Chronic Insomnia Is Associated With a Shift of Interleukin-6 and Tumor Necrosis Factor Secretion From Nighttime to Daytime

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Chronic insomnia, by far the most commonly encountered sleep disorder in medical practice, is characterized by difficulty falling or staying asleep at night and increased fatigue during the day. Interleukin-6 (IL-6) and tumor necrosis factor (TNF) are fatigue-inducing cytokines, and the daytime secretion of IL-6 is negatively influenced by the quantity and quality of the previous night's sleep. We hypothesize that the poor quality of insomniacs' sleep is associated with a hypersecretion of these 2 cytokines during the daytime, which, in turn, correlates with the fatigue experienced by these patients. Eleven young insomniacs (6 men and 5 women) and 11 (8 men and 3 women) age- and body mass index (BMI)-matched healthy controls participated in the study. Subjects were recorded in the sleep laboratory for 4 consecutive nights and serial 24-hour plasma measures of IL-6 and TNF were obtained during the 4th day. Insomniacs compared to controls slept poorly (sleep latency and wake were increased, whereas percentage sleep time was decreased during baseline nights, all $P < .05$). The mean 24-hour IL-6 and TNF secretions were not different between insomniacs and controls. However, the difference in the change (increase) of IL-6 plasma levels from midafternoon (2 PM) to evening (9 PM) between insomniacs and controls was significant ($P < .01$). Furthermore, cosinor analysis showed a significant shift of the major peak of IL-6 secretion from nighttime (4 AM) to evening (7 PM) in insomniacs compared to controls ($P < .05$). Also, while TNF secretion in controls showed a distinct circadian rhythm with a peak close and prior to the offset of sleep ($P < .05$), such a rhythm was not present in insomniacs. Finally, daytime secretion of TNF in insomniacs was characterized by a regular rhythm of 4 hours ($P < .05$); such a distinct periodicity was not present in controls. We conclude that chronic insomnia is 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 24-hour hypersecretion of cortisol, an arousal hormone, may explain the insomniacs' daytime fatigue and difficulty falling asleep.

CHRONIC INSOMNIA, by far the most commonly encountered sleep disorder in medical practice, is characterized by difficulty initiating or maintaining sleep, and loss of the restorative effects of sleep.[1,2] Insomnia is a difficult disorder to treat, and our knowledge in regard to its neurobiology and medical significance is limited. We recently reported that chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal (HPA) axis and that there is a positive correlation between the severity of insomnia and the degree of HPA axis activation, consistent with the view that insomnia is a disorder of behavorial and physiological hyperarousal.[3]

Interleukin-6 (IL-6) and tumor necrosis factor (TNF) are fatigue-inducing cytokines. These cytokines are elevated during the daytime in disorders of excessive daytime sleepiness (EDS)[4,5] or in experimentally induced sleepiness after a night of total sleep deprivation.[6] The daytime secretion of IL-6 is negatively influenced by the quantity and quality of the previous night's sleep.[6] Both IL-6 and TNF stimulate the activity of the HPA axis, and their secretion is suppressed by glucocorticoids.[7]

Insomniacs frequently report daytime fatigue, poor concentration, and inattention.[1,2] Interestingly, in objective daytime sleep testing (multiple sleep latency test [MSLT]), insomniacs are unable to fall asleep compared to normal sleepers.[8,9] We have previously attributed their difficulty falling asleep at night or during the day to activation of their stress system reflected by plasma cortisol concentrations that are increased around the clock.[3] The potential role of IL-6 and TNF secretion in mediating daytime fatigue in chronic insomnia has not been examined. The goal of this study was to examine the 24-hour quantitative and temporal pattern of IL-6 and TNF secretion in insomniacs and controls matched for age and body mass index (BMI). We hypothesized that insomnia would be associated with augmented daytime secretion of these 2 cytokines, which would explain the fatigue experienced by these patients.

MATERIALS AND METHODS

Eleven young insomniacs (6 men and 5 women) and 11 (8 men and 3 women) age- and BMI-matched healthy controls (mean ± SD age, 31.6 ± 6.7 *v* 27.1 ± 6.4 years, difference not significant [NS]; mean ± SD BMI, 25.0 ± 3.7 *v* 24.7 ± 3.4, NS) participated in the study.

Eligibility criteria for insomniacs included a history of difficulty falling asleep (taking 45 or more minutes to fall asleep) and/or staying asleep (obtaining fewer than 6.5 hours of total sleep time) at least 4 nights a week for at least 6 months. In addition, insomniacs had to demonstrate a sleep efficiency of less than 80% during a screening night in the sleep laboratory. Insomniacs were evaluated by a psychiatrist (A.N.V.,), and those who met the *Diagnostic and Statistical*

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Manual of Mental Disorders, ed 4 (DSM-IV) criteria of a current mental disorder, ie, major depression, psychosis, generalized anxiety disorder, panic disorder, or substance abuse, were excluded from the study.

Both insomniacs and controls were in good physical health, not using any medication for at least a month, or doing shift work, and had a battery of clinical tests that were negative for abnormal findings. All subjects were screened in the sleep laboratory for sleep-disordered breathing, nocturnal myoclonus, or other primary sleep disorders. Finally, controls had no sleep complaints, whereas during the screening night they had to demonstrate a sleep efficiency of greater than 85%. The study was approved by the institutional review board, and each subject signed a written informed consent.

Sleep Laboratory Recordings

Subjects were recorded in the sleep laboratory for 4 consecutive nights. The first night allowed for adaptation to the new sleeping environment and was not included in the analysis. Sleep laboratory recording was performed in a sound-attenuated, light- and temperature-controlled room that has a comfortable bedroom-like atmosphere. During this evaluation, each subject was monitored continuously for 8 hours. The sleep schedule in the sleep laboratory was similar to the subjects' normal sleep schedule, which was between 10 or 11 PM to 6 or 7 AM. Electroencephalographic, electro-oculographic, and electromyographic recordings were obtained in accordance with standard methods.[10] The sleep recordings were amplified using standard clinical polygraphs (Grass Instrument Co, Model 78d & e, Quincy, MA). The sleep records were scored independently of any knowledge of the experimental condition, according to standardized criteria.[10]

Sleep parameters, assessed from the sleep recordings, included sleep induction (sleep latency, or SL); sleep maintenance (wake time after sleep onset, or WTASO); TWT, which is the sum of SL and WTASO; total sleep time (ST) and percent ST (which is total ST, as percent of time in bed); percentage of the various sleep stages (rapid eye movement [REM], 1, 2, combined 3 and 4 for slow wave sleep [SWS], which is calculated as the minutes in each stage as the percent of total ST); and REM latency, which is the interval from sleep onset to the first REM period. Sleep onset was defined as the latency from lights out to the first occurrence of any stage sleep for a duration of 1 minute or longer. If, however, the initial stage of sleep was stage 1, then it had to be followed without any interfering wake, by at least 1 minute of any other stage.

Blood Drawing Technique

Twenty-four–hour blood sampling was performed serially every 30 minutes on the 4th day. An indwelling catheter was inserted in the antecubital vein about 30 minutes before the first blood draw. The catheter was kept patent with small amounts of heparin. During the sleep recording period, blood was collected outside the subjects' room through a perforation in the wall, via extended tubing, to decrease sleep disturbance from the blood drawing. During the daytime, the subjects were ambulatory, and they were allowed to watch television, play computer and table games, go to the bathroom, and engage in other similar activities. Also, they were instructed not to change their diet, and their 3 daily meals were at about 7 AM, noon, and 6 PM.

Hormone Assays

Blood collected from the indwelling catheter was transferred to an EDTA-containing tube and refrigerated until centrifugation (within 3 hours). The supernatant was frozen at –20 for the hormones until assay. Corticotropin (ACTH) and cortisol were measured every 30 minutes, and these findings have been previously reported.[3]

Hourly plasma TNF and IL-6 were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN). The intra- and interassay coefficients of variation ranged from 5.6% to 6.1% and 7.5% to 10.4%, respectively, for TNF and from 3.2% to 8.5% and 3.5% to 8.7% for IL-6. The lower detection limits for TNF and IL-6 were 0.18 and 0.094 pg/mL, respectively.

Statistical Analyses

Baseline sleep profiles were compared between insomniacs and controls using Student's *t* test. Twenty-four–hour serial plasma TNF and IL-6 levels were analyzed using multivariate analysis of variance (MANOVA). The change of IL-6 levels from midafternoon (2 PM) to evening (9 PM) was assessed using Student's *t* test. Data were transformed logarithmically to satisfy the normality assumption. In addition, the circadian rhythmicity of TNF and IL-6 secretion was assessed with cosinor-multiple-components rhythmometry by fitting a curve to each individual profile and the entire population profile.[11] This method allowed fitting a model with several cosine functions to the data. The data were analyzed after they were transformed to percent of the mean, which is the preferred approach to show predictable variability when data are obtained from different individuals.[12] Finally, for each group, the daytime TNF values were analyzed using PROC NLMIXED in SAS 8.0 (SAS Institute, Cary, NC) by fitting 2 components for each individual: a cosine wave with a periodic rhythmicity of 4 hours and 2 piecewise regression lines with the nadir point being 3 PM. The cosine waves describe the ultradian pattern while the regression lines describe the overall trend over time. The amplitude of the group was then tested against zero because a non-zero amplitude would suggest a rhythm be present. Variability estimates were expressed as SE, with the exception of age and BMI for which variability was expressed as SD.

RESULTS

Sleep Profiles of Insomniacs and Controls

Insomniacs compared to controls slept poorly during the baseline nights 2 and 3 (Table 1). Specifically, insomniacs demonstrated a longer SL, more WTASO and TWT, and less percentage of ST (all $P < .01$). Insomniacs and controls were not different in terms of sleep stage variables. Subjectively, insomniacs compared to controls reported longer SL, less amount of sleep, and lighter sleep (all $P < .05$).

Table 1. Baseline Sleep Patterns of Insomniac and Control Subjects

	Insomnia Subjects (n = 11)	Control Subjects (n = 11)
Sleep efficiency		
SL (min)	32.8 ± 5.2*	10.7 ± 2.0
WTASO (min)	50.9 ± 11.7†	23.3 ± 3.0
TWT (min)	83.7 ± 13.4*	34.0 ± 3.0
% ST	82.5 ± 2.8*	92.9 ± 0.6
Sleep stages		
% Stage 1	2.8 ± 0.3	3.4 ± 0.5
% Stage 2	66.9 ± 2.0	62.0 ± 2.3
% Slow wave sleep	8.7 ± 1.7	10.3 ± 1.8
% REM	21.5 ± 1.2	24.2 ± 1.2

NOTE. The data represent average values ± SE from nights 2 and 3. *P* values were derived using *t* test.

*$P < .01$.

†$P < .05$.

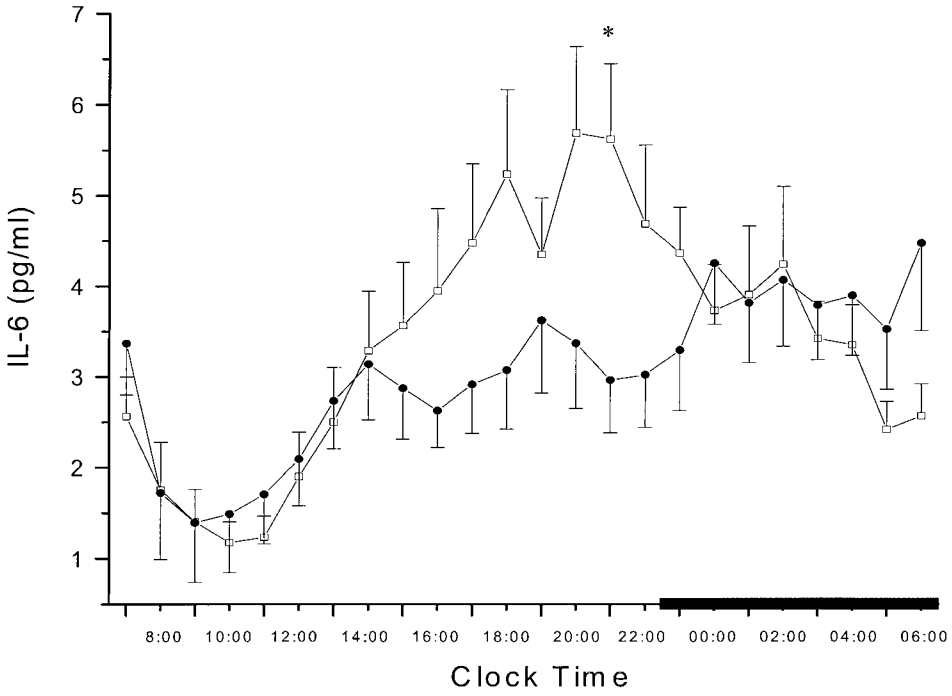

Fig 1. Twenty-four–hour circadian secretory pattern of IL-6 in insomniacs (□) and controls (●). The thick black line on the abscissa indicates the sleep recording period. Error bar indicates SE. *$P < .05$

Twenty-Four–Hour Secretion of IL-6 and TNF

The mean 24-hour IL-6 and TNF secretions were not different between insomniacs and controls (Figs 1 and 2). However, mean IL-6 levels were borderline significantly elevated in insomniacs compared to controls in the midafternoon and evening presleep period (2 to 11 PM, $P = .06$). Also, the difference in the change of the IL-6 levels from 2 PM to 9 PM between insomniacs and controls was significant ($P < .01$).

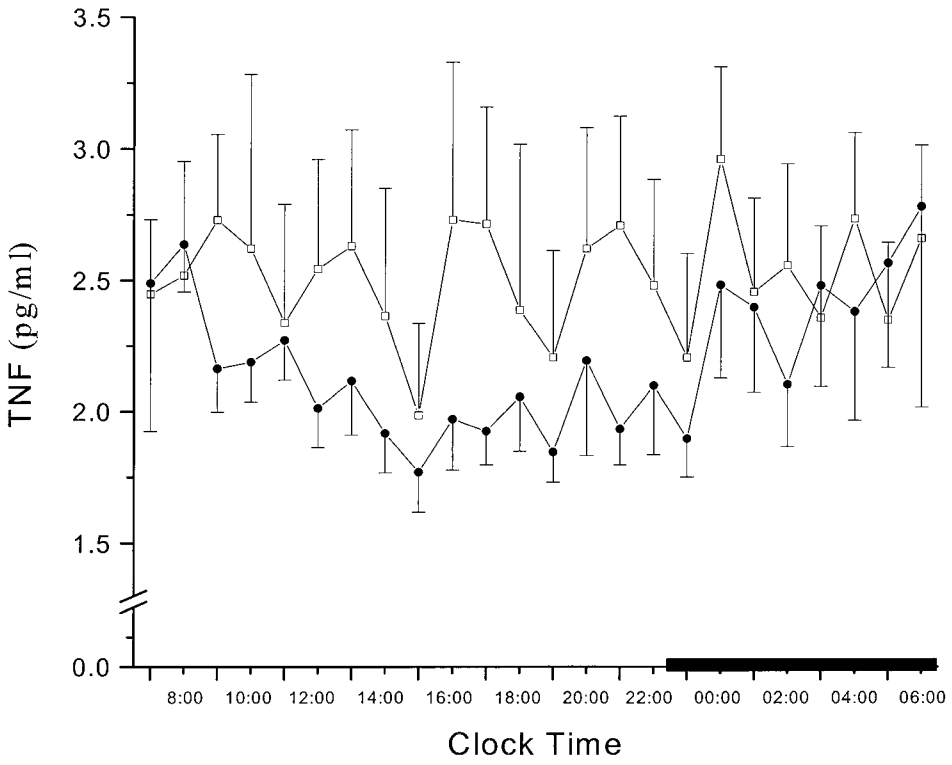

Fig 2. Twenty-four–hour circadian secretory pattern of TNF in insomniacs (□) and controls (●). The thick black line on the abscissa indicates the sleep recording period. Error bar indicates SE.

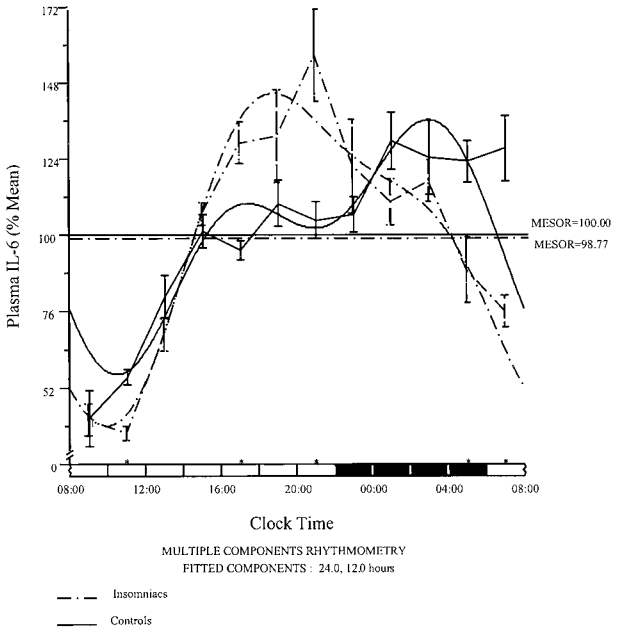

Fig 3. Multiple-component (24 and 12 hours) cosinor analysis of 24-hour plasma IL-6 in insomniacs (dotted line) and controls (solid line) expressed as percent variation from the mean. The thick black line on the abcissa represents the sleep recording period. MESOR, mid-line estimating statistic of rhythm or rhythm-adjusted mean; *$P < .05$.

Circadian/Ultradian Analysis

Cosinor analyses, both for the individual and population IL-6 data, indicated a significant circadian rhythm, with a multiple component curve, including periods with 12 and 24 hours both for insomniacs and controls ($P < .01$). In controls, there was a major peak at night (about 2 AM) and a secondary peak in the late afternoon (about 5 PM). In insomniacs compared to controls, there was a significant shift of the major peak from about 2 AM to about 7 PM ($P < .001$). The nadir point was temporally and quantitatively the same for both groups, about 9 AM (Fig 3).

Cosinor analyses, both for individual and population TNF data, indicated a significant circadian rhythm, with a multiple component curve, including periods with 12 and 24 hours only for controls ($P < .05$). The peak was close and prior to the offset of sleep (about 6 AM), whereas the nadir occurred in midafternoon (about 3 PM). Such a rhythm was not present in insomniacs (Fig 4).

Finally, TNF daytime secretion in insomniacs showed a periodic rhythmicity of 4 hours with an amplitude greater than zero ($P = .02$) (Fig 5). Such a rhythm was present but not significant in normal controls. The estimated amplitude for the insomniac group was 0.22 (95% confidence interval [CI], 0.047 to 0.392; $P = .02$), whereas the estimated amplitude for the control group was 0.047 (95% CI, -0.026 to 0.12; $P = .18$). Further, on an individual level, eight of the 11 insomniacs had an amplitude greater than zero, and 5 of these insomniacs had an estimated amplitude value greater than 0.5 pg/mL, whereas none of the controls had an estimated amplitude greater than 0.5 pg/mL.

DISCUSSION

This is the first controlled study to demonstrate that chronic, persistent insomnia is associated with a significant shift of IL-6 and TNF secretion from nighttime to daytime. We propose that the circadian alteration of the secretory pattern of these 2 cytokines may explain the daytime fatigue, poor concentration, and diminished performance of these patients.

We have previously reported that IL-6 and TNF are elevated in disorders of EDS, suggesting that these 2 cytokines may be mediators of sleepiness.[4,5] Also, we have shown that sleep deprivation increases daytime plasma IL-6 concentration and causes somnolence and fatigue during the next day.[6] In the latter condition, the increased daytime secretion of IL-6 is associated with no change or even decrease of cortisol secretion.[13] In contrast, in insomnia the shift of IL-6 and TNF secretion from nighttime to daytime was associated with a 24-hour cortisol hypersecretion.[3] This difference may explain why, in insomnia, fatigue is not associated with an ability to fall asleep when the subject is given the opportunity in contrast to the "true" sleepiness experienced by sleep-deprived normal sleepers.

Although in medical practice and literature, the terms sleepiness and fatigue are used interchangeably, 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 increased sleep propensity. Based on these definitions, sleep disorders or conditions associated with sleepiness include

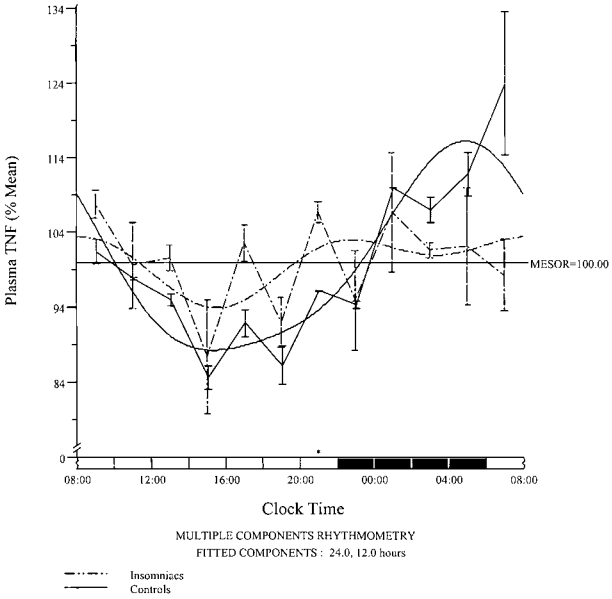

Fig 4. Multiple-component (24 and 12 hours) cosinor analysis of 24-hour plasma TNF in insomniacs (dotted line) and controls (solid line) expressed as percent variation from the mean. The thick black line on the abcissa represents the sleep recording period. MESOR, mid-line estimating statistic of rhythm or rhythm-adjusted mean; *$P < .05$.

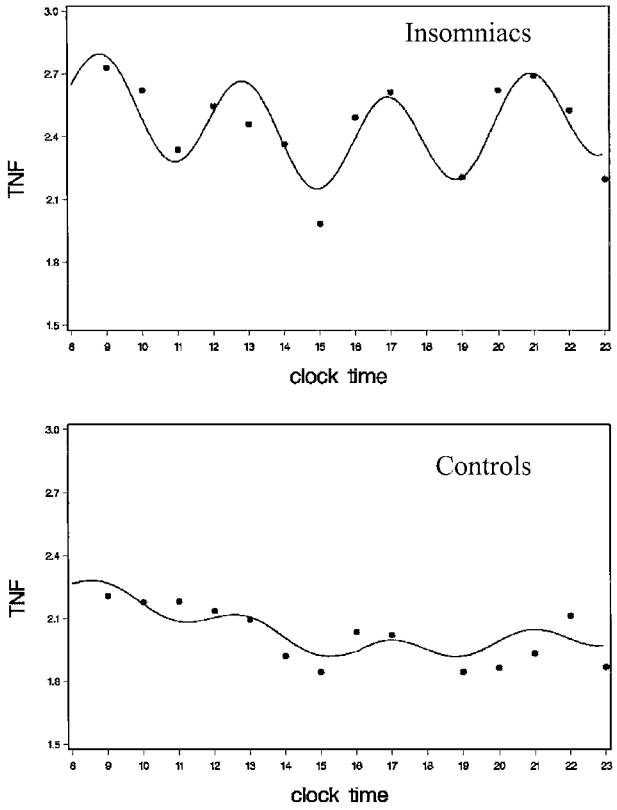

Fig 5. Analysis of daytime (8 AM to 11 PM) TNF ultradian secretory pattern by fitting a cosine wave in insomniacs (top) and controls (bottom).

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. Based on accumulated evidence from this study and previous studies,[3,6,13] 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.[6] On the other hand, we suggest that daytime cytokine hypersecretion and/or circadian alteration of cytokine secretion associated with HPA axis activation, eg, 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 corticotropin-releasing hormone, ACTH, and cortisol, during the early part of the night, induced by IL-6.[14] 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.[15]

In normal controls, IL-6 was secreted in a biphasic circadian pattern, with 2 nadirs at 8 AM and 9 PM and 2 zeniths at 5 PM and 1 AM, with the stronger peak at 2 AM. This pattern is very similar with our previous finding in young normal controls[6] and reinforces our early hypothesis that IL-6 circadian secretion coincides with the daily temporal pattern of sleepiness in humans.[16] Also, TNF peaked close and prior to the offset of sleep (about 6 AM), consistent with previous findings from studies using indirect measures, which showed that TNF secretion peaks during sleep.[17,18] Collectively, these findings support a role of TNF in sleep/wake regulation. Furthermore, we hypothesize that in insomniacs the low plasma levels of IL-6 and TNFα in the latter part of sleep (4 to 6 AM) may explain the early morning awakening/arousal of these patients.

An interesting finding of our study is that in chronic insomniacs, TNF daytime secretion follows a regular periodicity of about one pulse every 4 hours. A similar rhythm, which, however, was not significant, was present also in normal sleepers. It has been previously reported that in normal individuals, during wakefulness there is a recurrent fluctuation in alertness/drowsiness.[19] This phenomenon, which has been described as the basic rest-activity cycle (BRAC), has a reported periodicity of 2 to 4 hours and has been suggested to represent a diurnal extension of REM/non-REM cycle when the 24-hour sleep-wakefulness rhythm is fully established in the adult. It is possible that the augmented periodic daytime secretion of TNF in insomniacs may coincide with a stronger fluctuation in fatigue and alertness in this group of subjects.

The finding of an augmented daytime secretion of IL-6 and TNF in chronic insomniacs and its role in the subjective complaint of fatigue and poor performance may lead to some novel approaches in treating this disorder. For example, from anectodal reports, it is known that anti-inflammatory medications are used by insomniacs to fight fatigue and even to improve sleep. These reports are more frequent among elderly insomniacs, even in those without an active inflammatory disease. It appears that regularizing the 24-hour secretion of IL-6 and TNF, eg, through the use of antagonists or neutralizing antibodies, may improve nighttime sleep and decrease fatigue in individuals with chronic sleep disturbances.

In conclusion, chronic insomnia is 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 24-hour hypersecretion of cortisol, an arousal hormone, may explain the insomniacs' fatigue, which in contrast to disorders of EDS, is not associated with an increased sleep propensity at daytime or nighttime. These findings may lead to novel approaches in treating chronic insomnia.

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REFERENCES

1. Kales A, Kales JD: Evaluation and Treatment of Insomnia. New York, NY, Oxford University Press, 1984

2. American Psychiatry Association: Diagnostic and Statistical Manual of Mental Disorders (ed 4). Washington, DC, APA, 1994

3. Vgontzas AN, Bixler EO, Lin H-M, et al: 2001 Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: Clinical implications. J Clin Endocrinol Metab 86: 3787-3794, 2001

4. Vgontzas AN, Papanicolaou DA, Bixler EO, et al: Elevation of plasma cytokines in disorders of excessive daytime sleepiness: Role of sleep disturbance and obesity. J Clin Endocrinol Metab 82:1313-1316, 1997

5. Vgontzas AN, Papanicolaou DA, Bixler EO, et al: Sleep apnea and daytime sleepiness and fatigue: Relation to visceral obesity, insulin resistance, and hypercytokinemia. J Endocrinol Metab 85:1151-1158, 2000

6. Vgontzas AN, Papanicolaou DA, Bixler EO, et al: Circadian interleukin-6 secretion and quantity and depth of sleep. J Clin Endocrinol Metab 84:2603-2607, 1999

7. Chrousos GP: The hypothalamic-pituitary-adrenal axis in immune-mediated inflammation. N Engl J Med 332:1351-1362, 1995

8. Stepanski E, Zorick F, Roehrs T, et al; Daytime alertness in patients with chronic insomnia compared with asymptomatic control subjects. Sleep 11:54-60, 1988

9. Bonnet MH, Arand DL: The consequences of a week of insomnia. Sleep 19:453-461, 1996

10. Rechtschaffen A, Kales A (eds): A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects. Bethesda, MD, NIH publication 204, 1968

11. Mojón A, Fernández JR, Hermida RC: Chronolab: An interactive software package for chronobiologic time series analysis written for the MacIntosh computer. Chronobiol Int 9:403-412, 1992

12. Fernández JR, Hermida RC: Inferential statistical method for analysis of nonsinusoidal hybrid time series with unequidistant observations. Chronobiol Int 15:191-204, 1998

13. Vgontzas AN, Mastorakos G, Bixler EO, et al; Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: Potential clinical implications. Clin Endocrinol 51:205-215, 1999

14. Späth-Schwalbe E, Hansen K, Schmidt F, et al: Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men. J Clin Endocrinol Metab 83:1578-1579, 1998

15. Mullington JC, Korth D, Hermann A, et al: Dose-dependent effects of endotoxin on human sleep. Am J Physiol 278:R947-955, 2000

16. Lavie P: Ultrashort sleep-waking schedule, III. “Gates” and “forbidden zones” for sleep. Electroencephalogr Clin Neurophysiol 63:414-425, 1986

17. Gudewill S, Pollmächer T, Vedder H, et al: Nocturnal plasma levels of cytokines in healthy men. Eur Arch Psychiatr Clin Neurosci 242:53-56, 1998

18. Entzian P, Linnemann K, Schlaak M, et al: Obstructive sleep apnea syndrome and circadian rhythms of hormones and cytokines. Am J Respir Crit Care Med 153:1080-1086, 1996

19. Kleitman N: Sleep and Wakefulness (rev). Chicago, IL, University of Chicago Press, 1963