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Effect of Increased Corticosterone Levels Due to Chronic Stress on Body Weight Changes in *Rattus norvegicus*

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Abstract

Background: Corticosterone is a biomarker associated with chronic adaptation. Weight loss is associated with an increase in glucocorticoid hormones due to stress which affects the burning of brown fat so that calories are burned. Changes in body weight due to increased levels of the corticosterone hormone have not been widely studied.

Objective: Analyze the effect of increasing serum corticosterone levels due to chronic stress on body weight changes of *Rattus novergicus*.

Methods: True Experimental with Post-Test Only Control Group Design. *Rattus norvegicus* is divided into the control group (17 rats), which were not given stress treatment, and the treatment group (17 rats), given stress treatment using the CUMS method for 20 days. ELISA detected the serum corticosterone levels, and a measure of body weight changes was done twice before and after CUMS was given.

Results: The normality was tested using the Kolmogorov-Smirnov test and the Shapiro-Wilk test, followed by the independent T-Test parametric statistical test. Corticosterone in the treatment group was higher (72.84±64.03) than in the control group (23.29±8.42). Changes in body weight of the control group (14.62±4.98) were heavier than the treatment group (-10.33±11.24). Statistical test p=0.000 (p<0.05).

Conclusion: Increased serum corticosterone levels due to chronic stress on changes in body weight of *Rattus novergicus*.

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INTRODUCTION

Coronavirus 2019 (COVID-19) affects global mental health; a meta-analysis review among the general population in Asia and Europe showed that the prevalence of stress was 29.6%, anxiety at 31.9%, and depression at 33.7%. Another study conducted in the Philippines found that 16.3% of respondents rated the psychological impact of the outbreak as moderate to severe, 16.9% reported moderate to severe depressive symptoms, 28.8% had moderate to severe anxiety levels, and 13.4% had moderate to severe stress levels[1,2]. In Indonesia, mental health status during the COVID-19 pandemic was investigated and found the incidence of depression was 20.8%, anxiety at 34.6%, and stress at 25.4%. The incidence of moderate to severe anxiety is 12.4%, mild anxiety is 26.3%, and stress is 16%. Factors that affect mental health during the Covid-19 pandemic are career background in health and healthy behavior (washing hands after coughing, sneezing, and touching the nose)[3].

Stress and anxiety further affect the physical and psychological health status and result in adverse health outcomes. Stress can increase the risk of chronic disease and other health problems; dealing with chronic conditions and poor health can increase the amount of stress a person experiences. The 2011 APA survey showed that 39% reported skipping meals, and 29% of respondents reported overeating or eating unhealthy foods due to stress. In addition, 44% reported waking up at night due to stress[3,4]. Stress is divided into acute stress and chronic stress. Acute stress lasts for a short time but is quite strong, then disappears quickly. Chronic stress is stress that appears not too strong but can last for a long time, from days to months. Chronic stress experienced repeatedly can affect individual health and productivity[5]. Chronic stress is directly related to the stress endocrine system and affects an individual's brain structure, immune system, and behavior. Individuals exposed to chronic stress during their lifetime have a higher risk of developing cardiovascular disease, anorexia, obesity, cancer, immune disorders, and mental disorders such as depression[6].

Stressors in everyday life cannot be predicted in various forms; if individuals cannot cope with exposure to stress, exposure that occurs continuously causes chronic conditions. This study used the Chronic Unpredictable Mild Stress (CUMS) method. Chronic Unpredictable Mild Stress gives various treatments as stressors and resembles stressors of daily life that are not too heavy but continuous[7]. This method significantly increases corticosterone (cortisol in mice) levels within 20 days[8]. In rats, cortisol secretion is replaced by corticosterone[9]. Chronic stress increases the synthesis and secretion of glucocorticoids. Elevated glucocorticoids are biomarkers for stress and depressive disorders. Cortisol and corticosterone in rat serum are different and closely correlated with physiological conditions or stress dynamics. Corticosterone is a biomarker of chronic adaptation, whereas cortisol is more likely to reflect severe acute stress[9,10,11,12].

Stress makes the hormone corticosterone shift the balance of the body's metabolism through a catabolic process. The body deals with stress by increasing sugar availability in the blood through glycolysis and gluconeogenesis. Lipolysis is needed as a raw material for gluconeogenesis. Lipolysis increases the mobilization of

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fatty acids from adipose tissue. An increase in fatty acid oxidation processes in cells will reduce the transport of glucose needed for storage and maintain the number of triglycerides in fat cells. If this material is not present, the fat cells will release fatty acids. The breakdown of fatty acid reserves in the body causes weight loss[12].

Stress conditions cause two possibilities in changes in body weight; this is because each individual has a different stress response. Weight loss is associated with increased glucocorticoid hormones that affect the burning of brown fat so that calories can be burned[13]. However, according to Harding et al.[14], psychosocial stress is positively related to weight gain but not weight loss.

There has been no research on increased corticosterone due to chronic stress on changes in body weight. Existing research has only reached acute stress conditions and has not had consistent results; it is necessary to do further research on changes in the hormone corticosterone due to chronic stress on changes in body weight.

METHODS

This type of experimental research is True Experimental with Post Test Only Control Group Design. This research previously received a certificate of ethical feasibility from the ethics committee of the Faculty of Medicine, Universitas Airlangga Number: 129/EC/KEPK/FKUA/2019.

The number of sample units based on the Lemeshow formula was 30 female Wistar rats (*Rattus norvegicus*). The independent variable is corticosterone levels due to chronic stress, and the dependent variable is body weight change. There were two groups in this study: the negative treatment group or negative control group, namely rats that were not given the stressor (K), and the treatment group, namely the rats that were given the CUMS stressor (P). Rats were placed in cages and colonized 2 to 3 rats in a quiet, quiet, and ventilated room. Rats eat and drink given ad libitum. Group K rats were given lighting that adapts to outside light in the morning and evening without light and protected from stressful situations. P group rat was given stressor CUMS method according to Tabel 1.

Tabel 1. Chronic unpredictable mild stress (CUMS) protocol applied for 20 day

Day	Stresor (time)	Day	Stresor (time)
1	Foreign object (3h)	11	Restraint (1h)
2	Overcrowding (24h)	12	Without stressor
3	Immersion in cold water (3m)	13	restraint (1h)
4	Isolation (24h)	14	Immobilization (2h)
5	Immobilization (2h)	15	Overcrowding (24h)
6	Persistent light (24h)	16	Foreign object (3h)
7	Without stressor	17	Overcrowding plus Persistent light (24h)
8	Overcrowding plus Persistent light (24h)	18	Pain stressor (1h)
9	Pain stressor (1h)	19	Persistent light (24h)
10	Isolation (24h)	20	Immersion in cold water (3m)

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Examination of serum corticosterone levels using blood taken intracardially then examined using the ELISA method. Bodyweight is measured using a digital scale with 10^{-2} grams accuracy. The measurement of body weight of all study groups was carried out twice during the study. Measurements were made on the first day of the study, and the second measurement was carried out before termination.

Processing the collected serum corticosterone level and weight change data will be coded, edited, entered, and cleaned. Then grouped according to research variables and presented in tables, frequency distribution, crosstables, and graphs. The data obtained were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, followed by an independent T-Test parametric statistical test.

RESULTS

The research subjects in this study were 34 Rattus norvegicus divided into two groups, namely the control and treatment groups. There were 2 sample units on ELISA examination in the control group that did not detect corticosterone levels, so they were excluded. In the treatment group, there was one drop out because he died before the treatment was finished. The final number of sample units for each control group was 15, and the treatment group was 16.

a. Corticosterone Level

Measurement of corticosterone hormone levels using the Enzyme-Linked Immunoassay (ELISA) method. Corticosterone hormone levels in blood serum after 20 days of stressor administration. Based on Figure 1, it can be seen that the treatment group had higher serum corticosterone levels than the control group. The mean results of corticosterone hormone levels in *Rattus norvegicus* serum after 20 days of stressor administration in the treatment group (72.84 ± 64.03) were higher than in the control group (23.29 ± 8.42) as listed at Tabel 2.

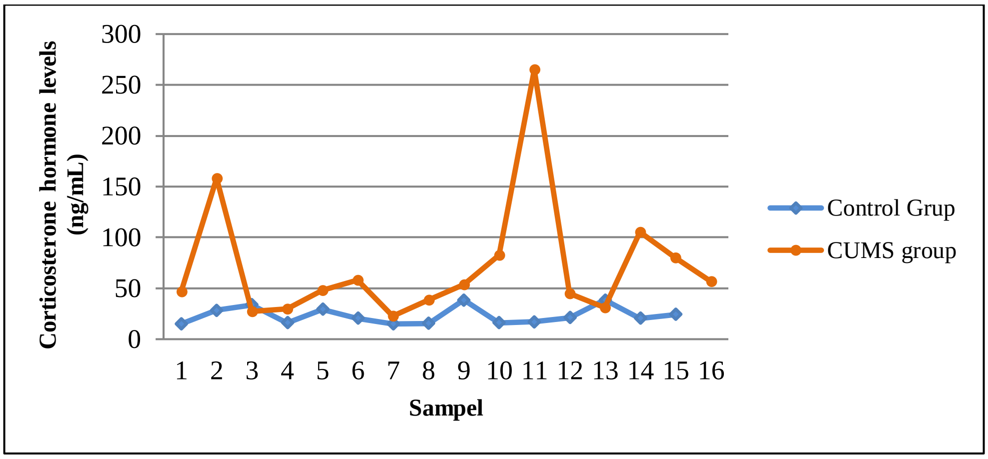

Figure 1. Corticosterone hormone levels in the serum of *Rattus norvegicus* after 20 days of stressor administration.

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Tabel 2. Mean and standard deviation of serum corticosterone levels

Group	n	Mean ± SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	p
Control	15	23.29 ± 8.42	15.004	38.604	<0.05
CUMS	16	72.84 ± 64.03	22.700	264.949	

The results of the normality test of serum corticosterone levels of *Rattus norvegicus* after 20 days of stressor were not normally distributed in both the control group (p=0.024, p>0.05) and the treatment group (p=0.000, p>0.05). Statistical analysis to look for differences was continued with the Mann-Whitney nonparametric test. The results of the Mann-Whitney test showed a significant difference between the serum levels of the hormone *Rattus norvegicus* in the control group and the treatment group with a p-value = 0.000 (p<0.05).

b. Bodyweight change

Bodyweight is measured using a digital scale that has an accuracy of up to 10-2 grams. Measurement of body weight of all study groups was carried out twice during the study. Measurements were made on the first day of the study, and the second measurement was carried out before termination.

Tabel 3. The average change in body weight of *Rattus norvegicus* before and after stressor administration

Group	Mean ± SD (ng/mL)	Min (ng/mL)	Max (ng/mL)	p
Control	14.62 ± 4.98	6.98	23.45	<0.05
CUMS	-10.33 ± 11.24	-25.33	7.50	

The average weight change of *Rattus norvegicus* before and after 20 days of stressor administration in the control group (14.62 ± 4.98) was higher than the treatment group (-10.33 ± 11.24). The control group experienced the most weight gain of 23.45 grams, and the treatment group only experienced the most significant increase in body weight of 7.50 grams. The treatment group is known to have decreased body weight up to 25.33 grams as listed at Table 3.

The normality test was carried out first using Shapiro Wilk with the results that all data were normally distributed (p>0.05). In the weight change of *Rattus norvegicus*, a statistical test was performed. The Independent t-test showed p = 0.000 (p <0.05), which means that there was a significant difference between the control and treatment groups. It can be concluded that the treatment group experienced weight loss after receiving a 20-day CUMS.

DISCUSSION

There was a significant difference in corticosterone levels between the two groups with a p-value = 0.000 (p<0.05), where the corticosterone levels in the treatment group were

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higher than in the control group. These results are in line with the research results conducted by Zhao[15], which found a significant increase in corticosterone levels in rats after 8 hours of restraint stress initiation. Wang[16], in his research on a mouse model of acute stress, found a significant increase in serum corticosterone concentrations.

Chronic stress boosts glucocorticoid production and secretion (cortisol in humans and corticosterone in rodents). Biomarkers for stress and depressive illnesses include elevated glucocorticoids[10]. Corticosterone, like cortisol in humans, is the principal glucocorticoid hormone in rodents. Under normal conditions, glucocorticoids will provide negative feedback by suppressing CRH secretion to prevent an excessive stress response. The phenomenon does not occur in chronic stress where the HPA axis continuous activity[9].

The sample unit in this study was *Rattus norvegicus*, a nocturnal animal[17]. All treatment activities were carried out in the morning - noon to change the circadian cycle of *Rattus norvegicus*. In this study, experimental animals were left awake during treatment. The treatment started at 09.00 am, and the length of the treatment varied. Sleep pattern disturbances applied to experimental animals cause circadian rhythm disturbances in the circadian rhythm regulation center in the hypothalamus, namely the Supra Chiasmatic Nucleus (SCN). Circadian rhythm is the body's biological clock for 24 hours a day. This system regulates hormone secretion and physiological processes, the awake sleep cycle[18]. States that sleep pattern disorders that are not addressed will fall into psychosocial stress conditions. In addition to the given physical stress, changes in circadian rhythms also impact psychological stress in experimental animals so that it exacerbates existing stress[19]. According to Gamble et al., the circadian cycle is an integral part of the regulation of the reproductive system; when this 24-hour program is not regular, the endocrine system can be disrupted[20]. The high corticosterone level in *Rattus norvegicus* in this study is in line with Lopez-Lopes et al., which stated that CUMS could increase corticosterone levels with a minimum of 20 days of treatment[8].

Changes in body weight and increases in the hormone corticosterone are used as unit parameters of the study sample who have experienced chronic stress. The average weight change during the treatment in the treatment group exposed to CUMS for 20 days was significantly different from the control group. There was a tendency to experience weight loss in the treatment group during the study. These results are by the results of a study conducted by Cordero, where the study results that the CUMS group experienced weight loss compared to the control group[21]. Research by Jeong got similar results to this study. This research found that stress affected body weight and food intake in mice. A reduction in daily food intake caused weight loss in rats subjected to restrained stress at the start of the study through modifying genes that regulate food intake. The sustained difference in body weight between the stress restraint and control groups is possible because of the influence of gene expression and regulation of the body's response to stress through the hormone corticosterone[22]. Another study found there was a relationship between chronic stress and eating disorders. It was found that there was a difference in gastrointestinal activity between rats exposed to electric shock stressors and rats that

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were not exposed. The feces size indicated that the startled mice had a larger volume of feces than the control mice[23].

Changes in body weight are one form of changes in body systems that were initially normal and are now disturbed. Stress can contribute to changes in eating behavior that lead to weight changes. This condition causes two possibilities for changes in body weight when stressed. Each individual has a different stress response. Weight loss is associated with an increase in glucocorticoid hormones that affect the burning of brown fat so that calories can be burned[13]. Repeated exposure to stress-sors can lead to several physiological and behavioral disorders, metabolic and eating disorders[23].

Weight loss in mice exposed to chronic stressors is a physiological correlation evoked by changes at the central level. The stress response is mediated by the 41-amino acid corticotrophin-releasing factor (CRF) peptide, synthesized in hypothalamic or extrahypothalamic nuclear neurons (i.e., paraventricular nucleus and central amygdala). CFR interacts with two significant receptors (CRF1 and CRF2), differentially expressed in different brain structures and peripheral tissues. Many studies have shown that central administration of CRF replicates signs of depression and anxiety in rodents, such as reduced exploration, increased hopelessness, decreased appetite, sleep disturbances, and weight loss[24].

The effects of stress on nutrition and the gastrointestinal (GI) system can affect appetite; this effect is associated with the involvement of the ventral tegmental area (VTA) or amygdala via the N-methyl-D-aspartate (NMDA) glutamate receptor. In addition, stress also affects the normal function of the GI tract. Stress affects absorption, intestinal permeability, mucus, gastric acid secretion, ion channel function, and GI inflammation. When stressed, the hormone corticosterone will shift the balance of the body's metabolism through a catabolic process. The body deals with stress by increasing sugar availability in the blood through glycolysis and gluconeogenesis. The breakdown of fat (lipolysis) is needed as a raw material for gluconeogenesis. Lipolysis increases the mobilization of fatty acids from adipose tissue. An increase in fatty acid oxidation processes in cells will reduce the transport of glucose needed for storage and maintain the number of triglycerides in fat cells. If this material is not present, the fat cells will release fatty acids. The breakdown of fatty acid reserves in the body causes weight loss[23, 25].

This study's weakness is that the cause of weight loss in the group of mice given the CUMS stressor was only the result of an increase in the hormone corticosterone or other factors such as changes in gene composition due to chronic stress. So that further research is needed to find out other causes that may contribute to weight loss in rats experiencing chronic stress.

CONCLUSION

Increased serum corticosterone levels due to chronic stress on changes in body weight of *Rattus novergicus*.

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