of ghrelin and serotonin levels on obesity in experimental animals treated with photoperiod, so as to determine the relationship between obesity caused by changes in circadian rhythm, which is known through the influence of ghrelin and serotonin levels.

MATERIALS AND METHODS

Samples

This experimental laboratory research adopted true experimental design with (post test control group design). The study was carried out at The Institute Tropical Disease (ITD) Universitas Airlangga and has received ethical approval from the Dental Committee of Airlangga University, Indonesia (No. 320/HRECC. FODM/VII/2020). Federer's formula was used to calculate the sample size. About 18 Wistar rats were randomly sampled and divided into three groups: a normal control group that received 12 hours of light, a dark treatment group that received 0 hours of light, and a light treatment group that received 24 hours of light. The inclusion criteria of the samples were 8-week-old male wistar rat, rat weighing 140–200 grams, with no anatomical defects, and active movement.

Preparation of the light exposure to animal model

All sample groups, namely the control group and the treatment group, were subjected to circadian treatment and dietary treatment. The circadian treatment was carried out with each group using a bright period placed in a lighting room that had a fluorescent lamp along with a timer switch for setting light duration in each group, namely the normal control group with 12 hours of light (12T) and the light treatment group with 24 hours of light (24T). The distance between the floor of the lighting room and the light source is ± 75 cm, so that the light received by the test animals ranges from 100-110 lux. Circadian treatment in the dark treatment group was a group with 0 hours of light or no light at all in the room. Each group will be given treatment for 8 weeks.

Diet treatment was carried out in the control group and treatment group by way of rats induced into obesity by increasing fat intake by 40-60% of total calories, feed, and drink given to rats ad libitum. Modified foods containing 65-70 percent carbohydrates, 20-25 percent protein, and 5-12 percent fat were given to rats. For 8 weeks, Wistar rats are given a drink containing 10-15% fructose solution in drinking water, which causes obesity (Wulansari, 2018). During the study, an analytical balance sheet was used to measure animal weight every 7 days with a precision of 0.1 grams.

Measurement of ghrelin and serotonin levels

After the circadian treatment and diet treatment, the samples' ghrelin and serotonin levels were measured by taking the rats serum, done by taking blood through the heart (intracardiac) as much as at least 3 mL, then accommodated into the vacutainer. The blood is then centrifuged at a speed of 3000 rpm for 10 minutes. Serum results from blood collected, then tested using Rat ELISA kit (Elab Science, USA) in accordance with factory rules.

Statistical analysis

Data analysis was tested by data normality test using Saphiro Wilk test, correlation test using Spearman Rho Correlation and different test using Repeated Measured Anova and Mann Whitney U test.

RESULTS

This study used experimental methods of in vivo laboratory with true experimental design with posttest control design to observe differences in initial data and results of control group experiments and treatment groups. This study was conducted with the aim of knowing that ghrelin and serotonin can be used as indicators of obesity in Wistar rat using experiments that have been divided into 3 circadian treatment groups, namely normal, dark and light groups with each group consisting of 6 sample numbers.

Before the test was conducted between groups, each group conducted a different test each group using statistical test Repeated Measured Anova. The results of the Normal Anova Repeated Measured test group showed no meaningful difference in Wistar rat weight from week 1 to week 9, due to overall p> 0.05 values.

Based on Figure, the results of the Repeated Measured Anova dark group test showed in week 2, there was weight loss but not significant (p> 0.05). Significant weight loss (p< 0.05) occurred in the 3rd week of 4 grams. This meant that during the 3 weeks the rat's weight was reduced by 4 grams. Week 4 of the rat's weight still experienced a significant decrease (p< 0.05) of 4.33 grams. This meant that during the 4 weeks there was a significant weight loss of 4.33 grams. Week 5 and the 6th weight of the rat began to increase although insignificant (p> 0.05). The 7th week later, there was a significant increase in weight (p< 0.05) of 33.67 grams of initial weight. In the 9th week the body weight of rat still experienced a significant decrease (p< 0.05) of 4.00 grams. This means that there is a significant weight loss of 4.00 grams judging by the weight of the 7th week against the 9th week.

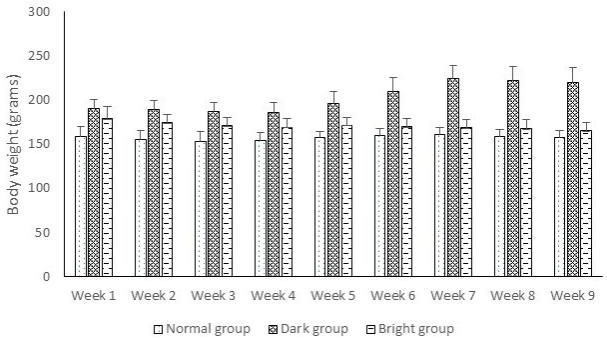

Fig. 1: The average of body weight over 8 weeks.

Repeated Measured Anova test results of the bright group showed from week 1 to week 8 there was a decrease in weight but not significant (p> 0.05), whereas if observations were observed from week 8 experienced significant weight loss on the 9th day (p< 0.05), amounting to 2,857 grams.

After conducting different tests each group then conducted tests between groups using statistics test Mann Whitney U test. The result was that there was a meaningful difference in Wistar rat weight between normal and dark group (p< 0.05). In the normal and light group (p> 0.05) it was found that there was no meaningful difference in Wistar rat weight. In the dark and light group (p< 0.05) there was a meaningful difference in the weight of Wistar rat (Table I).

Table I: The significant levels of body weight on circadian rhythm

Type of Data	Normal- Dark- (p-value)	Nomal-Bright (p-value)	Dark-Bright (p-value)
Weight	0,004	0,181	0,003

The average levels of ghrelin in Wistar rats showed that the dark group had higher levels than the light and normal groups. The average ghrelin level in the dark group rats is 923.673 ng/L, the light group rats are 837.014 ng/L and 768.390 ng/L in the normal group (Table II). Based on Table II, the average serotonin levels in Wistar rats showed that the dark group had higher levels than the light group and normal group. The average ghrelin level in rats with dark treatment is 30,412 ng/ml, in light group rats it is 28.291 ng/ml and in normal group rats is 27.299 ng/ml.

Table II: The average of ghrelin and serotonin levels

Sample groups	N	Mean ± SD	
		Ghrelin	Serotonin
Normal	6	768.390 ± 108.89	27.299 ± 0.797
Dark	6	923.673 ± 55.05	30.412 ± 1.220
Bright	6	837.011 ± 63.06	28.291 ± 1.634

Noted: SD: Standard Deviation; N: replication

After a different test was conducted Wistar mouse weight correlation test with ghrelin and serotonin using statistic test Spearman Rho Correlation. The result of Wistar rat weight correlation test with ghrelin obtained was p-value = 0.006 (p< 0.05), meaning that there was a significant relationship between weight and Ghrelin, with a value of r= 0.609 indicating that the relationship between the two was strong. The direction of the relationship is straight / positive, meaning the higher the weight, the higher ghrelin, and vice versa. The results of the Wistar mouse weight correlation test with serotonin obtained were p-value = 0.023 (p< 0.05), meaning that there was a significant relationship between weight and serotonin, with a value of r=0.517 indicating that the relationship between the two was moderate. The direction of the relationship is straight/positive, meaning the higher the BB, the higher the serotonin, and vice versa (Table III).

Table III: The correlation between body weight to ghrelin and serotonin levels

Type of Test	Weight -Ghrelin		Weight -Serotonin	
	p	r	p	r
Correlation Test Spearman's Rho	0.006	0.609	0.023	0.517

DISCUSSION

This study was conducted on Wistar rats, where the animal is a group of nocturnal animals that are active in the dark (10). Rats are nocturnal rodents that make the time of gene expression in contrast to 180o with diurnal animals (11, 12). Humans belong to the diurnal group who are accustomed to doing activities in the morning and rest at night which causes differences in the circadian rhythm of the mouse body with humans. Exposure to light at night received by humans causes circadian rhythm disturbances, as well as in rat the disorder can occur when obtained in groups that do not get any light at all or dark groups. Literature study conducted Tähkämu (13) states that many studies were found on the impact of light on human health, especially with regard to circadian rhythms.

5-HTP is a precursor compound in the production of the neurotransmitter serotonin. Serotonin is a hormone that is responsible for carrying messages between cells in the brain. Serotonin in the brain regulates anxiety, happiness, and mood (14). Elevated serotonin levels in the dark group occur due to circadian rhythm disturbances that cause serotonin that should be converted into melatonin to return to serotonin so that melatonin continues to decline. Melatonin decreases causing the body to be awake and experience a decrease in blood pressure, so it may experience sleep deprivation (10). Rat in the dark for a period of several weeks will show a change in behavior due to the absence of light to the center of the brain that controls mood disorders so that they may experience stress. Long-term lack of light can also exacerbate stress (15). Stress can trigger an increase in the hormone cortisol that begins with the release of stress hormones that secrete corticotrophin releasing factor (CRF) released from the hypothalamus so that it reaches the pituitary gland that is just below the hypothalamus which then stimulates the release of adenocorticotrophin hormone (ACTH) and when cortisol levels in the blood fall, the hypothalamus releases corticotropin-releasing hormone (CRH), which directs the pituitary gland to produce adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal glands to produce and release cortisol (16).

Rat in the dark tend to do activities continuously, so an increase in cortisol can lead to an increase in

the hormone ghrelin that motivates the body to eat. Research conducted by (17) said that ghrelin levels in serum are higher in stressful conditions compared to normal conditions (18), this is evidenced by measuring the weight of feed given to rat every day, that in the dark group has a more intake of 100 grams / day with the normal weight of feed given is 50 grams / day in each cage. This habit can be referred to as Dinner Syndrome (SMM) (19), according to the theory that one of the risk factors that support the occurrence of obesity is the disruption of the performance of circadian rhythms that act as regulators of the body's biolgis function as well as behavior (20). Tryptophan amino acids that are abundant in the body due to the presence of food intake can also be the cause of increased serotonin levels. Serotonin is a chemical in the brain that can affect mood. Eating foods that contain the essential amino acid known as tryptophan can help the body to produce more serotonin (21).

Serotonin is a precursor to melatonin, where serotonin increases it is also in line with the increase in melatonin. In bright conditions serotonin levels were also found to be increased but not higher compared to the dark group because the rat in the light state also experienced circadian rhythm disturbances. Exposure to received light can lead to decreased stimulation of norepinephrine in the pineal gland so that serotonin activity decreases (22). Over-available serotonin can lead to serotonin syndrome that can lead to excessive anxiety disorders that lead to stress to depression (23). Rat in a bright state will tend to do less activity so that the increase in ghrelin is not higher compared to the dark group, this is evidenced by the intake that is not always consumed in some cages in the light group.

CONCLUSION

Increased levels of ghrelin and serotonin due to circadian effects can be used as an indicator of obesity in Wistar rats.

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