Western Norway University of Applied Sciences

BACHELOR THESIS

Sleep deprivation and its consequences on health – with emphasis on metabolic and immunological functions

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We confirm that the work is self-prepared and that references/source references to all sources used in the work are provided, cf. Regulation relating to academic studies and examinations at the Western Norway University of Applied Sciences (HVL), § 12-1.

Abstract

Our short review elucidate evidence that sleep deprivation has profound deleterious effects on blood pressure and insulin sensitivity and glucose metabolism. Sleep deprivation-induced circadian misalignment can possibly lead to dysregulation of the bacterial population in the gastrointestinal tract (GIT). Moreover, upregulation of endocannabinoid system (ECS) may lead to increased hunger and appetite due to higher plasma levels of 2-arachidonoylglycerol (2-AG) as a result of restricted sleep. Overall, there is a growing body of scientific data suggesting a clear link between insufficient sleep duration and increased risk of diabetes, obesity and hypertension. Therefore, adequate sleep should be highly recommended as an important factor in addition to common treatment of obesity and diabetes. In addition, studies on immunological function demonstrate an association between dysregulation of inflammatory response and increased risk of infections and increased mortality, due to sleep deprivation and disturbances. There seem to be a bidirectional relationship between the sleep deprivation-induced enhancement in the immune response with increases in cytokines, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α) which in turn function to increase sleep drive. Furthermore, sleep deprivation may lead to dysregulation of cytokines, impaired immune response and higher serum levels of IL-6 and CRP followed by chronic low-grade inflammatory response. This leads to the suggestion that adequate sleep is recommended to sustain homeostasis, and that sleep disturbances may result in a variety of health problems.

Keywords: health, sleep deprivation, review, immune system, metabolism

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Abbreviations and definitions

Abbreviation	Definition
Aβ	Amyolid-β
ACTH	Adrenocorticotropic hormone
AEA	N-arachidonoylethanolamine
APC	Antigen presenting cell
BP	Blood pressure
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CNS	Central nervous system
CRH	Corticotrophin releasing hormone
CRP	C-reactive protein
DAMP	Danger associated molecular pattern
DBP	Diastolic blood pressure
EEG	Electroencephalogram
ECS	Endocannabinoid system
GIT	Gastrointestinal tract
HR	Heart rate
HPA-axis	Hypothalamic pituitary gland axis
ICAM-1	Intracellular cell adhesive molecule 1
IL	Interleukin
IFN-γ	Interferon γ
Mac-1	Macrophage associated antigen 1
NREM	Non-rapid eye movement
NTS	Nucleus tractus solitarius
PAMP	Pathogen associated molecular pattern
PG	Prostaglandins
PRR	Pattern recognition receptors
PSR	Partial sleep restriction
Pyrogen	An agent that induces fever
RA	Rheumatic arthritis
REM	Rapid eye movement

SBP	Systolic blood pressure
SCN	Suprachiasmatic nucleus
SD	Sleep deprivation
SNS	Sympathetic nervous system
Somnogen	An agent that induces sleep
SWS	Slow-wave-sleep
TCC	Terminal complement complex
TNF	Tumor necrosis factor
TSH	Thyroid-stimulating hormone
VCAM-2	Vascular cell adhesive molecule 2
Zeitgeber	External timing signals
2-AG	2-arachidonoylglycerol

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1. Introduction

Sleep is a biological need in every living organism. The amount of adequate sleep varies, but it is recommended to obtain 6 to 9 hours of sleep each night (Ursin, 1996). Human adults sleep on average 7,5 hours each night, but studies have proven that short sleep duration may be adequate in some individuals to maintain important functions of the body. One of the important functions of sleep is maintaining homeostasis, which among others include a metabolic and an immunologic element. Circadian rhythms throughout the body regulates both metabolic and immunological systems, and sleep. It can be defined as endogenous rhythms that consists for about 24 hours and lasts in the absence of external timing signals (zeitgeber) (Thomas Bollinger, Bollinger, Oster, & Solbach, 2010). These external timing signals include light-dark cycle, body chore temperature and social rhythms.

Sleep is divided into five phases, including phase 1 to 4, and rapid eye movement (REM) sleep. Phase 1 to 4 is defined as non-rapid eye movement (NREM) sleep, where phase 3 and 4 are slow-wave sleep (SWS). The phases are mainly divided based on wavelength measured with electroencephalogram (EEG), where delta-activity is the most important parameter that covers the need for sleep through homeostatic effects and enforcement. Delta-activity is generated in the relay cells in thalamus and inhibits the transfer of sensory information to the cortex (Ursin, 1996). The inhibition mainly occurs in sleep stage 3 and 4, which is the main reason for SWS and the state of deep sleep.

Sleep deprivation is another description for sleep loss or sleep disruption, where the individual fails to obtain adequate sleep amount and/or quality. The extent of the sleep deprivations severity varies along with the situation, based on if the condition is acute or chronic. Total sleep deprivation over time is unfavorable and may cause adverse health consequences. For example, growth hormone and prolactin, which are released during phase 3 and 4 of NREM sleep, are hormones that take part in many important functions in the body (Ursin, 1996).

Sleep is essential to maintain metabolic- and hormonal processes in the body. The metabolic processes taking place during sleep is not fully known in detail, however, sleep deprivation is presumed to cause dysregulation in metabolism through hormonal imbalance, countless pathways involving sympathetic overstimulating, subclinical inflammation and hormonal

imbalance. In accordance with our increasingly sleep deprived society, the prevalence of diabetes, cardiovascular diseases and obesity is growing proportionally. Even though factors such as physical activity, diet and stress contribute to the risk of obesity, inadequate sleep and its impact on metabolic functions is becoming increasingly acknowledged. Sleeps impact on health may contribute to potential countermeasure and clinical treatment.

As well as metabolic functions, sleep also contributes with a crucial part for optimal function of the central nervous system (CNS) and the immune system (Luciana Besedovsky, Lange, & Haack, 2019; Thomas Bollinger, Bollinger, Oster, & Solbach, 2010; Lorton et al., 2007). There is a crosstalk between brain and the immune system, where CNS modulates immune functions through, among others, inflammatory response and cytokine secretion. Interleukin (IL)-1β and tumor necrosis factor (TNF)- α are sleep modulators that are secreted by different immune cells during immune responses. Consistently, sleep deprivation may cause dysregulation of immune response. Excessive immune response is not beneficial for humans, as it may have detrimental health effects. In the same way, impaired immune functions may lead to increased infections risk and mortality. All in all, researchers suggest that sleep enhances immunological functions, while long term sleep deprivation may result in severe health consequences.

Multiple studies have proven a correlation between sleep loss and dysregulation of metabolic and immunological functions, but the mechanisms are yet not fully understood. This short review article will discuss different studies that have been conducted in consistency with these functions and sleep deprivation, to get a better understanding of the biological mechanisms.

2. Material and method

In this short review article, a selection of the findings reported in mainly review articles in the selected topic; sleep deprivation and its main consequences on health with special emphasis on metabolic and immune functions, are used to accomplish a short literature review. The aim is to be able to sum up the main and newest findings regarding knowledge about sleep deprivation and its main consequences on health with special emphasis on metabolic and immune functions.

The majority of the original and review articles was assigned by Dr. Radhika Basheer from Section of Molecular Neuroscience, Laboratory of Neuroscience at Harvard Medical School, Department of Psychiatry, Harvard University and V.A. Boston Healthcare System, Boston, US and Dr. Alvhild Alette from the Department of Safety, Chemistry and Biomedical Laboratory Sciences, Faculty of Engineering and Science at Western Norway University of Applied Sciences, Bergen, Norway. Following key terms were used for search in databases PubMed and Web of Science: *Health, sleep deprivation*, *review*, *immune system and metabolism/metabolic*. In various ways, the terms were combined with "AND" in the effort to achieve the most narrowly search. We only used and referred to internationally published articles written in English.

Several procedures were followed to guarantee high quality review of the literature on "Sleep deprivation and its consequences on health with emphasis on metabolic- and immunological functions". First, the source had to be in line with the purpose of the review based on the research topic of the article. Second, the articles chosen where per-reviewed to ensure high quality.

3. Sleep deprivations effect on metabolic and immunologic functions

There is a growing body of observational evidence suggesting a significant link between insufficient sleep duration and increased risk of diabetes, cardiac morbidity, caloric intake, hypertension and obesity. In this short review, we want to elucidate the mechanism that link insufficient sleep to obesity, by emphasizing the findings of increased health risk factors related to metabolic and immunological functions as a result of sleep deprivation.

3.1 Sleep deprivation and its effects on blood pressure

Maintaining blood pressure (BP) within normal limits is essential for normal health and lower risk of cardiovascular diseases. BP varies and can be affected by many activities (Shahoud & Aeddula, 2019). BP is physiologically regulated through many endogenous, homeostatic mechanisms. Baroreflex is one of the homeostatic mechanisms in the body that helps maintain a constant level of BP through a rapid negative-feedback system. The pressure sensors, baroreceptors, located in the carotid sinus and aortic arch, are activated by changes in arterial pressure. The carotid sinus baroreceptors are innervated by a branch of glossopharyngeal nerve (IX cranial) and the aortic arch baroreceptors are innervated by the aortic nerve that combines with the vagus nerve (X cranial), both of which terminate at a sleep-wake regulating node, nucleus tractus solitarius (NTS) (Cottle, 1964). Therefore, when BP mechano-sensitive ion channels in response to changes in aortic pressure transmit information to NTS, they can potentially influence sleep and vice versa. The exact role of NTS in sleep-wake regulation is still debated (Anaclet and Fuller, 2017). Some reports demonstrated that low frequency electrical stimulations NTS produces cortical synchronization indicative of slow wave sleep (Magnes, Moruzzi, & Pompeiano, 1961), whereas lesions of NTS produce desynchronization of the EEG. A recent study in cats showed that electrical stimulations of NTS enhance EEG theta and beta frequency power and increased wakefulness (Martínez-Vargas, Valdés-Cruz, Magdaleno-Madrigal, Fernández-Mas, & Almazán-Alvarado, 2017). Either way, NTS does play a role in regulating sleep-wake.

The normal pattern of BP displays small increase in BP before the termination of night-time sleep and a reduction of approximate 15% in systolic blood pressure (SBP) and of lesser amount in diastolic blood pressure (DBP) during sleep relative to waking. However, BP is generally lower during the night at sleep compared to waking either during night or day. Individuals with this normal nighttime reduction are also called dippers (Berbari & Mancia,

2018). Reduction in physical and mental activity, and lower autonomic nervous system activity is associated with the BP decrease during sleep. BP is lowest during slow-wave-sleep, and highest during REM sleep. Individuals with poorer sleep quality and quantity have been reported with absent reductions in nighttime BP (Mansoor, 2002). The underlying mechanisms of the nocturnal reduction in BP are not completely understood, however, individuals with no dipping BP pattern have shown to have increased sympathetic nervous system activity with decreased parasympathetic nervous system activity, and higher levels of norepinephrine and epinephrine, compared with individuals with normal reduction in BP during night (Sherwood, Steffen, Blumenthal, Kuhn, & Hinderliter, 2002).

Sleep deprivation in general result in higher BP during the night. During increased work expenditure and stress, an increase in sympathetic activation and decrease parasympathetic activation are seen (Janet M Mullington, Haack, Toth, Serrador, & Meier-Ewert, 2009). When sleep is of inadequate quality or quantity, similar autonomic changes in combination with stress response may also occur. Furthermore, investigators have observed a link between insufficient sleep and increased resting BP (Kato et al., 2000; Meier-Ewert et al., 2004; Ogawa et al., 2003; Tochikubo, Ikeda, Miyajima, & Ishii, 1996). Based on this, Mullington and colleagues hypothesized that during resisting phase of an extended wakefulness, BP is increased due to activated autonomic nervous system (Janet M Mullington et al., 2009). Several findings suggest increased BP with autonomic nervous system activation in sleep deprived subjects. However, the findings were indeed varied, possibly due to non-identical experimental conditions that causes the mechanisms that maintain cardiovascular system to respond differently (Janet M Mullington et al., 2009).

Others in the literature report that sleep deprivations (SD) are involved in increased sympathetic activation and higher BP, with decreased baroreflex sensitivity. In a study of 6 nights of partial SD, elevation in sympatho-vagal balance by SD during morning and through mid-day hours was reported (K. Spiegel, Leproult, & Van Cauter, 1999). According to Zhong and colleagues, BP variability and sympathetic nervous system were both significantly increased in 18 healthy individuals (age 19-36), undergoing continuously SD under controlled conditions of ambient temperature and fluid intake (Zhong et al., 2005). After 36 h of wakefulness, BP variability and heart rate (HR) were increased, and baroreflex sensitivity was decreased. Both studies involved participants avoiding recumbent posture during the vigil period. In contrast, other studies reported a decrease in sympathetic activity after SD. Both

Kato and colleagues and Holmes and colleagues suggest that increased BP is due to baroreflex setpoint change and that sympathetic outflow is decreased as a protective response (Holmes, Burgess, & Dawson, 2002; Kato et al., 2000). These studies involved recumbent posture through the sleep deprivation-period, and it was relatively short for this reason.

The studies have used different methods and experimental conditions, with resulting different outcomes. Environmental conditions may affect study results, like body posture, stress, ambient temperature and fluid and food intake, which influence regulating factors such as catecholamines and hormones that control blood pressure and could therefore be difficult to compare. However, these studies present results showing that sleep deprivation increase blood pressure that is known to increase the risk of cardiac morbidity, despite the unknown mechanism of the increased BP.

3.2 The impact of endocannabinoid system on hedonic feeding in sleep deprived subjects

Endocannabinoid system (ECS) is a widespread lipid signaling system, recognized as a major system not only in the brain, but in the peripheral tissues as well. In short terms, ECS plays a significant role in a wide range of physiologic behaviors and processes including CNS, synaptic plasticity, immune function, energy homeostasis, metabolism, emotional state, sleep and arousal, reward processing and in response to environmental and endogenous insults (Ronan, Wongngamnit, & Beresford, 2016).

The ECS consist of various integrated mechanisms, such as cannabinoid receptors, enzymes responsible for the degradation and synthesis of the endocannabinoids, also referred to as endogenous cannabinoids, synthesized in our body (Lu & Mackie, 2016). The endogenous agonist 2-arachidonoylglycerol (2-AG) and anandamide (N- arachidonoylethanolamine, AEA), derived from arachidonic acid (Zadalla & Vasiliki, 2009), bind to the currently two known subtypes of cannabinoid receptors type 1 (CB1) and -type 2 (CB2) (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). In CNS, the CB1 receptors are abundant, especially in cerebellum, cortex, basal ganglia and hippocampus (Mackie, 2005). Despite ECS's importance, this complex system has been an unknown part of the human body function until 1992 when Raphael Mechoulam, Roger Pertwee and colleagues first described AEA (Devane

et al., 1992). Later, the biologically important endocannabinoid 2AG was discovered, as a second major endocannabinoid (Kendall & Alexander, 2009).

The ECS has emerged as a possible regulator of the hedonic or uncontrolled feeding in sleep deprived subjects. Previous studies have reported a link between reduced sleep and rise in appetite and hunger. Broussard and colleagues refer to their own study with Hanlon and colleagues, consisting of fourteen healthy non-obese participants, reporting large circadian variation of 2-AG. Variation of 2-AG results in mediated appetite-enhancing effects of endocannabinoids (Broussard & Cauter, 2016; Hanlon et al., 2016). Investigators measured circulating endocannabinoids for 3 nights on the 24 h daily profile, with restricted sleep (4,5 h sleep per night) versus 8,5 h sleep per night. The 2-AG serum concentration had nearly threefold increase from early morning to lunchtime after only 4,5 h sleep pr. night, with a nadir concentration around the middle of the sleep period, followed by a continuous increase in the concentration in the early afternoon. The findings of this study suggest that activity of the ECS is deeply modulated by circadian rhythmicity and regulation of hedonic food intake is suppressed during sleep. As a response to sleep restriction, in the same individuals, an activation of the ECS occurred at daytime, whit both an elevation and delay of the 2-AG peak. The individuals also reported increased appetite and hunger as well as afternoon elevation of 2-AG concentration. The result suggests that activation of the endocannabinoid system may be linked to excessive food intake during insufficient sleep and can possibly contribute to the increase risk of obesity (Hanlon et al., 2016).

Zhu and colleagues presents two studies measuring circulating levels of 2-AG to evaluate the effect of sleep restriction (Zhu, Shi, Park, Zhao, & Reutrakul, 2019). Both findings reported higher plasma levels of 2-AG after restricted sleep (Cedernaes et al., 2016; Hanlon et al., 2016). In Cedernaes and colleague's study, 2-AG plasma concentration was measured in 16 normal-weight men with three nights of partial sleep deprivation (4,25-h sleep opportunity) versus normal sleep (8,5-h sleep opportunity). In addition, subjective hunger and stress were measured. The result showed an 80% higher plasma concentration of 2-AG after 1,5 h awakening compared with normal sleep, when participants were sleep deprived. This corresponds with 25% increased hunger ratings after sleep deprivation compared to normal sleep. The results support the fact that changes in hedonic aspects of food consumption after acute sleep loss, may partially result from activation of endocannabinoid-signaling pathways, and did indeed correlate with the findings of Broussard and colleagues (Broussard & Cauter,

2016; Cedernaes et al., 2016). Weljie and colleagues reported an increase in lipid metabolism after SD in both humans and rats. Even though the study did not show a significant change in arachidonic acid, which 2-AG derived from, the overall study present main changes in lipid metabolites (Weljie et al., 2015). However, even if most of the reported studies support changes in the endocannabinoid system towards increased feeding after sleep deprivation, knowledge of endocannabinoid as a regulator of hedonic feeding among sleep deprived subjects requires further research.

3.3 Gut microbiome and circadian misalignments

The human gastrointestinal tract (GIT) harbors a diverse milieu of microorganisms, most of which belong to the domain Bacteria, that play a key role in the metabolic homeostasis of the host organism (Haller, 2018). The gut microbiome, as it is collectively referred to, has emerged as a key modulator in both maintaining human health and pathogenesis of diseases, involved in metabolic and immune functions (Kimmel & Rosenberg, 2020). However, the diet of the host plays a critical role in shaping the gut microbiota an also in shaping its ability to regulate immune-function and metabolism (Fava, 2015).

Experimental circadian misalignments potentially caused by periods of sleep deprivation can lead to alteration in the presence of bacterial communities, which may further promote increased energy balance due to increased energy absorption from food ingestions (Broussard & Cauter, 2016). In the comprehensive review of Broussard and colleagues, they present findings of Thaiss and colleagues showing that intestinal mycobiome, in both humans and mice, exhibits diurnal oscillations that are influenced by feeding rhythms. Induction of jet lag, e.g. circadian misalignments, leads to microbial imbalance in the body as an impaired microbiota. Thus, jet-lag-generates dysbiosis or microbial imbalance in both humans and mice may possibly promotes obesity and glucose intolerance (Thaiss et al., 2014). Weljie and colleagues found that gut metabolism was elevated in sleep-restricted humans. One of the elevated metabolites after sleep restriction was pipecolic acid, which is a metabolite of lysine (Weljie et al., 2015). Previous studies have reported that the concentration of pipecolic acid in several sites in the brain fluctuates depending on feeding conditions and is suggested to be an endogenous suppressor of food intake which acts via GABAergic system (Takagi et al., 2003). Based on these findings, it is reasonable to believe that there is an interplay between insufficient sleep, gut microbiome and the host metabolism.

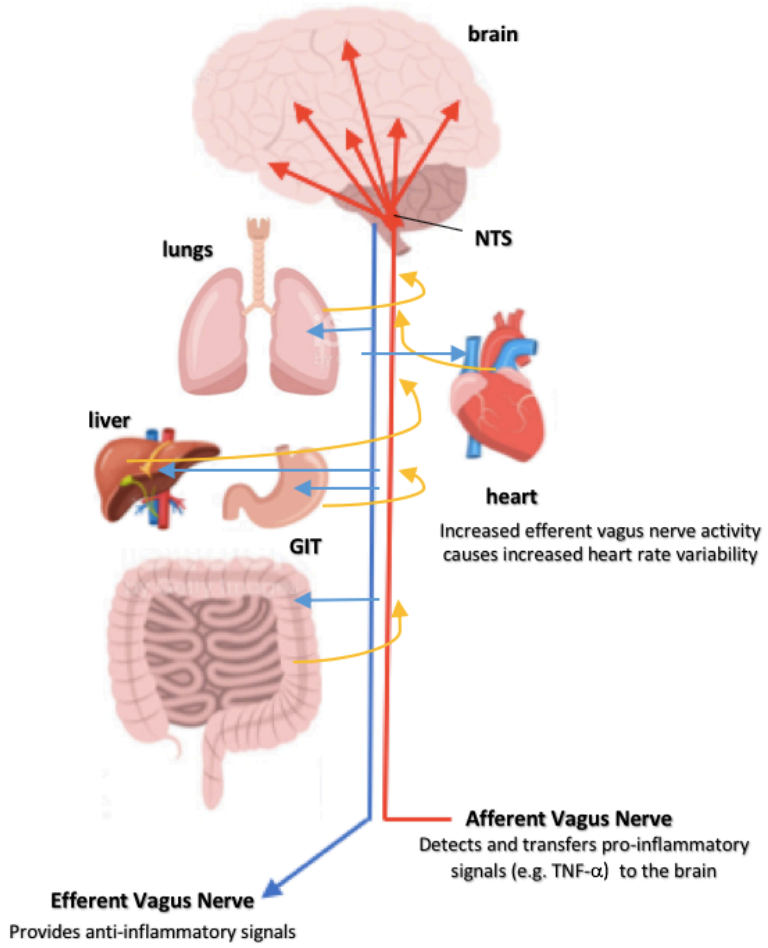

Figure 1: Connection between the brain and the gastrointestinal tract (GIT). The vagus nerve establish one of the important connections between the brain and the GIT. Via afferent fibers it sends information about the inner organs to the brain. The vagus nerve plays an important role in the brain, the gut, and immune system, as the gut is an important control center of the immune system and the vagus nerve has immunomodulatory properties (Breit, Kupferberg, Rogler & Hasler, 2018). Inflammatory response is regulated through efferent and afferent vagus nerves, impacted by environmental factors, stressors and pathogens (Modified from Zielinski et. Al. 2019).

3.4 Effects of sleep deprivation on appetite-regulating hormone leptin and ghrelin

Leptin and ghrelin are two hormones that have been recognized to influence satiety and hunger. Leptin signalize energy sufficiency and satiety to the brain (J. M. Mullington, 2009). Ghrelin on the other hand, is an appetite stimulating hormone (Klok, Jakobsdottir, & Drent, 2007). Leptin shows a regular diurnal rhythm, with maximal levels at night during sleep. Leptin amplitude is reduced with a lowered nocturnal peak concentration during total acute and prolonged partial sleep deprivation. Additionally, ghrelin levels and appetite are elevated during reduced sleep (J. M. Mullington, 2009).

Broussard and colleagues found significantly elevated breakfast- and dinner-related ghrelin levels peaks during sleep restriction compared to normal sleep, in nineteen healthy men. There was also a positive correlation between the increased evening level of ghrelin and higher consumption of calories. On the other hand, sleep restriction did not alter the 24 h leptin profile. In the review article of Mullington with colleagues, they reported a significant increase of ghrelin during partial sleep deprivation in a study of 12 healthy men. Sustained partial- and acute sleep deprivation led to reduced diurnal rhythm amplitude and peak amount of leptin in peripheral circulation (Janet M Mullington et al., 2009; Karine Spiegel, Tasali, Penev, & Cauter, 2004).

Lately, newer studies and reviews including meta-analysis have reported differently. Zhu and colleague review a comprehensive meta-analysis of studies conducting profile analyses of ghrelin and leptin. The majority of the studies in the meta-analysis (consisting of 11 studies) did not find a significant effect of sleep restriction on hourly profiles of leptin (Zhu et al., 2019). Similarly, no corresponding changes in ghrelin were observed. It is worth mentioning that several factors may account for differences between studies in results related to leptin and ghrelin, such as diet, energy balance and sex. However, only two studies reported an increased ratio of ghrelin to leptin under partial sleep restriction (PSR). Spiegel and colleagues is one of the two studies showing a significant increase of ghrelin and decrease of leptin referred to in Zhu and colleagues comprehensive review (Karine Spiegel et al., 2004; Zhu et al., 2019).

As shown, the effect of sleep restriction on ghrelin and leptin levels has been conflicting. Even though Mullington and colleagues reported an increase in ghrelin and decrease in leptin, Zhu and colleagues did not find significant impacts of restricted sleep on total appetite-regulating hormones in young, healthy adults. By this, it is not possible to say whether leptin or ghrelin is altered due to sleep restriction.

3.5 Sleep deprivation-induced reduction in glucose metabolism and increased insulin sensitivity

Normally, glucose levels in blood are tightly regulated with a narrow range to avoid hyper-/hypoglycemia and the associated impairment of the central nervous system, as well as to prevent the resulting adverse and eventually life-threatening effects. To maintain glucose

homeostasis, there is a balance between glucose utilization by tissues such as the brain and muscles and glucose production by the liver (K. Spiegel, Knutson, Leproult, Tasali, & Van Cauter, 2005). Insulin is essential for glucose homeostasis as it allows cells digest and then metabolize glucose. In the absence of insulin, plasma glucose concentration may be high but the cells are effectively glucose deprived as the glucose cannot enter the cell (Hinson, Raven, & Chew, 2010). Insulin resistance, or reduced insulin sensitivity occurs when higher levels of insulin are needed to reduce blood glucose levels after the administration of the same amount of exogenous glucose as in normal condition.

In normal individuals, blood levels of glucose remain stable or fall minimally during overnight sleep (Van Cauter, Polonsky, & Scheen, 1997). On the other hand, short-term physiological studies have reported an association between inadequate sleep and slowed glucose metabolism, suggesting that sleep is an important homeostatic regulator of factors contributing to increased risk for development of cardiovascular disease and metabolic syndrome (Gonzalez-Ortiz, Martinez-Abundis, Balcazar-Munoz, & Pascoe-Gonzalez, 2000; J. M. Mullington et al., 2003). Insufficient sleep duration has been associated with impaired glucose tolerance in woman and men, and the development of diabetes in women (Ayas et al., 2003). According to Gonzalez-Ortiz and colleagues, glucose metabolism is slowed down during both total- and partial sleep deprivation (Gonzalez-Ortiz et al., 2000; J. M. Mullington et al., 2003). Of notes, one night of sleep deprivation is reported to decrease waking glucose metabolism in multiple brain regions (Zhu et al., 2019). Reduced glucose utilization in important brain regions could possibly result in increased perception of hunger (Chaput & Tremblay, 2009). Based on these findings, it may indicate that nocturnal glucose levels are lower during sleep deprivation than during normal sleep.

Yet another mechanism for decreased insulin sensitivity may be via the changes in the thyroid stimulating hormone (TSH). The thyroid gland produces and secretes thyroid hormones that influence many aspects of reproduction, cell differentiation, growth, and metabolism (Norris & Carr, 2013). TSH is the major regulator of thyroid gland growth and function (Gordon & Ridgway, 2016). As is described elsewhere in this review, sleep plays an important role in thermoregulation. Sleep deprivation represents not only loss of sleep, but also reduction in the normal nocturnal drop in both BP and body temperature. Short term sleep deprivation seems to elevate TSH (Janet M Mullington et al., 2009). In addition to regulate metabolic rate, heart contractility and cardiac output, the thyroid hormones raise the rate at which the GIT absorbs

glucose, which may increase insulin resistance. In a comprehensive review of Zhu and colleagues, PSR resulted in a significant decrease in insulin sensitivity in five studies. Five of eight studies resulted in a significant increase in insulin resistance (Zhu et al., 2019). Zhu and colleagues support Mullington and colleague's suggestion that sleep deprivation may increase insulin resistance. Even though the increased TSH level is small, it may nonetheless contribute to development of disease in general (Janet M Mullington et al., 2009).

3.6 Sleep deprivation and the immune system

Sleep is a biological need, and adequate sleep duration and quality help maintain immune health and homeostasis. It is impacted by crosstalk between the brain and the immune system, where cytokines alter sleep patterns during infections, e.g. inducing sleep to conserve energy for the host defense (Lorton et al., 2007). Anti-inflammatory signals are provided from the brain and NTS to organs throughout the body via efferent nerves, while afferent nerves detect and transfers pro-inflammatory signals to the brain (figure 1). Of notes, studies have shown that infections increase sleep through induction of pro-inflammatory cytokines, while anti-inflammatory cytokines have the opposite effect (Bryant, Trinder, & Curtis, 2004), and that sleep protects the organism from being infected by bacteria and normally harmless bacteria from the natural flora of the body (Luciana Besedovsky et al., 2019). The CNS is in charge of signaling target cells of the immune system through autonomic and neuroendocrine pathways, and in this manner, it modulates the immune system and cytokine secretion. Interestingly, cytokines from the immune system, particularly interleukin (IL)-1β and tumor TNF-α, also regulates sleep by signaling neuroendocrine, autonomic, limbic and cortical areas of CNS to modify behavior, by hormone release and autonomic function (Lorton et al., 2007).

IL-1β and TNF-α are considered sleep regulators. To become sleep regulators, the substances have to fulfill certain demands. These demands include that the substance and its receptor oscillates with sleep propensity, when substance is administrated the sleep amount either increase or decrease, the substance changes sleep if natural production/activity is inhibited, the substance levels are changed during infectious state, and it acts on known regulatory sleep circuits. Many chemical substances fill several of these demands, but only few cover them all (Clinton, Davis, Zielinski, Jewett, & Krueger, 2011). IL-1β and TNF-α have been proven to be sleep regulating substances by administration of IL-1β- and TNF-α-antagonists to inhibit their biological action. Administration of IL-1β- and TNF-α-antagonist resulted in reduction

in physiological NREM-sleep amount or NREM rebound sleep after sleep deprivation. Interestingly, NREM-sleep amount and intensity was increased, and REM-sleep amount was suppressed, when promoting IL-1β and TNF-α availability (Luciana Besedovsky et al., 2019). Hence, IL-1β and TNF-α are sleep regulator substances.

Muramyl peptide is a decomposition product from bacterial cell walls, and functions both as a somnogen and pyrogen. The somnogen is produced during phagocytosis of the macrophage, which results in IL-1β and TNF-α secretion (Majde & Krueger, 2005). Decomposition of normal flora gut bacteria increases circulating muramyl peptide, suggesting that the somnogen are acting as physiological regulators of sleep (Krueger & Majde, 1994). Of notes, animal models proved that muramyl peptide contribute to homeostatic regulation of SWS, and IL-1β and TNF-α mediates infections challenge response through increased SWS (Majde & Krueger, 2005). TNF-α role in NREM sleep has been studied thoroughly by Takahashi and colleagues, and they have shown that TNF-α induces SWS in a variety of species (Takahashi, Kapas, & Krueger, 1996). In addition, when animals are treated with anti-TNF-antibodies, TNF-binding proteins, soluble TNF receptors or TNF receptor fragments, SWS decreases in normal animals (Takahashi, Kapas, Fang, & Krueger, 1995). These findings support the sleep regulating role of TNF-α increasing NREM sleep and SWS. Studies also show that, by assessing TNF-α mRNA and proteins, TNF-α follows circadian rhythm, while IL-1 mRNA and proteins in brain and plasma follows diurnal patterns and sleep wake-cycle (Lorton et al., 2007). In addition, after prolonged wakefulness, IL-1β mRNA and proteins are elevated which creates the suggestion that IL-1β takes part in the homeostatic sleep process (Mackiewicz, Sollars, Ogilvie, & Pack, 1996). Accordingly, mentioned studies give the impression that muramyl peptide regulates SWS and NREM sleep, inducing secretion of IL-1β and TNF-α which are suggested to take part in the homeostatic sleep process. A recent study by Zielinski et al., (2017) reported that sleep deprivation enhances the nucleotide-binding domain and leucine-rich repeat protein-3 (NLRP3) inflammasome protein complex formation and in turn caspase 1 enzyme is activated which converts inactive IL-1β to its active form. This might be one of the mechanisms by which the expression of IL-1β is increased following sleep deprivation and promotes homeostatic sleep response. NLRP3 knockout mice fail to show such response (Zielinski et al., 2017).

Sleep response to infections challenges is mainly triggered by IL-1β and TNF-α, and as key mediators, they upregulate activity and intensity [i.e. slow-wave activity (SWA)] of NREM sleep and downregulates REM sleep (Majde & Krueger, 2005). Furthermore, endotoxin injection studies found that increased SWA in NREM sleep is response dependent, whereas a mild infection response will enhance NREM sleep and a strong immune response may lead to fever and sleep disruption (Haack, Schuld, Kraus, & Pollmacher, 2001; J. Mullington et al., 2000).

All in all, NREM sleep is of great significance when it comes to regulating immunological homeostasis and functions (Zielinski, Systrom, & Rose, 2019). SWS induces endocrine and immunological changes via an optimal endocrine milieu that supports the adaptive immune system with optimal conditions for, among others, antigen presenting cell (APCs)-T cell interaction and T cell migration (Luciana Besedovsky et al., 2019). Consistently, growth hormone and prolactin release is also elevated during SWS and nocturnal sleep, which support cell mediated immunity through Th1-cell promoting effects (Matera, Mori, Geuna, Buttiglieri, & Palestro, 2000).

3.7 Sleep disturbances and detrimental effects

Chronic sleep disturbances have harmful effects on health and life expectancy. According to Bollinger et al. there is an association between long sleep duration and reduced levels of infections, and that sleep improves the immune response (Thomas Bollinger et al., 2010). This has been supported by a study of Cohen and colleagues, where participants with reported short sleep duration in the weeks before administration of nasal drops containing rhinovirus, had an increased risk of acquiring a clinical cold (Cohen, Doyle, Alper, Janicki-Deverts, & Turner, 2009). Furthermore, the findings have been similar in other studies where short sleep duration was measured objectively with actigraphy, with results as increased susceptibility to the common cold, influenza and gastroenteritis (Orzech, Acebo, Seifer, Barker, & Carskadon, 2014; Prather, Janicki-Deverts, Hall, & Cohen, 2015). On the contrary, a recent cohort study showed no association between shorter sleep duration and quality and self-reported upper-respiratory tract infections (Ghilotti et al., 2018). In addition to short sleep duration, sleep duration for more than 9 hours has also been reported to increase risk of infection (Patel et al., 2012), but not enough studies have yet been conducted to make any conclusion, and the increased risk may descend from subclinical conditions. All in all, adequate sleep duration

can improve infection outcome and is associated with reduced infection and diseases risk (Luciana Besedovsky et al., 2019).

One of the reasons sleep disturbance and SD has detrimental health effects, is immune function alterations. Prolonged wakefulness increases, among others, IL-1β mRNA and proteins in brain and plasma (Mackiewicz et al., 1996), and dysregulation of cytokines and sleep regulators may alter nighttime sleep and give daytime fatigue and sleepiness (Lorton et al., 2007). Acute SD alters immune functions by affecting natural killer (NK)-cell activity, antigen uptake, phagocytosis, mitogen responses, circulating immune complexes and IgG (immunoglobulin G) levels, secondary antibody response, the number of T cell subset and cytokine production (Luciana Besedovsky et al., 2019; Thomas Bollinger et al., 2010; Lorton et al., 2007).

Furthermore, the complement system has an important immunological function consisting of plasma proteins that through a cascade of reactions creates the terminal complement complex (TCC) to attack pathogens. One of the steps in the cascade is the split of complement factor 3 (C3) into C3a and C3b. C3b takes a part in building the TCC, while C3a operates as an anaphylatoxin. Hence, inappropriate activation of the complement system is unfortunate, and studies has shown an association between complement activation and diseases such as rheumatic arthritis (RA), Alzheimer's disease and sepsis (Holers, 2014). After acute SD in a human study, C3 levels in plasma were found to be elevated (Hui, Hua, Diandong, & Hong, 2007). Similarly, an animal study showed elevated C3 levels after REM-sleep deprivation for 96 hours (Zager, Andersen, Ruiz, Antunes, & Tufik, 2007). However, another study in humans found that plasma levels of C3 seemed to be more elevated during sleep compared to nocturnal wakefulness (Reis et al., 2011). In contrast, other studies did not find elevated C3 levels or any association between sleep disturbance and inappropriate complement activation (Reis et al., 2011; Ruiz et al., 2012) , and no conclusion with respect to this matter can be drawn.

Cell adhesion molecules play an important part in cell-to-cell and cell-to-extracellular matrix interactions. In the immune system, where leukocyte migration is a significant process, cell adhesion molecules contribute to circulating immune cells and endothelial cell interactions. Studies show that soluble cell adhesive molecules as E-selectin and intracellular cell adhesive molecule-1 (ICAM-1) was increased following one night without sleep, while vascular cell

adhesive molecule-2 (VCAM-2) remained unchanged (Frey, Fleshner, & Wright, 2007; Sauvet et al., 2010). Sauvet et al. suggest that the increases are enhancement of pro-inflammatory response and endothelial activity and can be connected to vascular disease development (Sauvet et al., 2010). Another study found that sleep deprivation between 11PM and 3AM prevented increase of macrophage associated antigen (Mac-1) positive lymphocytes, this may imply that sleep restriction may impair immune cells ability to migrate to current sites after infection. L-selectin positive lymphocyte and monocyte availability was on the other hand enhanced during nocturnal sleep disturbances compared to normal sleep (Redwine, Dang, & Irwin, 2004). The lower levels of L-selectin during normal sleep may be caused by cell activation, because the immune cells shed L-selectin when they are activated. However, L-selectin is the first step in the migration cascade, and reduced cell expression may also impair the cells migration potential. All in all, sleep disturbances may impair immune functions through reduced migration capacity, but different cellular expressions should be interpret with caution (Luciana Besedovsky et al., 2019).

Sleep deprivations effects on the immune system can also be seen through vaccination response. Studies show that normal sleep patterns reduce TNF-α producing CD8 positive T cells, and enhance vaccine response through primary antibody production (Dimitrov, Lange, Tieken, Fehm, & Born, 2004; Lange, Perras, Fehm, & Born, 2003). In other words, there are less produced antibodies after sleep deprivation, as shown in a study based on sleep deprivation and Hepatitis A vaccination (Lange et al., 2003). Furthermore, another study found that sleep restriction for four days prior to Influenza vaccination resulted in impaired antibody response (Thomas Bollinger et al., 2010). An Influenza vaccine study was also performed with mice, where the mice were kept gently awake for seven hours after recent immunization. The sleep deprived mice had lower Influenza specific antibody-titer compared to mice with normal sleep, and in addition, sleep deprived mice were not able to achieve viral clearance when exposed to virus (Brown, Pang, Husband, & King, 1989). These findings suggest that sleep contributes to the memory phase of the immune system, but studies show that sleep affects the effector phase as well. Thus, sleep enhances numbers of antigen specific CD4 positive T cell and their production of effector cytokine interferon-γ (IFN-γ) (Lange, Dimitrov, Bollinger, Diekelmann, & Born, 2011). The cytokine IFN-γ plays and important role in modulating and inducing immune response. And lastly, some studies did not find any alterations in vaccine response after sleep deprivation (Renegar, Crouse, Floyd, & Krueger,

2000; K. B. Renegar, R. Floyd, & J. M. Krueger, 1998; K. B. Renegar, R. A. Floyd, & J. M. Krueger, 1998; Toth & Rehg, 1998). This leads to the suggestion that sleep supports the development of immunological memory to a new antigen, instead of interfering with the existing immunity (Luciana Besedovsky et al., 2019).

Chronic sleep disturbances and habitual short sleep duration are linked to a variety of diseases such as: cardiovascular diseases, metabolic diseases, chronic pain conditions, some forms of cancer, and neuropsychiatric disease (M. R. Irwin, 2015). All of these conditions have immune dysregulation in common, e.g. upregulating inflammatory factors such as IL-6 and C-reactive protein (CRP) (Luciana Besedovsky et al., 2019). For longer periods of time, sleep loss enhances activation of inflammatory pathways and may reduce immune cells functional capacity (Aho et al., 2013; Moller-Levet et al., 2013; Pellegrino et al., 2012). Interestingly, studies have proven that sleep needs are individual, and that short sleep duration may be optimal for the immune functions in some individuals. For instance, a study on participants with less than 5 hours of habitual sleep, reporting complaints about poor sleep quality, had an increased risk of developing respiratory infections compared with participants with 7-8 hours of habitual sleep (Patel et al., 2012). Simultaneously, participants with less than 5 hours of habitual sleep, which had no complaints about sleep quality, did not have higher risk of containing infection. The study was conducted through self-reported habitual sleep and questionnaires. In the end, the different studies may imply that there is a link between habitual short sleep duration and increased infection risk, as self-reported short term-sleep increases risk of respiratory infection, including elevated inflammatory factors and impaired immune cell capacity. However, other studies findings create the suggestion that sleep needs are individual, and that habitual short sleep duration may be adequate for some individuals.

3.8 Circadian clocks and signaling

The master circadian clock is located in the suprachiasmatic nucleus (SCN) and interplays with peripheral immune cells through the sympathetic nervous system (Thomas Bollinger et al., 2010). The SCN communicates with other circadian clocks located in immune cells through hormonal release (e.g. melatonin, glucocorticoids) and body temperature rhythms, signaling via the autonomous system (Thomas Bollinger et al., 2010). Studies shows that leukocytes have a rhythmic expression of clock genes that are associated with sleep-wake-patterns (Archer, Viola, Kyriakopoulou, von Schantz, & Dijk, 2008; Arjona & Sarkar, 2006;

Hayashi, Shimba, & Tezuka, 2007). The function of the biological clocks is that every cell has clock-related genes coding for mRNA and their respective clock-proteins making them able to set time – this is mainly regulated by neurons in the SCN. However, the details of the communication setting the time in every cell in the body as well as the brain, is not fully known. Schibler and Sassone-Corsi suggest that cells in central and peripheral tissues are synchronized with external light/dark cycle (zeitgeber), and are reset by non-photic cues as food-availability, ambient temperature and social interactions (Paul, Pyter, Freeman, Galang, & Prendergast, 2009; Schibler & Sassone-Corsi, 2002). Nonetheless, it is known that sleep deprivation and disturbances disrupt this process, and the immune-related cell clocks might become desynchronized, followed by dysregulated immune response.

At molecular levels, the cellular clocks are generated by autonomous cellular oscillators and transcriptional/ translational auto-regulatory feedback loops, where protein products of clock genes negatively feedback on their own transcription (Reppert & Weaver, 2002). Mutation of clock genes may result in sleep disorders, e.g. a study on animal models and cell cultures found expression of light-induced genes in rat-1 fibroblast that normally accumulates in SCN and/or liver, when cell cultures of rat-1 fibroblast was treated with high concentrations sera (here: nutritious media containing 50% serum from horse, pig, rat or cow) (Balsalobre, Damiola, & Schibler, 1998). The current genes were *rper1* and *rper2* [rat PER (Period circadian protein) 1 and 2], and were recorded with RNase protection 72 hours following the serum treatment. At first. the expression was induced, and then it was repressed. The study shows the existence of circadian clocks in peripheral tissue cells, and that factors in serum can reset or adjust peripheral clocks. PER 1 and 2 are transcriptional repressors, acting as circadian modulators of physiological functions including metabolism, sleep, body temperature, blood pressure, endocrine-, immune-, cardiovascular- and renal function. Due to PER 1 and 2 important functions, it is clear that interference in gene expressions gives consequences, and might lead to sleep disorders.

The circadian rhythm of sleep is regulated by complex interactions between neuronal, endocrine and immune system (Lorton et al., 2007), where immune cells are able to modulate sleep and circadian clocks (Thomas Bollinger et al., 2010). However, this work both ways, as sleep and circadian clocks also modulates immune cells and function, for instance, sleep restriction has the ability to affect the circadian rhythm of genes with respect to immune and inflammatory responses (Archer & Oster, 2015; Moller-Levet et al., 2013). Leukocyte

distribution is very affected by circadian rhythm, and the alterations in leukocyte counts is a part of the homeostatic mechanism (Cermakian et al., 2013; Scheiermann, Kunisaki, & Frenette, 2013). This has been shown through studies where total monocyte counts, and lymphocyte counts and subsets [i.e. B cells, CD4 and CD8 T cells, and natural killer (NK)-cells] has been reduced by sleep and/or increased with sleep deprivation, compared with acute or prolonged sleep reduction (L. Besedovsky, Dimitrov, Born, & Lange, 2016; Born, Lange, Hansen, Molle, & Fehm, 1997; Dimitrov, Lange, Nohroudi, & Born, 2007; Ingram, Simpson, Malone, & Florida-James, 2015; M. Irwin et al., 1996; Ruiz et al., 2012; Wilder-Smith, Mustafa, Earnest, Gen, & Macary, 2013). Other studies have found leukocyte counts unaltered (Boudjeltia et al., 2008; Chennaoui et al., 2017; Ricardo et al., 2009). Furthermore, studies with measurements in the evening following a night of normal sleep, have also found increased numbers of CD4 T cells, CD8 T cells and NK-cells (van Leeuwen et al., 2009; Wilder-Smith et al., 2013). The alterations are normally leukocyte redistribution to different tissues and organs, because the changes are detectable after 3 hours of sleep, whereas proliferation changes need more time to become measurable (Luciana Besedovsky et al., 2019). However, leukocyte alterations are clearly detectable, suggesting a link between circadian rhythms and immune functions.

As previously mentioned, sleep deprivation might desynchronize circadian immune clocks, which is detrimental for immunological and metabolic homeostasis, as sleep is an important regulator of these functions (Hastings, O'Neill, & Maywood, 2007; Vgontzas et al., 2004). Bollinger et al. predict that immunity on cellular levels are driven by rhythms sustained by the sympathetic nervous system and body core temperature, synchronized by peripheral circadian clocks. Furthermore, the peripheral immune clocks need signals from SCN to keep up clock synchrony with other cells such as leukocyte sub-populations (Thomas Bollinger et al., 2010). Short term sleep deprivation/disruption will only have minor effects on immune cell clock synchrony, due to excessive stabilization of the clocks by synchronizing factors in the sympathetic nervous system, e.g. body core temperature. However, the circadian immune rhythm will be affected through sleep-modulated signals (Hastings et al., 2007), i.e. hormonal priming of immune cells by prolactin and growth hormone. Bollinger et. al. also suggest that long-term sleep deprivation/restriction may desynchronize the rhythm between immune clocks, resulting in disarranged immune function and, consequently, deregulated immune responses (Thomas Bollinger et al., 2010). Hence, disrupted circadian rhythms at immune cell levels are a consequence of sleep deprivation and restrictions.

Sleep disturbances affects both endocrine and physiological circadian rhythms, which several studies have investigated. For instance, studies found circadian rhythms modulated by sleep in immune cells, such as neutrophils, monocytes, dendritic cells, NK-cells, B cells, T cells, and regulatory T cells, and other studies found no modulation of neutrophils and regulatory T cells (T. Bollinger et al., 2009; Dimitrov et al., 2007; Lange, Dimitrov, Fehm, Westermann, & Born, 2006). However, most studies found that the existing circadian rhythm was flatten by sleep deprivation/restriction. Another study found that the rhythmic activity that suppress detrimental immune response, with respect to regulatory T cells, was only found during normal sleep compared to sleep deprivation, as cytokine secretion by T cells are not altered by sleep, but follows circadian rhythm (Thomas Bollinger et al., 2010; T. Bollinger et al., 2009). Consequently, unessential immune response over a certain amount of time might lead to chronic low-grade inflammation, which will be discussed after the next section. Furthermore, the study measured alterations between normal sleep and 1 night of sleep deprivation on T cell proliferation, and serum levels of prolactin, cortisol and IL-2. Findings indicated that circadian rhythms of prolactin and T cell proliferation are significantly influenced by sleep, in contradistinction to circadian rhythms of cortisol and IL-2 (Thomas Bollinger et al., 2010)

3.9 Sleep and the stress system

The hypothalamic pituitary gland (HPA)-axis represents the central stress system in mammals, and its main function is to maintain homeostasis (Lorton et al., 2007). Environmental impacts operate as stressors and activate the hypothalamic pituitary gland axis (HPA-axis) and sympathetic nervous system (SNS) activity, resulting in increased circulating stress hormones. Furthermore, the immune response regulates internal homeostasis through stress mediators, immune cells and regulatory cytokines. One of the main regulators of the HPA-axis is corticotrophin releasing hormone (CRH). CRH released from the paraventricular nucleus results in the enhancement of adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, followed by induced secretion of the stress hormone, cortisol (Lorton et al., 2007).

Acute sleep deprivation is considered a stressor and a potent stimulus of stress hormones and dysregulation of the stress system (Lorton et al., 2007). Normally, SWS conduct an inhibitory effect on the HPA-axis and cortisol secretion (Weitzman, Zimmerman, Czeisler, & Ronda,

1983). Consistently, a study on nocturnal cortisol secretion in healthy men, exhibited elevated plasma cortisol levels following disrupted sleep by frequent arousal (Spath-Schwalbe, Gofferje, Kern, Born, & Fehm, 1991). When the HPA-axis is activated, it may also support or suppress inflammatory response and adaptive immune response in humans. The intervention is dependent on other factors, such as if the activation was before or after the immunological challenge (Rohleder, 2012), but all in all, the stress systems part in suppressing inflammatory processes is crucial, and prolonged sleep loss might impair this function (Meerlo, Sgoifo, & Suchecki, 2008). Studies provide results from the effect of pro-longed sleep loss, where the interplay between inflammatory- and stress markers at cell level was dysregulated, i.e. IL-6 levels where elevated due to monocytes were less sensitive to counter-inflammatory cortisol. Normally, cortisol partly operates as a negative feedback mechanism to execute appropriate termination of stress response via inhibition of monocytes and other immune cells, in the production of inflammatory markers, e.g. IL-6 (Simpson et al., 2016). In other words, inadequate immune response will not be suppressed, because as Simpson et al. suggest, for optimal adaptation to stressors, sleep is required. Also, if the reactivity to physiological or psychological challenges is inadequate, increased disease vulnerability may occur (Luciana Besedovsky et al., 2019). Sleep disturbances might have an alternating effect on inflammatory response, because of how inflammatory markers are activated by stressful challenges (Meerlo et al., 2008). For example, poorer sleep quality was associated with higher IL-6 levels following psychosocial stress in postmenopausal women (Prather, Epel, Cohen, Neylan, & Whooley, 2013). In addition, another study found that higher levels of IL-6 was reported in men and postmenopausal women age 50 years and older after a series of cognitive challenges, who also reported poor sleep quality (Heffner et al., 2012). Finally, there is an apparent connection between the activation of the HPA-axis and increased inflammatory response through IL-6 and CRP secretion. Considering acute sleep deprivation is a stressor, it may also be presumed that there is a connection between sleep deprivation and increased inflammatory response as well.

3.10 Chronic low-grade inflammation

It is known that acute sleep disturbance has detrimental effects on the immune system and health in general. Therefore, it is no surprise that chronic sleep disturbances may lead to severe health consequences and are associated with diseases such as RA, fibromyalgia, chronic fatigue syndrome, obesity and diabetes type 2. During sleep, the body is kept at

homeostasis through a variety of biological mechanisms and processes, and this include the immunological homeostasis. Homeostatic mechanisms normally terminate immune response after the clearance of a pathogen or whenever tissue damage has been repaired, and provide homeostasis through restoration and regulation of natural fluctuations in cytokine production and sleep (Lorton et al., 2007). However, if inflammatory response consists due to fail in obtaining homeostasis, chronic low-grade inflammation may occur. Sleep counteracts low-grade inflammation, which makes sleep loss a cause of inflammatory dysregulation, with increase in leukocyte counts and pro-inflammatory cytokines, and resulting in low-grade systemic inflammation (Luciana Besedovsky et al., 2019).

Sleep disturbance, a stressor and a trigger of low-grade inflammation, is related to cellular stress and activation of pattern recognition receptors (PRRs) on immune cells. PRRs are located at tissue resident macrophages and dendritic cells (DCs) and recognize immune challenges through innate immune sensors that are encoded in the germline, and activate immune response through pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). PAMPs are conserved from microbes like bacteria or viruses [e.g. bacterial lipopolysaccharide (LPS); viral stranded ribonucleic acid (RNA)], while DAMPs are released with stress or cell injury [e.g. heat-shock proteins (HPS)] (Chu & Mazmanian, 2013). When immune cells are activated, PRRs activate inflammatory signaling pathways, followed by release of acute phase cytokines, such as IL-1β, IL-6, and TNF-α, interferons (IFN), vasoactive mediators like prostaglandins (PG), and chemotaxis (chemokines that attract leukocytes) (Luciana Besedovsky et al., 2019). Throughout the wake or waking state, the individual comes across multiple environmental factors that may initiate inflammatory response through release of PAMPs and DAMPs. Besedovsky and colleagues suggests, through their review article, that sleep loss promotes low-grade inflammation as a result of more activated PRRs in wakeful state than during sleep. This suggestion is supported by the fact that amyloid-β (Aβ) is a DAMP type of molecule (Clark & Vissel, 2015) released from neurons regulated by synaptic activity, which increases during the day and decreases during the night (Kang et al., 2009). Besedovsky and colleagues also mention that the decrease of Aβ may be caused by SWS-dependent processes e.g. increased sleep or deep-sleep/NREM that counteract inflammation in the brain (Luciana Besedovsky et al., 2019). During sleep, it is assumed that the glymphatic system flushes away the current DAMP from the brain parenchyma, which is a system that eliminates soluble metabolites and proteins from

the CNS especially at night when at sleep. Sleep disturbance may cause inadequate clearance of such DAMP type of molecules as Aβ is in the glymphatic system, hence lead to neuro-inflammation and eventually neurodegenerative diseases, such as Alzheimer's disease (Boespflug & Iliff, 2018). All in all, sleep supports the immunological homeostasis, while sleep loss and disturbances prompts excessive inflammatory response.

Inflammatory markers that are frequently used while assessing low-grade inflammation are, among others, IL-6 and CRP. CRP is a response to IL-6 secretion, and IL-6 secretion is a result in inflammatory response to sleep loss. Several studies have reported elevated IL-6 and/or CRP levels with shorter sleep duration or habitual short sleep (Luciana Besedovsky et al., 2019). For instance, a cohort study based on the longitudinal Whitehall II study in over 5000 middle aged adults reported higher levels of IL-6 and CRP associated with shorter sleep duration (Ferrie et al., 2013; M. R. Irwin, Olmstead, & Carroll, 2016). Dysregulation of the inflammatory markers are assumed to be linking sleep duration and mortality, if assuming inflammatory markers are independent predictors of mortality risk in older populations (Bruunsgaard et al., 2003). Another association that has been reported is between dysregulation of cytokines, including IL-6, and fatigue, as can be seen in the result of developing diseases such as chronic fatigue syndrome (J. M. Mullington, Hinze-Selch, & Pollmacher, 2001). CRP is also a clinically useful marker for cardiovascular risk prediction, as there is an association between deficient sleep and increased cardiovascular diseases (Grandner et al., 2016).

4. Conclusion

Growing evidence from several experimental and epidemiological studies have shown that metabolic functions is tightly linked to sleep quantity and quality. Studies have provided greater insight into the mechanism underlying the relationship between insufficient sleep and human metabolism. In this short review, we present data showing a significant increase in blood pressure as a result of sleep deprivation. However, the underlying mechanism of increased BP is not fully known, and further research is required. Previous findings also suggest that activation of the endocannabinoid system could potentially be linked to excessive food intake during restricted sleep and might possibly contribute to the increase risk of obesity. Additionally, it is reasonable to believe that there is a correlation between gut microbiome and metabolic imbalance as a result of insufficient sleep in the host organism. However, not enough studies have been conducted to make any conclusion on this issue. Even though the effect of sleep restriction on appetite-regulating hormones, leptin and ghrelin, have been conflicting, newer studies report no significant impact of restricted sleep on total appetite-regulating hormone level in young, healthy adults. Furthermore, a majority of epidemiological and laboratory studies have suggested that nocturnal glucose levels were lower during sleep deprivation compared to normal sleep, which may increase the risk of developing diabetes. Consequently, studies lead to the suggestion that sleep is an important homeostatic regulator of factors contributing to increased risk for development of diabetes and metabolic syndrome. Together this presumed mechanism may act in concert to result in the increased risk of obesity and diabetes in sleep deprived subjects. Thus, adequate sleep should be highly recommended to maintain good health among people, particularly to those at risk for obesity and diabetes.

Adequate sleep is also favorable when it comes to immunological functions. Sleep and the immune system are bi-directionally related, where adequate sleep quality and duration help maintain immune health and homeostasis. There is a correlation between slow-wave-sleep (SWS) and pro-inflammatory endocrine milieu, whereas many important immunological functions occur during SWS. IL-1β and TNF-α are considered sleep regulators and are involved in regulation of both SWS and NREM. Manipulation of sleep can affect immune markers, such as leukocyte migration and distribution, cytokine production, leukocyte activity and proliferation, complement activation, expression of cell adhesion molecules and immune related genes. In addition, sleep disruption results in less produced antibodies as vaccine

response, hence normal sleep enhances TNF-α producing CD8 positive T cells and antibody production. Leukocytes have a rhythmic expression of circadian clock genes that are associated with sleep-wake patterns. The clocks are genes coding for mRNA and proteins to set the time in peripheral immune cells, which are regulated by neurons in the master circadian clock, SCN. How the SCN communicates with peripheral cells are not fully know, however, studies have proven that leukocyte distribution is very affected by circadian rhythm, and that disruption of circadian processes through sleep loss desynchronize immune clocks, resulting in dysregulated immune response. Dysregulated immune response also occurs when the hypothalamic-pituitary-adrenal (HPA)-axis of the stress system is activated excessively. SWS conduct an inhibitory effect on the stress system, where the HPA-axis´ job is to either support or suppress inflammatory response. Consequently, sleep disturbances lead to inappropriate activation of immune response through the stress system, and disease vulnerability may increase. Adequate response to different challenges is important in every situation in the body, to such a degree that homeostasis is maintained. Homeostatic mechanisms terminate immune response after meeting immunological challenges, because if inflammatory response consists, low-grade inflammation may occur. Hence, sleep disturbances are linked to dysregulation of inflammatory markers and increased risk of disease.

Recommended sleep amount is 6 to 9 hours to maintain metabolic and immunologic homeostasis, and to sustain essential biological processes in the body. Sleep disturbances may lead to reduced glucose metabolism, dysregulation of the endocrine control of appetite and hunger, and dysregulation of host defense, immune responses, and increased infection risk. Consequently, sleep disturbances may result in a variety of lifestyle and/or autoimmune diseases. However, some studies have proven that sleep needs are individual, and that short sleep duration is adequate for some. Nonetheless, adequate amount of sleep is necessary to maintain an optimal functioning body, but all the details of why sleep is so important, is not yet fully known and more studies need to be conducted.

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