Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (5): 537-544, 2023
DOI: 10.9775/kvfd.2023.29955

RESEARCH ARTICLE

Melatonin Treatment Affects Leptin and Nesfatin-1 Levels but not Orexin-A Levels in REM Sleep Deprived Rats

Aysen CAKIR [1 (*)] Sevda SEHZADE [1] Cansu KOC [2] Nevzat KAHVECI [1]

[1] Bursa Uludag University School of Medicine, Department of Physiology, TR-16059 Bursa - TÜRKİYE
[2] Bursa Uludag University School of Medicine, Department of Medical Pharmacology, TR-16059 Bursa - TÜRKİYE

[(*)] **Corresponding author:** Aysen CAKIR
Phone: +90 224 2954014
E-mail: aysencakir@uludag.edu.tr

How to cite this article?
Cakir A, Sehzade S, Koc C, Kahveci N: Melatonin treatment affects leptin and nesfatin-1 levels but not orexin-A levels in REM sleep deprived rats. *Kafkas Univ Vet Fak Derg,* 29 (5): 537-544, 2023.
DOI: 10.9775/kvfd.2023.29955

Article ID: KVFD-2023-29955
Received: 01.06.2023
Accepted: 04.09.2023
Published Online: 08.09.2023

ABSTRACT

Sleep contributes to the energy balance of body. This study aims to investigate how rapid eye movement (REM) sleep deprivation (SD) or recovery sleep affects rat weight and the serum levels of Nesfatin-1, Orexin-A, and Leptin; additionally, seeks to determine the impact of melatonin administration on these parameters. Male, Sprague Dawley rats were randomized into two groups (n=9). REMSD was induced using the modified multiple platform method (MMPM). Melatonin was used as a treatment (20 mg/kg). Study Group I was created to investigate the effectiveness of the treatment during REMSD. Study Group II was established to analyze the possible therapeutic role of melatonin and recovery sleep after REMSD-induced damage. The rats' weights were recorded during the experiments. Blood samples were collected from all rats via decapitation after experiments. The levels of serum Nesfatin-1, Orexin-A, and Leptin were analyzed using the ELISA method. REMSD affected weight of the rats and altered the levels of serum Nesfatin-1 and Leptin. Melatonin administration influenced weight gain and affected Nesfatin-1 and Leptin levels. REMSD or melatonin did not affect Orexin-A levels. REMSD and melatonin play significant roles in the body's energy balance. This study will contribute to elucidating the role of SD in metabolic processes and will play a role in assessing the impact of melatonin, a commonly used treatment in human and veterinary medicine.

Keywords: Leptin, Melatonin, Nesfatin-1, Orexin-A, Sleep deprivation

INTRODUCTION

We spend one-third of our lives sleeping. Sleep influences every system in the body and is crucial for physical, emotional, and mental well-being. The American Academy of Sleep Medicine and Sleep Research Society considers 7-8 h of sleep per night important for maintaining people's overall health [1]. It is also well known that sleep contributes to the homeostasis of energy [2]. Many people experience sleep deprivation (SD), especially due to changing living and working conditions [3]. Research indicate that sleep deprivation is associated with elevated risk of cardiovascular diseases, metabolic disorders, cerebrovascular pathologies, and accidents [4]. It is suggested that changes in metabolism resulting from sleep deprivation may cause these health issues. Some of these are hyperphagia, weight loss, high energy expenditure, elevated plasma catecholamines, hypothyroidism, deterioration in physical appearance, low anabolic hormone levels, and weakened immune system [5]. Furthermore, it has been discovered that light exposure can suppress melatonin production and DNA damage repair capability, thereby raising the risk of developing chronic illnesses [6].

Animal studies have shown that the effects of SD on body weight differ from those reported in humans. Sleeping less than 7 hours per night causes weight gain in humans [7]. On the other hand, rapid eye movement (REM) SD causes weight loss despite increased food intake (hyperphagia) in rats [8]. Hyperphagia was thought to be linked to higher oxygen consumption and increased energy metabolism [9]. The occurrence of weight loss despite increased food intake suggests that SD has a negative effect on energy balance [10].

The hypothalamus and brain stem regulate eating behavior [11]. There are also many hormones responsible for the control of body weight and nutrition. One of them, Leptin, known as the satiety hormone. The impact of sleep deprivation on Leptin levels appears to vary, with a study suggesting an increase [12] while another indicate no change [13]. Additionally, it was shown that insufficient sleep leads to higher energy expenditure and reduced Leptin levels [14], also it has been determined that Leptin

levels decrease as a result of sleep deprivation [5,15]. Leptin is thought to affect Orexin neuronal activity to control energy homeostasis [16]. Orexin receptors are found throughout the central nervous system (CNS), particularly in the hypothalamus. Orexin-A can cross the blood-brain barrier and promotes adipocyte glucose uptake [17]. Additionally, melatonin has been shown to induce sleep by inhibiting Orexin neurons [18]. Another hormone, Nesfatin-1 is an anorexigenic hormone that suppresses appetite [19]. Nesfatin-1 is strongly associated with various diseases, including neurogenic diseases, certain psychiatric disorders, diabetes, and obesity [20]. Nesfatin-1 expression has been shown to decline after 72 hours of REM sleep deprivation and it rises again after 3 hours of rebound sleep. Furthermore, intracerebroventricular Nesfatin-1 injection has been shown to reduce REM sleep temporarily while increasing slow-wave sleep [21]. Nesfatin also plays a crucial role in sleep regulation, and Nesfatin neurons are primarily located in the dorsolateral hypothalamus lateral hypothalamic and perifornical regions of the CNS [22]. These areas significantly contribute to the sleep-wake cycle [23]. Nesfatin-1 is believed to reduce appetite independently to the Leptin pathway by activating the melatonin system [24].

Melatonin promotes healthy sleep and plays an important role in regulating human sleep [25], it has also been shown to increase REM sleep percentage [26]. Sleep loss alters both melatonin cycle and eating behavior [27]. It has been demonstrated that REM sleep stabilizes the hypothalamic representation of feeding behavior and influences future food intake. This highlights the significant impact of sleep and SD on food intake [28]. Although various studies have unveiled the role of melatonin in the pathologies arising from SD and the impact of SD or recovery sleep on metabolic processes, no investigation has been found that examining the connection and underlying mechanisms between these factors. The purpose of this study is to investigate the effects of REM SD or recovery sleep on the weight of rats and the serum levels of Nesfatin-1, Orexin-A, and Leptin. In addition, we scoped to determine the impact of melatonin administration on these parameters.

Material and Methods

Ethical Statement

The study has been approved by the Bursa Uludag University Local Ethics Committee on Animal Research under decision number 2022-17/07.

Animals and Groups

Experiments were conducted in accordance with the NRC Guide for the Care and Use of Laboratory Animals. The rats were acclimatized to laboratory conditions for 2 days before the onset of the experiments. The room was temperature-controlled (23-25°C) with a 12 h light-dark cycle. Male, 8-12-week-old Sprague Dawley rats were randomly assigned to groups (n=9). The modified multiple platform method (MMPM) was used to induce REMSD. This was achieved by placing 3 animals on 6 platforms inside a large tank for 6 consecutive days. The tanks were filled with water at 24°C and platforms (6.5 cm diameter) were placed 2 cm above the water surface. When the animals lost muscle tone during REM sleep, they fell into the water, thereby waking up and experiencing REMSD. For the animals in the control group, same-size tanks were used, but grids were placed on the platforms to prevent the animals from falling into the water when they entered REM sleep. This ensured that the control group was exposed to the same environmental conditions without SD. Melatonin was used as a treatment and the dosage of melatonin (20 mg/kg) was determined based on the previous study showing its effectiveness [29]. The melatonin was dissolved in dimethyl sulfoxide (DMSO) and intraperitoneally (i.p.) administered at 08:00 am, adjusted as the end of the night cycle. The animals were divided into the following groups:

Study Group I: These groups were created to investigate the molecular changes that occur during REMSD and the effectiveness of the treatment. The animals in these groups were monitored in their appropriate cages for 6 days with daily i.p. injections, and on the 7th day, they were decapitated *(Fig 1)*. The animals in Control Groups were placed on grids according to MMPM, were provided access to food and water under optimal conditions: C+S: Saline treated control group (n=9); C+DMSO: DMSO treated control group (n=9); C+MEL: Melatonin treated control group at a dose of 20 mg/kg (n=9). The animals in REMSD Groups were placed on small platforms (6.5 cm diameter) according to MMPM, were provided access to food and water under optimal conditions: REMSD+S:

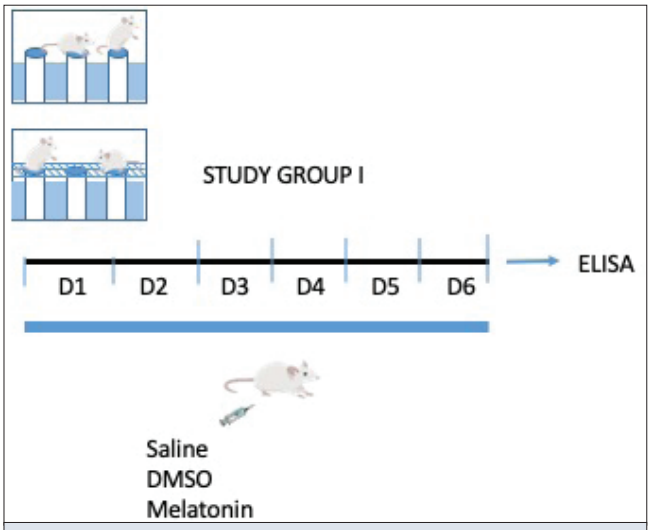

Fig 1. Schematic diagram of Study Group I

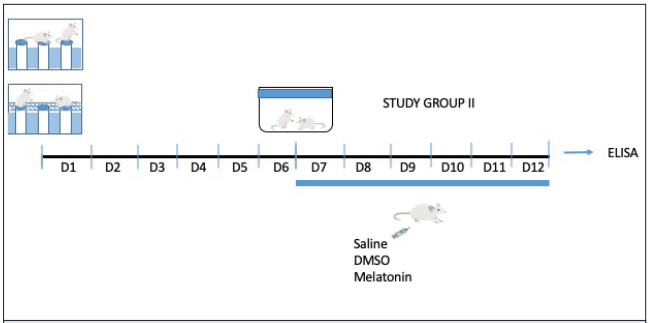

Fig 2. Schematic diagram of Study Group II

Saline treated REMSD group (n=9); REMSD+DMSO: DMSO treated REMSD group (n=9); REMSD+MEL: Melatonin treated REMSD group at a dose of 20 mg/kg (n=9).

Study Group II: These groups were created to analyze the possible therapeutic role of treatment and recovery sleep after damage caused by REM SD. Animals were housed in appropriate cages (according to MMPM) for 6 days, and then followed in standard laboratory cages from days 7-12. Intraperitoneal injections were administered once daily on days 7-12 and animals were decapitated on 13th day *(Fig. 2)*. Animals in Control Groups were placed on grids according to the MMP method, provide optimal access to food and water: RC+Saline: Saline treated recovery control group (n=9); RC+DMSO: DMSO treated recovery control group (n=9); RC+MEL: Melatonin treated recovery control group at a dose of 20 mg/kg (n=9). Animals in REM Sleep Deprivation Groups were placed on small platforms (6.5 cm in diameter) according to the MMPM, provide optimal access to food and water: RREMSD+S: Saline treated recovery REMSD group (n=9); RREMSD+DMSO: DMSO treated recovery REMSD group (n=9); RREMSD+MEL: Melatonin treated recovery REMSD group at a dose of 20 mg/kg (n=9).

Enzyme-Linked Immunosorbent Assay (ELISA) Analyses

Rats were anesthetized using sevoflurane and blood was obtained from all rats after decapitation between 09.00-11.00 am and collected in glass centrifuge tubes. Blood was centrifuged for 15 min at 3000 r.p.m., serum was collected and stored at -80°C until used. Serum Leptin, Orexin-A, and Nesfatin-1 levels were analyzed spectrophotometrically following the commercial kit protocols based on the ELISA principle (BT-LAB, Shanghai Korain Biotech Co., Ltd, People's Republic of China).

Statistical Analyses

Analyses were performed using Sigma Plot version 12.5. All values are reported as the Means ± SEM. Statistical analyses for weight of the animals in Study Group I were performed using One-way ANOVA followed by Holm-Sidak test, for weight of the animals in Study Group II were performed using One-way ANOVA RM. In addition, statistical analyses for ELISA tests were performed using One-way ANOVA followed by Holm-Sidak test. The correlation between OD values scored with ELISA was measured by the Pearson correlation. Statistical significance level was set at P<0.05.

Results

Weights of the Animals

Animal weights were recorded before and after the experiments. The data and statistical analyses for weight-related measurements of rats were presented in *Table 1* and *Table 2*. No significant differences were observed in the weights of animals in the Control Groups. However, a statistically significant decrease in weights on day 7 compared to day 1 was observed in rats subjected to REMSD in Study Group I (at least P<0.01) *(Table 1)*.

The weights of animals in Study Group II were compared at the beginning of the experiment (day 1), the day of transfer to normal laboratory cages (day 7), and the end of the experiment (day 13). A statistically significant increase in weights on day 7 compared to day 1 was observed in Control Groups (at least P<0.01). Rats in RC+S and RC+DMSO continued to gain weight, and the weight

Table 1. *Weights of animals in Study Group I at the beginning (day 1) and end of the experiment (day 7)*

Groups	Day 1	Day 7
C+S	248.9±8.7	242.2±8.0
C+DMSO	271.1±7.5	266.7±8.5
C+MEL	381.7±7.5	363.3±4.2
REMSD+S	292.2±4.0	241.1±4.5***
REMSD+DMSO	296.7±5.5	247.8±8.3***
REMSD+MEL	383.3±8.7	343.3±6.5**

***P<0.01, ***P<0.001 compared to day 1, n=9, mean±standard error*

Table 2. *Weights of animals in Study Group II at the beginning (day 1), transferred to normal laboratory cages (day 7) and end of the experiment (day 13)*

Groups	Day 1	Day 7	Day 13
RC+S	394.4±2.4	408.9±2.0***	413.3±2.4***
RC+DMSO	364.4±1.8	387.8±2.2***	390.0±2.9***
RC+MEL	344.0±2.2	358.0±2.5**	350.0±3.0
RREMSD+S	342.2±5.7	322.2±2.2***	338.9±3.5^{+++}
RREMSD+DMSO	316.7±2.4	302.2±2.8**	313.3±3.3^{++}
RREMSD+MEL	316.7±2.4	297.8±2.8***	304.4±2.4**

***P<0.01, ***P<0.001 compared to day 1, $^{++}$P<0,01, $^{+++}$ P<0,001 compared to day 7, n=9, mean±standard error*

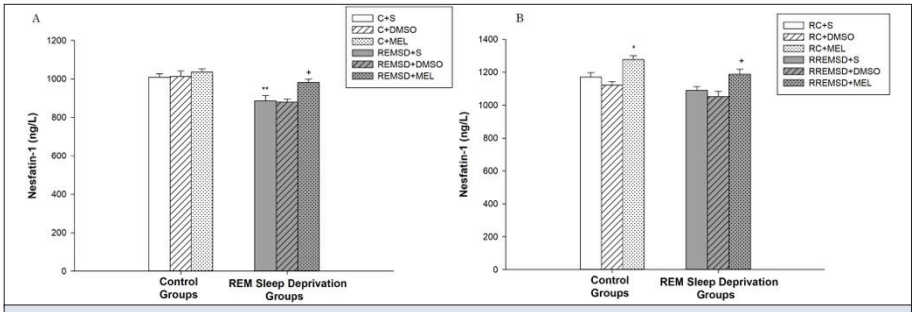

Fig 3. A- Serum Nesfatin-1 levels in Study Group-I, **P<0.01 indicates significant differences compared to the C+S group, +P<0.05 indicates significant differences compared to the REMSD+S group. **B**- Serum Nesfatin-1 levels in Study Group-II, *P<0.05 indicates significant differences compared to the RC+S group, +P<0.05 indicates significant differences compared to the RREMSD+S group. Values are presented as mean ± SEM. n=7

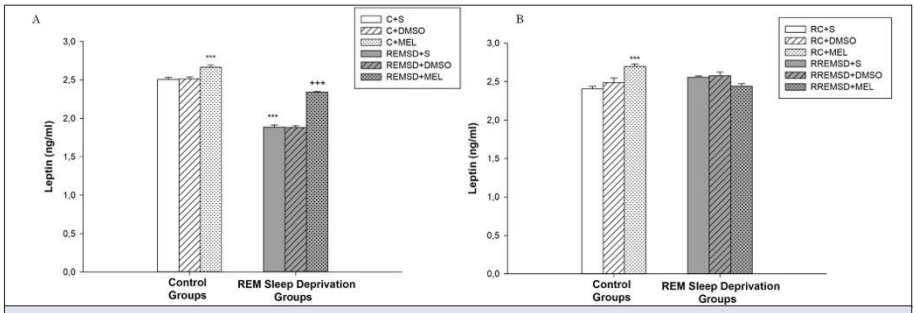

Fig 4. A- Serum Leptin levels in Study Group-I, ***P<0.001 indicates significant differences compared to the C+S group, +++P<0.001 indicates significant differences compared to the REMSD+S group. **B**- Serum Leptin levels in Study Group-II, ***P<0.001 indicates significant differences compared to the RC+S group. Values are presented as mean ± SEM. n=7

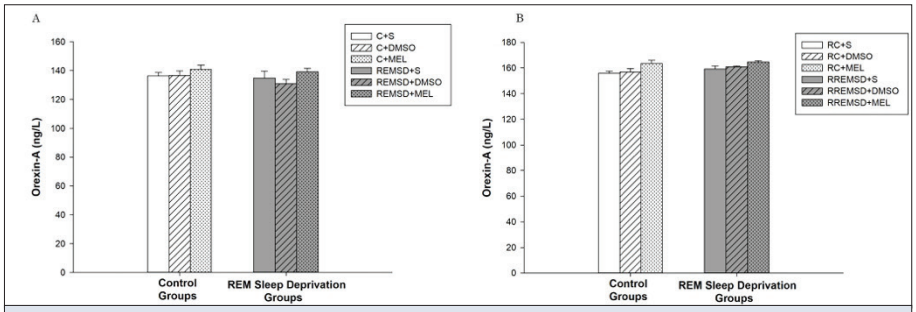

Fig 5. A- Serum Orexin-A levels in Study Group-I. **B**- Serum Orexin-A levels in Study Group-II. Values are presented as mean ± SEM. n=7

on day 13 was significantly higher than that of day 1 (P<0.001). Although not statistically significant, but there was a decreasing trend of weight on day 13 compared to day 7 in rats in RC+MEL group *(Table 2)*. On the other hand, rats subjected to REMSD showed a statistically significant decrease in weights on day 7 compared to day 1 (at least P<0.01). In addition, rats treated with Saline and DMSO in RREMSD groups showed a significant increase in weights on day 13 compared to day 7 (respectively, at least P<0.001 and P<0.01) *(Table 2)*.

ELISA Analyses

1- Serum Nesfatin-1 Levels

Since there was no significant difference between the animals treated with saline and DMSO, all comparisons were made according to the Saline group. In Study Group I, there was a significant decrease in serum Nesfatin-1 levels in REMSD+S group in comparison to C+S group (P<0.01). In addition, serum Nesfatin-1 level was higher in REMSD+MEL group compared to REMSD+S group (P<0.05) *(Fig.3-A)*. Melatonin treatment increased serum Nesfatin-1 levels compared to those received saline (P<0.05) in Study Group II *(Fig. 3-B)*.

2- Serum Leptin Levels

There was a significant increase in serum Leptin levels of C+MEL group (P<0.001), while there was a significant decrease in serum levels of REMSD+S group in comparison to C+S group (P<0.001). In addition, serum Leptin level was higher in REMSD+MEL group compared

to REMSD+S group (P<0.001) *(Fig. 4-A)*. Melatonin treatment also increased serum Leptin levels of RC+MEL group compared to those received saline (P<0.001) in Study Group II *(Fig. 4-B)*.

3- Serum Orexin-A Levels

There was not any significant difference in serum levels of Orexin-A between the groups *(Fig. 5-A,B)*.

4- Correlation of Nesfatin-1, Leptin and Orexin-A Levels

When the results of the Pearson correlation analyses were reviewed, it was determined that there were no significant correlations between Nesfatin-1 and Orexin-A, Nesfatin-1 and Leptin, or Orexin-A and Leptin in any of the groups.

Discussion

The present study provides novel information on the changes of serum Leptin, Orexin-A, and Nesfatin-1 levels following REMSD and recovery sleep and explains the connection between weight changes observed during melatonin treatment.

Sleep plays a crucial role in energy homeostasis [30]. According to the results of our study, a notable decrease was observed in the weight of rats subjected to REMSD. However, after a period of recovery sleep, the rats' body weight significantly increased. In a sleep restriction research, it was shown that while weight loss was observed at the beginning, there was weight gain afterwards [15]. The results of our study, in parallel with the study [31], show that SD causes weight loss and recovery sleep reverses this loss.

When the effects of melatonin on body weight were examined, the C+MEL group experienced a higher percentage weight loss than the C+S group. This result is likely related to the reduced appetite caused by an increase in Leptin levels. Furthermore, the group subjected to REMSD and treated with melatonin (REMSD+MEL) exhibited less weight loss compared to the REMSD group without melatonin treatment (REMSD+S). It is known that animals lose weight during REMSD despite increased food intake due to a negative energy balance. It is thought that nutrition increases due to increased energy needs [31]. The reduced weight loss with melatonin treatment may be attributed to the positive effects of melatonin on negative energy balance. The significant increase in Nesfatin-1 and Leptin levels in animals in the REMSD+MEL group compared to the REMSD+S group also supports these findings.

In the comparison of recovery sleep results between the 7th and 13th days, it was observed that there was weight loss in the RC+MEL group and weight gain in the other groups. This weight loss is thought to be related to the increased levels of Nesfatin-1 and Leptin which have an anorexigenic effect. Similarly, it was concluded that the less weight gain in the RREMSD+MEL group compared to the RREMSD+S group was related to increased levels of Nesfatin-1. Melatonin treatment is thought to regulate energy balance by reducing oxidative stress [32] through its antioxidant properties [33] in animals subjected to SD.

Melatonin administration has been shown to reduce body weight and body mass index in people with metabolic syndrome [34]. It has been observed that the administration of melatonin in rats with fructose-induced metabolic syndrome leads to weight loss [35]. A recent meta-analysis reported that melatonin has a weight-reducing effect [36]. Melatonin increases brown adipose tissue activity and mass [37]. When the effects of melatonin on nutrition and weight were examined, it was shown that animals supplemented with 0.4 µg/mL melatonin in their drinking water and followed for 12 weeks did not show any differences in food intake compared to the control group, but a significant decrease in weight was observed [38]. These findings support previous research [35-38] indicating that melatonin supplementation leads to a decrease in body weight.

Nesfatin-1 is an important anorexigenic peptide produced in the hypothalamus, defined as the satiety hormone, and plays a significant role in nutrition and glucose metabolism [19,39]. Nesfatin-1 has a primary effect on reducing appetite, independent of the leptin pathway, leading to decreased food intake and weight gain, as well as increased glucose uptake into tissues [40]. It has been shown that Nesfatin-1 mRNA and protein expression are decreased in the dorsolateral hypothalamus of rats after 72 hours of REMSD [21] . Consistent with these findings, the results of our study showed that Nesfatin-1 levels decreased in the REMSD group compared to the Control group.

Leptin receptors are present in both the CNS and peripheral tissues [41]. The results regarding changes in Leptin levels following sleep restriction are controversial. A previous study showed no change in Leptin levels [13], while another indicate an increase [12], or a decrease [42]. In animal studies, SD has been found to reduce Leptin levels [14,43]. Similarly, our study revealed a decrease in Leptin levels due to SD. A previous study has demonstrated that melatonin reduces Leptin levels [44], while another shows that it increases Leptin expression [45]. In our study, melatonin increased Leptin levels in both the control and SD groups.

Orexin plays an important role in normal sleep, energy metabolism and food intake. Orexin is a peptide produced from the lateral hypothalamus and basically increases food intake [46]. While Orexin release is low during the daytime wakefulness, it is high at night. Orexin receptor

subtype has a varied role in regulating NREM and REM sleep [47]. In the study in which 7 days of REMSD was created with the multi-platform method, values of Hypocretin-immunoreactive neurons were investigated and no significant difference was observed in Wistar rats compared to the control group, while an increase was observed in Wistar-Kyoto type rats, used as a depression model, compared to the control group [48]. Another study analyzed changes in Orexin-A levels in different brain regions after 96 h of REMSD. While there was no significant difference in the hippocampus and pedunculo-pontine area, Orexin-A levels significantly increased in the locus coeruleus, hypothalamus and cortex, and returned to normal level after recovery [49]. Similarly, in a study with mice, in which both Orexin-A and Orexin-B levels were examined in the whole brain after 96 hours of REM SD and 24 hours of recovery sleep, it was shown that neither SD nor recovery sleep changed Orexin levels [50]. The results of our study also showed that Orexin-A levels did not change with SD or recovery sleep, as reported in the previous studies [48-50]. In addition, no effect of melatonin treatment was observed on these parameters. There were no significant correlations between Nesfatin-1 and Orexin-A, Nesfatin-1 and Leptin, or Orexin-A and Leptin in any of the groups. The absence of a significant correlation was attributed to the limited sample size.

In conclusion, our study provides insights into the effects of REMSD, recovery sleep, and melatonin treatment on weight regulation and hormone levels related to the body weight change and nutrition. The most important limitation of this study is the inability to monitor the feeding of animals in this SD method. This study will shed light on future studies on sleep and nutrition and has added new information to research on the effects of melatonin, which is frequently used in treatment. Furthermore, this study will contribute to elucidating the role of sleep deprivation on eating behavior, evaluating the effects of melatonin metabolic processes, which is a widely used treatment in human and veterinary medicine, in accordance with the one health concept. The results obtained from this study will aid in predicting the effects that may occur on metabolic processes following the treatment.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author (A. Cakir).

Acknowledgements

We would like to thank the Scientific and Technological Research Council of Türkiye (TUBITAK) and Bursa Uludag University Scientific Research Projects for supporting our research.

Funding Support

This study was funded by the Scientific and Technological Research Council of Türkiye (TUBITAK) ARDEB 1001 Grant No:220S210 and Bursa Uludag University Scientific Research Projects Grant No: THIZ-2023-1383.

Ethical Statement

The study has been approved by the Bursa Uludag University Local Ethics Committee on Animal Research under decision number 2022-17/07.

Competing Interest

The authors declared that there is no conflict of interest.

Author Contributions

A. C.: Conceptualization; Methodology; Investigation; Data curation; Writing original draft, Writing - Review & Editing and Funding acquisition. S. S.: Methodology; Investigation; Data curation; and Writing original draft. C. K.: Methodology; Investigation; Data curation; and Writing original draft, Writing - Review & Editing. N. K.: Conceptualization; Methodology; Writing - original draft; Writing - Review & Editing; Supervision; and Funding acquisition.

References

1. Watson NF, Badr MS, Belenky G, Bliwise DL, Buxton OM, Buysse D, Dinges DF, Gangwisch J, Grandner MA, Kushida C, Malhotra RK, Martin JL, Patel SR, Quan SF, Tasali E, Twery M, Croft JB, Maher E: Recommended amount of sleep for a healthy adult: A joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society. *Sleep*, 38 (6): 843-884, 2015. DOI: 10.5665/sleep.4716

2. Cirelli C: Cellular consequences of sleep deprivation in the brain. *Sleep Med Rev*, 10 (5): 307-321, 2006. DOI: 10.1016/j.smrv.2006.04.001

3. Tarokh L, Saletin JM, Carskadon MA: Sleep in adolescence: Physiology, cognition and mental health. *Neurosci Biobehav Rev*, 70, 182-188, 2016. DOI: 10.1016/j.neubiorev.2016.08.008

4. Chattu VK, Manzar MD, Kumary S, Burman D, Spence DW, Pandi-Perumal SR: The global problem of insufficient sleep and its serious public health implications. *Healthcare (Basel),* 7 (1):1, 2019. DOI: 10.3390/healthcare7010001

5. Everson CA, Crowley WR: Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. *Am J Physiol Endocrinol Metab*, 286 (6): E1060-E1070, 2004. DOI: 10.1152/ajpendo.00553.2003

6. Noguti J, Ribeiro DA: Sleep deprivation and carcinogenesis: The role of melatonin. *Sleep Sci*, 5 (2): 65-67, 2012.

7. Patel SR, Malhotra A, White DP, Gottlieb DJ, Hu FB: Association between reduced sleep and weight gain in women. *Am J Epidemiol*, 164 (10): 947-954, 2006. DOI: 10.1093/aje/kwj280

8. Koban M, Stewart CV: Effects of age on recovery of body weight following REM sleep deprivation of rats. *Physiol Behav*, 87 (1): 1-6, 2006. DOI: 10.1016/j.physbeh.2005.09.006

9. Koban M, Swinson KL: Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. *Am J Physiol Endocrinol Metab*, 289 (1): E68-E74, 2005. DOI: 10.1152/ajpendo.00543.2004

10. Rechtschaffen A, Bergmann BM: Sleep deprivation in the rat: An update of the 1989 paper. *Sleep*, 25 (1): 18-24, 2002. DOI: 10.1093/sleep/25.1.18

11. Farr OM, Li CR, Mantzoros CS: Central nervous system regulation of eating: Insights from human brain imaging. *Metabolism,* 65 (5): 699-713, 2016. DOI: 10.1016/j.metabol.2016.02.002

12. Porkka-Heiskanen T, Van Leeuwen WMA, Hublin C, Sallinen M, Härmä M, Hirvonen A: Prolonged sleep restriction affects glucose metabolism in healthy young men. *Int J Endocrinol*, 2010:108641, 2010. DOI: 10.1155/2010/108641

13. St-Onge MP, O'Keeffe M, Roberts AL, RoyChoudhury A, Laferrère B: Short sleep duration, glucose dysregulation and hormonal regulation of appetite in men and women. *Sleep*, 35 (11): 1503-1510, 2012. DOI: 10.5665/sleep.2198

14. Barf RP, Van Dijk G, Scheurink AJW, Hoffmann K, Novati A, Hulshof HJ, Fuchs E, Meerlo P: Metabolic consequences of chronic sleep restriction in rats: Changes in body weight regulation and energy expenditure. *Physiol Behav*, 107 (3): 322-328, 2012. DOI: 10.1016/j.physbeh.2012.09.005

15. Venancio DP, Suchecki D: Prolonged REM sleep restriction induces metabolic syndrome-related changes: Mediation by pro-inflammatory cytokines. *Brain Behav Immun*, 47, 109-117, 2015. DOI: 10.1016/j.bbi.2014.12.002

16. Zhu Y, Yamanaka A, Kunii K, Tsujino N, Goto K, Sakurai T: Orexin-mediated feeding behavior involves both leptin-sensitive and -insensitive pathways. *Physiol Behav*, 77 (2-3): 251-257, 2002. DOI: 10.1016/S0031-9384(02)00843-0

17. Wang C, Wang Q, Ji B, Pan Y, Xu C, Cheng B, Bai B, Chen J: The orexin/receptor system: Molecular mechanism and therapeutic potential for neurological diseases. *Front Mol Neurosci*, 11:220, 2018. DOI: 10.3389/fnmol.2018.00220

18. Sharma R, Sahota P, Thakkar MM: Melatonin promotes sleep in mice by inhibiting orexin neurons in the perifornical lateral hypothalamus. *J Pineal Res*, 65 (2):e12498, 2018. DOI: 10.1111/jpi.12498

19. Oh-I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M: Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature*, 443 (7112): 709-712, 2006. DOI: 10.1038/nature05162

20. Dai H, Li X, He T, Wang Y, Wang Z, Wang S, Xing M, Sun W, Ding H: Decreased plasma nesfatin-1 levels in patients with acute myocardial infarction. *Peptides*, 46, 167-171, 2013. DOI: 10.1016/j.peptides.2013.06.006

21. Vas S, Ádori C, Könczöl K, Kátai Z, Pap D, Papp RS, Bagdy G, Palkovits M, Tóth ZE: Nesfatin-1/NUCB2 as a potential new element of sleep regulation in rats. *PLoS One*, 8 (4):e59809, 2013. DOI: 10.1371/JOURNAL.PONE.0059809

22. Goebel-Stengel M, Wang L, Stengel A, Taché Y: Localization of nesfatin-1 neurons in the mouse brain and functional implication. *Brain Res*, 1396, 20-34, 2011. DOI: 10.1016/j.brainres.2011.04.031

23. Tortorella S, Rodrigo-Angulo ML, Núñez A, Garzón M: Synaptic interactions between perifornical lateral hypothalamic area, locus coeruleus nucleus and the oral pontine reticular nucleus are implicated in the stage succession during sleep-wakefulness cycle. *Front Neurosci*, 7:216, 2013. DOI: 10.3389/fnins.2013.00216

24. Stengel A, Taché Y: Nesfatin-1 - Role as possible new potent regulator of food intake. *Regul Pept*, 163 (1-3): 18-23, 2010. DOI: 10.1016/j.regpep.2010.05.002

25. Costello RB, Lentino C V., Boyd CC, O'Connell ML, Crawford CC, Sprengel ML, Deuster PA: The effectiveness of melatonin for promoting healthy sleep: A rapid evidence assessment of the literature. *Nutr J*, 13 (1): 106, 2014. DOI: 10.1186/1475-2891-13-106

26. Kunz D, Mahlberg R, Müller C, Tilmann A, Bes F: Melatonin in patients with reduced REM sleep duration: Two randomized controlled trials. *J Clin Endocrinol Metab*, 89 (1): 128-134, 2004. DOI: 10.1210/jc.2002-021057

27. Gomes S, Ramalhete C, Ferreira I, Bicho M, Valente A: Sleep patterns, eating behavior and the risk of noncommunicable diseases. *Nutrients*, 15 (11):2462, 2023. DOI: 10.3390/nu15112462

28. Oesch LT, Gazea M, Gent TC, Bandarabadi M, Herrera CG, Adamantidis AR: REM sleep stabilizes hypothalamic representation of feeding behavior. *Proc Natl Acad Sci U S A*, 117 (32): 19590-19598, 2020. DOI: 10.1073/pnas.1921909117

29. Jameie SB, Mesgar S, Aliaghaei A, Raoofi A, Amini M, Khodagholi F, Danyali S, Sorraya M, Sadeghi Y: Neuroprotective effect of exogenous melatonin on the noradrenergic neurons of adult male rats' locus coeruleus nucleus following REM sleep deprivation. *J Chem Neuroanat*, 100:101656, 2019. DOI: 10.1016/j.jchemneu.2019.101656

30. Meyhöfer S, Wilms B, Oster H, Schmid SM: Importance of sleep and circadian rhythm for energy metabolism. *Internist*, 60 (2): 122-127, 2019. DOI: 10.1007/s00108-018-0543-x

31. Koban M, Site L V, Le WW, Hoffman GE: Sleep deprivation of rats: The hyperphagic response is real. *Sleep*, 31 (7): 927-933, 2008. DOI: 10.5665/sleep/31.7.927

32. Szewczyk-Golec K, Rajewski P, Gackowski M, Mila-Kierzenkowska C, Wesołowski R, Sutkowy P, Pawłowska M, Woźniak A: Melatonin supplementation lowers oxidative stress and regulates adipokines in obese patients on a calorie-restricted diet. *Oxid Med Cell Longev*, 2017:8494107, 2017. DOI: 10.1155/2017/8494107

33. Zhang HM, Zhang Y: Melatonin: A well-documented antioxidant with conditional pro-oxidant actions. *J Pineal Res*, 57 (2): 131-146, 2014. DOI: 10.1111/jpi.12162

34. Bahrami M, Cheraghpour M, Jafarirad S, Alavinejad P, Cheraghian B: The role of melatonin supplement in metabolic syndrome: A randomized double blind clinical trial. *Nutr Food Sci*, 49 (5): 965-977, 2019. DOI: 10.1108/NFS-01-2019-0018

35. Cardinali DP, Scacchi Bernasconi PA, Reynoso R, Reyes Toso CF, Scacchi P: Melatonin may curtail the metabolic syndrome: Studies on initial and fully established fructose-induced metabolic syndrome in rats. *Int J Mol Sci*, 14 (2): 2502-2514, 2013. DOI: 10.3390/ijms14022502

36. Delpino FM, Figueiredo LM: Melatonin supplementation and anthropometric indicators of obesity: A systematic review and meta-analysis. *Nutrition*, 91:111399, 2021. DOI: 10.1016/j.nut.2021.111399

37. Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ: Significance and application of melatonin in the regulation of brown adipose tissue metabolism: Relation to human obesity. *Obes Rev*, 12 (3): 167-188, 2011. DOI: 10.1111/j.1467-789X.2010.00756.x

38. Wolden-Hanson T, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM, Rasmussen DD: Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology*, 141 (2): 487-497, 2000. DOI: 10.1210/endo.141.2.7311

39. Anwar GM, Yamamah G, Ibrahim A, El-Lebedy D, Farid TM, Mahmoud R: Nesfatin-1 in childhood and adolescent obesity and its association with food intake, body composition and insulin resistance. *Regul Pept*, 188, 21-24, 2014. DOI: 10.1016/j.regpep.2013.12.001

40. Feijóo-Bandín S, Rodríguez-Penas D, García-Rúa V, Mosquera-Leal A, Otero MF, Pereira E, Rubio J, Martínez I, Seoane LM, Gualillo O, Calaza M, García-Caballero T, Portolés M, Roselló-Lletí E, Diéguez C, Rivera M, González-Juanatey JR, Lago F: Nesfatin-1 in human and murine cardiomyocytes: Synthesis, secretion, and mobilization of GLUT-4. *Endocrinology*, 154 (12): 4757-4767, 2013. DOI: 10.1210/en.2013-1497

41. Martínez-Sánchez N: There and back again: Leptin actions in white adipose tissue. *Int J Mol Sci*, 21 (17): 1-26, 2020. DOI: 10.3390/ijms21176039

42. Spiegel K, Leproult R, L'Hermite-Balériaux M, Copinschi G, Penev PD, Van Cauter E: Leptin levels are dependent on sleep duration: Relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *J Clin Endocrinol Metab*, 89 (11): 5762-5771, 2004. DOI: 10.1210/jc.2004-1003

43. Moraes DA, Venancio DP, Suchecki D: Sleep deprivation alters energy homeostasis through non-compensatory alterations in hypothalamic insulin receptors in Wistar rats. *Horm Behav*, 66 (5): 705-712, 2014. DOI: 10.1016/j.yhbeh.2014.08.015

44. Suriagandhi V, Nachiappan V: Protective effects of melatonin against obesity-induced by leptin resistance. *Behav Brain Res*, 417:113598, 2022. DOI: 10.1016/j.bbr.2021.113598

45. Albreiki MS, Shamlan GH, BaHammam AS, Alruwaili NW, Middleton B, Hampton SM: Acute impact of light at night and exogenous melatonin on subjective appetite and plasma leptin. *Front Nutr*, 9:1079453, 2022. DOI: 10.3389/fnut.2022.1079453

46. Barson JR, Leibowitz SF: Orexin/hypocretin system: Role in food and drug overconsumption. *Int Rev Neurobiol*, 136, 199-237, 2017. DOI: 10.1016/bs.irn.2017.06.006

47. Mieda M, Hasegawa E, Kisanuki YY, Sinton CM, Yanagisawa M,

Sakurai T: Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep. *J Neurosci*, 31 (17): 6518-6526, 2011. DOI: 10.1523/JNEUROSCI.6506-10.2011

48. Allard JS, Tizabi Y, Shaffery JP, Manaye K: Effects of rapid eye movement sleep deprivation on hypocretin neurons in the hypothalamus of a rat model of depression. *Neuropeptides*, 41 (5): 329-337, 2007. DOI: 10.1016/j.npep.2007.04.006

49. Mehta R, Khanday MA, Mallick BN: REM sleep loss associated changes in orexin-A levels in discrete brain areas in rats. *Neurosci Lett*, 590, 62-67, 2015. DOI: 10.1016/j.neulet.2015.01.067

50. Pedrazzoli M, Hipólide D, D'Almeida V, Tufik S: The effects of REM sleep deprivation and REM sleep rebound on hypocretin levels in mice brains. *Sleep Sci*, 2 (1): 21-23, 2009.