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Differences in body weight post-induction sleep deprivation and sleep recovery in white male rats (*Rattus norvegicus*)

Perbedaan berat badan pasca-induksi sleep deprivation dan sleep recovery pada tikus putih (Rattus norvegicus) jantan

Fitranto Arjadi[1*], Wahyudin[2], Joko Setyono[3]

[1] Departemen Anatomi, Fakultas Kedokteran Universitas Jenderal Soedirman, Purwokerto, Indonesia.
E-mail: fitranto.arjadi@unsoed.ac.id
[2] Departemen Anatomi, Fakultas Kedokteran Universitas Jenderal Soedirman, Purwokerto, Indonesia.
E-mail: wahyuwahyudin@unsoed.ac.id
[3] Departemen Biokimia, Fakultas Kedokteran Universitas Jenderal Soedirman, Purwokerto, Indonesia.
E-mail: joko.setyono1907@unsoed.ac.id

***Correspondence Author:**
Departemen Anatomi, Fakultas Kedokteran Universitas Jenderal Soedirman, Jl. Dr. Gumbreg, No.1, Purwokerto Selatan, Banyumas, Jawa Tengah, Indonesia.
E-mail: fitranto.arjadi@unsoed.ac.id

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Abstract

Sleep Deprivation (SD) reduced leptin levels, increased ghrelin levels, and it caused were multifactorial, so research was conducted on experimental animals to prove whether SD was the single factor causing changes in body weight (BW). The study's objectives were to know the difference in BW after induction of paradoxical and total SD and to observe the improvement in sleep recovery (SR). This study was true experimental with posttest only, and a control group design used 25 male albino rats randomly shared into five groups; control, PSD, TSD, PSD+SR, dan TSD+SR on August – September 2021. The weight is measured by OHAUS® balance. Statistical analysis used by One-way ANOVA and paired t-test denoted no significant difference after SD (p=0,277) and SR (p=0,297), a significant difference in the TSD+SR and TSD between before (p=0,014), after SD (p=0,008), and after SR (p=0,034). Sleep deprivation increases BW through raised ghrelin, and SR reverses the effects by increasing the antioxidant. Results must be confirmed by measuring ghrelin levels and leptin orexin type 1 and 2 receptors. In conclusion, that was a significant difference in the TSD+SR and TSD between pre and post-sleep deprivation and the TSD+SR between pre and post-SR.
Keywords: Body weight, sleep deprivation, sleep recovery

Abstrak

Sleep deprivation (SD) menurunkan kadar leptin dan meningkatkan dan ghrelin, penyebabnya bersifat multifaktorial sehingga diperlukan penelitian pada hewan coba untuk membuktikan apakah SD merupakan faktor tunggal penyebab perubahan berat badan. Penelitian bertujuan mengetahui perbedaan berat badan pascainduksi paradoxical SD dan total serta melihat perbaikannya dengan sleep recovery (SR). Penelitian eksperimental *post-test only with control group design* memakai 25 ekor tikus putih Wistar yang secara acak dibagi menjadi 5 kelompok; kontrol, PSD (20 jam SD/hari 5 hari), TSD (24 jam SD/hari 5 hari), PSD+SR, dan TSD+SR pada Agustus sampai dengan September 2021. Pengukuran berat badan menggunakan timbangan analitik OHAUS®. Uji statistika menggunakan uji One-way ANOVA dan paired t-test yang menunjukkan tidak terdapat perbedaan pasca SD (p=0,277) maupun SR (p=0,297), perbedaan signifikan pada kelompok TSD+SR dan TSD antara pra (p=0,014) dengan pasca SD (p=0,008), serta pasca SR (p=0,034). *Sleep deprivation* meningkatkan berat badan melalui peningkatan *ghrelin* dan SR memulihkan dampaknya dengan meningkatkan antioksidan. Hasil penelitian perlu dikonfirmasi dengan mengukur kadar *ghrelin, leptin* dan *reseptor orexin* tipe 1 dan 2. Kesimpulan terdapat perbedaan pada kelompok TSD+SR dan TSD antara pra dan pasca SD, serta kelompok TSD+SR antara pra dan pasca SR.
Kata Kunci: Berat badan, sleep deprivation, sleep recovery

Introduction

Sleep deprivation (SD) is a total lack of sleep for a particular duration or a lack of the expected optimal sleep duration. It causes a variety of adverse health impacts caused by an individual contemporary lifestyle and work-related factors such as shift work (Lateef & Akintubosun, 2020). According to the Family Life Survey (IFLS-5) of 2015, 11,0% of the population in Indonesia suffer from sleep deprivation (Peltzer & Pengpid, 2019). Sleep deprivation disrupts the body's metabolism, increases the risk of obesity, changes in metabolic genes and hormones, and triggers inflammatory responses (Mosavat et al., 2021).

Sleep deprivation reduces levels of the leptin hormone that reduces appetite by 18% and increases the hormone ghrelin that triggers a desire for food by 28% (Papatriantafyllou et al., 2022). The harmful effects of SD can be corrected with sleep recovery (SR), a method to make the animal try to restore standard sleep time (Agatri et al., 2020). Sleep deprivation in mice has been to indicate weight loss and increased activity of the hypothalamic-hypothesis-adrenal axis (HPA), accompanied by increased food intake and energy consumption by increasing hunger and appetite by disrupting metabolic and endocrine functions, decreasing insulin sensitivity and leptin levels, and increasing cortisol and ghrelin levels (Timper & Brüning, 2017). Sleep deprivation causes the metabolic response of an increase in total daily energy expenditure ± 2x the baseline rate. Even though mice increase their daily calorie intake, weight loss remains due to high metabolism. Sleep recovery can change the reduced availability of orexin and its receptors that reduce thermogenesis and reduce energy expenditure to contribute to weight gain. (McHill & Wright, 2017).

Several methodologies can evaluate the effects of lack of sleep: Paradoxical SD (PSD) as a short-term disturbance, total sleep depravation (TSD), as a long-term disorder, and can be corrected with SR after sleep deprivation. (Arjadi et al., 2022). The different types of sleep disorders that can affect eating behavior and weight can represent species differences between mice and humans, and this makes the researchers interested in observing the weight differences of post-induced SD and SR in white mice (*Rattus norvegicus*). Based on this background, the study aims to determine the difference in animal BW after SD induction and total SD and see its improvement with SR.

Methods

The research was conducted in the Anatomy Laboratory of the Faculty of Medicine of the University of General Soedirman in August – September 2021 and obtained ethical approval from the Medical Research Ethics Commission FK Unsoed on May 10, 2021, with the reference number: 097/KEPK/V/2021. The type of research is an experimental posttest with a control group design on 25 white rats (Rattus norvegicus) male Wistar of the Integrated Research and Testing Laboratory (LPPT) 4 University of Gadjah Mada. The free variable of the study is the stress model of sleep deprivation-SR , and the bound variable is BW.

The use of biological research tools is the OHAUS® analytical scale commonly used in the laboratory to measure the mass and weight of an object whose measurement is valid and smooth, five cages of mice with the size of 60 x 30 x 30 cm, twenty tanks size 34 x 26 x 29 cm that is equipped with the muscle atonia tool and the materials used are animal feed is Comfeed AD II as standard animal feed and drink animal testing is AQUA® mineral water given in the same type, composition, and quantity ad libitum. The criteria for inclusion of trial animals are 200-300 grams, aged 3-4 months, normal and healthy condition, and excluded if illness and weight decrease>10% after acclimatization. The determination of the number of trial animals in each group was determined using the Federer formula that (t-1)(n-1) > 15 with t = the amount of treatment, n = a number of repetitions and obtained the result that at least each group consists of 5 test animals and because there are 5 groups so requires a trial animal of 25 animals (Charan & Kantharia, 2013).

Acclimatization for 7 days was performed on test animals at a temperature of 28 ± 2 °C, humidity of 75 ± 5% and natural lighting 12 hours dark (19.00-07.00 WIB) and 12 hours light (07.00-19.00 WIB) (Capdevila et al., 2007). The SD method uses the modified multi-platform method (MMPM) by placing mice on a stainless steel tank with a size of 34 x 26 x 29 cm, a diameter of 6,5 cm, between platforms a distance

of 10 cm, the base of the tank is filling with water with a height of 1 cm, and the water is replaced daily (Mahmoudi et al., 2017). The tank is equipt with an atonia muscle shock device that automatically turns on every 10 minutes so that the test animal will stay awake and the mice move freely in the tank platform.

The division of the treatment group is a healthy control group (KI) that various models of SD stress do not induce. The treatment I (KII) is inducted by PSD stress for 20 hours (11.00-07.00 WIB), returned to the cage and given sleep time 4 hours (07.00-11.00 WIB) which is carried out continuously for five days and continued SR during five days from 11:00-11.00, treatment II (K-III) is an animal attempted induction of total SD stress (TSD) for five full days from 11.00-11.00 WIB and continued SR for five days from 11:00-11.00, treatment III (K-IV) which is induced PSD stress for 20 hours (11.00-07.00 WIB), returned to the cage and given sleep time 4 hours (07.00-11.00.00 WIB) that is carried out continuously during five days, and treatment IV (K-V) is an animal attempted to induce TSD stress for five whole days from 10.00-11.30 WIB (Arjadi et al., 2022). Wistar male rats' weight was weighed before SD treatment, after SD treatment and specifically for the PSD+SR and TSD+SR groups after SR treatment.

The data was analyzed univariately to show the mean, median, and standard deviation distribution, the normality of the data was tested with Shapiro-Wilk and the homogeneity of data variance was checked with Levene's test. When the data distribution is homogeneous, and the data variance is normal, bivariate analysis is continued using the one-way Analysis of Variance (ANOVA) and Paired T-Test tests. The ANOVA test is used to examine the rarity difference between more than two groups. At the same time, the Paired T-Test is oxidized to see the difference in rarity between two pairs of samples with a density of 95% ($\alpha = 0,05$).

Result and Discussion

The statistical tests show each treatment's minimum, maximum, and median values in Table 1. The weight measurement of the test animals was carried out prior to the treatment of sleep deprivation, after the treatment for SD in the PSD and TSD groups, and after the SR treatment in PSD+SR and T SD+SR groups. The control group showed an increase in BW of 0,2 ± 5,63 grams, and the PSD + SR, TSD +SR, PSD, and TSD groups showed weight loss after treatment which was in sequence of 11,4 ± 22,95; 28,4 ≤ 13,16; 0,2± 31.47; and 69,6 ± 37,5 grams.

The tests of normality and homogeneity of the test animals are listed in Table 2. The paired t-test showed significant differences in the treatment of TSD+SR (p=0,014), TSD between before and after SD (p =0,008) and TSD +SR treatment between before sleep depravation and after SR (P=0,034). Anova's one-way tests showed no significant differences in the animal's weight before sleep deprivation, after sleep deprivation, and after SR (Table 3).

After the induction of sleep deprivation, only the control group whose weight increased while the other treatment group experienced weight loss. Weight gain in the control group of 0,2 ± 5,63 grams occurred due to higher energy intake from food intake compared to energy expenditure due to metabolism in the body and physical activity. Weight gain in the control group can also occur because the mice do not emit excess energy due to stress, which is a sympathetic response of fight or flight so that the food given can be deposited in the form of fat (Arora et al., 2019).

Weight loss in the group with SD stress treatment PSD, TSD, PSD+SR, and TSD+SR showed a decrease in BW in the trial animals, with significant weight decreases occurring in the TSD and T SD+SR groups. During sleep deprivation, rats experienced increased food intake with weight loss (Orzel-Gryglewska, 2010) and the mechanism is associated with a decrease in the level of leptin circulated and in the nucleus of the hypothalamic arcuate that suppresses appetite (Muthmainah et al., 2021).

Reduced leptin levels stimulate the release of the hormone ghrelin, which triggers agouti-related peptide (AGRP) and neuropeptide Y (NPY) regulation and inhibits the regulation of anorexigenic-melanocyte stimulating hormone (a-MSH), which further activates the pathway to stimulate hunger (Wilkinson & Imran, 2019). The hormones ghrelin and leptin are essential in controlling appetite, which can affect BW. Leptin is secreted by the fat cells that reduce hunger, while ghrelin is secreted by cells in the stomach layer to increase the feeling of hunger. Increased levels of ghrelin occur through the Growth Hormone Secretagogue-Receptor (GHS-R), while

increased leptin levels occur through Obesity Receptor (Ob-R) (Kojima & Kangawa, 2006). The decrease in leptin levels caused by SD leads to hyperphagia associated with increased levels of oxygen and neuropeptide-Y (NPY), as well as decreases in levels of anorexigenic and α-melanocyte stimulating hormone (α-MSH). Weight loss during hyperphagia conditions associated with increased appetite is affected by increased energy expenditure through gene expression uncoupling protein 1 in the mitochondria of brown adiposa tissue. (Schmid et al., 2008). The condition of hyperphagia during the induction of SD disappears after SR because SR can restore the reduction in leptin levels caused by SD (Brondani et al.,2012).

Table 1. Body weight (BW) data for experimental animals (n= 5)

Group	BW Before SD	BW After SD	Δ BW after SD	BW after SR	Δ BW after SR
	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$	$\bar{X} \pm \sigma$
Controlled (gram)	259,8 ± 35,05	260 ± 39,15	0,2 ± 5,63		
PSD+SR (gram)	263 ± 46,78	251,6 ± 51,36	11,4 ± 22,95	258,2 ± 49,74	6,6 ± 6,46
TSD+SR (gram)	248,8 ± 56,75	220,4 ± 44,51	28,4 ± 13,16	225,2 ± 43,70	4,8 ± 4,44
PSD (gram)	234,2 ± 66,48	234 ± 56,45	0,2 ± 31,47		
TSD (gram)	267 ± 24,8	197,4 ± 27,87	69,6 ± 37,5		

Table 2. The p-value of normality and homogeneity test results (n= 5)

Group	Test of Normality (*Shapiro-Wilk*)			Test of Homogeneity (*Levene's test*)		
	Before SD	After SD	After SR	Before SD	After SD	After SR
Control	0,721	0,378				
PSD+SR	0,436	0,389	0,525			
TSD+SR	0,689	0,452	0,540	0,318	0,820	1,000
PSD	0,570	0,878				
TSD	0,254	0,190				

Table 3. One Way ANOVA statistical analysis results

Group	n	p[a] Before & After SD	p[a] Before & After SR	p[a] Before SD & After SR	p[b] BW After SD	p[b] BW After SR
Control	5	0,941				
PSD+SR	5	0,329	0,111	0,527		
TSD+SR	5	0,008*	0,097	0,034*	0,227	0,297
PSD	5	0,989				
TSD	5	0,014*				

Note: [a]paired T-Test, [b] One-Way Anova test, *Significant (p < 0,05)
PSD= Paradoxical Sleep Deprivation, TSD= Total Sleep Deprivation
BW= Body weight, SR= Sleep Recovery, SD= Sleep Deprivation

A decrease in weight during SD is explained by increased energy expenditure and an increase in the basal metabolism that is strongly stimulated by the expression of the protein gene of uncoupling protein-1 in the mitochondria of brown fat tissue. Weight loss is due to mice being unable to offset increased energy expenditure with more food intake (Martins et al., 2006). Stress Conditions for SD treatment increases metabolic rate due to the decomposition of endogenous proteins and fat reserves in the body of mice due to nitrogen excretion that causes the BW of the animal to decrease or tend to settle (Astutik & Kuswati, 2014). Although rats experience hyperphagia during sleep deprivation, food intake becomes inefficient and is not stored as a fat reserve because it is used to meet the body's energy needs (Gurnida & Rosifah, 2011).

Weight loss in the TSD and TSD+SR groups showed a significant decrease compared to other

groups with higher energy expenditure and more significant TSD-induced oxidative stress compared with the PSD group (Lim & Foldvary-Schaefer, 2016). Lipid peroxidase occurs, as a cause of oxidative stress, due to the continuous work of the mitochondria without any rest resulting in increasingly massive damage, and produces a residual ROS metabolism that increases (Süer et al., 2011).

The PSD+SR and TSD+SR groups treated for SR experienced insignificant weight gain of 6,6 ± 6,46 grams and 4,8 ± 4,44 grams. Sleep recovery is estimated to cause improvement effects after the animal tries to experience stress due to sleep deprivation, so there is an increase in BW (Hipólide et al.,2006) and SR can restore the body's metabolism to normal and restore food intake ad libitum within one day (Wilkinson & Imran, 2019). Glutathione and melatonin levels are thought to have influenced improvement from SD treatments (Everson & Crowley, 2004). Glutathione is known to minimize lipid peroxidation from cell membranes and other target cells due to oxidative stress caused by SD (Kerksick & Willoughby, 2005). Melatonin in the body also increases BW and food intake by limiting the oxidative stress processes that cause lipid degradation by acting as a free radical catcher (Campos et al., 2019).

Sleep recovery after SD also reduces the availability of orexin and its receptors, thereby reducing thermogenesis (both stimulated by the sympathetic nervous system and non-exercise activity) because orexin plays an essential role in energy expenditure. Reduced energy expenditure due to decreased function of orexin combined with increased eating behavior during PSD can contribute to weight gain (Muthmainah et al., 2021). The findings in this study are consistent with the research (Barf et al. 2012), which showed that at the end of SR for five days, the rat's BW remained significantly lower than the control, as the rats did not increase their food intake despite the supposed decrease in leptin and insulin levels due to differences in neuropeptide Y mRNA, orexin/hypocretins mRNA levels in the hypothalamus, as well as leptin and insulin levels to induce hyperphagia (Muthmainah et al., 2021).

Treatment of SD in animals attempted to trigger a stress response that can be observed, such as an increase in self-grooming behavior, i.e. self-cleansing such as licking and scratching the body associated with adaptive behaviour to the stress response as a result of the distortion of normal behaviours of rats (Pires et al., 2013). Sleep deprivation in humans produces short-term health changes, such as stress and psychosomatic and long-term adverse effects, such as obesity, diabetes mellitus, cancer, and cardiovascular disorders, to death. Mechanisms occur through increased catecholamines, cortisol, ACTH, ghrelin, appetite, inflammatory mediators (TNF-, Il-1, Il-6, CRP), and ROS. In addition, it disrupts O2 consumption, CO2 production, and the rhythm of life and decreases the sensitivity of insulin, leptin and melatonin sensitivity (Medic et al., 2017). Sleep recovery in humans is necessary to restore the impact of SD because it reduces lipid peroxidase, increases the antioxidant glutathione and restores the balance between pro-inflammatory and anti-inflammatory molecules at a systemic level (Ibarra-Coronado et al., 2015).

Conclusion

There are significant differences in the TSD+SR and TSD group between before and after SD and the T SD+SR group between prior to SD and after SR .

This study may prove that sleep disorders/SD can reduce weight in trial animals, and sleep replacement/repair measures can restore the trial animal weight, although not significant. The results of this study need to be confirmed by measuring levels of ghrelin, leptin and the hormone melano corticotropin as hormones that affect eating behavior as well as its neurological circuit pathways in the brain by examining orexin type 1 and type 2 receptors.

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