Molekul

by Fk Unsoed

Submission date: 23-Nov-2022 07:20PM (UTC+0700)
Submission ID: 1961995521
File name: 6218-Fitranto-_386-395.pdf (230.31K)
Word count: 6399
Character count: 35001

Molekul, Vol. 17. No. 3, November 2022: 386 – 395

MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2022.17.3.6218

Levels of Cortisol and Inflammatory Cytokines after The Induction of Various Sleep Deprivation Stress Models in Male Wistar Rats

Fitranto Arjadi[1*], Sindhu Wisesa[1], Nor Sri Inayani[2], Catharina Widiartini[1], Prasetyo Tri Kuncoro[1]

[1]Departement of Anatomy, Faculty of Medicine, Universitas Jenderal Soedirman Purwokerto 53112, Indonesia
[2]Departement of Biochemistry, Faculty of Medicine, Universitas Jenderal Soedirman Purwokerto 53112, Indonesia

*Corresponding author email: fitranto.arjadi@unsoed.ac.id

Received June 23, 2022; **Accepted** September 20, 2022; **Available online** November 20, 2022

ABSTRACT. Sleep deprivation (SD) can modulate the production of various cytokines, including pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ, and anti-inflammatory cytokines such as IL-10. Paradoxical sleep deprivation (PSD) increases the risk of inflammation but can be reversed by sleep recovery (SR). This study aimed to determine the differences in levels of cortisol and inflammatory cytokines (IL-6, IL-10, TNF-α, dan IFN-γ) in male Wistar rats (*Rattus norvegicus*) after induction of various sleep deprivation stress models. Twenty-five of male Wistar rats were randomly divided into five groups: control, PSD (20 hours of SD/day for five days), Total Sleep Deprivation or TSD (24 hours of SD/day for five days), PSD+SR (PSD followed by SR), and TSD+SR (TSD followed by SR). The plasma cortisol levels were measured with ELISA, and inflammatory cytokine levels were measured with immunoassay and calculated with fold change. Mean cortisol levels were significantly increased in treatment groups compared to the control group (p=0.029). Multivariate analysis showed no statistically significant difference in inflammatory cytokine levels of IL-6 (p=0.658), IL-10 (p=0.065), TNF-α (p=0.399), and IFN-γ (p=0.283) in all groups. In conclusion, various sleep deprivation stress models affect cortisol levels but not inflammatory cytokine levels of IL-6, IL-10, TNF-α, and IFN-γ among male Wistar rats.

Keywords: cortisol, inflammatory cytokines, sleep deprivation, sleep recovery

INTRODUCTION

Sleep plays a vital role in life by maintaining the optimal physiological function of the human body. It combines stimulation of afferent nerve delivery to the brain and activation of functional neurons in specific brain areas (Chokroverty et al., 2017). Good sleep is determined by the quality of sleep, how deep a person sleeps, and the quantity of sleep, amount of time as a person sleeps (Patrick et al., 2017). According to the National Sleep Foundation, a young adult (18-25 years) or adult (26-64 years) needs 7-9 hours of sleep daily, but not less than 6 hours or more than 11 hours for young adults or 10 hours for adults. Otherwise, an older adult (65 years and over) requires at least 7-8 hours of sleep daily, but not less than 5 hours or more than 9 hours (Lichtenstein, 2015). People who live in developed countries are chronically sleep-deprived because of their cultural and socioeconomic environments. Incidence of symptoms related to sleep deprivation has recently increased, suggesting its long-term detrimental health effects are more abundant than expected (Chokroverty & Ferini-Strambi, 2017).

A study by Hirshkowitz et al. (2015) showed that > 30% of men and women between the ages of 30-64 years sleep less than 6 hours per day, and 5-15% of the world population suffer from sleep disorders. Another study indicated that 40-70% of older adults have chronic sleep disorders that are more prominent in people with medical or psychiatric comorbidity (Praharaj et al., 2018). The National Sleep Foundation stated that 70% of Indonesian people experience sleep disturbances at least once a week, and 30 million people have sleeping difficulty every night (Lestarianto, 2014). A survey of the healthy lifestyle index by American International Assurance (AIA) in 2013 demonstrated that Indonesian people have an average sleep duration of 6.8 hours per day due to increased activity and decreased sleep duration from its normal range, indicating many Indonesian people are suffered from sleep deprivation (Putri et al., 2017).

The relationship between sleep duration and the inflammation process has not been studied extensively, despite showing a similar correlation between sleep duration and mortality. People with long sleep duration (>8 hours/night) and short sleep duration (<7 hours/night) have a 30% and 12% greater risk of death, respectively, compared to people with moderate sleep duration (7-8 hours/night). Physiologically, sleep affects two major effector systems, the Hypothalamus-Pituitary-Adrenal (HPA) axis and the sympathetic nervous system, which

simultaneously increases the release of pro-inflammatory cytokines and markers of systemic inflammation through β-adrenergic activation. The activity of sympathetic nervous system decreases when a person is sleeping, which may explain the relationship between sleep disorder, short sleep duration, and elevation of inflammatory markers (Chen et al., 2017). Patients that pathologically tend to fall asleep during daytime and easily get tired exhibit an increase in IL-6, a pro-inflammatory cytokine, in the circulatory system due to activation of the HPA Axis. People suffering from sleep deprivation produce more IL-6 during the day and less IL-6 at night, whereas good sleep reduces pro-inflammatory exposure to tissues. Furthermore, sleep deprivation upregulates pro-inflammatory cytokines through NF-kB activation, generating a pro-inflammatory state. Overexposure to the pro-inflammatory cytokines caused by sleep deprivation will increase tissue damage, decrease brain function, and increase the expression of IL-6 (Vgontzas et al., 2000).

Sleep deprivation correlates with the increase in TNF-α, indicating a pathological condition caused by sleep disorders (Chennaoui et al., 2011). Moreover, TNF-α is expressed in neurons and plays a role in brain neuroplasticity (Rockstrom et al., 2018). On the other hand, a clinical trial showed that IFN-γ was found in cases of fever, flu, and drowsiness, whereas IFN-γ can be somnogenic in the presence of TNF-α. Therefore, IFN-γ has a significant role in sleep regulation during viral infection (Van Dongen et al., 2011).

Studies suggest that cortisol levels as a physiological stress marker and systemic inflammatory marker may increase in sleep-deprived conditions. Sleep deprivation triggers the release of adrenocorticotropic hormone (ACTH) via the HPA axis, inducing the adrenal gland to release cortisol. Along with other stimulations, including norepinephrine and radical oxygen species (ROS), cortisol triggers inflammatory activation in the brain and peripheral immune cells by increasing the expression of pro-inflammatory cytokine genes such as IL-1, IL-6, and TNF-α via transcriptional regulator of pro-inflammatory gene expression such as NF-κB. These pro-inflammatory cytokines then enter the systemic circulation inducing the increase of leukocytes, mainly neutrophils, CD4+ T-cells, B-cells, and monocyte leading to inflammatory reactions (Garbarino et al., 2021).

Sleep deprivation (SD) can be categorized into paradoxical sleep deprivation (PSD) and total sleep deprivation (TSD), and studied using the Modified Multiple Platform Method (MMPM). While both types can induce stress, total sleep deprivation causes poor decision-making control, triggers repetitive errors in working memory, and abolishes individual spontaneity in communication, making the subjects appear lazy, lethargic, and unmotivated (Gunn et al., 2017).

Previous studies showed that sleep deprivation affects cortisol and inflammatory cytokine levels in rats or humans by interfering with homeostatic sleep patterns and disrupting HPA axis activation (Chennaoui et al., 2011; Medic et al., 2017; Neto et al., 2010; Wright et al., 2015). While sleep recovery may restore rat deteriorating conditions caused by sleep deprivation, no studies clearly define the effect of sleep recovery on pro-inflammatory and anti-inflammatory cytokine levels. This study directly compared cortisol and inflammatory cytokine level among rats treated with PSD, TSD, and sleep recovery after PSD and TSD treatment. Considering that many people have experienced sleep deprivation, this study is essential as initial research to determine the effect of sleep deprivation and sleep recovery on the levels of cortisol and inflammatory cytokines that impair various metabolic functions in mammals.

EXPERIMENTAL SECTION

Research Design

This study was an experimental study using a post-test only with control group design.

Subject

Wistar rats (*Rattus norvegicus*) with characteristics of male, 8-12 weeks old, and 200-300 grams of weight were obtained from the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) 4 Universitas Gajah Mada. Sleep deprivation experiments were performed using the Modified Multiple Platform Method (MMPM) to prevent the rats from sleeping. A total of 25 rats were randomly divided into five groups containing five rats in each group: control (no SD), PSD (20 hours of SD per day at 11.00 a.m. - 07.00 a.m. for 5 days and 4 hours rest at 07.00 a.m. - 11.00 a.m.), TSD (24 hours of SD per day at 11.00 a.m. - 11.00 a.m. for 5 days without rest), PSD+SR (PSD treatment for 5 days followed by sleep recovery (SR) for 5 days at 07.00 a.m. - 07.00 a.m.), and TSD+SR (TSD treatment for 5 days followed by sleep recovery for 5 days at 11.00 a.m. - 11.00 a.m.).

Research Procedure

Modified Multiple Platform Method (MMPM) tanks measuring 123 x 44 x 35 cm were used for the sleep deprivation stress model. The tanks were filled with water and equipped with 12 platforms of 6.5 cm in size with a distance of 10 cm between the platforms as a foothold for the rats that are instinctively avoiding water. Each tank contains five experimental rats equipped with a muscle atonia device, an instrument that gives an automatic shock effect every 10 minutes to keep the rats awake. The experiments were conducted in the Anatomy Laboratory of the Faculty of Medicine of Jenderal Soedirman University from August to September 2021.

Observation

Cortisol levels were measured by drawing 2 ml of rat blood for each group at 07.00-09.00 a.m. via retroorbital vein using a microhematocrit pipette into an EDTA tube, centrifuging for 15 minutes at 2,000-3,000 rpm, and storing in -20 °C. A total of 50 mL of collected plasma was examined with the Enzyme-Linked Immunosorbent Assay (ELISA) method and read by the ELISA reader in 450 nm absorbance (Zabidi et al., 2015).

For inflammatory cytokines level measurement, rat blood plasma samples were collected from the retroorbital plexus as much as 55 mL. The levels of inflammatory cytokines of IL-6, IL-10, TNF-α, and IFN-γ were then measured using multiplex bead-based immunoassay from MILLIPLEX® MAP kit with Luminex technology in the Integrated Laboratory of Faculty of Medicine, Universitas Indonesia. Briefly, the frozen samples were thawed, stirred thoroughly with a vortex, and centrifuged to remove particulates before the immunoassay. The processed samples were then placed on the plate, added with conjugated beads from the MILLIPLEX® MAP kit, and incubated for 2 hours at 20-24 °C or overnight at 4-8 °C. After incubation, the beads were washed, incubated in biotinylated-detection antibodies, added with Streptavidin-PE (SAPE), and further incubated. The beads were then washed, resuspended in an appropriate buffer, and placed on Luminex technology to measure cytokine levels. The plasma cytokine levels were analyzed based on the fold change value by comparing the treatment and the control group.

Statistical analysis

Normality analysis was performed by the Shapiro-Wilk test, and data homogeneity was analyzed by Levene's test. The multivariate analysis was then performed by the One-Way ANOVA and Post-hoc Least Significant Difference (LSD) to determine differences between groups. Kruskal-Wallis and Mann-Whitney tests were performed for non-parametric analysis.

All procedures were approved by The Medical Research Ethics Commission of The Faculty of Medicine of Jenderal Soedirman University on May 10, 2021 (reference number: 097/KEPK/V/2021).

RESULT S AND DISCUSSION

Measurement and analysis results of mean cortisol levels among all groups are shown in **Figure 1**. Since the plasma cortisol levels were not normally distributed, non-parametric tests were used for further analysis. Kruskal-Wallis analysis showed substantial differences in cortisol levels between at least two groups for the assessed variables ($p=0.029$). Further post-hoc analysis with the Mann-Whitney test obtained significant results between every intervention group and the control ($p<0.05$). PSD for 20 hours/day increased cortisol levels in the afternoon and evening in rats by two times compared to control (Olayaki et al., 2015). Cortisol levels increased in the first six hours after PSD induction caused by HPA axis activation to maintain metabolism changes that were occurred during sleep deprivation (Galvão et al., 2009). Consistent with a study by Olayaki et al. (2015) demonstrating that TSD for five days increased cortisol levels compared to the control, this study pointed out that the TSD group had the highest mean cortisol levels among all groups (56.23±26.29 ng/mL).

The PSD group showed lower mean cortisol levels than the TSD group because PSD has a sleep period daily, thus the negative impact of sleep deprivation can be relieved. Two hours of an afternoon nap after one night of sleep deprivation decreased the secretion of cortisol hormone caused by inhibition of the HPA axis at the slow wave sleep (SWS) phase that reduces cortisol levels (Pejovic et al., 2013). The effects of sleep deprivation that impair HPA function cause an increase in evening cortisol levels as a result of an elevation in its secretory pulses amplitude, suggesting that the negative feedback of glucocorticoid mediated by hippocampal function may be affected by sleep-deprived condition (Hirotsu et al., 2015). Cortisol levels were higher in the subjects who stayed awake than in subjects who slept sufficiently, indicating that sleep deprivation affects the cortisol levels as a result of stress responses (Bassett et al., 2018). Cauter et al. (2008) emphasized that the initial consequence of partial sleep loss is a cortisol level increase in the evening. While under normal conditions, cortisol levels decrease rapidly and reach the minimum level just before sleeping time. Kantasa et al. (2016) stated that cortisol can suppress the immune system and induce an inflammatory pathway, making the body vulnerable to various diseases.

PSD + SR group exhibited lower cortisol levels than TSD + SR, and both PSD + SR and TSD + SR showed lower cortisol levels than PSD and TSD. A study by Mattice et al. (2011) showed that subjects induced by TSD for 24 hours and 48 hours followed by sleep recovery for 24 hours relieved their sleep deprivation effect for 72% and 42%, respectively. Sleep recovery for three days decreases cortisol levels and ameliorates the impact of sleep deprivation by restoring the HPA axis interaction (Pejovic et al., 2013). Sleep recovery also decreases lipid peroxidase and free radical production by increasing glutathione and other enzymatic antioxidants (Hirotsu et al., 2015). Glutathione increases after sleep recovery for two days, indicated by the elevation of 6-PGD, an enzyme that acts on the pentose phosphate pathway in the carbohydrate metabolism that protects cells from oxidative stress in the form of NADPH, thus inhibiting stress oxidative that has a role in cortisol increase (Kim et al., 2022). Taken together, these results indicate that sleep recovery is able to decrease cortisol levels which increase after induction of PSD or TSD. Results of data analysis showing the value of minimum,

maximum, mean, median, and standard deviation for each group are shown in **Table 1.**

Normality analysis by the Shapiro-Wilk test and homogeneity analysis by the Levene's test showed a p-value >0.05 in all groups, indicating data of inflammatory cytokine levels were normally distributed and homogenous. Multivariate analysis using the One-Way ANOVA test demonstrated a p-value >0.05 in all groups, indicating no significant difference in inflammatory cytokine levels among the treatment groups, as shown in **Table 2**. The inflammatory cytokine levels were then compared among the treatment and control groups using fold change. The results demonstrated the highest increase in inflammatory cytokine levels was in the TSD treatment group (**Figure 2**). However, the multivariate analysis of the fold change of inflammatory cytokine levels using the One-Way ANOVA test did not show a significant difference ($p > 0.05$) in IL-6 ($p=0.658$), IL-10 ($p=0.085$), TNF-α ($p= 0.313$), and IFN-γ ($p=0.283$).

Sleep deprivation triggers a stress response in animal models by increasing pro-inflammatory cytokines and activates the main stress axis in humans, the HPA axis. IL-6 can stimulate cortisol secretion directly in the adrenal gland or via activation of the hypothalamus that induces the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. On the other hand, a slight elevation of IL-6 level in plasma induces the anti-inflammatory cytokines, IL-1ra and IL-10, along with c-reactive protein (CRP). Recent studies stated that apart from injury and infection, sleep deprivation triggers the pro-inflammatory response through the increase of pro-inflammatory cytokines secretion (Medic et al., 2017; Simpson & Dinges, 2007; Sukendra, 2015).

Activation of inflammatory responses caused by sleep deprivation can increase the levels of anti-inflammatory cytokines to prevent excessive inflammatory responses. IL-10, a potent anti-inflammatory cytokine, inhibits the activation of macrophages and dendritic cells, leading to the reduction of cytokine levels produced by T-helper1 (Th1) cells (Welsh et al., 2011). IL-10 also potently inhibits the production of IL-10 itself, IL-12, L-1α, IL-1β, IL-6, macrophage-colony stimulating factor (M-CSF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), leukemia inhibitory factor (LIF), and platelet-activating factor (PAF) from activated monocytes and macrophages (Saraiva & O'Garra, 2010).

This study showed that sleep deprivation did not significantly affect levels of IL-10 in each variable ($p = 0.065$). This result is consistent with the study of Neto et al. (2010), showing that sleep deprivation did not impair levels of anti-inflammatory cytokines, including IL-10. Otherwise, they also observed sleep deprivation increased IL-10 levels in different adipose tissue depots and decreased the TNF-α levels in the brain. Therefore, the profile of systemic anti-inflammatory cytokines after sleep deprivation remains controversial.

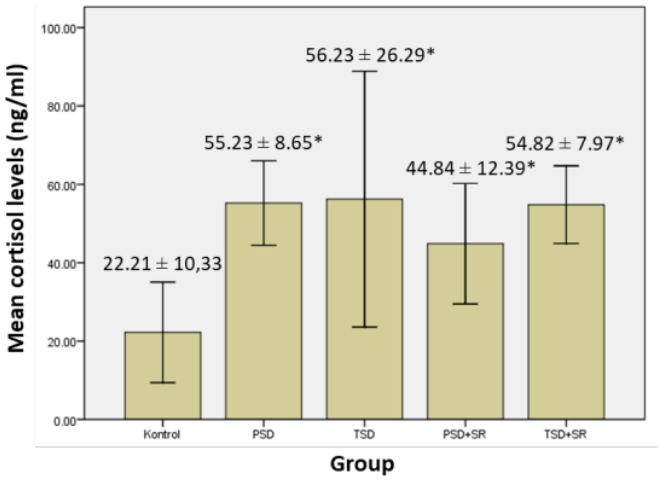

Figure 1. Mean cortisol levels in male Wistar rat (*Rattus norvegicus*). Control: no SD; PSD: 20 hours of SD with 4 hours rest for five days; TSD: 24 hours of SD for five days; PSD+SR: PSD followed by SR; TSD+SR: TSD followed by SR. SD: sleep deprivation, SR: sleep recovery, PSD: paradoxical sleep deprivation, TSD: total sleep deprivation. Error bars represent 95% confidence intervals. Statistical analysis was performed using Kruskal-Wallis test ($p = 0.029$) and Mann-Whitney test for post-hoc analysis. *Post-hoc Mann-Whitney test compared to control, $p < 0.05$.

Table 1. Levels of inflammatory cytokine in male Wistar rat (pg/mL).

Group	N	Min.				Max.				Mean±SD			
		IL-6	IL-10	TNF-α	IFN-γ	IL-6	IL-10	TNF-α	IFN-γ	IL-6	IL-10	TNF-α	IFN-γ
Control	5	311.14	15.14	2.89	736.59	1706.25	83.31	12.06	1375.77	974± 504	34.42±11.92	6.91±3.28	1038.08±272.26
PSD+SR	5	452.10	17.52	4.54	754.54	3103.86	93.76	23.89	1001.67	1281±1056	53.07±12.98	9.75±8.05	847.45±102.73
TSD+SR	5	311.14	57.89	6.54	718.80	2518.29	317.89	26.13	1001.67	1578±928	87.06±47.99	14.17±8.17	865.83±112.95
PSD	5	520.98	44.84	6.90	781.54	1858.94	74.98	12.24	1385.65	1192±544	59.47±5.44	9.39±2.62	1046.28±239.32
TSD	5	382.22	33.52	1.76	700.85	1365.64	178.02	14.17	1824.71	952±373	68.06±25.53	8.01±4.39	1167.39±432.52

Table 2. P-value of normality, homogeneity, and One-Way ANOVA test in the levels of inflammatory cytokines in male Wistar rat.

Group	Shapiro-Wilk (p-value)				Levene's (p-value)				One-Way Anova (p-value)			
	IL-6	IL-10	TNF-α	IFN-γ	IL-6	IL-10	TNF-α	IFN-γ	IL-6	IL-10	TNF-α	IFN-γ
Control	0.832	0.941	0.389	0.640	0.256	0.352	0.697	0.075	0.658	0.065	0.399	0.283
PSD+SR	0.884	0.482	0.081	0.409								
TSD+SR	0.812	0.909	0.096	0.921								
PSD	0.067	0.845	0.200	0.610								
TSD	0.622	0.500	0.615	0.750								

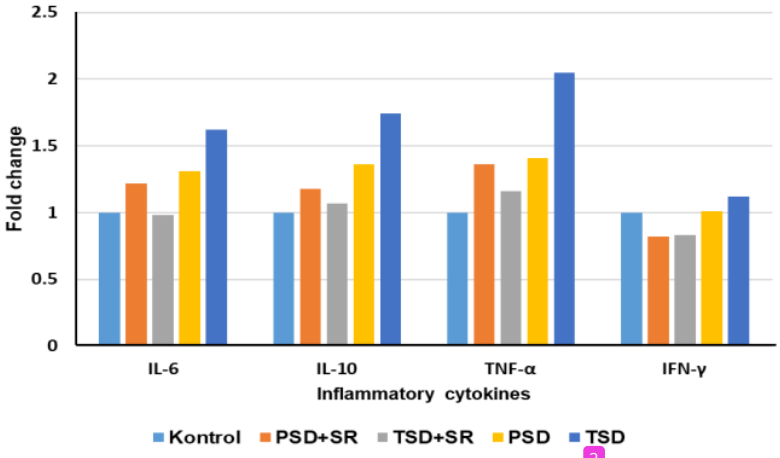

Figure 2. Mean of the fold change of inflammatory cytokine levels of IL-6, IL-10, TNF-α, and IFN-γ in male Wistar rat (*Rattus norvegicus*). Control group: no SD; PSD+SR: PSD followed by SR; TSD+SR: TSD followed by SR; PSD: 20 hours of SD with 4 hours rest for five days; TSD: 24 hours of SD for five days. SD: sleep deprivation, SR: sleep recovery, PSD: paradoxical sleep deprivation, TSD: total sleep deprivation.

A study by Patel et al. (2009) also demonstrated that sleep deprivation did not affect levels of anti-inflammatory cytokines such as IL-1 and IL-10. They explained that the different responses of sleep deprivation on the levels of anti-inflammatory cytokines may depend on the differences in the effect of sleep on inflammatory components in each individual, the half-life of each inflammatory component, and the instrument performance for cytokine measurement. Intriguingly, anti-inflammatory cytokine levels increase in chronic sleep deprivation that lasts over weeks, months, or years.

Wright et al. (2015) demonstrated that sleep deprivation significantly increased levels of IL-10 on day 26 to day 28 measured with ELISA. Sleep deprivation induces circadian rhythm impairment that, over weeks, will increase levels of anti-inflammatory cytokine (IL-10), the pro-inflammatory protein (TNF-α), and CRP. Otherwise, cortisol levels increase during acute sleep deprivation, indicating that acute and chronic sleep deprivation provoke different responses of circadian rhythm impairment. Therefore, acute sleep deprivation is associated with physiological stress or metabolic response of increased cortisol levels, whereas chronic sleep deprivation is associated with physiological adaptation as a response to decreased cortisol levels followed by increased pro-inflammatory and anti-inflammatory cytokines.

No significant difference in TNF-α levels after induction of various sleep deprivation stress models in this study ($p = 0.366$) is consistent with the study by Ruiz et al. (2012), showing that total sleep deprivation for two days or paradoxical sleep deprivation for four days followed by sleep recovery for three days did not alter TNF-α levels. Other studies also demonstrated that ten days of sleep deprivation did not increase TNF-α or its receptors, regardless of sex characteristics (Shearer et al., 2001; Xu et al., 2015). In contrast, a study by Irwin et al. (2006) exhibited that sleep deprivation increased TNF-α production. Sleep deprivation probably induced TNF-α cellular expression through the activation of nuclear factor NF-kB, a key component in controlling the cellular expression of pro-inflammatory cytokines.

In agreement with the study by Crooks et al. (2019) that demonstrated no significant result between sleep quality and IL-6 levels, this study approved that there was no significant result between the levels of pro-inflammatory cytokines, IL-6, with various sleep deprivation stress models ($p = 0.658$). Conversely, a study by Siregar (2017) showed a significant result between sleep quality and IL-6 levels. Sleep deprivation may affect components of the immune system through modification of CD4+ cells, CD8+ cells, NK cells, and levels of pro-inflammatory cytokines such as TNF-α, INF-γ, and IL-6 (Ibarra-Coronado et al., 2015). Collectively, these results indicate that pure TSD and PSD or TSD and PSD followed by sleep recovery did not affect plasma cytokine levels of IL-6 and TNF-α.

Multivariate analysis among the treatment groups showed no significant difference between sleep deprivation and IFN-γ levels ($p=0.283$). This result is consistent with the study by Hirotsu et al. (2012), demonstrating that there was no significant difference in IFN-γ levels in psoriasis rats model induced by paradoxical sleep deprivation for 48 hours. IFN-γ production is generally unaffected by sleep

deprivation, instead is increased at the end of sleep recovery. These insignificant results may be affected by sample processing that caused cytokine stability disruption, cytokine degradation by protease, or cytokine binding to its soluble cellular receptors. These alterations may also explain the decrease in cytokine levels over time during sample storage (Hennø et al., 2017).

The main limitation of this study is that it only examined the level of inflammatory cytokine levels in plasma but not in the tissue resulting in an insignificant increase in inflammatory cytokine levels after sleep deprivation treatment. Induction of inflammatory cytokine release is likely more prominent in the brain or other tissues, which are initially released by immune and glial cells in the brain after activation of the HPA axis (Garbarino et al., 2021). Another limitation is that the stress induction protocol by sleep deprivation in this study was not enough to induce an increase in the cytokine level in plasma. Various sleep deprivation protocols may also cause different results in inflammatory cytokine levels among studies. Furthermore, variation in the circadian cycle may also affect the production of various cytokines, which confounds the levels of circulating cytokines in this study (Ruiz et al., 2012).

To date, studies concerning sleep deprivation and inflammatory cytokines remain elusive. Sleep deprivation indeed increases the main stress hormone, cortisol, which plays a role in various disease development, such as hypertension and cardiovascular disease (Sá Gomes e Farias et al., 2022). On the other hand, some studies indicated that people with sleep disorders showed an alteration in circulating levels of TNF-α and IL-6. However, other studies failed to demonstrate the change in TNF-α and IL-6 levels between normal and sleep-deprived subjects (Crooks et al., 2019; Irwin et al., 2006; Shearer et al., 2001; Siregar, 2017). Although this study showed insignificant results, sleep deprivation also has a potency to increase the pro-inflammatory cytokine expression that is associated with inflammatory reaction-related diseases such as cancer, neurodegeneration, and cardiovascular disease (Garbarino et al., 2021). Establishing how far sleep deprivation can affect cortisol and inflammatory cytokine levels and how much sleep recovery is required to alleviate the physiological disruption caused by sleep deprivation is essential to making recommendations for a healthy lifestyle. Furthermore, in this modern era, night shift job is increasing in various sectors, and the future development of this study can be used to make recommendations for working regulation to create work-life balance.

CONCLUSIONS

Stress induction by the various models of sleep deprivation modifies cortisol levels but not inflammatory cytokines levels of IL-6, IL-10, TNF-α, and IFN-γ in male Wistar rats (*Rattus norvegicus*). These results indicate cortisol levels can be used as a stress indicator induced by sleep deprivation, but inflammatory cytokines levels of IL-6, IL-10, TNF-α, and IFN-γ may not be used for this purpose. Furthermore, although not statistically different, sleep recovery restores the levels of cortisol and inflammatory cytokines after PSD or TSD treatment, suggesting sleep recovery able to bring back homeostasis conditions after stress. Further exploration of sleep deprivation and sleep recovery protocols is required to get valuable outcomes.

ACKNOWLEDGEMENTS

The authors sincerely thank the Director of Jenderal Soedirman University and the Head of the LPPM Jenderal Soedirman University for providing research grants with the 2021 Institutional Research scheme with the research contract letter no. T/452/UN23.18/PT01.03/2021, the Head of the Integrated Laboratory of Faculty of Medicine of Universitas Indonesia, and Delta Iswara, Mochammad Haikal Alhamdi, Tyara Rahma Astari, and Ashlabiellah Nur Safah Lubis for the research data.

REFERENCES

Bassett, S. M., Lupis, S. B., Gianferante, D., Rohleder, N., & Wolf, J. M. (2018). Sleep quality but not sleep quantity effects on cortisol responses to acute psychosocial stress. *Revista Del Colegio Americano de Cardiologia*, *72*(23), 2964–2979. https://doi.org/10.3109/10253890.2015.1087503.Sleep

Cauter, E. V., Spiegel, K., Tasali, E., & Leproult, R. (2008). Metabolic consequences of sleep and sleep loss. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1016/S1389-9457(08)70013-3

Chen, L., Huang, J., Yang, L., Zeng, X. A., Zhang, Y., Wang, X., Chen, M., Li, X., Zhang, Y., & Zhang, M. (2017). Sleep deprivation accelerates the progression of alzheimer's disease by influencing Aβ-related metabolism. *Neuroscience Letters*, *650*, 146–152. https://doi.org/10.1016/j.neulet.2017.04.047

Chennaoui, M., Sauvet, F., Drogou, C., Van Beers, P., Langrume, C., Guillard, M., Gourby, B., Bourrilhon, C., Florence, G., & Gomez-Merino, D. (2011). Effect of one night of sleep loss on changes in tumor necrosis factor alpha (TNF-α) levels in healthy men. *Cytokine*, *56*(2), 318–324. https://doi.org/10.1016/j.cyto.2011.06.002

Chokroverty, S., & Ferini-Strambi, L. (2017). Oxford textbook of sleep disorders. *Oxford University Press*.

Crooks, E., Hansen, D. A., Satterfield, B. C., Layton, M. E., & Van Dongen, H. P. A. (2019). Cardiac

autonomic activity during sleep deprivation with and without caffeine administration. *Physiology & Behavior*, *210*, 112643. https://doi.org/10.1016/j.physbeh.2019.112643.Cardiac

Galvão, M. de O. L., Sinigaglia-Coimbra, R., Kawakami, S. E., Tufik, S., & Suchecki, D. (2009). Paradoxical sleep deprivation activates hypothalamic nuclei that regulate food intake and stress response. *Psychoneuroendocrinology*, *34*(8), 1176–1183. https://doi.org/10.1016/j.psyneuen.2009.03.003

Garbarino, S., Lanteri, P., Bragazzi, N. L., Magnavita, N., & Scoditti, E. (2021). Role of sleep deprivation in immune-related disease risk and outcomes. *Communications Biology*, *4*(1). https://doi.org/10.1038/s42003-021-02825-4

Gunn, B. G., Cox, C. D., Chen, Y., Frotscher, M., Gall, C. M., Baram, T. Z., & Lynch, G. (2017). The endogenous stress hormone CRH modulates excitatory transmission and network physiology in hippocampus. *Cerebral Cortex*, *27*(8), 4182–4198. https://doi.org/10.1093/cercor/bhx103

Hennø, L. T., Storjord, E., Christiansen, D., Bergseth, G., Ludviksen, J. K., Fure, H., Barene, S., Waage-Nielsen, E., Mollnes, T. E., & Brekke, O. L. (2017). Effect of the anticoagulant, storage time and temperature of blood samples on the concentrations of 27 multiplex assayed cytokines – Consequences for defining reference values in healthy humans. *Cytokine*, *97*(May), 86–95. https://doi.org/10.1016/j.cyto.2017.05.014

Hirotsu, C., Rydlewski, M., Araújo, M. S., Tufik, S., & Andersen, M. L. (2012). Sleep loss and cytokines levels in an experimental model of psoriasis. *PLoS ONE*, *7*(11), 1–9. https://doi.org/10.1371/journal.pone.0051183

Hirotsu, C., Tufik, S., & Andersen, M. L. (2015). Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. *Sleep Science*, *8*(3), 143–152. https://doi.org/10.1016/j.slsci.2015.09.002

Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., DonCarlos, L., Hazen, N., Herman, J., Katz, E. S., Kheirandish-Gozal, L., Neubauer, D. N., O'Donnell, A. E., Ohayon, M., Peever, J., Rawding, R., Sachdeva, R. C., Setters, B., Vitiello, M. V., Ware, J. C., & Adams Hillard, P. J. (2015). National sleep foundation's sleep time duration recommendations: Methodology and results summary. *Sleep Health*, *1*(1), 40–43. https://doi.org/10.1016/ j.sleh.2014.12.010

Ibarra-Coronado, E. G., Pantaleón-Martínez, A. M., Velazquéz-Moctezuma, J., Prospéro-García, O., Méndez-Díaz, M., Pérez-Tapia, M., Pavón, L., & Morales-Montor, J. (2015). The Bidirectional relationship between sleep and immunity against infections. *Journal of Immunology Research*, *2015*, 678164. https://doi.org/10.1155/2015/678164

Irwin, M. R., Wang, M., Campomayor, C. O., Collado-Hidalgo, A., & Cole, S. (2006). Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Archives of Internal Medicine*, *166*(16), 1756–1762. https://doi.org/10.1001/archinte.166.16.1756

Kantasa, V. D., Kusumawardani, B., & Herniyati. (2016). Efek Stresor rasa sakit renjatan listrik terhadap limfosit dan makrofag pada gingiva tikus sprague dawley (The Effect of electrical shock stressor on lymphocytes and macrophages in gingival tissue of sprague dawley rats). *Pustaka Kesehtan*, *4*(1), 48–54.

Kim, J., Yun, K. sang, Cho, A., Kim, D. H., Lee, Y. K., Choi, M. J., Kim, S. hyung, Kim, H., Yoon, J. W., & Park, H. C. (2022). High cortisol levels are associated with oxidative stress and mortality in maintenance hemodialysis patients. *BMC Nephrology*, *23*(1), 1–9. https://doi.org/10.1186/s12882-022-02722-w

Lestarianto, J. A. (2014). Hubungan antara internet addiction dan tingkat stres dengan kejadian insomnia pada mahasiswa keperawatan Fakultas Ilmu Kesehatan Universitas Nuhammadiyah (Correlation between internet addiction and stress levels with the incidence of insomnia in nursing students at the Faculty of Health Sciences of University of Muhammadiyah). *Diss. Universitas Muhammadiyah Purwokerto*. http://repository.ump.ac.id/id/eprint/5503

Lichtenstein, G. R. (2015). The Importance of sleep. *Gastroenterology & Hepatology*, *11*(12), 790.

Mattice, C. D., Brooks, R., & Lee-Chiong, T. (2011). Fundamentals of sleep technology: Second edition. *Philadelphia: Lippincott Williams & Wilkins*.

Medic, G., Wille, M., & Hemels, M. E. H. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, *9*, 151–161. https://doi.org/10.2147/NSS.S134864

Neto, J. C. R., Lira, F. S., Venancio, D. P., Cunha, C. A., Oyama, L. M., Pimentel, G. D., Tufik, S., Do Nascimento, C. M. O., Santos, R. V. T., & De Mello, M. T. (2010). Sleep deprivation affects inflammatory marker expression in adipose tissue. *Lipids in Health and Disease*, *9*(1), 125. https://doi.org/10.1186/1476-511X-9-125

Olayaki, L. A., Sulaiman, S. O., & Anoba, N. B. (2015). Vitamin C prevents sleep deprivation-induced elevation in cortisol and lipid peroxidation in the rat plasma. *Nigerian Journal of Physiological Sciences : Official Publication of the Physiological Society of Nigeria*, *30*(1–2), 5–

9.

Patel, S. R., Zhu, X., Storfer-Isser, A., Mehra, R., Jenny, N. S., Tracy, R., & Redline, S. (2009). Sleep duration and biomarkers of inflammation. *Sleep*, *32*(2), 200–204. https://doi.org/10.1093/sleep/32.2.200

Patrick, Y., Lee, A., Raha, O., Pillai, K., Gupta, S., Sethi, S., Mukeshimana, F., Gerard, L., Moghal, M. U., Saleh, S. N., Smith, S. F., Morrell, M. J., & Moss, J. (2017). Effects of sleep deprivation on cognitive and physical performance in university students. *Sleep and Biological Rhythms*, *15*(3), 217–225. https://doi.org/10.1007/s41105-017-0099-5

Pejovic, S., Basta, M., Vgontzas, A. N., Kritikou, I., Shaffer, M. L., Tsaoussoglou, M., Stiffler, D., Stefanakis, Z., Bixler, E. O., & Chrousos, G. P. (2013). Effects of recovery sleep after one work week of mild sleep restriction on interleukin-6 and cortisol secretion and daytime sleepiness and performance. *American Journal of Physiology - Endocrinology and Metabolism*, *305*(7), 890–896. https://doi.org/10.1152/ajpendo.00301.2013

Praharaj, S. K., Gupta, R., & Gaur, N. (2018). Clinical practice guideline on management of sleep disorders in the elderly. *Indian Journal of Psychiatry*, *60*(7), S383–S396. https://doi.org/10.4103/0019-5545.224477

Putri, D. N. E., Nasrul, E., & Masri, M. (2017). Pengaruh kurang tidur terhadap berat badan pada tikus Wistar jantan (effect of sleep deprivation on body weight in male Wistar rats). *Jurnal Kesehatan Andalas*, *6*(1), 78–82. https://doi.org/10.25077/jka.v6i1.655

Rockstrom, M. D., Chen, L., Taishi, P., Nguyen, J. T., Gibbons, C. M., Veasey, S., & Krueger, J. M. (2018). Tumor necrosis factor alpha in sleep regulation. *Sleep Medicine Reviews*, *40*, 69–78. https://doi.org/10.1016/j.smrv.2017.10.005. Tumor

Ruiz, F. S., Andersen, M. L., Martins, R. C. S., Zager, A., Lopes, J. D., & Tufik, S. (2012). Immune alterations after selective rapid eye movement or total sleep deprivation in healthy male volunteers. *Innate Immunity*, *18*(1), 44–54. https://doi.org/10.1177/1753425910385962

Sá Gomes e Farias, A. V., de Lima Cavalcanti, M. P., de Passos Junior, M. A., & Vechio Koike, B. del. (2022). The association between sleep deprivation and arterial pressure variations: a systematic literature review. *Sleep Medicine: X*, *4*, 100042. https://doi.org/10.1016/j.sleepx.2022.100042

Saraiva, M., & O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nature Reviews Immunology*, *10*(3), 170–181. https://doi.org/10.1038/nri2711

Shearer, W. T., Reuben, J. M., Mullington, J. M., Price, N. J., Lee, B. N., O'brian Smith, E., Szuba, M. P., Van Dongen, H. P. A., & Dinges, D. F. (2001). Soluble TNF-α receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight. *Journal of Allergy and Clinical Immunology*, *107*(1), 165–170. https://doi.org/10.1067/mai.2001.112270

Simpson, N., & Dinges, D. F. (2007). Sleep and inflammation. *Nutrition Reviews*, *65*(SUPPL.3), S244–S252. https://doi.org/10.1111/j.1753-4887.2007.tb00371.x

Siregar, E. M. H. (2017). Korelasi kualitas tidur dengan kadar interleukin-6 serum pada pasien hemodialisis regular (Correlation of sleep quality with interleukin-6 serum levels in regular hemodialysis patients). In *Thesis Universitas Sumatera Utara*.

Sukendra, D. M. (2015). Efek olahraga ringan pada fungsi imunitas terhadap mikroba patogen: infeksi virus dengue (effects of light exercise on immune function against microbial pathogens: dengue virus infection). *Media Ilmu Keolahragaan Indonesia*, *5*(2), 57–65.

Van Dongen, H. P. A., Belenky, G., & Krueger, J. M. (2011). A Local, Bottom-up perspective on sleep deprivation and neurobehavioral performance. *Current Topics in Medicinal Chemistry*, *11*(19), 2414–2422. https://doi.org/doi: 10.2174/156802611797470286.

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Hopper, K., Lotsikas, A., Lin, H. M., Kales, A., & Chrousos, G. P. (2000). Sleep apnea and daytime sleepiness and fatigue: Relation to visceral obesity, insulin resistance, and hypercytokinemia. *Journal of Clinical Endocrinology and Metabolism*, *85*(3), 1151–1158. https://doi.org/10.1210/jcem.85.3.6484

Welsh, P., Murray, H. M., Ford, I., Trompet, S., De Craen, A. J. M., Jukema, J. W., Stott, D. J., McInnes, I. B., Packard, C. J., Westendorp, R. G. J., & Sattar, N. (2011). Circulating interleukin-10 and risk of cardiovascular events: A prospective study in the elderly at risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(10), 2338–2344. https://doi.org/10.1161/ATVBAHA.111.231795

Wright Jr, K. P., Drake, A. L., Frey, D. J., Fleshner, M., Desouza, C. A., Gronfier, C., & Czeisler, C. A. (2015). Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Physiology & Behavior*, *47*, 24–34. https://doi.org/10.1016/j.bbi.2015.01.004.Influence

Xu, C., Wu, X., Hack, B. K., Bao, L., & Cunningham, P. N. (2015). TNF causes changes in glomerular endothelial permeability and

morphology through a Rho and myosin light chain kinase-dependent mechanism. *Physiological Reports*, *3*(12), 1–16. https://doi.org/10.14814/phy2.12636

Zabidi, L., Supriatna, M., Departemen, M. M., Kesehatan, I., Fk-Undip, A., Rsup, /, Semarang, K., & Mexitalia, M. (2015). Kadar kortisol serum sebagai indikator prognosis sepsis pada anak (serum cortisol level as prognostic indicator for pediatric sepsis). *Sari Pediatri*, *17*(2), 101–106.

Molekul

ORIGINALITY REPORT

10%	%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Handbook of Immunosenescence", Springer Science and Business Media LLC, 2019 Publication	3%
2	Masihullah Radmanish, Olfa Khalfallah, Nicolas Glaichenhaus, Anne Forhan et al. "Sleep duration trajectories associated with levels of specific serum cytokines at age 5: A longitudinal study in preschoolers from the EDEN birth cohort", Brain, Behavior, & Immunity - Health, 2022 Publication	2%
3	Irwin, Michael R.. "Why Sleep Is Important for Health: A Psychoneuroimmunology Perspective", Annual Review of Psychology, 2015. Publication	1%
4	"Neuroimmunology of Sleep", Springer Science and Business Media LLC, 2007 Publication	1%
5	Marco Mozzati. "Influence of Superpulsed Laser Therapy on Healing Processes Following	1%

Tooth Extraction", Photomedicine and Laser Surgery, 06/2011
Publication

---

6 Shirin Arjmandi-Rad, Mahshid Ebrahimnejad, Mohammad-Reza Zarrindast, Salar Vaseghi. "Do Sleep Disturbances have a Dual Effect on Alzheimer's Disease?", Cellular and Molecular Neurobiology, 2022 — 1 %
Publication

---

7 Wright, Kenneth P., Amanda L. Drake, Danielle J. Frey, Monika Fleshner, Christopher A. Desouza, Claude Gronfier, and Charles A. Czeisler. "Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance", Brain Behavior and Immunity, 2015. — 1 %
Publication

---

8 ZaenalAdi Susanto, Wahyu Siswandari, Lantip Rujito. "Cd60 (GTG>GAG)/Hb Cagliari mutation was found in scanning of β-thalassemia alleles from patients of East Kalimantan, Indonesia", Molecular Genetics and Metabolism Reports, 2020 — 1 %
Publication

---

9 "Sleep Disorders Medicine", Springer Science and Business Media LLC, 2017 — 1 %
Publication

---

10 "Handbook of nutrition, diet and sleep", Wageningen Academic Publishers, 2013 1%
Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Fk Unsoed
Assignment title:	Molekul
Submission title:	Molekul
File name:	6218-Fitranto-_386-395.pdf
File size:	230.31K
Page count:	10
Word count:	6,399
Character count:	35,001
Submission date:	23-Nov-2022 07:20PM (UTC+0700)
Submission ID:	1961995521

Molekul, Vol. 17. No. 3, November 2022: 386 – 395

MOLEKUL eISSN: 2503-0310

Articles

https://doi.org/10.20884/1.jm.2022.17.3.6218

Levels of Cortisol and Inflammatory Cytokines after The Induction of Various Sleep Deprivation Stress Models in Male Wistar Rats

Fitranto Arjadi[1*], Sindhu Wisesa[1], Nor Sri Inayani[2], Catharina Widiartini[1], Prasetyo Tri Kuncoro[1]

[1]Departement of Anatomy, Faculty of Medicine, Universitas Jenderal Soedirman Purwokerto 53112, Indonesia
[2]Departement of Biochemistry, Faculty of Medicine, Universitas Jenderal Soedirman Purwokerto 53112, Indonesia

*Corresponding author email: fitranto.arjadi@unsoed.ac.id

Received June 23, 2022; **Accepted** September 20, 2022; **Available online** November 20, 2022

ABSTRACT. Sleep deprivation (SD) can modulate the production of various cytokines, including pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ, and anti-inflammatory cytokines such as IL-10. Paradoxical sleep deprivation (PSD) increases the risk of inflammation but can be relieved by sleep recovery (SR). This study aimed to determine the differences in levels of cortisol and inflammatory cytokines (IL-6, IL-10, TNF-α, dan IFN-γ) in male Wistar rats (Rattus norvegicus) after induction of various sleep deprivation stress models. Twenty-five of male Wistar rats were randomly divided into five groups: control, PSD (20 hours of SD/day for five days), Total Sleep Deprivation or TSD (24 hours of SD/day for five days), PSD+SR (PSD followed by SR), and TSD+SR (TSD followed by SR). The plasma cortisol levels were measured with ELISA, and inflammatory cytokine levels were measured with immunoassay and calculated with fold change. Mean cortisol levels were significantly increased in treatment groups compared to the control group ($p=0.029$). Multivariate analysis showed no statistically significant difference in inflammatory cytokine levels of IL-6 ($p=0.658$), IL-10 ($p=0.065$), TNF-α ($p=0.399$), and IFN-γ ($p=0.283$) in all groups. In conclusion, various sleep deprivation stress models affect cortisol levels but not inflammatory cytokine levels of IL-6, IL-10, TNF-α, and IFN-γ among male Wistar rats.

Keywords: cortisol, inflammatory cytokines, sleep deprivation, sleep recovery

INTRODUCTION

Sleep plays a vital role in life by maintaining the optimal physiological function of the human body. It combines stimulation of afferent nerve delivery to the brain and activation of functional neurons in specific brain areas (Chokroverty et al., 2017). Good sleep is determined by the quality of sleep, how deep a person sleeps, and the quantity of sleep, amount of time as a person sleeps (Patrick et al., 2017). According to the National Sleep Foundation, a young adult (18-25 years) or adult (26-64 years) needs 7-9 hours of sleep daily, but not less than 6 hours or more than 11 hours for young adults or 10 hours for adults. Otherwise, an older adult (65 years and over) requires at least 7-8 hours of sleep daily, but not less than 5 hours or more than 9 hours (Lichtenstein, 2015). People who live in developed countries are chronically sleep-deprived because of their cultural and socioeconomic environments. Incidence of symptoms related to sleep deprivation has recently increased, suggesting its long-term detrimental health effects are more abundant than expected (Chokroverty & Ferini-Strambi, 2017).

A study by Hirshkowitz et al. (2015) showed that > 30% of men and women between the ages of 30-64 years sleep less than 6 hours per day, and 5-15% of the world population suffer from sleep disorders. Another study indicated that 40-70% of older adults have chronic sleep disorders that are more prominent in people with medical or psychiatric comorbidity (Praharaj et al., 2018). The National Sleep Foundation stated that 70% of Indonesian people experience sleep disturbances at least once a week, and 30 million people have sleeping difficulty every night (Lestarianto, 2014). A survey of the healthy lifestyle index by American International Assurance (AIA) in 2013 demonstrated that Indonesian people have an average sleep duration of 6.8 hours per day due to increased activity and decreased sleep duration from its normal range, indicating many Indonesian people are suffered from sleep deprivation (Putri et al., 2017).

The relationship between sleep duration and the inflammation process has not been studied extensively, despite showing a similar correlation between sleep duration and mortality. People with long sleep duration (>8 hours/night) and short sleep duration (<7 hours/night) have a 30% and 12% greater risk of death, respectively, compared to people with moderate sleep duration (7-8 hours/night). Physiologically, sleep affects two major effector systems, the Hypothalamus-Pituitary-Adrenal (HPA) axis and the sympathetic nervous system, which

386