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Elevation of Plasma Cytokines in Disorders of Excessive Daytime Sleepiness: Role of Sleep Disturbance and Obesity

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ABSTRACT

Excessive daytime sleepiness (EDS) and fatigue are frequent symptoms in the general population and the chief complaint of the majority of patients at Sleep Disorders Centers. There is evidence that the inflammatory cytokines tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and IL-6 are involved in physiological sleep regulation and that their administration to humans is associated with sleepiness and fatigue. To explore whether plasma levels of TNFα, IL-1β, and IL-6 are elevated in patients with EDS, we measured morning plasma levels of TNFα, IL-1β, and IL-6 in 12 sleep apneics, 11 narcoleptics, 8 idiopathic hypersomniacs, and 10 normal controls. TNFα was significantly elevated in sleep apneics and narcoleptics compared to that in normal controls ($P < 0.001$ and $P = 0.001$, respectively). Plasma IL-1β concentrations were not different between sleep disorder patients and controls, whereas IL-6 was markedly and significantly elevated in sleep apneics compared to that in normal controls ($P = 0.028$). The primary factor influencing TNFα values was the degree of nocturnal sleep disturbance, whereas the primary determinant for IL-6 levels was the body mass index. Our findings suggest that TNFα and IL-6 might play a significant role in mediating sleepiness and fatigue in disorders of EDS in humans. (*J Clin Endocrinol Metab* **82:** 1313–1316, 1997)

EXCESSIVE DAYTIME sleepiness (EDS) occurs in about 5% of the general population and is the chief complaint of the majority of patients evaluated at Sleep Disorders Centers (1, 2). Sleep apnea, narcolepsy, and idiopathic hypersomnia are the three most common sleep disorders associated with EDS. No information exists with regard to potential circulating endogenous factors whose alterations could lead to pathological sleepiness in any of these disorders.

The inflammatory cytokines, tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1) appear to be involved in the regulation of sleep in animals and humans, whereas the exogenous administration of IL-1β to patients has been associated with somnolence and/or increased sleep (3–5). IL-6 was reported to be pyrogenic, but not somnogenic, in rabbits (6). However, in a recent study on sleeping sickness (African trypanosomiasis), plasma concentrations of IL-6 were found to be significantly elevated during the course of the illness (7). Also, exogenous administration of IL-6 in patients with cancer (8) or increased production of endogenous IL-6 (9) were associated with increased sleepiness and fatigue, suggesting that IL-6 may also be related to sleep and sleepiness in man.

In this study, we explored whether circulating TNFα, IL-1β, and IL-6 play a potential pathogenetic role in the EDS associated with sleep apnea, narcolepsy, and idiopathic hypersomnia.

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Subjects and Methods

Subjects

The study population and their clinical profiles are summarized in Table 1. Twelve patients with obstructive sleep apnea, 11 with narcolepsy, 8 with idiopathic hypersomnia, and 10 normal controls were evaluated.

To qualify for the study, apneic patients had to have obstructive apnea of sufficient severity to warrant recommendation for treatment, as previously described (10).

Narcoleptics were defined as those that present with EDS and cataplexy or a sleep-onset rapid eye movement (REM) period during at least one of the morning naps. A sleep-onset REM period was determined as the occurrence of REM sleep within the first 10 min after sleep onset. Finally, patients that presented with EDS and did not meet the criteria for the diagnosis of any other daytime sleepiness disorder (including sleep apnea, narcolepsy, hypersomnia due to a mental disorder, severe snoring, and sleep deprivation) were diagnosed with idiopathic hypersomnia (2).

To avoid potential effects of aging on plasma cytokine levels (11), patients above the age of 55 yr were excluded from the study. Patients who received treatment with methylphenidate (Ritalin; Ciba Pharmaceutical, Summit, NJ) or sympathomimetic or sympatholytic medications were excluded. All patients were asked to abstain from nonsteroidal antiinflammatory medications for 1 week before the study. Patients with any active infection, including the common cold, or any inflammatory diseases, such as rheumatoid arthritis, were excluded.

Control subjects were recruited from the community. They were in good general health, had no sleep complaints, had normal sleep laboratory findings, and were not taking any medications.

TABLE 1. Demographic and nocturnal sleep data in normal controls and patients with EDS

	Controls	Sleep apneics	Narcoleptics	Idiopathic hypersomniacs
Total no.	10	12	11	8
Males	10	11	7	3
Females	0	1	4	5
Age	24.1 ± 0.8	40.9 ± 2.2[a]	28.9 ± 4.0	38.4 ± 3.2[a]
BMI	24.6 ± 0.7	40.5 ± 3.2[a]	32.0 ± 3.7	28.5 ± 2.7
Sleep latency (min)	23.7 ± 5.2	52.8 ± 16.1	14.2 ± 4.9	11.3 ± 3.1
Wake time after sleep onset (min)	29.4 ± 5.7	103.2 ± 16.2[a]	46.9 ± 14.3	54.2 ± 7.4
Total wake time (min)	53.1 ± 6.8	155.9 ± 21.8[a]	61.2 ± 15.0	65.5 ± 7.6
% Sleep time	88.9 ± 1.4	67.3 ± 4.6[a]	87.4 ± 3.1	86.3 ± 1.6
% Stage 1	7.9 ± 2.0	62.7 ± 7.2[a]	17.2 ± 3.5	11.6 ± 1.2
% Stage 2	63.7 ± 2.0	24.6 ± 7.0[a]	57.7 ± 3.0	65.3 ± 1.7
% Slow wave	4.3 ± 1.5	0.0 ± 0.0	4.5 ± 1.6	3.4 ± 1.4
% REM	24.1 ± 1.8	12.7 ± 2.1[a]	20.6 ± 1.3	19.7 ± 1.9
Apnea/hypopnea index	0.0 ± 0.0	63.7 ± 10.3[a]	2.7 ± 2.0	0.0 ± 0.0
Minimum O_2 saturation	96.7 ± 0.6	70.1 ± 3.4[a]	92.2 ± 2.3	95.9 ± 0.4

Data are presented as the mean ± SE.
[a] $P < 0.01$ *vs.* controls.

Sleep laboratory procedures

A thorough medical assessment, including physical examination, laboratory tests, and sleep history, was completed for each patient and control subject. All of the patients and control subjects were evaluated in the sleep laboratory for 1 night for 8 h according to standard polysomnographic procedures (12). In addition, narcoleptic and hypersomniac patients were recorded in the sleep laboratory for two daytime naps after nighttime polysomnography (13). The sleep records were scored independently of any knowledge of the experimental conditions according to standardized criteria (12). Throughout the night, respiration was monitored by thermocouples at the nose and mouth (model TCT 1R, Grass Instrument Co., Quincey, MA) and thoracic strain gauges. All-night recordings of hemoglobin oxygen saturation (S_aO_2) were obtained using a cardiorespiratory oximeter (model 8800, Nonin Medical Inc., Plymouth, MN) attached to the finger. The respiratory data were quantified as previously described (10).

Cytokine assays

Single blood samples for measurement of plasma TNFα, IL-1β, and IL-6 were drawn between 0600–0700 h after the completion of the nocturnal sleep laboratory recording. Blood samples were placed on ice immediately and were centrifuged no later than 3 h after the blood was drawn. Plasma was stored at −70 C until assay. All samples were processed in the same manner. Plasma TNFα, IL-1β, and IL-6 were measured by ELISA (R&D Systems, Minneapolis, MN). The intra- and interassay coefficients of variation were from 5.6–6.1% and 7.5–10.4%, respectively, for TNFα; 11.3% and 18% for IL-1β; and from 3.2–8.5% and 3.5–8.7% for IL-6. The lower detection limits for TNFα, IL-1β, and IL-6 were 0.18, 0.1, and 0.094 pg/mL, respectively.

Statistical analyses

The results of parametric values are expressed as the mean ± SE. For comparisons of parametric values between the control group and disorders of EDS, the Dunnett multiple comparison two-tailed test was employed. For comparisons of nonparametric values, we used Fisher's exact test. The statistical confidence level selected for all analyses was $P < 0.05$.

To assess the strength of the association between cytokines and the intensity of EDS, we employed the Pearson product-moment correlation within those subjects with nap recordings. Specifically, we calculated the correlation between cytokine levels and the mean nap sleep latency employed as a measure of the intensity of EDS. We further calculated the correlation between cytokine levels and possible confounding factors, *i.e.* age, body mass index (BMI), minimum S_aO_2, and nocturnal sleep disturbance (percentage of sleep time).

To assess the relative strength of association of EDS as well as possible confounding factors with cytokines, we employed a stepwise multiple regression in the entire group, including the controls. In this analysis, we used cytokines as the dependent variables and evaluated the order of inclusion in the model of the following independent variables: EDS (a categorical variable, *i.e.* patients with EDS *vs.* controls), BMI, minimum S_aO_2, nocturnal sleep disturbance (percentage of sleep time), and age. Finally, to indicate the overall amount of variability accounted for by the multiple regression analysis, we report the final variability (r_{xy}^2).

Results

Cytokines and daytime sleepiness

The plasma TNFα concentration was significantly elevated in apneics and narcoleptics ($P < 0.001$ and $P = 0.001$, respectively) compared to that in normal controls (Table 2 and Fig. 1A). No significant difference was detected among the different groups with regard to plasma IL-1β levels. Plasma IL-6 was markedly elevated only in sleep apneics compared to controls ($P = 0.028$; Table 2 and Fig. 1B).

When we evaluated the association between the plasma cytokine concentration and a categorical measure of EDS, both TNFα and IL-6 were positively correlated with the pres-

TABLE 2. Cytokine levels in normal controls and patients with EDS

	Controls (n = 10)	Sleep apneics (n = 12)	Narcoleptics (n = 11)	Idiopathic hypersomniacs (n = 8)
TNFα	1.17 ± 0.10	2.51 ± 0.13[a]	2.09 ± 0.25[a]	1.57 ± 0.12
IL-1β	0.60 ± 0.29	0.39 ± 0.14	0.50 ± 0.23	0.11 ± 0.23
(% detected)	50.0	66.7	72.7	14.3
IL-6	1.02 ± 0.42	3.25 ± 0.76[b]	1.72 ± 0.58	1.40 ± 0.34

Data are presented as the mean ± SE. Cytokine values are presented as picograms per mL.
[a] $P < 0.01$ *vs.* controls.
[b] $P < 0.05$ *vs.* controls.

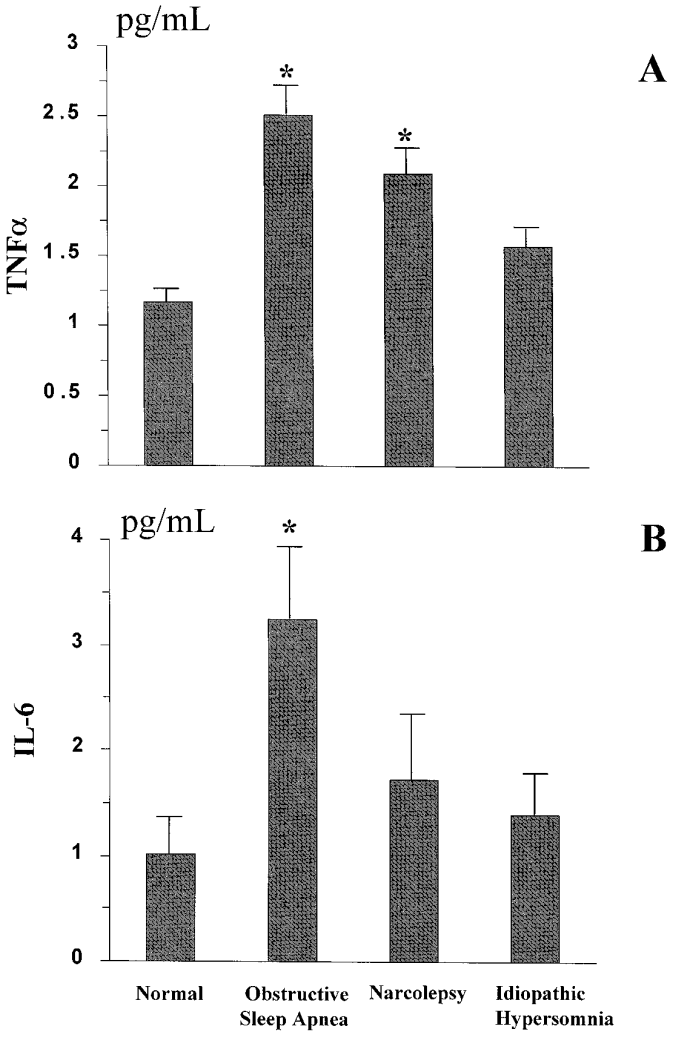

FIG. 1. Plasma TNFα and IL-6 levels in normal subjects and patients with EDS. A: *, $P < 0.001$ *vs.* normal. B: *, $P = 0.028$ *vs.* normal.

ence of EDS ($r_{xy} = 0.59$; $P < 0.001$ and $r_{xy} = 0.28$; $P = 0.044$, respectively).

When we evaluated the association between the cytokine concentration and the intensity of sleepiness using a continuous measure of EDS (mean sleep latency during naps), plasma IL-6 concentrations correlated positively with intensity of sleepiness in the group of narcoleptics ($r_{xy} = -0.70$; $P = 0.017$) and tended to do so in the combined group of narcoleptics and hypersomniacs ($r_{xy} = -0.42$; $P = 0.079$). Plasma TNFα was not correlated with this index of daytime sleepiness.

Cytokines and factors affecting daytime sleepiness

TNFα values were positively correlated with the degree of nocturnal sleep disturbance (percent sleep time; $r_{xy} = -0.50$; $P = 0.001$) and the degree of hypoxia (minimum S_aO_2; $r_{xy} = -0.46$; $P = 0.002$), but not with BMI or age.

IL-6 values were positively correlated with BMI ($r_{xy} = 0.63$; $P < 0.001$; Fig. 2), degree of hypoxia (minimum S_aO_2; $r_{xy} = -0.48$; $P = 0.001$), and degree of nocturnal sleep disturbance

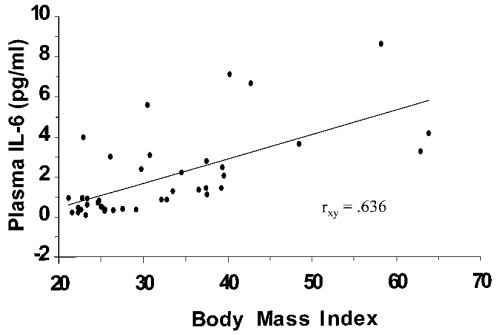

FIG. 2. BMI and IL-6 plasma levels are positively correlated.

(percentage of sleep time; $r_{xy} = -0.40$; $P = 0.006$), but not with age, in the entire group of controls and patients.

Evaluation of the relative strength of association using stepwise multiple regression between TNFα and EDS as well as the other possible confounding variables showed that EDS and nocturnal sleep disturbance were the two variables that were included in the model ($r_{xy}^2 = 0.46$; $P < 0.001$). When this same analysis was completed using IL-6 as the dependent variable, only BMI was included in the model ($r_{xy}^2 = 0.40$; $P = 0.014$). Finally, when IL-1β was employed as the dependent factor in this analysis, no variable was strong enough to be included in the model.

Discussion

Plasma levels of TNFα and/or IL-6 were elevated in patients with disorders associated with excessive daytime sleepiness. These elevations were significant in sleep apnea (TNFα and IL-6) and narcolepsy (TNFα), but not in idiopathic hypersomnia. The lack of significance in the latter group may be due to the small sample size of this cohort and/or to the lesser degree of daytime sleepiness in these patients than in the other patient groups. There was no association between IL-1β and disorders of EDS, which may be attributed to insufficient sensitivity of the currently available IL-1β assay to allow measurements within the normal range.

Plasma inflammatory cytokines follow a circadian rhythm, with peaks between 0100–0200 h, when slow wave sleep usually occurs, suggesting a potential physiological role of these cytokines in normal sleep (14–16). In our EDS patients, we observed elevations during the morning (0600–0700 h), also suggesting a pathophysiological role for these cytokines. The 2- to 3-fold increases in inflammatory cytokines in the plasma of patients with EDS may, in fact, reflect much higher elevations in the production and/or target sites of these cytokines, in this case, we presume, the central nervous system. The underlying mechanisms of the plasma TNFα and/or IL-6 elevations in EDS disorders are not clear. The strong positive correlation between IL-6 and BMI is interesting. Also, IL-6 and TNFα were highest in the sleep apnea group, which had the highest BMI among the patient groups studied. Plasma TNFα concentrations are significantly elevated in obese animals, and its levels best correlate with massive obesity and insulin resistance (17). Our results confirmed this in humans and, in addition, indicated that IL-6 secretion is increased in obese men and women. Interestingly, obesity, even in the absence of sleep apnea, is more

frequently associated with subjective complaints of fatigue, EDS, and nocturnal sleep disturbance (10) as well as with higher degrees of objective EDS than those in age- and sex-matched controls (Vgontzas, A., manuscript in preparation). Our results suggest that inflammatory cytokines, particularly IL-6, may be associated with the increased fatigue and sleepiness exhibited by obese subjects.

Recently, in animal and human studies, IL-6 secretion was found to be regulated positively by catecholamines through β-adrenergic receptors (18, 19). The known increased peripheral sympathetic activity in sleep apnea (20) and obesity (21) could explain the high levels of IL-6 observed in our patients. Furthermore, our data provide evidence that sleep deprivation and, to a lesser extent, hypoxia might play a role in inflammatory cytokine elevation, particularly of TNFα, in disorders of EDS and are consistent with previous findings that sleep deprivation in humans leads to elevations in TNFα (22).

In conclusion, our study suggests that TNFα and IL-6 may play a significant role in mediating sleepiness and fatigue in disorders of EDS in humans. Also, our data point to several potential underlying factors affecting cytokine levels, with obesity playing the strongest role in IL-6 elevation, and sleep disturbance being the strongest factor in TNFα elevation.

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