International Journal of
Disabilities Sports and Health Sciences
e-ISSN: 2645-9094

RESEARCH ARTICLE

Chronic Unpredictable Mild Stress Affects Weight Changes Through Circadian Cycle Mechanism

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Abstract
Everyday stress is unpredictable and comes in many different forms. The person is constantly exposed to stress because they cannot handle it. Chronic stress results from this illness. Chronic stress can affect changes in the body's standard mechanisms, including body weight modulated by the circadian cycle. Due to the fact that each person's response to stress is unique, stressful situations might result in two different variations in body weight. The purpose of this study is tobserve the effect of the CUMS on weight changes in Rattus novergicus through the circadian cycle mechanism. 16 chronic stress models of the Rattus norergicus were split into control and treatment groups. The chronic stress was provided using the Chronic Unpredictable Mild Stress (CUMS) approach. The control group did not receive any treatment but was still given maintenance such as providing food, drink and measuring their body weight every day. While the treatment group received CUMs for 20 days. Every morning before the stressor exposure, body weight was recorded. And the measurement of corticosterone levels using the Enzyme-Linked Immunoassay (ELISA) method with the Duplo technique. Before termination, blood is taken at the heart. Corticosterone levels varied across the groups (p = 0.032). The study's changes in body weight (p = 0.00) revealed a difference between the control and treatment groups, with the treatment group's weight being -11.15 ± 7.91. Chronic stress can alter how the body normally functions, mainly how the circadian cycle regulates body weight. Since each person's response to stress is unique, stressful situations might result in two different variations in body weight.

Keywords
Chronic Unpredictable Mild Stress, Circadian Cyle, Weight Changes

INTRODUCTION

Stress in daily life is unpredictable and takes many forms. The individual's inability to cope with stress causes exposure to stress to occur continuously. This condition causes chronic stress.

The mechanism of the body's response to stress begins with a stimulus that can come from internal or external and then forwarded to the limbic system as an adaptation center. The anterior pituitary will create ACTH (adrenocorticotropin-releasing hormone) and TRF (thyrotrophine releasing factor) in response to the hypothalamus's release of CRF (corticotrophin-releasing factor). Through the bloodstream, the adrenocorticotropin hormone reaches the adrenal glands, which secrete glucocorticoid hormones, namely cortisol (Willy, 2016).

Typically, cortisol will give negative feedback by reducing CRH secretion to stop overreacting to stress. However, this is not the case with chronic stress, where the hypothalamus-

Received: 17 October 2023 ; **Revised** ; 16 March 2024 ; **Accepted:** 21 March 2024; **Published:** 25 March 2024

How to cite this article: Puriastuti, A.C., Maramis, M.M., Annas, J.J., I'Thisom, R., Rejeki, P.S. and Sulistiawati, S. (2024). Chronic Unpredictable Mild Stress Affects Weight Changes Through Circadian Cycle Mechanism. *Int J Disabil Sports Health Sci*;7(2):469-474. https://doi.org/10.33438/ijdshs.1376508

pituitary-adrenal (HPA) axis is always activated (Willy, 2016).

Chronic stress can raise cortisol levels, which can impact a number of the body's regular processes, including changes in body weight, which the circadian cycle also regulates. According to Xumin Peng (2023) and Teruel (2022), the circadian cycle is a biological clock that controls several natural bodily processes, including the sleep-wake cycle, blood pressure, core body temperature, hormone secretion, and metabolism.

Stressful conditions cause two possibilities in weight change, increase or decrease. The weight change is because each individual has a different stress response. Numerous studies' findings indicate that stress affects circadian cycle modifications. Significant alterations in the body are impacted by disruptions in the circadian cycle, particularly those about the development of fat cells (Teruel, 2022).

Stress damages the body through complex network signaling cascades interacting and regulating vulnerability and severity. Repetitive stress can cause broader changes and adaptations, especially related to changes in energy metabolism (Koch et al. 2023). The mechanism of weight change in individuals who experience chronic stress can be caused by various mechanisms, one of which is the circadian cycle. Chronic stress can indirectly affect changes in the circadian cycle. According to Koch et al. (2023), alterations in the HPA axis' activity and glucocorticoid levels, as well as metabolic diseases and serious depression, are all linked to disruptions of the circadian clock.

Ghrelin and leptin, two hormones that control hunger, can be impacted by sleep. Leptin conveys satiety, while ghrelin promotes hunger. When the body lacks sleep, the circadian rhythm is thrown off, causing ghrelin levels to rise and leptin levels to fall. As a result, you feel more hungry and want to consume delicious meals (Scheer et al., 2013).

Increases in glucocorticoid hormones, which influence brown fat burning to burn calories, are linked to weight loss (Symonds, 2016). However, psychological stress is positively associated with weight growth but not weight loss, claim Harding et al. (2018). The effects of chronic psychological stress are more varied. Harris (2016) assumes that further studies regarding phenotypic differences between humans and experimental animals in response to chronic and acute stress are needed.

Chronic stress conditioning in this study used the Chronic Unpredictable Mild Stress (CUMS) method. CUMS is the administration of various treatments as stressors, which are similar to everyday stress but not too heavy and carried out for at least three weeks (López-López, 2016). Numerous treatments are given for chronic unpredictable mild stress, which reflects the stresses of daily life, which are not too heavy but continuous (Maramis, 2015). This method has significantly increased corticosterone levels within 20 days (López-López, 2016). This study aims to observe the effect of the CUMS on weight changes in Rattus novergicus through the circadian cycle mechanism.

MATERIALS AND METHODS

In this lab experiment, a chronic stress model called Rattus novergicus was used. In 16 Rattus Novergicus, control and treatment groups were created, and this research was authorized by the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University.

The Chronic Unpredictable Mild Stress (CUMS) approach was employed in this study to measure chronic stress. CUMS is the administration of various treatments as stressors, which are similar to everyday stress but not too heavy and carried out for at least three weeks (López-López, 2016).

The experimental animals were 16 female rats (Rattus norvegicus), Wistar strain, aged 5–6 months. All rats were adapted and acclimatized for seven days, then randomly divided into two groups, namely the control group and the treatment group. The maintenance room between the control and treatment groups was separated to prevent stress in the control group. Rats were caring in a quiet, calm, ventilated research room with room temperature and a 12-hour lighting cycle.

The treatment group did not receive any treatment but still received animal care. Both groups had their feed, remaining feed, and body weight balanced daily.

Treatment was given for 22 days, it shows in “Table 1”, and the rats were sacrificed 24 hours after the last treatment to avoid the effects of acute stress.

Table 1. The Stressor in 22 days

Day of Treatment	Stressor (time)	Day of Treatment	Stressor (time)
1	Overcrowding or placed in one cage containing 5 mice (24 hours)	12	Immobilize with wire mesh restrainer (2 hours)
2	Isolation in a narrow and dark space (24 hours)	13	Tie the tail with thread (1 hours)
3	Tie the tail with thread (1 hours)	14	Isolation in a narrow and dark space (24 hours)
4	The cage is tilted 45º (5 hours)	15	The tail is pierced with a needle ± 2.5 cm long (1 hours)
5	Immobilize with wire mesh restrainer (2 hours)	16	Immobilize with wire mesh restrainer (2 hours)
6	Swimming in cold water 4ºC (3 menit)	17	Exposure to noise and loud sounds (85 – 90 dB) (3 hours)
7	Without stressor	18	Exposure to bright light of 300 – 400 lux (4 watts) is carried out twice a day (each 45 minutes)
8	Overcrowding or placed in one cage containing 5 mice (24 jam)	19	Overcrowding or placed in one cage containing 5 mice (24 hours)
9	Exposure to bright light of 300 – 400 lux (4 watts) is carried out twice a day (each 45 minutes)	20	Swimming in cold water 4ºC (3 menit)
10	Exposure to noise and loud sounds (85 – 90 dB) (3 hours)	21	The tail is pierced with a needle ± 2.5 cm long (1 hours)
11	Without Stressor	22	Swim 4ºC (3 menit)

Source : (López-López, 2016; Puriastuti, 2017 ; Brouček, 2014)

Body weight was measured every morning before exposure to the stressor. To prevent the consequences of acute stress, rats were euthanized 24 hours after the last treatment using an excessive amount of Eter. The intracardial blood sample was the reason the excessive ether killed approach was selected over the cervical spine dislocation method.

After that, the serum and other blood components were separated for an hour by being stored in a test tube with a label. Cold centrifugation were used, with a speed of 3000 rpm for ten to fifteen minutes.The serum was separated and then put into an Eppendorf tube that had been labeled so that it could be examined using the Enzyme-Linked Immunoassay (ELISA) technique with the Duplo technique (Maramis, 2015).

RESULTS

Increased Corticosterone Levels

After performing an independent t-test, "Table 1" shows that there was a difference in corticosterone levels between the control and treatment groups ($p = 0.032$). Corticosterone levels were greater in the treatment group than in the control group.

Change (Δ) Body Weight

Weight measurements are carried out daily with digital scales with an accuracy of up to 10^{-2} grams.

Tabel 2. Rattus norvegicus corticosterone levels 24 hours after 21 days of CUMS treatment

	Group	N	Mean ± SD (ng/mL)	Average (ng/mL)	Lowest (ng/mL)	Highest (ng/mL)	p
C	Control	8	13.36 ± 4.31	15.85	7.07	20.34	0.032*
T	Treatment	8	33.56 ± 21.28	26.61	17.28	82.40	

Tabel 3. Change (∆) body weight

	Group	N	Mean ± SD (gr)	Average (gr)	Lowest (gr)	Highest (gr)	p
BW Before	Control	8	156.6 ± 13.7	156.4	137.4	175.20	0.58
	Treatment	8	174.2 ± 22.4	168.9	147.0	223.0	
BW After	Control	8	171.2 ± 15.7	170.1	145.2	194.0	0.35
	Treatment	8	163.0	162.5	134.9	201.1	
Δ *BW* During data collection	Control	8	14.55 ± 5.06	15.4	6.00	19.70	0.00
	Treatment	8	-11.15 ± 7.91	-9.00	- 21.90	0.00	

BW (Body Weight)

Based on "Table 3", statistical tests using an independent t-test showed no difference between the control and treatment groups before the treatment (p = 0.58) and after the study (p = 0.35). These results indicate that the difference between the two groups became greater after being given CUMS. On the other hand, changes in body weight (∆) over the course of the trial revealed a distinction between the control and treatment groups (p = 0.00), with the treatment group's weight being -11.15 ± 7.91. The treatment group, it can be inferred, underwent weight fluctuations and tended to lose weight.

DISCUSSION

Correlation with HPA Axis

Corticosterone levels between the two groups were significantly different. Namely, the treatment group was higher than the control group. Similar to how cortisol predominates in humans, corticosterone is the primary glucocorticoid hormone in rodents (Guyton, 2016).

When under stress, the hypothalamus releases CRF (corticotropin-releasing factor), which causes the anterior pituitary to release ACTH (adrenocorticotropin hormone) and TRF (Thyrotrophine releasing factor). Adrenocorticotropin hormone, through the bloodstream, reaches the adrenal glands, which make the adrenal glands secrete glucocorticoid hormones, namely cortisol. In order to minimize excessive stress responses, cortisol will normally provide negative feedback by squelching CRH secretion. However, this is not the case with chronic stress, where the hypothalamus-pituitary-adrenal (HPA) link is always activated (Willy, 2016).

Correlation to the Circadian Cycle

Stress is a process of adaptation of the body that arises to support adjustments to environmental stimuli that influence the development and stimulation of expression. Stress can be induced by changes in plasticity in brain function and behavior (Bohus, 1995). However, if stress continues and occurs continuously, it can cause disturbances in various body systems. These disturbances can be in the form of changes in the immune system, neurochemistry, and even pathological conditions (Pasiak et al. 2005). Rattus norvegicus, a nocturnal animal (Kusumawati, 2004) that served as the study's sample unit, underwent all treatment procedures from the morning until the afternoon. Rattus norvegicus' circadian cycle is subsequently altered.

Numerous ideas propose prolonged stress as a cause of abnormalities in reproductive function. Disrupted circadian cycles are further investigated in this study, in addition to the activity of CRH and glucocorticoids, which will be studied next, as well as the impact on gonadotropin hormones. The circadian cycle allows organisms to anticipate regular and daily recurring events that occur at almost the same time (Gamble et al. 2013); therefore, forms of stress with the CUMS method also disrupt this cycle. The circadian cycle is a crucial regulatory component of the reproductive system, and when this 24-hour rhythm is irregular, the endocrine system can be affected (Gamble et al. 2013; Mahoney, 2010). The circadian clock regulates several biological processes, such as glucose and lipid homeostasis, energy expenditure, and hormone secretion. Environmental misalignments, such as abnormal light/dark cycles, and behavioral misalignments, including feeding, sleep-wake cycles, and activity, can cause circadian disruption, contributing to obesity-related metabolic disorders (Peng, 2023; Teruel, 2022). Behavioral misalignments, such as shift work, jet lag, and sleep disturbances, can accelerate the onset and development of obesity and related metabolic disorders in both animal models and humans. The existence of circadian rhythms in thermogenic fats also suggests that they play a role in obesity-related circadian disruption and relevant metabolic complications (Peng, 2023).

Change (∆) Body Weight

Corticosterone levels and changes in body weight were the variables used as the research sample unit. Weight change is a form of modification in the bodily system that was previously normal but has turned abnormal, according to Pasiak (2005). Due to the fact that everyone reacts to stress differently, stress can result in weight fluctuations in two different ways. In order to burn calories, brown fat must be burned, which is impacted by an increase in glucocorticoid hormones (Symonds, 2016). But according to Harding et al. (2014), psychosocial stress is linked to weight growth rather than weight loss. Haris (2015) contends that further research is required to understand how experimental animals and people react to short-term and long-term stress. Changes in body weight due to disruption of the circadian cycle can occur due to the development of thermogenic fat, namely brown and beige adipocytes, which are controlled by transcriptional regulation.

Circadian disturbance, such as lack of sleep or exposure to continuous light, is a contributing factor in many cases of obesity and related disorders. Thermogenic fat, like the majority of other tissues, exhibits robust circadian oscillation due to the intricate interaction between local and central clocks (Peng, 2023). The thermogenic clock is influenced by circadian factors such as eating, light, and clock genes. The circadian clock plays a vital role in the differentiation and development of thermogenic fat. In order to maintain metabolic homeostasis, most thermogenic fat functions, such as thermogenesis and glucose and lipid metabolism, are regulated by the circadian rhythm (Teruel, 2022).

Conflict of interest

The authors declared there were no conflicts of interest. Additionally, no financial assistance was given.

Ethics Committee

Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University. (Approvel Nummer: 2.KE.054.03.2018).

Author Contributions

Study Design, Data Collection, Statistical Analysis, Data Interpretation, Manuscript Preparation, and Literature Search were all planned by the author. The manuscript's author has read and approved the published version.

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