

# Effects of Sleep and Sleep Deprivation on Interleukin-6, Growth Hormone, Cortisol, and Melatonin Levels in Humans\*

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## ABSTRACT

The objective of this study was to evaluate the effects of nocturnal sleep, partial night sleep deprivation, and sleep stages on circulating concentrations of interleukin-6 (IL-6) in relation to the secretory profiles of GH, cortisol, and melatonin. In 31 healthy male volunteers, blood samples were obtained every 30 min during 2 nights: uninterrupted, baseline sleep and partial sleep deprivation-early night (awake until 0300 h). Sleep was measured by electroencephalogram polysomnography.

Sleep onset was associated with an increase in serum levels of IL-6 ( $P < 0.05$ ) during baseline sleep. During PSD-E, the nocturnal increase in IL-6 was delayed until sleep at 0300 h. Sleep stage analyses indicated that the nocturnal increase in IL-6 occurred in association

with stage 1–2 sleep and rapid eye movement sleep, but levels during slow wave sleep were not different from those while awake. The profile of GH across the 2 nights was similar to that of IL-6, whereas the circadian-driven hormones cortisol and melatonin showed no concordance with sleep.

Loss of sleep may serve to decrease nocturnal IL-6 levels, with effects on the integrity of immune system functioning. Alternatively, given the association between sleep stages and IL-6 levels, depressed or aged populations who show increased amounts of REM sleep and a relative loss of slow wave sleep may have elevated nocturnal concentrations of IL-6 with implications for inflammatory disease risk. (*J Clin Endocrinol Metab* 85: 3597–3603, 2000)

**S**LEEP IS COMMONLY viewed as a restorative process that influences the homeostatic regulation of the autonomic, neuroendocrine, and immune systems (1–3). During normal human sleep, there is a redistribution of circulating lymphocyte subsets and an increase in some aspects of cellular immunity (4). In contrast, in clinical populations who show disordered sleep due to stress or a variety of diseases, including depression, alcohol dependence, and human immunodeficiency virus (HIV) infection, decrements in natural and cellular immune function coincide with disturbances of sleep architecture and loss of sleep (5–8). Moreover, in experimental studies that induce a night of partial sleep loss that resembles the kind of sleep loss found in many clinical samples, suppression of natural killer cell activity and cellular immunity is found (9). Thus, it is thought that disordered sleep and loss of sleep lead to alterations in immune functions that might adversely affect host resistance to infectious disease (10), increase cancer risk (11, 12), and alter inflammatory disease progression (13).

The production and release of proinflammatory cytokines such as IL-6 are essential in the function and regulation of peripheral blood mononuclear cells (14). Interleukin-6 (IL-6) and other proinflammatory cytokines are secreted by mac-

rophages in response to infectious challenge and play a key role in the differentiation, maturation, and proliferation of T and B cells. In addition, IL-6 is a potent stimulator of the hypothalamic-pituitary-adrenal axis, stimulating ACTH and cortisol (15) as well as GH secretion (14).

Accumulating data suggest an interaction among sleep, circadian rhythms, and IL-6. Circulating concentrations of IL-6 show a periodicity, with low values during the daytime and maxima at night (16). IL-6 is proposed to have somnogenic effects, and one study of healthy volunteers found that the administration of IL-6 induced decreases in  $\delta$  sleep during the first half of the night and increases during the second half (15). Finally, disordered sleep and sleep loss are suggested to alter the release of IL-6. Daytime elevations of IL-6 are associated with disordered sleep (*i.e.* sleep apnea) (17), and sleep deprivation is reported to lead to daytime oversecretion and nighttime undersecretion of IL-6 (18). Although one study reported that amounts of slow wave sleep negatively correlate with daytime levels of IL-6 (18), the relationship between electroencephalogram (EEG)-recorded sleep and IL-6 secretion has not been extensively studied (18). Moreover, two other studies failed to detect changes in circulating levels of IL-6 in relation to sleep deprivation and/or circadian rhythms (3, 4). Hence, the few studies performed in humans have not yielded consistent results, and it is not known whether nocturnal IL-6 concentrations are related to sleep activity or sleep stages.

To address these questions regarding the effects of nocturnal sleep and sleep loss on the nocturnal secretion of IL-6, we studied changes in circulating levels of IL-6 in human volunteers over 2 nights: baseline and partial night sleep

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deprivation. In addition, we monitored sleep EEG and assayed circulating concentrations of GH, cortisol, and melatonin. GH is thought to be sleep dependent (19, 20), whereas cortisol and melatonin are proposed to be driven primarily by circadian processes (21, 22). We hypothesized that sleep onset would be associated with an increase in IL-6, and that during the night of early night partial sleep deprivation, the increase in IL-6 would be delayed until sleep onset. The profile of secretion of IL-6 across the baseline and partial sleep deprivation nights is hypothesized to be parallel to that of GH, but not that of cortisol or melatonin.

## Subjects and Methods

### Subjects

Male volunteers ( $n = 31$ ) were selected using recruitment procedures of the Mental Health Clinical Research Center (MH-CRC), which involved a standardized search strategy of the San Diego area employing advertisements in local newspapers and university newsletters. Before giving informed consent for the present study, the volunteers underwent a rigorous psychiatric and medical evaluation by MH-CRC psychiatric research fellow-physicians. This assessment included psychiatric and medical histories, physical examination, screening laboratory examination (chemistry panel, complete blood cell count, thyroid function tests, and HIV test), and formal structured psychiatric diagnostic interview using the Schedule for the Clinical Interview (DSM-III-R or DSM-IV) (23). After consensual diagnosis by at least three MH-CRC psychiatrists, all subjects were found to have no lifetime history of a DSM-III-R or DSM-IV mental disorder such as major depression or substance dependence (24). Medical history, physical examination, and laboratory tests revealed that the men were in good medical health; subjects did not have a history of recent (<10 days) viral infection or of diseases (e.g. autoimmune disorders or cancer) that could affect immune function. In addition, none of the men reported using psychotropic medications or other medications such as PG inhibitors (*i.e.* aspirin or nonsteroidal antiinflammatory agents) or  $\beta$ -blockers, which are known to affect sleep structure and/or immune function within a 7-day period before study enrollment. Screening laboratory tests, including complete blood cell count, chemistry panel, liver and thyroid function, and HIV tests, were within normal limits.

The average age of the subjects was 35.8 yr (SD, 10.12; range, 25–65 yr), and they had achieved a mean educational level of 16.3 yr (SD = 1.9; range, 14–21 yr). The ethnic composition of the sample was 81% white, 7% Philippino, 7% Native American, 3% black, and 3% Asian; marital characteristics of the sample showed that 45% were single, 39% were married, and 16% were divorced/separated.

Vgontzas *et al.* suggested that better quantity and depth of sleep are associated with the secretion of IL-6 (17). Thus, threshold criteria for sleep quality during the baseline night were implemented for comparison of IL-6 between the baseline and partial sleep deprivation-early night (PSD-E) nights, and analyses of IL-6 data were limited to those subjects who slept more than 5 h and had a sleep efficiency greater than 85% during the baseline night ( $n = 12$ ). The characteristics of the subsample was similar to the total sample; average age was 34.6 yr (SD, 9.8; range, 25–60 yr) with the following ethnic composition: 75% White, 8% Native American, 8% Philippino, and 8% Asian. Marital characteristics of the subsample were also similar to the total sample: 50% were married, 25% were single, and 25% were separated/divorced. For GH, cortisol, and melatonin, subsample results were identical to those obtained on the total sample.

### Procedures

Two weeks before entry into the study, sleep-wake activity diaries were obtained; all volunteers were regularly sleeping between the 2200–0700 h with average total sleep of 7.0 (SD, 0.7) h/night. Subjects participated in a 3-night sleep protocol. On the first night in the sleep laboratory, subjects adapted to the conditions of the laboratory. During this adaptation night, recordings of oxygen desaturation and tibial myoclonus were obtained to exclude subjects with either sleep apnea or

nocturnal myoclonus. On the second night (baseline night), sleep EEG recordings and nocturnal blood samples were obtained during an uninterrupted period of nocturnal sleep. On the third night (PSD-E night; awake time, 2200–0300 h), sleep EEG recordings and nocturnal blood samples were again obtained.

On the 2 experimental nights, subjects arrived at the laboratory between 2000–2100 h. Subjects then readied for sleep and had electrodes placed for EEG, electrooculography, and submental electromyography recordings. Between 2030 and 2130 h, an iv catheter was inserted into a forearm vein, and subjects rested in a supine horizontal position in an individual bedroom at the MHCRC. Subjects remained awake during the initial 30 min, with the first blood sample obtained at the end of this interval. We previously demonstrated that immunoregulatory cell numbers as well as circulating levels of catecholamines reach a stable, resting baseline within 30 min after iv placement and supine rest (25). Lights were turned off between 2300–2400 h, and subjects were, on the average, asleep at 2330 h on the baseline night and at 0300 h on the PSD-E night. On the baseline night, subjects were awakened at 0630 h after the last blood draw if they were not already awake. On the PSD-E night, subjects were kept awake by a sleep technician who monitored subject behavior and EEG until the 0300 h blood draw. All subjects included in the present analyses remained supine throughout the entire nocturnal period on the baseline and PSD-E nights; a bedside urinal was used if subjects needed to urinate during the night.

For blood sampling, the iv catheter was connected to a long thin plastic tube that enabled blood collection from an adjacent room without disturbing the subject's sleep. Between blood samplings, continuous heparinized isotonic saline was infused, totaling about 1000 mL across each of the nocturnal periods. Blood was sampled every 30 min beginning at 2100–2200 h and continuing until 0600 h; the volume of blood removed totaled no more than 150 mL each experimental night. Immediately after blood was obtained, it was put into heparinized and non-heparinized tubes. Heparinized tubes were immediately placed on ice and centrifuged for acquisition of plasma. Serum was obtained by allowing the blood samples to clot at 10 C. All samples were then stored at  $-80$  C until assay.

Sleep EEG measures were obtained during continuous recordings between approximately 2200–0630 h during the adaptation, baseline, and PSD-E nights. Sleep data from the first night were not used in the analyses. Sleep records were visually scored according to the criteria of Rechtschaffen and Kales (26). Data from each 30-s epoch were entered into a computer program that tallies the summary statistics for each subject. Sleep onset was defined as the first minute of stage 2 or rapid eye movement (REM) sleep followed by at least 8 min of sleep in the next 9 min. A REM period was defined by not less than 3 consecutive min of REM sleep. Sleep efficiency was the ratio of total sleep time to the time in bed multiplied by 100. Sleep architecture was defined as the duration of time spent asleep in non-REM sleep, stages 1–4. REM density was an estimate of the number of eye movements per min of REM sleep, scored on a scale of 0–4/30-s epoch, but expressed on a scale of 0–8/min. Sleep research technicians were regularly tested on scoring reliability, and high standards were maintained: sleep latency ( $r = 0.96$ ), REM latency ( $r = 0.99$ ), REM density ( $r = 0.91$ ), amounts of stages 3 and 4 ( $r = 0.85$ ), and total sleep time ( $r = 0.99$ ).

### Assays

**IL-6.** Blood samples across the entire baseline and PSD-E nights were available for assay for serum concentrations of IL-6 in 12 subjects. Serum levels of IL-6 were quantified using Quantikine High Sensitivity human IL-6 kits (R&D Systems, Inc., Minneapolis, MN) with an intraassay coefficient of variation of 4% and an interassay coefficient of variation of 10%. The minimal detectable dose of IL-6 is 0.156 pg/mL, well below levels identified in our subjects.

**GH, cortisol, and melatonin.** Blood samples across the entire baseline and PSD-E nights were available for assay for plasma concentrations of these hormones in 31 subjects. For GH, RIA kits were used to quantify plasma concentrations of GH (ICN Biomedicals, Inc., Carson, CA); the intraassay coefficient of variation is 5%, with an interassay coefficient of variation of 8%. Commercially available RIA kits were also used to quantify plasma concentrations of cortisol (Diagnostic Products, Los Angeles, CA) with an intraassay coefficient of variation of 4% and an interassay

coefficient of variation of 6%. To quantify plasma concentrations of melatonin, RIA kits were also used (DiagnosTech International, Inc., Osceola, WI), with an intraassay coefficient of variation of 6% and an interassay coefficient of variation of 10%.

### Statistical analyses

To evaluate specific hypotheses regarding changes in circulating IL-6 and hormone levels in relation to sleep onset on the baseline night, circulating levels of IL-6 and hormone levels were profiled in reference to the individual time of sleep onset, and planned comparisons using paired *t* tests were conducted. Planned comparisons are considered the most appropriate test for evaluating specific hypotheses and allow for the extraction of information critical to the status of the research question (27). Thus, for example, to test whether an increase in circulating IL-6, GH, cortisol, or melatonin occurred after sleep onset, paired *t* tests compared average awake and nocturnal peak IL-6 and hormone levels. Additional planned comparisons were conducted in reference to chronological time and compared average levels of IL-6, GH, cortisol, and melatonin between awake and early and late parts of the baseline and PSD-E nights. To evaluate changes in hormone concentrations from waking to sleep,  $\delta$  scores (early night – awake; late night – awake) for IL-6, GH, cortisol, and melatonin were calculated based on awake levels of the individual baseline and PSD-E nights.

### Results

Table 1 shows the means for the various EEG sleep measures obtained during the baseline and partial sleep deprivation nights. The subjects slept an average of 6.2 h, and this amount of total sleep was reduced to about 3.3 h on the PSD-E night. During the PSD-E night, sleep was more consolidated, as evidenced by increased sleep efficiency, shorter sleep latency, and a relative increase in stage 3 and 4 sleep at the expense of stage 1 and 2 sleep compared to baseline sleep. Aside from a shorter REM latency during the PSD-E night, relative amounts of REM sleep and REM density were similar on the two nights.

### Sleep onset and IL-6, GH, cortisol, and melatonin levels during the baseline night

Compared to levels during the awake period ( $1.1 \pm 0.6$  pg/mL), nocturnal levels of IL-6 increased as early as 30 min after sleep onset ( $1.4 \pm 0.5$  pg/mL;  $t = -3.0$ ;  $P < 0.05$ ) and peaked 2.5 h after sleep ( $2.3 \pm 1.6$  pg/mL) across individuals who were time locked to sleep onset (Fig. 1A). Analyses on the basis of chronological time revealed similar results. On the average, the subjects fell asleep at 2330 h. IL-6 levels were higher during the early (2330–0300 h;  $1.6 \pm 0.8$  pg/mL) and late (0330–0600 h;  $1.6 \pm 0.8$  pg/mL) parts of the night compared to those during the awake period before sleep (2200–2300 h;  $1.1 \pm 0.7$  pg/mL;  $t = -7.1$ ,  $P < 0.001$ ;  $t = -2.6$ ,  $P < 0.05$ ).

Compared to awake levels ( $2.1 \pm 2.2$  pg/mL), circulating concentrations of GH were elevated 30 min after falling asleep ( $4.0 \pm 4.1$  pg/mL;  $t = -2.2$ ;  $P < 0.05$ ), peaked 1 h after sleep onset ( $5.7 \pm 5.1$  pg/mL), and then returned to levels comparable to those during the awake period 2 h after sleep onset (Fig. 1B). Analyses in reference to chronological time confirmed that GH levels were significantly elevated during the early ( $3.4 \pm 1.6$  pg/mL;  $t = -2.4$ ;  $P < 0.05$ ), but not the late ( $1.9 \pm 1.4$  pg/mL;  $t = 1.2$ ;  $P = 0.2$ ) part of the night compared to those during the awake period before sleep ( $2.3 \pm 1.9$  pg/mL).

Cortisol levels reached a concentration ( $7.02 \pm 5.8$  pg/mL) 2.5 h after sleep onset that was higher than awake levels ( $4.9 \pm 4.5$  pg/mL;  $F = 5.3$ ;  $P < 0.05$ ), and this hormone continued to increase throughout the remainder of the nocturnal period, with all subsequent cortisol levels significantly higher ( $P < 0.01$ ) than awake levels (Fig. 1C). Analyses on the basis of chronological time showed that cortisol levels were similar during the early part of the night ( $5.8 \pm 3.5$ ;  $t =$

**TABLE 1.** EEG sleep measures during baseline and partial sleep deprivation-early night

	Baseline night [mean (SD)]	Partial sleep deprivation- early night [mean (SD)]	Significance	
			<i>t</i>	<i>P</i>
Sleep continuity				
Total sleep time (min)	372.2 (63.3)	195.4 (16.4)	17.6	0.001
Sleep efficiency (%)	83.4 (12.4)	91.0 (5.6)	-3.8	0.001
Sleep latency (min)	19.3 (19.0)	6.8 (4.0)	4.2	0.001
Sleep architecture				
Stage 1 sleep				
Min	33.3 (17.1)	11.0 (6.7)	7.7	0.001
%	9.8 (7.5)	5.6 (3.4)	3.2	0.002
Stage 2 sleep				
Min	213.7 (45.5)	101.2 (21.2)	14.0	0.001
%	57.4 (8.0)	51.8 (9.8)	3.2	0.003
Stage 3 sleep				
Min	30.8 (22.8)	25.4 (13.8)	1.6	0.1
%	7.9 (5.5)	13.0 (7.1)	-5.0	0.001
Stage 4 sleep				
Min	14.7 (17.2)	14.2 (5.4)	0.2	0.9
%	3.7 (9.4)	7.5 (9.4)	-3.4	0.002
REM measures				
REM sleep				
Min	79.6 (27.3)	43.5 (15.6)	7.8	0.001
%	21.0 (5.4)	22.1 (7.1)	-0.7	0.5
REM latency				
Min	74.0 (29.6)	41.9 (33.6)	5.7	0.001
REM density				
1st period	1.7 (0.8)	1.8 (0.8)	-1.7	0.1

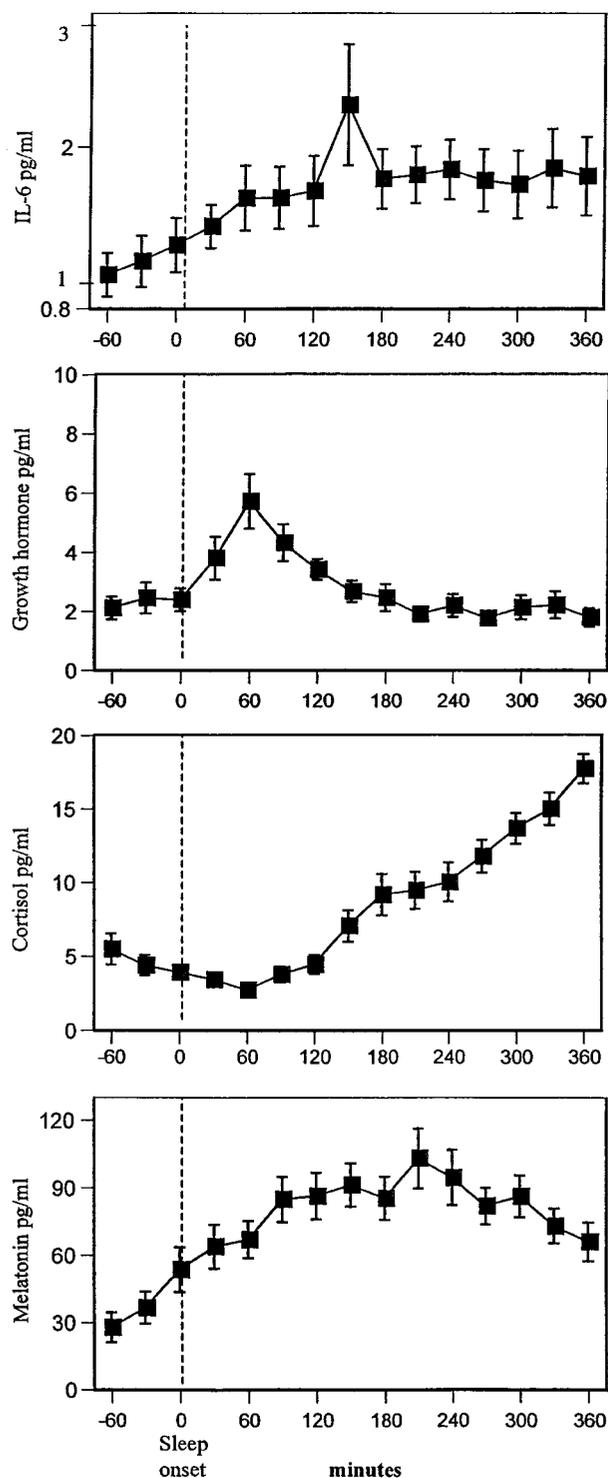


FIG. 1. Mean ( $\pm$ SEM) circulating levels of IL-6 (A), GH (B), cortisol (C), and melatonin (D) in subjects during the baseline night. Profiles were averaged across individuals time locked to sleep onset. The vertical dashed line indicates the average time that subjects were asleep. Blood was drawn every 30 min.

$-0.873$ ;  $P = 0.39$ ), but were higher during the late part of the night ( $15.4 \pm 4.1$  pg/mL;  $t = -8.13$ ;  $P < 0.001$ ) compared to those during the awake period.

Melatonin levels reached a concentration ( $61.8 \pm 57.3$  pg/

mL) immediately after sleep onset that was higher than awake levels ( $37.2 \pm 38.8$ ;  $t = -4.4$ ;  $P < 0.001$ ). All subsequent melatonin levels obtained during sleep were higher ( $P < 0.01$ ) than awake levels, with a peak concentration 3.5 h after sleep onset ( $102.8 \pm 74.3$  pg/mL). Analyses in reference to chronological time showed that melatonin levels were higher during the early ( $79.7 \pm 51.4$  pg/mL;  $t = -6.4$ ;  $P < 0.001$ ) and late ( $75.2 \pm 42.3$  pg/mL;  $t = -3.9$ ;  $P < 0.01$ ) parts of the night compared to those during the awake period.

#### Sleep deprivation and IL-6, GH, cortisol, and melatonin levels

In contrast to those on the baseline night, early night IL-6 levels ( $1.6 \pm 0.5$  pg/mL) were similar to awake levels ( $1.4 \pm 0.8$  pg/mL;  $t = -1.0$ ;  $P = 0.33$ ) during the PSD-E night. However, once subjects fell asleep during the late part of the night, IL-6 levels increased and were higher than those while awake ( $2.0 \pm 1.0$ ;  $t = -2.3$ ;  $P < 0.05$ ; Fig. 2A). During PSD-E, GH levels showed a similar pattern; GH levels were not different between the awake period and the early part of the night ( $2.1 \pm 2.0$  pg/mL;  $t = -1.04$ ;  $P = 0.31$ ), but increased after sleep onset in the late part of the night ( $3.8 \pm 2.6$  pg/mL;  $t = -4.03$ ;  $P < 0.001$ ; Fig. 2B). However, in contrast with the delay in onset of IL-6 and GH secretion during PSD-E, the secretory profile of cortisol and melatonin showed increases during the early part of the PSD-E night before sleep onset. Compared to the level while awake ( $3.8 \pm 1.6$  pg/mL), cortisol levels were higher in early ( $5.9 \pm 3.2$  pg/mL;  $t = -4.24$ ;  $P < 0.001$ ) and late ( $11.8 \pm 3.1$  pg/mL;  $t = -12.95$ ;  $P < 0.001$ ) parts of the night (Fig. 2C). Likewise, melatonin levels in the early ( $61.1 \pm 46.61$ ;  $t = -6.4$ ;  $P < 0.001$ ) and late ( $72.7 \pm 44.5$  pg/mL;  $t = -6.0$ ;  $P < 0.001$ ) parts of the night were higher than levels while subjects were awake ( $29.0 \pm 28.2$  pg/mL; Fig. 2D).

#### Comparison of IL-6, GH, cortisol, and melatonin between baseline and PSD-E

To further evaluate the effects of sleep and sleep deprivation on IL-6, GH, cortisol, and melatonin levels between the 2 nights, change scores were calculated for each time point in relation to awake levels. For IL-6, the average change in the early part of the night was higher during the baseline compared to the PSD-E night ( $t = 2.4$ ;  $P < 0.05$ ). In the late part of the night, IL-6 change scores showed similar elevations on both baseline and PSD-E nights ( $t = -0.11$ ;  $P = 0.91$ ; Fig. 3A) For GH, the average change in the early part of the night was not statistically different between nights ( $t = 0.92$ ;  $P = 0.37$ ; Fig. 3B). In contrast, late night change scores were elevated on the PSD-E night compared to baseline ( $t = -3.9$ ;  $P < 0.01$ ), consistent with the idea that there is pulsatile release of GH after sleep onset and that increases in GH are not maintained throughout the duration of the nocturnal period. For cortisol, the average change during the early night showed no difference between the baseline and PSD-E nights ( $t = -0.98$ ;  $P = 0.34$ ), although late night change scores were decreased on the PSD-E compared to baseline ( $t = 6.7$ ;  $P < 0.001$ ). For melatonin, the average changes during the

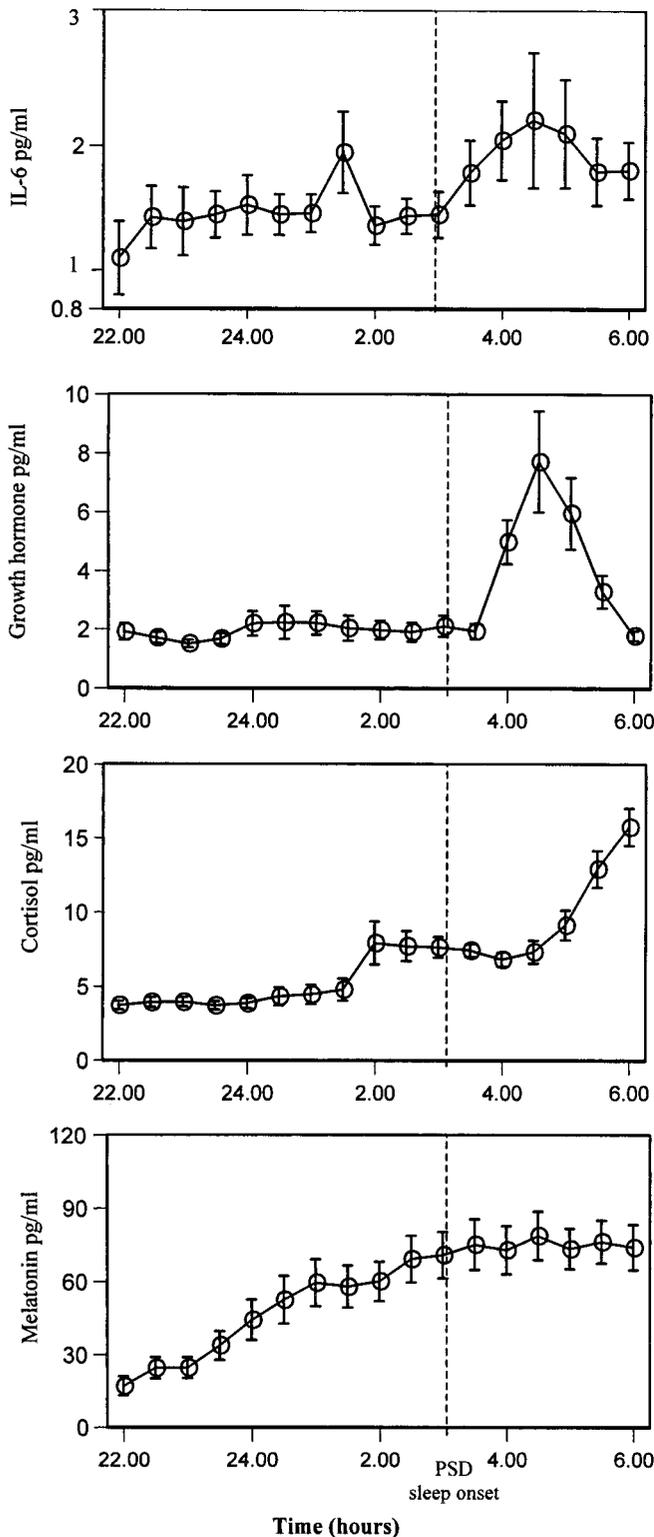


FIG. 2. Mean ( $\pm$ SEM) circulating levels of IL-6 (A), GH (B), cortisol (C), and melatonin (D) in subjects during the PSD-E night. The vertical dashed line at 0300 h indicates the time of sleep onset.

early and the late parts of the night were similar on the PSD-E and baseline nights ( $t = 1.45$ ,  $P = 0.16$ ;  $t = -0.54$ ,  $P = 0.59$ ).

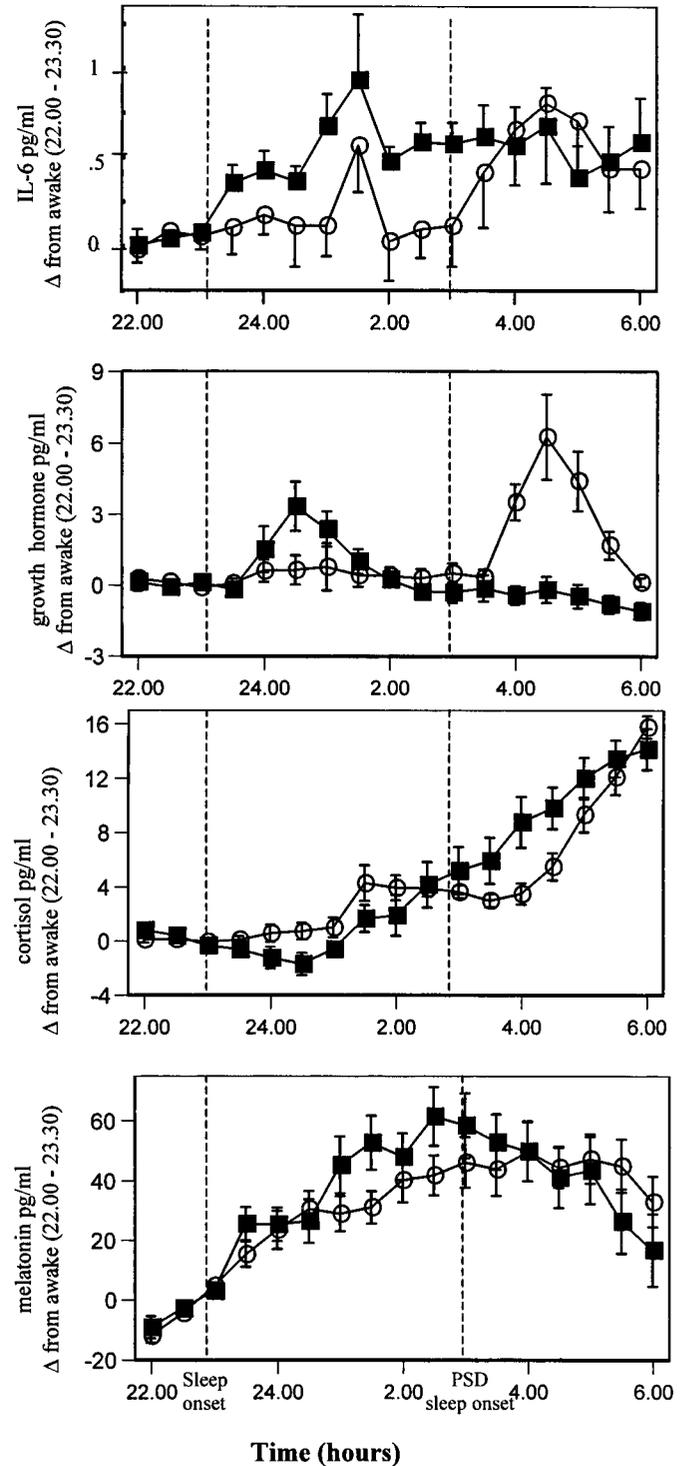


FIG. 3. Averaged change scores from the awake period ( $\pm$ SEM) for circulating levels of IL-6 (A), GH (B), cortisol (C), and melatonin (D) in subjects during baseline (■) and PSD-E (○) nights. The vertical dashed line after 2300 h indicates the average time that the subjects were asleep on the baseline night; the vertical dashed line at 0300 h indicates the time that the subjects were asleep on the PSD-E night.

#### Sleep stages and IL-6 levels

Levels of IL-6 were compared between awake and different sleep stages over the nocturnal period. A general factorial

univariate ANOVA revealed that circulating concentrations of IL-6 were different across sleep stages ( $F = 8.9$ ;  $P < 0.001$ ; Fig. 4). *Post-hoc* comparisons demonstrated that circulating concentrations of IL-6 were higher during stages 1–2 sleep ( $t = 3.2$ ;  $P < 0.01$ ) and REM sleep ( $t = 3.6$ ;  $P < 0.01$ ) compared to average levels during the awake period. Levels of IL-6 were similar between the awake period and stages 3–4 ( $t = -0.365$ ;  $P = 0.72$ ).

### Discussion

Sleep onset is associated with an increase in circulating levels of IL-6. Across the night, concentrations of IL-6 obtained during sleep were higher than those while awake, with peak values occurring 2.5 h after sleep onset. Furthermore, during partial night sleep deprivation when the onset of sleep was delayed, the nocturnal increase in IL-6 did not occur until after sleep onset at 0300 h. Finally, increased secretion of IL-6 during sleep was associated with stages 1–2 and REM sleep. In contrast, IL-6 secretion during slow wave sleep was comparable to the levels found while awake.

These data implicate sleep in the nocturnal regulation of IL-6 secretion. First, the increase in IL-6 occurred after sleep onset, similar to the profile found for the sleep-dependent hormone, GH. Second, experimental induction of sleep loss resulted in a delay in the nocturnal elevation of IL-6 and GH. In contrast, sleep deprivation had no effect on the nocturnal secretory profile of cortisol or melatonin, which, taken together, suggest that sleep, rather than a circadian pacemaker, influences nocturnal IL-6 and GH secretion. Third, nocturnal elevations of IL-6 occurred during periods of stage 1–2 sleep and REM sleep, but not slow wave sleep. The present observations linking decreases in nocturnal IL-6 to the onset of slow wave sleep are consistent with those of Vgontzas *et al.*, who found a negative correlation between slow wave sleep and IL-6 release (18). Moreover, Vgontzas *et al.* found that IL-6 was decreased during a night of recovery sleep, which is typically associated with increased relative amounts of slow wave sleep (18).

There is considerable heterogeneity in studies linking sleep and the secretion of IL-6. Some studies have failed to find nocturnal increases in IL-6 or effects of sleep loss on IL-6 (3, 4), whereas others have reported increases in IL-6 during the nocturnal period and/or increases in IL-6 during the

daytime, postsleep deprivation period (16, 18). These discrepant results may be explained in part by differences in sampling rate, as suggested by Vgontzas *et al.* (18). A longer sampling interval (3 h) may not provide as reliable an estimate of circulating IL-6 as shorter sampling periods and may miss changes in IL-6 that occur during sleep periods or sleep stages that are short in duration. Differences in assay sensitivity may also explain the heterogeneity in results linking sleep and IL-6; the cytokine assay used in the present study is comparable to that used by Vgontzas *et al.* (18) and is more sensitive than those used in prior studies (3, 4).

Our data suggest that sleep amounts and depth of sleep play a role in the nocturnal secretion of IL-6. The increases in IL-6 during stage 1–2 sleep and REM sleep along with the short and infrequent periods of slow wave sleep translate into an overall increase in IL-6 during sleep in middle-aged adults. Similar to these findings concerning IL-6, nocturnal elevations of IL-2 are reportedly related to sleep, rather than to a circadian pacemaker (4). Alternatively, cytokines such as IL-6 are hypothesized to have a regulatory influence on sleep. Exogenous doses of IL-6 are associated with decreased REM sleep and decreased amounts of slow wave sleep in the first part of the night, followed by increases in the second half (15). In rabbits, central infusion of IL-6 is also reported to induce a decrease in non-REM sleep, but this effect is small and not statistically significant, possibly due to the use of human IL-6 and the species-specific action of this cytokine (28). However, other animal studies have revealed that proinflammatory cytokines (*e.g.* IL-1 $\beta$  and TNF- $\alpha$ ) affect sleep physiology by enhancing slow wave sleep. Importantly, the somnogenic effects of IL-1 $\beta$  and TNF- $\alpha$  are independent of the pyrogenic activity of these cytokines; low doses of IL-1 and TNF enhance sleep without inducing fever, whereas blockade of fever has no effect on their somnogenic action (2). Taken together, these observations suggest a possible bidirectional interaction between sleep and cytokines, in which sleep regulates cytokine expression and cytokine release alters sleep.

IL-6 is known to have a stimulating influence on the secretory activity of the HPA axis (15, 29, 30). However, in studies involving the exogenous administration of IL-6, doses were higher than might be achieved under physiological conditions and/or the subjects displayed advanced malignancies (31). In the present study we found that a delay in secretion of IL-6 during PSD-E is associated with a delay in onset of cortisol release. These findings together with the observations of Crofford *et al.* in rheumatoid arthritis patients suggest that endogenous IL-6 may be one of several physiological factors stimulating adrenal cortisol production during the night (13).

Among the mechanisms underlying the relation between sleep architecture and nocturnal IL-6 secretion, the release of catecholamines needs to be considered. Catecholamines stimulate IL-6 secretion via the  $\beta$ -adrenergic receptor (32, 33), and exercise-induced release of epinephrine and norepinephrine correlates with increases in IL-6 (34). During sleep, sympathetic neural activity increases during REM sleep (35) and decreases during slow wave sleep (36). Indeed, increases in norepinephrine during REM may account for the increase in IL-6 during this sleep stage. Nevertheless, compared to awake levels, average circulating levels of catecholamines

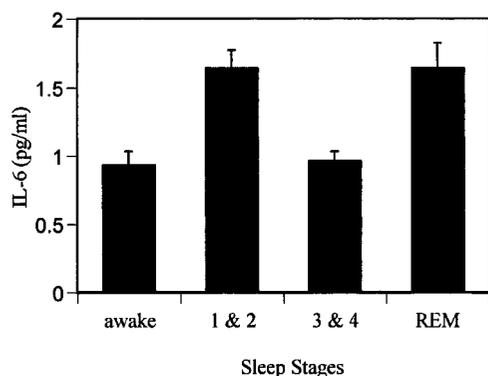


FIG. 4. Mean ( $\pm$ SEM) serum levels of IL-6 across the various sleep stages.

decrease during sleep, and it not known what accounts for the average increase in IL-6 during sleep. GH and melatonin have been reported to promote proinflammatory and T cell function (37–39), but there was no correlation between IL-6 levels and these neuroendocrine hormones in the present study.

Consistent with the idea that sleep has a restorative function, normal sleep is associated with enhancement of IL-6 release as well as other immune processes (4). Thus, loss of sleep during part of the night may be one factor exacerbating the immunological alterations found in persons who experience psychological stress or suffer from a psychiatric disorder and commonly complain of insomnia (40, 41). In addition, given the association between increases in IL-6 and REM sleep, disturbances of sleep architecture may lead to abnormalities in the sleep-related secretion of IL-6. For example, in conditions such as aging and depression, where there is a relative increase in REM sleep at the expense of slow wave sleep (40), it is possible that abnormal increases in IL-6 may occur during sleep, with implications for inflammatory and cardiovascular disease risk in these populations (14, 42).

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