

### HPA AXIS AND SLEEP

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#### ABSTRACT

Sleep is an important component of mammalian homeostasis, vital for our survival. Sleep disorders are common in the general population and are associated with significant adverse behavioral and health consequences. Sleep, in particular deep sleep, has an inhibitory influence on the hypothalamic-pituitary- adrenal (HPA) axis, whereas activation of the HPA axis or administration of glucocorticoids can lead to arousal and sleeplessness. Insomnia, the most common sleep disorder, is associated with a 24-hour increase of ACTH and cortisol secretion, consistent with a disorder of central nervous system hyperarousal. On the other hand, sleepiness and fatigue are very prevalent in the general population, and studies have demonstrated that the pro- inflammatory cytokines IL-6 and/or TNF-alpha are elevated in disorders associated with excessive daytime sleepiness, such as sleep apnea, narcolepsy and idiopathic hypersomnia. Sleep deprivation leads to sleepiness and daytime hypersecretion of IL-6, whereas daytime napping following a night of total sleep loss appears to be beneficial both for the suppression of IL-6 secretion and for the improvement of alertness. These findings suggest that the HPA axis stimulates arousal, while IL-6 and TNF-alpha are possible mediators of excessive daytime sleepiness in humans. It appears that the interactions of and disturbances between the HPA axis and inflammatory cytokines determine whether a human being will experience deep sleep/sleepiness or poor sleep/fatigue.

#### NORMAL SLEEP

Sleep is an important component of mammalian homeostasis, vital for the survival of self and species. We, humans spend at least one third of our lives asleep, yet we have little understanding of why we need sleep and what mechanisms underlie its capacities for physical and mental restoration. Sleep has been proposed to play a fundamental role in the conservation, utilization, and reallocation of energy to maintain cellular homeostasis, anabolism, proper immune function, and normal neural plasticity (1, 2). In addition, sleep contributes substantially to the achievement of synaptic homeostasis by renormalization of the potentiation of synaptic connections that occurs during wakefulness, in order to support learning and memory functions (2-4). In spite of this though, there has been a significant increase of empirical knowledge that is useful in the evaluation and management of most sleep complaints and their underlying disorders (5). The interaction of circadian effects, i.e., usual time to go to sleep, and amount of prior wakefulness (homeostatic response), determines the onset and amount of sleep (6). Regulated by a strong circadian pacemaker, free running natural sleep-wake rhythms cycle at about 25 hours rather than coinciding with the solar 24-hour schedule (7). However, cues from the environment (zeitgebers) entrain sleep's rhythm to a 24hour schedule. As a result, persons depend on external cues to keep their diurnal cycle "on time." The normal diurnal clock resists natural changes in its pattern by more

than about 1 hour per day, which explains the sleep difficulties that usually accompany adaptation to new time zones or switches in work shifts.

Individuals differ considerably in their natural sleep patterns. Most adults in non-tropical areas are comfortable with 6.5 to 8 hours daily, taken in a single period. Children and adolescents sleep more than adults, and young adults sleep more than older ones. Normal sleep consists of four to six behaviorally and electroencephalographically (EEG) defined cycles, including periods during which the brain is active (associated with rapid eye movements, called REM sleep), preceded by four progressively deeper, quieter sleep stages graded 1 to 4 on the basis of increasingly slow EEG patterns (8) (Figure 1). Deep sleep or slow wave sleep (SWS) (stages 3 and 4) gradually lessens with age and usually disappears in the elderly.



Figure 1. Effects of age on normal sleep cycles. REM sleep (darkened area) occurs cyclically throughout the night at intervals of approximately 90 minutes in all age groups. REM sleep decreases slightly in the elderly, whereas stage 4 sleep decreases progressively with age, so that little, if any, is present in the elderly. In addition, the elderly has frequent awakenings and a notable increase in wake time after sleep onset.

#### SLEEP DISORDERS

Sleep disorders are common in the general population and are associated with significant behavioral and health consequences (9, 10). Insomnia, the most common sleep disorder, is often associated with psychologic difficulties (11-13) and significant cardiometabolic morbidity and mortality (14-24). Excessive daytime sleepiness is the predominant complaint of most patients evaluated in sleep disorders clinics and often reflects organic dysfunction. Sleep apnea, narcolepsy, and idiopathic hypersomnia are the most common disorders associated with excessive daytime sleepiness. Sleep apnea occurs predominantly in middle-aged men and post-menopausal women and is associated with obesity and cardiovascular complications, including hypertension, while narcolepsy and idiopathic hypersomnia are chronic brain disorders with an onset at a young age (5). In the general population, excessive daytime sleepiness and fatigue are frequent complaints of patients with obesity (13, 25, 26), depression (13, 27, 28) parasomnias, and diabetes (27). The includina sleepwalking, night terrors, and nightmares, have benign implications in childhood, but often reflect psychopathology or significant stress in adolescents and adults and organic etiology in the elderly.

#### SLEEP AND THE STRESS SYSTEM

In mammalian organisms, including human beings, the stress system consists of central and peripheral components, whose main function is to maintain homeostasis, both in the resting and stress states (29-31). The central components include: (i) the paraventricular nuclei (PVN) of the hypothalamus, which secrete corticotropin-releasing hormone (CRH and arginine vasopressin (AVP); ii) the CRH neurons of the paragigantocellular and parabranchial nuclei of the

medulla and (iii) the noradrenergic nuclei of the medulla and pons, including the locus caeruleus (LC), regulating arousal, and other nuclei mostly secreting norepinephrine (NE) and regulating the systemic sympathetic and adrenomedullary, as well as the parasympathetic nervous systems. The peripheral components include (i) the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis; (ii) the efferent systemic sympathetic and adrenomedullary (SNS) and parasympathetic nervous systems (31).

## Normal Sleep and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Although the association between sleep and stress has been noted for hundreds of years, a more systematic approach in the relation between several features of sleep and stress system activity has only taken place in the last two decades (Figure 2). In 1983, Weitzman and colleagues reported that sleep, in particular SWS, appears to have an inhibitory influence on the HPA axis and cortisol secretion (32). Since then, several studies have replicated this finding. In turn, central (intracerebroventricular) administration of CRH (33, 34) or systemic administration of glucocorticoids (35) can lead to arousal and sleeplessness. In normal individuals, wakefulness and stage 1 sleep (light sleep) accompany cortisol increases (36), while slow wave sleep or deep sleep is associated with declining plasma cortisol levels (37). In addition, in normals, induced sleep disruption (frequently repeated arousals) is associated with significant increases of plasma cortisol levels (38). Furthermore, mean 24-hour plasma cortisol levels are significantly higher in subjects with a shorter total sleep time than those with a longer total sleep time (39).





Figure 2. A simplified, heuristic model of the interactions between central and peripheral components of the stress systems with sleep and REM sleep. ACTH: corticotropin; AVP: arginine vasopressin; CRH: corticotropin-releasing hormone; GH: growth hormone; LC/NE: locus caeruleus-norepinephrine/sympathetic nervous system. A solid green line denotes promotion/stimulation; a dashed red line denotes suppression.

The amount of REM sleep, a state of central nervous system activation that resembles unconscious wakefulness (paradoxic sleep), appears to be associated with a higher activity of the HPA axis. An early study showed that 24-hour urinary 17-hydroxycorticoids were increased during REM epochs in urological patients (40). More recently, a study in healthy, normal sleepers showed that the amount of REM sleep was positively correlated with 24-hour urinary free cortisol excretion (41). These results are consistent with the co-existence of HPA axis activation, and REM sleep increases in patients with melancholic depression (30).

## Corticotropin Releasing Hormone and ACTH in Sleep/Wake Regulation

CRH produced and released from parvocellular neurons of the paraventricular nucleus is the key regulator of the HPA axis (31). Release of CRH is followed by enhanced secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary and cortisol from the adrenal cortex. In addition, CRH exerts various influences on behavior, including cerebral activation and waking maintenance, through activation of CRH receptors, which are expressed in the basal prosencephalic areas, thalamus, hypothalamus, mesencephalus, brainstem, and pons (42).

In animals, central (intracerebroventricular) administration of CRH induces increased waking (34, 43, 44), decreased NREM and REM sleep (44, 45), and altered locomotor activity (44, 46). Also, specific CRH antisense oligodeoxynucleotides caused a reduction in spontaneous wakefulness during the dark period, but not during the light period in rats (47). Several reports indicate that CRH is excitatory in the locus caeruleus (LC), amygdala, hippocampus, cerebral cortex, and some portions of the hypothalamus (30, 31). Spontaneous discharge rates in the LC are highest during arousal and lowest during sleep.

In humans, the majority of the studies suggest that the sleep of young individuals is rather resistant to the arousing effects of CRH (48-51). In contrast, middle-aged individuals responded to an equivalent dose of CRH with significantly more wakefulness and suppression of slow wave sleep compared to baseline (51). Based on these findings, we concluded that middle-aged men show increased vulnerability of sleep to stress hormones, possibly resulting in impairments in the quality of sleep during periods of stress. These findings suggest that changes in sleep physiology associated with middle- age play a significant role in the marked increase of prevalence of insomnia in middle-age. Also, peripheral administration of CRH is associated with a REM suppression, which is stronger in the young than in the middle aged.

The administration of ACTH and its analogues in humans has been associated with general CNS activation consisting of a decreased sleep period time and sleep efficiency, and an increase of sleep latency (39, 44). Continuous administration of ACTH produced a marked reduction in NREM sleep (44).

#### **Glucocorticoid Effects On Sleep**

The administration of glucocorticoids causes a robust suppression of REM sleep (44, 52). In addition to the wellestablished decrease of REM sleep in some studies, the continuous or pulsatile nocturnal administration of cortisol was paradoxically associated with a modest increase of SWS (48,53). It has been suggested that this effect of cortisol on SWS compared to CRH may be mediated by a feedback inhibition of CRH by cortisol. A study in Addisonian patients demonstrated that an evening replacement dose of hydrocortisone was necessary for proper expression of REM sleep, (vide infra) suggesting that glucocorticoids have some permissive action for this sleep parameter, possibly reflecting an inverse u-shaped dose response curve (54).

In clinical practice, the use of pharmacologic doses of glucocorticoids is associated with sleep disturbance. In fact, in a multicenter, placebo-controlled study in which steroids were used on a short-term basis, insomnia was one of the most common side effects (35).

In addition to their direct effects on sleep, glucocorticoids might also modulate sleep indirectly by influencing the activity of the circadian clock system (from the Latin "circa diem" meaning "approximately a day"), a highly conserved timekeeping system that creates internal rhythmicity under the influence of day/night cycles (55-57). At the cellular and molecular level, glucocorticoids influence peripheral clocks at multiple sites through their intracellular receptor, the glucocorticoid receptor (GR), which functions as a ligand-activated transcription factor (58). Besides this molecular cross-talk, accumulating evidence suggests that glucocorticoids might play a fundamental role in the entrainment of wake-sleep cycle rhythmicity through regulation of behavioral adaptation to phase shifts, possibly through an indirect feedback to the SCN (44, 59).

#### AGING, HPA AXIS AND SLEEP

Old age is associated with marked sleep changes consisting of increased wake, minimal amounts of SWS, declining amounts of REM sleep, and earlier retiring and rising times. Some studies have shown that older adults have elevated cortisol levels at the time of the circadian nadir and have higher basal cortisol levels than younger adults (60-62). It is difficult to discern whether the latter changes are associated with aging or increased medical morbidity common in this group. It has been suggested that the effect of aging on the levels and diurnal variation of human adrenocorticotropic activity could be involved in the etiology of poor sleep in the elderly (60). Higher evening cortisol concentrations are associated with lower amounts of REM sleep (60, 62), and increased wake (62). More recently, it was shown that older women without estrogen replacement therapy (ERT), when subjected to

mild stress, showed greater disturbances in sleep parameters than women on ERT (63).

#### SLEEP DEPRIVATION AND HPA AXIS

If sleep is important for our sense of well-being, then it is conceivable that sleep deprivation represents a stressor to human bodies and should be associated with activation of the stress system (64). However, several studies that have assessed the effects of one night's sleep deprivation on the HPA axis have shown that cortisol secretion is either not or minimally affected by sleep following prolonged wakefulness (65-67). More recent studies have reported somewhat antithetical results, with some studies showing that cortisol secretion is elevated the next evening following sleep deprivation (68-71) and the other studies showing a significant decrease of plasma cortisol levels the next day (72-74). Additionally, the study by Vgontzas et al (72) indicated that this inhibition of the HPA axis activity was associated with an enhanced activity of the growth hormone axis. Also, similarly to these inconsistent results from studies of total sleep deprivation, partial sleep loss (4 hours of sleep for 6 days) has been reported to be associated with evening cortisol elevation (75) while a modest restriction of sleep to 6 hours per night for one week was associated with a significant decrease of the peak cortisol secretion (76). Newer studies using different sleep restriction experimental protocols have also found inconsistent results, with the majority of them reporting no effect of sleep restriction on the HPA axis (77-82). Methodological differences primarily related on the way subjects were handled during deprivation may explain these opposing findings. The finding that sleep deprivation leads to lower cortisol levels post- deprivation (primarily during the subsequent night of sleep) suggests that lowering the level of HPA activity, which is increased in depression, may be the mechanism through which sleep deprivation improves the mood of depressed individuals. In one study, we assessed the effects of a 2-hour midafternoon nap following a night of total sleep deprivation on sleepiness, proinflammatory cytokines (IL-6, TNFα) and cortisol levels. Parameters of interest (subjective feeling of sleepiness, psychomotor vigilance-PVT, IL-6, TNFa and cortisol levels) were measured on the fourth (predeprivation) and sixth days (postdeprivation). We observed a marked and significant drop of cortisol levels during napping, which was followed by a transient increase during the postnap period (83). These findings suggest that sleep and particularly SWS has an inhibiting effect on cortisol secretion and that wake and alertness are associated with higher levels of cortisol.

Prolonged sleep deprivation in rats results in increased plasma norepinephrine levels, higher ACTH and corticosteroid levels at the later phase of sleep deprivation (84). It is postulated that these increases are due to the stress of dying from septicemia rather than to sleep loss. The effects of prolonged sleep deprivation on the HPA axis in humans have not been studied. It is possible that there is activation of stress response when a certain tolerability threshold has been reached.

#### SLEEP DISORDERS AND HPA AXIS

Although sleep disorders/disturbances with their various physical and mental effects on the individual should be expected to affect the stress system, information regarding the effects of sleep disorders/disturbances on this system is limited.

#### Insomnia and HPA Axis

Insomnia, a symptom of various psychiatric or medical disorders, may also be the result of an environmental disturbance or a stressful situation. When insomnia is chronic and severe, it may itself become a stressor that affects the patient's life so greatly that it is perceived by the patient as a distinct disorder itself. Either way, as a manifestation of stress or a stressor itself, insomnia is expected to be related to the stress system. Few studies have measured cortisol levels in "poor" sleepers or insomniacs, and those results are inconsistent. The majority of these studies reported no difference between controls and poor sleepers in 24-hour cortisol or 17-hydroxysteroid excretion (85). In a 1998 study in 15 young adult insomniacs, 24-hour urinary free cortisol (UFC) excretion levels were positively correlated with total wake time (86). In addition, 24-hour urinary levels of catecholamines and their metabolites DHPG and DOPAC were positively correlated with percent stage 1 sleep and wake time after sleep onset. However, the total amount of the 24-hour UFC or catecholamine excretion was not different from normative values.

These preliminary findings were confirmed and extended in a controlled study in which objective sleep testing and frequent blood sampling was employed; the 24-hour ACTH and cortisol plasma concentrations were significantly higher in insomniacs than matched normal controls (87). Within the 24-hour period, the greatest elevations were observed in the evening and first half of the night (Figure 3). Also, insomniacs with a high degree of objective sleep disturbance (% sleep time < 70) secreted a higher amount of cortisol, compared to those with a low degree of sleep disturbance. Pulsatile analysis revealed a significantly higher number of peaks per 24h in insomniacs than in controls (p < 0.05), while cosinor analysis showed no differences in the circadian pattern of ACTH or cortisol secretion between insomniacs and controls. Thus, insomnia is associated with an overall increase of ACTH and cortisol secretion, which, however, retains a normal circadian pattern. Also, this increase relates positively to the degree of objective sleep disturbance. These findings are consistent with a disorder of CNS hyperarousal not only during the night but during the day as well, rather than one of sleep loss, which is usually associated with no change or a decrease in cortisol secretion, or a circadian disturbance. Increased evening and nocturnal cortisol peripheral concentrations have been reported in one study (88), while another study that included insomniacs without evidence of objective sleep disturbance did not report differences between insomniacs and controls (89).



Figure 3. Twenty-four-hour plasma cortisol concentrations in insomniacs (■) and controls (O). The thick black line indicates the sleep recording period. The error bar indicates SE, P < 0.01.

It appears that the difference between these two groups of studies is the degree of polysomnographically documented sleep disturbance. For example, in the study by Rodenbeck et al (88) the correlation between the area under the curve (AUC) of cortisol and % sleep efficiency was -0.91 suggesting that high cortisol levels are present in those insomniacs with an objective short sleep duration. In contrast, in the study by Riemann et al (89), in which no cortisol differences were observed between insomniacs and controls, the objective sleep of insomniacs was very similar to that of controls (88.2% Sleep efficiency vs 88.6%). In another study that applied constant routine conditions, all indices of physiological arousal were increased but not to a significant degree due to lack of power and controls not being selected carefully (90).

Interestingly, in the latter study a visual inspection of cortisol data suggested an elevation of cortisol values of 15% to 20% in the insomnia group, a difference similar to that reported in the study by Vgontzas *et al.* (87) and which should be considered of clinical significance. These preliminary findings on the role of objective sleep disturbance, were recently corroborated by newer studies in experimental or community samples of insomnia patients using polysomnography or actigraphy to objectively assess nighttime sleep (91-93). In all these three studies, insomnia coupled with objective short sleep duration was associated with higher cortisol levels both in adults (91, 92), as well as in children (93).

Based on our observations from the studies on Insomnia and HPA axis, that cortisol levels are higher in those with objective short sleep duration (Figure 4) we expected that insomnia with short sleep duration should be associated with significant medical morbidity and mortality. A study, which used a large general random sample of men and women (n=1,754), demonstrated that insomnia with objective short sleep duration (<5h nighttime sleep) entailed the highest risk for hypertension, followed by the insomnia group who slept 5-6 hours, compared to the normal sleeping and >6h sleep duration group (14). In the same population sample, chronic insomnia with objective short sleep duration was also found to be associated with increased odds for type 2 diabetes (15), as well as neuropsychological deficits in speed processing, attention, visual memory and verbal fluency (16). In order to examine the mortality risk in this sample, we followed up men and women for 14 and 10 years respectively. After controlling for several confounders, the mortality rate in insomniac men with objective short sleep duration was four times higher than in control normal sleepers (17).



Time (Clock Hours)

Figure 4. Twenty-four-hour plasma cortisol concentrations in insomniacs, with low total ST (■) vs. those with high total ST (O) (MANOVA). The thick black line indicates the sleep recording period. The error bar indicates SE, P < 0.01.

More recently, from the same random, general population sample of the Penn State Cohort, 1395 adults were followed up after 7.5 years (22). All of the subjects underwent 8-hour polysomnography. We used the median polysomnographic percentage of sleep time to define short sleep duration (i.e. < 6 hours). Compared with normal sleepers who slept  $\geq 6$  hours, the highest risk for incident hypertension was in chronic insomniacs with short sleep duration. This study was the first longitudinal study to have examined the association of insomnia with objective short sleep duration with incident hypertension usina polysonomnography (22).

Finally, another cross-sectional study on a research sample on chronic insomniacs, showed that chronic insomnia (based on standard diagnostic criteria with symptoms lasting  $\geq 6$  months) when associated with physiological hyperarousal, (as defined by long MSLT values) is associated with a high risk for hypertension (19). Collectively, the above data further support that objective sleep measures in insomnia are an important index of the medical severity of the disorder, and they also point out the need for validation of practical, feasible, inexpensive methods, such as actigraphy, to measure sleep duration outside of the sleep laboratory. From a clinical standpoint, these data suggest that the therapeutic goal in insomnia

should not be just to improve the quality or quantity of nighttime sleep. Rather, they suggest that the common practice of prescribing only hypnotics for patients with chronic insomnia at most is of limited efficacy. Furthermore, the focus of psychotherapeutic and behavioral modalities, including sleep hygiene measures, should not be to just improve the emotional and physiological state of the insomniac pre- or during sleep, but rather to decrease the overall emotional and physiologic hyperarousal and its underlying factors, present throughout the 24-hour sleep/wake period.

It is possible that medications that suppress the activity of the HPA axis, such as antidepressants (94), could be a promising tool in our pharmacologic approaches. The effects of antidepressants on sleep, as well as on the daytime function and well-being of insomniacs, have not been assessed systematically yet some preliminary studies have reported improvement on sleep (95, 96). The potential usefulness of antidepressants in insomnia was further supported by a study by Rodenbeck et al. (97), who demonstrated the beneficial role of doxepin (a TCA) on both sleep and cortisol secretion in patients with primary insomnia without a clinically diagnosed depression. Moreover, two other studies have also underlined the efficacy and the safety of small doses of doxepin in adults suffering from primary insomnia, as well as in a model of transient insomnia (98, 99).

In conclusion, more studies are needed in order to confirm the possible therapeutic role of antidepressants on chronic insomnia as well as to clarify their underlying sleep-promoting mechanisms.

#### DISORDERS OF EXCESSIVE DAYTIME SLEEPINESS AND THE HPA AXIS

Obstructive sleep apnea (OSAS), the most common sleep disorder associated with excessive daytime sleepiness and fatigue, is accompanied by nocturnal hypoxia and sleep fragmentation. The latter conditions should be expected to be associated with an activation of the stress system. Indeed, it has been shown that urinary catecholamines, as well as plasma catecholamines measured during the nighttime, are elevated in sleep apneics compared to controls (100). Also, using microneurography, it has been shown that obstructive apneic events are associated with a surge of sympathetic nerve activity (101). In addition, another study lately demonstrated the beneficial effect of CPAP treatment on the stress system of sleep apneic patients (102). It has also been proposed that sympathetic activation in sleep apnea is one of the mechanisms leading to the development of hypertension, a condition commonly associated with sleep apnea. This proposal was supported by a study which showed that 3-month CPAP therapy could moderate hypertension in obese apneic men, an effect that may be attributed to the normalizing actions of CPAP on the stress system by eliminating chronic intermittent hypoxia and repetitive microawakenings (103).

Similarly, it was expected that sleep apnea would also be associated with an activation of the HPA axis. However, findings from different studies are inconsistent. Few studies that have assessed the plasma cortisol levels in sleep apneics have failed to show any differences between sleep apneics and controls (104-107), or more recently, sleep apnea was even reported to be associated with relative hypocortisolemia (108). In addition, no differences were reported in the plasma or urinary free cortisol levels following the abrupt withdrawal of CPAP, the most commonly recommended treatment for sleep apnea (109). However, another study reported that CPAP corrected preexisting hypercortisolemia, particularly after prolonged use (110). In accordance with the latter study, a more recent one demonstrated an association between OSAS and a mild but significant at night elevation of cortisol levels, in obese apneics compared to obese nonapneic controls. The increased levels of cortisol were corrected after the 3-month use of CPAP (111). These results were confirmed later by Henley et al., who showed that untreated compared to treated OSAS was associated with marked disturbances in ACTH and cortisol secretory dynamics (112). Two other studies lately reported increased cortisol levels in sleep apnea (113, 114). This latter study by Kritikou and collaborators showed that sleep apnea in non-obese men was associated with HPA axis activation, similar albeit stronger compared with obese individuals with sleep apnea. The same study was also the first to show that women, similarly to men, suffer from the same degree of the HPA axis activation. Finally, similarly to our previous study in obese men (111), shortterm CPAP use had a significant effect on cortisol levels compared with baseline.

Of interest is also that, in nondepressed, normally sleeping, nonapneic obese men, plasma levels of cortisol were lower than those in nonobese controls and exogenous administration of CRH provoked an enhanced ACTH response (111). These results suggest that in obesity there is hyposecretion of hypothalamic CRH, associated with hypotrophic adrenal cortices requiring compensatorily elevated amounts of ACTH to produce normal amounts of cortisol (111).

The association of the two other major organic disorders of excessive daytime sleepiness, narcolepsy and idiopathic hypersomnia, with the stress system has not been assessed. Preliminary data from a study that assessed the responsiveness of plasma cortisol and ACTH to the exogenous administration of ovine CRH in idiopathic hypersomniacs was associated with a normal or reduced plasma cortisol response, while the ACTH response to CRH tended to be significantly higher in the patients than controls (115). These preliminary findings suggested a subtle hypocortisolism and an inferred hypothalamic CRH deficiency in patients with idiopathic hypersomnia. A central CRH deficiency in these patients is consistent with their clinical profile of increased daytime sleepiness and deep nocturnal sleep (generalized hypoarousal). Consistent with these findings, it was reported that narcolepsy is associated with reduced basal ACTH secretion and a putative reduction of central CRH (116).

## FATIGUE, SLEEPINESS AND SLEEP APNEA, AND ADRENAL FUNCTION

Patients with Cushing's syndrome frequently complain of daytime fatigue and sleepiness. In one controlled study, it was demonstrated that Cushing's syndrome was associated with increased frequency of sleep apnea (117). In that study, about 32% of patients with Cushing's syndrome were diagnosed with at least mild sleep apnea, and 18% had about significant sleep apnea (apnea/hypopnea index > 17.5 events per hour). Interestingly, those patients with sleep apnea were not more obese or different in any craniofacial features compared to those patients without sleep apnea. These findings are interesting in light of the new findings that visceral obesity, which is prominent in Cushing's syndrome, is a predisposing factor to sleep apnea (118). Also, Cushing's syndrome in the absence of sleep apnea was associated with increased sleep fragmentation, increased stage 1 and wake, and decreased delta sleep (119).

Adrenal insufficiency or Addison's disease is associated also with chronic fatigue. A study indicated that untreated patients with adrenal insufficiency demonstrated increased sleep fragmentation, increased REM latency, and decreased amount of time in REM sleep, findings that may patients' fatigue (54). These explain the sleep abnormalities were reversed following treatment with a replacement dose of hydrocortisone. These results suggest that cortisol secretion may be needed to facilitate both initiation and maintenance of REM sleep. It should be noted that in normal individuals. exogenous glucocorticoids have been found to reduce REM sleep (52). The authors interpreted their findings that the inhibitory role of glucocorticoids on REM sleep in normals, along with their permissive role in Addison's patients, demonstrate that some cortisol is needed for REM sleep, with excess cortisol inhibiting REM sleep, perhaps indirectly by suppression of CRH.

Also, fatigue and excessive daytime sleepiness are prominent in patients with secondary adrenal insufficiency, e.g., steroid withdrawal, and African trypanosomiasis. The latter conditions are associated with decreased adrenocortical function and elevated TNF $\alpha$  and/or IL-6 levels (120, 121), which may be the mediators of the excessive sleepiness and fatigue associated with primary or secondary adrenal insufficiency, as well of the profound somnolence of patients with African trypanosomiasis.

#### SLEEP AND CYTOKINES

Research has shown a strong interaction between the HPA axis and the immune system (29). Cytokines have a strong stimulating effect on the HPA axis, whereas cortisol, the end-product of the HPA axis, suppresses secretion (Figure 2). In this section, we will describe what is known on the role of cytokines in sleep regulation and sleep disorders, as well as the interaction effect of cytokines and HPA axis on sleep and its disorders.

Feelings of fatigue and sleepiness are common symptoms associated with infectious diseases and many other physical and mental pathologic conditions. Physicians for millennia have advised their patients to sleep during the course of an illness. However, it is only within the past 20 years that there has been a systematic study on changes in sleep that occur with infection or following microbial product-induced cytokine production (reviewed in 122).

The first reports of the effects of IL-1 on sleep in animals were published in 1984 (123, 124). Following the observation that astrocytes (neuroglia) produce IL-1 (125), which provided the basis to explore whether IL-1 was somnogenic, several studies by Krueger and collaborators established the role of this cytokine in the sleep physiology of the rabbit. These studies (123, 126, 127) indicated that: 1) administration of IL-1 increased electroencephalogram (EEG) slow wave activity in the delta frequency band; 2) the effects of IL-1 on sleep were dose-related; and 3) the enhancement of induction of slow wave sleep (SWS) by IL-1 was not merely a byproduct of fever because pretreatment of animals with anisomycin abolished IL-1-induced fever but not IL-1-induced SWS. Since then, IL-1 has proven to be somnogenic in species other than rabbits, including rats, mice, cats, and monkeys.

The same group of investigators demonstrated that TNF $\alpha$  induced SWS in several species (126, 128), whereas TNF $\alpha$  mRNA (129) and protein in brain (130), exhibit circadian rhythms that coincide with sleep-wake activity. Also, direct intervention with the TNF system by the use of antibodies, binding proteins, or soluble receptors or receptor fragments reduced SWS in otherwise normal animals (131). In addition, mice that lack the 55 kDa TNF

receptor sleep less than background strain controls (132). The effects of IL-6 on sleep in animals have not been assessed systematically.

## Normal Sleep in Humans and Circadian Secretion of Cytokines

There are several reports that in normal people plasma levels of cytokines are related to the sleep-wake cycle. Moldofsky et al. first described such relations in human beings, showing that IL-1 activity was related to the onset (133). slow-wave sleep Subsequently, other of investigators showed that plasma levels of TNFa vary in phase with EEG slow wave amplitudes (134), and that there is a temporal relation between sleep and IL-1b activity (135, 136). Other studies, using indirect ex vivo methods or direct in vivo measures of plasma concentrations, have demonstrated that IL-6 and TNFa levels in young healthy individuals peak during sleep (103, 136, 137).

Specifically, in the study by Vgontzas *et al.* (137), at baseline, IL-6 is secreted in a biphasic circadian pattern, with two nadirs at 08.00 and 21.00 h and two zeniths at about 19.00-20.00 and 04.00-05.00 h, with the stronger peak at 05.00 h (Figure 5). Previous studies noted the circadian pattern of IL-6 secretion and its late-night peak (138-140). That these studies did not report on the daytime zenith of IL-6 at about 19.00-20.00 h could be attributable to their using infrequent sampling or a small number of subjects.



# Figure 5. Twenty-four-hour plasma IL-6 concentrations pre- and post-sleep deprivation in eight healthy young men. Each data point represents the mean + SE. \*, P < 0.05 indicates statistical significance from the peak value within 24 h for each condition (MANOVA followed by Dunnett, post hoc test). The darkened area indicates the sleep recording period.

In the same study, we demonstrated that daytime IL-6 levels are negatively related to the amount of nocturnal sleep (137). Thus, decreased overall secretion of IL-6 is associated with a good night's sleep and a good sense of well-being the next day, and good sleep is associated with decreased exposure of tissues to the proinflammatory and potentially detrimental actions of IL-6 on the cardiovascular system, insulin sensitivity, and bones (141, 142).

These findings on the circadian pattern of IL-6 secretion were also illustrated in two studies that assessed the effects of modest sleep restriction (76) (Figure 6) as well as the effects of a 2-hour midafternoon nap following a night of total sleep loss (83) (Figure 7) on sleepiness, psychomotor performance and finally plasma levels of proinflammatory cytokines (IL-6, TNF $\alpha$ ) and cortisol.



Figure 6. Twenty-four-hour circadian secretory pattern of IL-6 Before ( $\diamond$ ) and after (•) partial sleep restriction. Bar indicates SE. The thick black bar on the abscissa represents the sleep recording period during baseline. The open bar on the abscissa represents the sleep recording period during partial sleep restriction. \*, P<0.05.



Figure 7. Twenty-four-hour IL-6 values pre-(  $\star$  ) and post-( $\Box$ ) sleep deprivation in the no-nap group (top) and the nap group (bottom). Thick black lines on the abscissa indicate nighttime and nap recording periods. Significant reduction of IL-6 during the nap period (1400-1600), P<0.05

Recently, we assessed the effects of recovery sleep after one week of mild sleep restriction on IL-6, sleepiness and performance (79). Serial 24-h IL-6 plasma levels increased significantly during sleep restriction and returned to baseline after recovery sleep (Figure 8). Subjective and objective sleepiness increased significantly after restriction and returned to baseline after recovery. In contrast, performance deteriorated significantly after restriction and did not improve after recovery.



**Clock Time** 

Figure 8. Serial 24-h IL-6 values at baseline (♦), restriction (■), and recovery (▲). Thick white, gray, and black lines on the abscissa indicate the nighttime sleep recording period at baseline, restriction, and recovery, respectively.

The view that IL-6 is involved in sleep regulation is further supported by the observation that exogenous administration of IL-6 in humans caused profound somnolence and fatigue (143), while in another study, its administration was associated with an increase of SWS in the second half of the night, suggesting a direct action of IL-6 on central nervous system sleep mechanisms (144). The sleep-disturbing effect of exogenous IL-6 noted in the first half of the night might be attributed to increased secretion of CRH, ACTH and cortisol induced by IL-6 during the early part of the night. An alternative, not mutually exclusive, hypothesis is that high levels of IL-6 per se may compromise early nighttime sleep.

#### Sleep Disturbance, Cytokines and Normal Aging

In a study in which we compared older adults to young subjects, the mean 24-h IL-6 and cortisol secretion was significantly higher in older adults (P < 0.05) (62) (Figure

9). IL-6 secretion in older adults was increased both during the daytime and nighttime, whereas cortisol secretion was more pronounced during the evening and nighttime periods. TNFa secretion in young adults showed a statistically significant circadian rhythm with a peak close to the offset of sleep; such a rhythm was not present in older adults. Both IL-6 and cortisol levels were positively associated with total wake time. The effect of IL-6 on wake time was markedly stronger for the older group than for the young group. The combined effect of cortisol and IL-6 on wake time was additive. IL-6 had a negative association with REM sleep only in the young, while cortisol was associated negatively with REM sleep both in the young and old, with a stronger effect in the young. These results suggest that in healthy adults, age-related alterations in nocturnal wake time are associated with elevation of both plasma IL-6 and cortisol concentrations, while REM sleep declines with age is primarily associated with cortisol increases.





Figure 9. Twenty-four-hour plasma concentrations of IL-6 (top), TNF $\alpha$  (middle), and cortisol (bottom) in healthy young ( $\Box$ ) and old (**a**) individuals. Each data point represents the mean **±** SE. The darkened area indicates the sleep recording period.



It has been previously suggested that increased HPA axis activity associated with aging is a result of the "wear and tear" of lifelong exposure to stress (53, 54). An alternative, not mutually exclusive, explanation is that the significant alteration of HPA axis activity associated with age is at least partially secondary to the hypersecretion of IL-6, whose peripheral levels are a good marker of increased morbidity and mortality (145). The source of IL-6 hypersecretion in the elderly is not known. However, we know that IL-6 peripheral levels correlate negatively with sex-steroids levels, positively with the amount of adipose tissue, are decreased after a good night's sleep, and are chronic pain/inflammatory syndromes elevated in (62,137,142,146-148). Old age is associated with decreased sex-steroid concentrations, increased proportional body fat, decreased quantity and quality of sleep, and frequent chronic pain/inflammatory conditions. Reducing the secretion of IL-6 in elderly, either by (a) administration of sex steroids, (b) decreasing fat through diet and exercise, (c) improving nighttime sleep, and (d) controlling adequately chronic pain and inflammation with nonsteroidal anti-inflammatory agents, may improve sleep, daytime alertness, and performance, and decrease the risk of common ailments of old age, e.g., metabolic and cardiovascular problems, cognitive disorders, and osteoporosis (118, 149, 150).

## Cytokines as Potential Mediators of Pathological or Experimentally Induced Excessive Daytime Sleepiness

Excessive daytime sleepiness (EDS) occurs in about 5-9% of the general population (9, 13, 151) and is the chief complaint of the majority of patients evaluated at sleep disorders centers. EDS is one of the major physiological consequences of obstructive sleep apnea. Besides the obvious effects of daytime sleepiness on patients' occupational and social life, daytime sleepiness appears to be a major concern of public safety.

There has been a number of studies of cytokine profiles in patients with excessive daytime sleepiness (pathologic or experimentally- induced) within the last 20 years. In one of the first studies in 1996, Entzian *et al.* studied 10 hospitalized patients requiring therapy for obstructive sleep

apnea (104). Blood samples were collected every 4 hours during the day (08.00 to 20.00 h) and at 2-h intervals during nighttime sleep. Whole blood cultures stimulated with lipopolysaccharide were used to determine cytokine release. The circadian rhythm of TNFa was significantly altered in sleep apnea patients; the peak concentrations that occurred during the night in normal control subjects were not present in sleep apnea patients. Rather, sleep apnea patients exhibited increased TNFα concentrations in the afternoon, the time period during which concentrations in normal control subjects are at a minimum. It is also interesting to note that in spite of a lack of statistical differences due to inherent inter-individual variability, infrequent sampling and a small sample size, absolute IL-1 concentrations in the sleep apnea patients were more than twice those obtained from normal controls, and IFN concentrations were more than three times those of normal controls. Finally, IL-6 in sleep apneics reached maximum concentrations in the evening, in contrast to normal subjects where IL-6 peaked at about 2.00 a.m.

In 1997, we published a study in which cytokine profiles were obtained from several patient populations with disorders of excessive daytime sleepiness (152). Three populations were studied; those with obstructive sleep apnea (n = 12); narcoleptics (n = 11); and idiopathic hypersomniacs (n = 8). Single blood samples were drawn in the morning after the completion of the nighttime sleep laboratory recordings. Plasma concentrations of IL-1β, TNFa, and IL-6 were determined by ELISA. Relative to control subjects, plasma IL-1 concentrations did not differ between the three groups. TNFa was elevated in sleep apnea patients and narcoleptics, and IL-6 was elevated only in sleep apnea patients. Correlational analyses indicated that TNFa and IL-6 correlated positively with measures of excessive daytime sleepiness; TNF was positively correlated with the degree of nocturnal sleep disturbance, and the degree of hypoxia, whereas IL-6 concentrations were correlated with degree of nocturnal sleep disturbance, degree of hypoxia, and body mass index. The potential role of IL-6 as a mediator of daytime sleepiness was further suggested in a study by Hinze-Selch et al. that showed that IL-6 secretion by monocytes was higher in narcoleptics than controls and plasma levels of IL-6 were non-significantly higher in patients compared to controls (153).

The results of our first study prompted us to study further the role of IL-6 and TNFα as potential mediators of EDS in disorders of EDS, i.e., sleep apnea, and in conditions of experimentally-induced daytime sleepiness following sleep deprivation. Those preliminary findings were later corroborated by several studies by us or other researchers that showed that: (a) Single and 24-hour TNF $\alpha$  and IL-6 plasma levels are elevated in adults and children with sleep apnea independently of obesity (103, 118, 154-158) (Figure 10); (b) body mass index (BMI) positively correlates with both TNFa and IL-6 levels, suggesting that these two cytokines may play a role in daytime sleepiness experienced by obese individuals in the absence of sleep apnea (25); (c) daytime levels of IL-6 and TNF $\alpha$  are elevated in healthy humans experiencing somnolence and fatigue as a result of total sleep deprivation (137) or even after a modest sleep loss by restricting sleep to 6 hours a night per week (76,159); and (d) a midafternoon nap following a night of total sleep deprivation is beneficial for both the suppression of IL-6 secretion and for the improvement of alertness (83). In the latter studies, greater

pre-sleep deprivation amounts of slow wave (deep) sleep rendered the subjects resistant to the effect of sleep deprivation. It is common experience that individuals differ significantly in terms of their ability to sustain sleep loss or curtailment. Those with greater amounts of SWS are inherently more capable of tolerating sleep loss, possibly avoiding exposure to the potentially harmful effects of increased IL-6 secretion. Other studies have confirmed these findings by showing that IL-6 and TNF $\alpha$  are elevated following an 88-hour period of wakefulness (160) and that the nighttime rise of IL-6 levels is delayed during partial sleep deprivation (161). TNFa plasma levels are also elevated in other diseases associated with excessive daytime sleepiness, such as chronic fatigue syndrome, post-dialysis fatigue, HIV patients (162). In 2004, a pilot, placebo- controlled, double-blind study further supported the somnogenic actions of IL-6 and TNFa. In this study, researchers tested the results of etanercept, a TNFa antagonist in eight obese male apneics suffering from excessive daytime sleepiness. Both sleepiness and AHI (number of apneas/hypopneas per hour) were reduced significantly by the drug compared to the placebo. IL-6 levels were also significantly decreased (163).



Figure 10. Plasma TNF, IL-6, and leptin levels in sleep apneics and BMI-matched obese and normal weight controls. A \*, P <0.01 vs. normal weight (nl wt) controls, B \*, P < 0. 5 vs. nl wt controls, C \*, < 0.05 vs. obese and lean controls.

These studies collectively provide evidence that cytokines are elevated in individuals suffering from a disorder of EDS, e.g., apnea, or healthy individuals experiencing EDS secondary to acute or short-term sleep loss and support the hypothesis that EDS (pathologic or experimentallyinduced) may be mediated in part by somnogenic cytokines.

#### Insomnia and Cytokines

Chronic insomnia, by far the most commonly encountered sleep disorder in medical practice, is characterized by long

sleep latencies or increased wake time during the night and increased fatigue during the day, although in objective daytime sleep testing, insomniacs are unable to fall asleep (164, 165).

In a 2002 study, we demonstrated that the mean 24-hour IL-6 and TNF secretions were not different between insomniacs and controls. However, mean IL-6 levels were significantly elevated in insomniacs compared to controls in the mid-afternoon and evening pre-sleep period (15.00-23.00, P < 0.05) (146) (Figure 11). Furthermore, cosinor analysis showed a significant shift of the major

peak of IL-6 secretion from early morning (05.00) to evening (20.00) in insomniacs compared to controls. Also, TNF $\alpha$  secretion in controls showed a statistically significant

circadian rhythm with a peak close to the offset of sleep; such a rhythm was not present in insomniacs (Figure 12).



Figure 11. Twenty-four-hour circadian secretory pattern of IL-6 in insomniacs ( $\circ$ ) and controls ( $\bullet$ ). The thick black line on the abscissa indicates the sleep recording period. Error bar indicates SE, \* P < .05.

Moreover, the daytime secretion of TNF in insomniacs was associated with a regular periodicity of about 4 hours, and its amplitude was significantly different from zero. Controls showed a similar rhythm, which, however, was not significant.



Figure 12. Twenty-four-hour circadian secretory pattern of TNF $\alpha$  in insomniacs ( $\circ$ ) and controls ( $\bullet$ ). The thick black line on the abscissa indicates the sleep recording period. Error bar indicates SE.



Based on these findings, we concluded that chronic insomnia was associated with a shift of IL-6 and TNF secretion from nighttime to daytime, which may explain the daytime fatigue and performance decrements associated with this disorder. The daytime shift of IL-6 and TNF secretion, combined with a 24h hypersecretion of CRH and cortisol, both arousal hormones, may explain the insomniacs' daytime fatigue and difficulty falling asleep during the daytime and/or the nighttime.

In conclusion, despite the fact that the above findings show abnormal secretion patterns of  $TNF\alpha$  and IL-6 in insomnia, further studies are needed in order to get better insight into the association between cytokine secretion pattern and chronic insomnia.

## Sleepiness vs. Fatigue: The Role of the Interaction of HPA Axis with Cytokines

From our previous studies, it became evident that cytokines are elevated both in disorders of deep sleep/EDS as well as in disorders of poor sleep/fatigue. These seemingly inconsistent findings can be better understood if we clarify the terms sleepiness vs. fatigue and understand the effects of cytokines on sleep/sleepiness in terms of their interaction with HPA axis.

"Sleepiness" and "fatigue" have been considered to be either the same state, different states on a continuum or finally fundamentally different states. In medical practice and literature, these terms are often used interchangeably; however, there is enough clinical evidence to propose a separate definition for these 2 terms in sleep disorders medicine. Sleepiness is a subjective feeling of physical and mental tiredness associated with increased sleep propensity. Fatigue is also a subjective feeling of physical and/or mental tiredness; however, it is not associated with

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increased sleep propensity. Based on these definitions, sleep disorders or conditions associated with sleepiness include sleep apnea, narcolepsy, and sleep deprivation. On the other hand, sleep disorders associated with fatigue include chronic insomnia, sleep disturbances in the elderly, and psychogenic hypersomnia. This distinction between "sleepiness" and "fatigue" was adopted unanimously as useful for the field of insomnia research by an expert panel of 25 sleep researchers who convened in Pittsburgh on March 10-11, 2005 (166).

Based on our studies, we propose that daytime cytokine hypersecretion and/or circadian shift of cytokine secretion not associated with HPA axis activation leads to sleepiness and deeper sleep, and a good example of this is sleep deprivation. On the other hand, we suggest that daytime cytokine hypersecretion and/or circadian alteration of cytokine secretion associated with HPA axis activation, e.g., insomnia, leads to fatigue and poor sleep.

Such a model, which combines cytokine secretion and HPA axis function to explain sleepiness and increased sleep versus fatigue and poor sleep, is supported by experiments on the effects of exogenous activation of the host defense system on sleep in humans. For example, exogenous administration of IL-6 in healthy humans in the evening was associated with both fatigue and a sleep disturbing effect in the first half of the night, most likely due to increased secretion of corticotrophin-releasing hormone, ACTH, and cortisol, during the early part of the night, induced by IL-6 (144). Also, in dose-response experiments using endotoxin, it was shown that subtle host defense activation not associated with HPA axis activation and increased body temperature enhanced the amount of non-REM sleep, whereas higher doses associated with increased cortisol secretion and increased body temperature, resulted in reduced non-REM sleep and increased wakefulness (167).

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