

Soluble TNF- α receptor 1 and IL-6 plasma levels in humans subjected to the sleep deprivation model of spaceflight

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Background: The extent to which sleep loss may predispose astronauts to a state of altered immunity during extended space travel prompts evaluation with ground-based models. **Objective:** We sought to measure plasma levels of selected cytokines and their receptors, including the putative sleep-regulation proteins soluble TNF- α receptor (sTNF- α R) I and IL-6, in human subjects undergoing 2 types of sleep deprivation during environmental confinement with performance demands. **Methods:** Healthy adult men ($n = 42$) were randomized to schedules that varied in severity of sleep loss: 4 days (88 hours) of partial sleep deprivation (PSD) involving two 2-hour naps per day or 4 days of total sleep deprivation (TSD). Plasma samples were obtained every 6 hours across 5 days and analyzed by using enzyme-linked immunoassays for sTNF- α RI, sTNF- α RII, IL-6, soluble IL-2 receptor, IL-10, and TNF- α . **Results:** Interactions between the effects of time and sleep deprivation level were detected for sTNF- α RI and IL-6 but not for sTNF- α RII, soluble IL-2 receptor, IL-10, and TNF- α . Relative to the PSD condition, subjects in the TSD condition had elevated plasma levels of sTNF- α RI on day 2 ($P = .04$), day 3 ($P = .01$), and across days 2 to 4 of sleep loss ($P = .01$) and elevated levels of IL-6 on day 4 ($P = .04$). **Conclusions:** Total sleep loss produced significant increases in plasma levels of sTNF- α RI and IL-6, messengers that connect the nervous, endocrine, and immune systems. These changes appeared to reflect elevations of the homeostatic drive for sleep because they occurred in TSD but not PSD, suggesting that naps may serve as the basis for a countermeasures approach to prolonged spaceflight. (*J Allergy Clin Immunol* 2001;107:165-70.)

Key words: Spaceflight model, sleep deprivation, TNF- α RI, IL-6

Conditions of isolation, confinement, and sleep loss are among the most common factors that may predispose astro-

Abbreviations used

PSD: Partial sleep deprivation
sTNF- α R: Soluble TNF- α receptor
TSD: Total sleep deprivation

nauts to a state of altered immunity during extended space travel. In planning for long-duration spaceflight, it is therefore important to study these unique stressors of the space environment with ground-based models to permit the careful and controlled assessment of their possible harmful effects on human immune responses. Sleep loss is documented to occur in spaceflight,¹⁻⁵ and ground-based experiments of sleep deprivation have revealed alterations of cellular immune responses.⁶⁻⁹ Moreover, there is a growing awareness that immunologic alterations coincide with sleep disturbances in a variety of debilitating conditions, including schizophrenia,¹⁰ depression,¹¹ and aging.¹²

There is mounting evidence from animal research to indicate that the cytokines regulating inflammatory processes, IL-1 β and TNF- α ,¹³ IL-4,¹⁴ and IL-10,^{15,16} are intricately involved in sleep-wake regulation. TNF- α has 2 soluble receptors, soluble TNF- α receptor (sTNF- α R) I and sTNF- α RII; there is evidence to suggest that sleep is regulated by sTNF- α RI¹⁷ and not by sTNF- α RII.¹⁸ High plasma levels of TNF- α upregulate the T-cell/monocyte surface expression of sTNF- α RI and sTNF- α RII, as well as the release of these receptors in soluble form, thereby serving a homeostatic function. In addition to TNF- α and IL-1 β , IL-4 and IL-10 are activated by nuclear factor κ B, a DNA-binding protein involved in transcription.¹³ Nuclear factor κ B has been reported to be activated in the cerebral cortex during sleep deprivation in mice,¹⁹ raising the possibility of cytokine alterations as a consequence of sleep deprivation in human subjects.

Research on human subjects has also demonstrated a clear sensitivity of the sleep-wake system to activation of host-response mechanisms. When healthy volunteers receive low-dose endotoxin challenge, they show increased nonrapid eye movement sleep and slow-wave sleep.²⁰ The sleep enhancement is most prominent when cytokine levels are elevated to a subpyrogenic level that also fails to raise plasma cortisol levels.²¹ Not only does sleep respond to host immune activation, it appears to modulate host response itself because after sustained

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Supported by National Aeronautics and Space Administration cooperative agreement NCC 9-58 through the National Space Biomedical Research Institute (W.T. Shearer, D.F. Dinges, and J.M. Mullington), by the Air Force Office of Scientific Research grant F49620-95-1-0388 (D.F. Dinges), and by National Institutes of Health grant RR-00040.

Received for publication August 23, 2000; revised September 25, 2000; accepted for publication October 11, 2000.

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0091-6749/2001 \$35.00 + 0 1/84/112270

doi:10.1067/mai.2001.112270

wakefulness of approximately 40 hours, the body temperature response to low-dose endotoxin challenge is reduced.²² In healthy volunteers plasma IL-6 levels have been negatively correlated with the amount of nocturnal sleep,²³ and IL-6 administration has been linked to increased fatigue.²⁴ It may be that there is a stronger relationship between IL-6 and fatigue than sleepiness per se because 2 studies have found negative correlations between IL-6 levels and the amount of nonrapid eye movement sleep during daytime naps.^{25,26}

Although there have been reports of significant alterations in the number of immunocompetent cells in circulation during sleep loss^{6,9} and of a proliferative response to stimulation during sleep loss,^{6,27-29} little is known about the effects of sleep loss on plasma levels of cytokines and their receptors.^{7,23} Furthermore, there are no studies investigating the putative sleep-regulation proteins sTNF- α RI and IL-6 in human subjects undergoing sleep deprivation during conditions of environmental confinement, social isolation, and performance demands characteristic of spaceflight. This experiment was undertaken (1) to determine whether the severity of sleep deprivation would alter the plasma levels of certain cytokines and cytokine receptors that are known to regulate sleep and (2) to explore the extent to which a sleep schedule consisting of intermittent naps could serve as a possible countermeasure to immune changes induced by sleep loss during long-duration space travel.

METHODS

Web site video

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Subjects

A total of 58 men between 21 and 47 years of age (mean, 28.7 years) gave informed consent for the protocol, which was approved by the Institutional Review Board of the University of Pennsylvania. Subjects were screened to ensure they met the following criteria: healthy and free of active infection and symptoms; within normal limits on all parameters in one or more prestudy complete blood cell counts and clinical chemistry tests; medication free and drug free, as determined by toxicology; no medical history of cancer, hepatitis, or other serious conditions; and within age-appropriate normal limits in physical and psychologic health, sleep-wake pattern, and ease of blood draw. Their sleep-wake behavior was monitored by using wrist actigraphy and sleep logs to ensure regular sleep-wake timing for approximately 2 weeks before the laboratory study.

Procedures

Subjects participated in a 10-day laboratory study in the National Institutes of Health General Clinical Research Center of the Hospital of the University of Pennsylvania. After one adaptation day and 2 baseline days with bedtimes from 11:30 PM until 7:30 AM, subjects were randomly assigned to either 4 days (88 hours) of partial sleep deprivation (PSD) involving two 2-hour nap opportunities per day (ie, every 12 hours at 2:45 PM to 4:45 PM and 2:45 AM to 4:45 AM) or 4 days of total sleep deprivation (TSD). Within the PSD and TSD conditions, subjects were further randomized to a placebo

or low-dose caffeine condition. Plasma samples were available from 42 subjects: 9 TSD plus placebo; 12 TSD plus caffeine; 10 PSD plus placebo; and 11 PSD plus caffeine.

During the 6-day period centered on the 88-hour sleep deprivation time, subjects were confined to a standard hospital room in pairs; maintained in ambient light below 50 lux; fed isocaloric meals adjusted to body mass at 8:40 AM, 12:40 PM, 6:40 PM, and 10:40 PM; prohibited from exercise; socially isolated from events in the outside world and from contact with anyone other than project staff; instrumented for continuous physiologic monitoring of electroencephalographic, electro-oculographic, and electrocardiographic signals and core body temperature; and required to perform a computerized neurobehavioral test battery (performance, mood, and symptoms) for 30 minutes every 2 hours throughout all waking periods. The results of the neurobehavioral, electrophysiologic, and temperature data will be reported elsewhere. After the 88-hour sleep deprivation period, subjects stayed in the laboratory for 3 recovery days.

Subjects in both the TSD and PSD conditions were randomized to receive either a caffeine pill (0.3 mg/kg body weight) or a placebo pill every hour (except during the naps of the PSD condition), starting 22 hours into the 88 hours of sleep deprivation and continuing for the remaining 66 hours. The 24-hour dose of caffeine was equivalent to approximately 540 mg of caffeine cumulatively across a 24-hour day for a 75-kg (165-lb) subject; one cup of coffee contains between 75 and 150 mg of caffeine, making the cumulative dose administered per day equivalent to between 3 and 7 cups of coffee. This nested, double-blind, randomized, placebo-controlled trial of sustained low-dose caffeine was conducted to determine how nap and caffeine strategies individually and in combination would affect neurobehavioral and physiologic outcomes in sleep-deprived subjects. From the final baseline night until the end of the first recovery day (ie, 5 days), blood samples were taken by a nurse at 1.5-hour intervals by means of an indwelling intravenous catheter for hormone and immune assessments. For studies of plasma cytokine and cytokine receptor levels, 4.5 mL of blood was obtained every 6 hours (ie, at 9 PM, 3 AM, 9 AM, and 3 PM) through the indwelling catheter. Plasma was obtained by centrifugation, stabilized with Na-EDTA (1 mg/mL blood) and aprotinin (300 kallikrein inhibiting units/mL blood), and frozen at -70°C until thawed for analysis.

Cytokine and cytokine receptor analysis

Plasma samples were analyzed for cytokines and cytokine receptors by using enzyme-linked immunoassays that were blinded to experimental conditions (PSD vs TSD, caffeine vs placebo, and day and hour). The frozen plasma samples were rapidly thawed and brought to room temperature and assayed for the presence of IL-6, sTNF- α RI, and sTNF- α RII by using Quantikine ELISA kits (R&D Laboratories, Minneapolis, Minn), according to the manufacturer's instructions. Serial dilutions of known concentrations of IL-6, sTNF- α RI, and sTNF- α RII were used to construct a standard curve for each of the analytes. The concentrations of IL-6, sTNF- α RI, and sTNF- α RII in samples were interpolated from their respective standard curves. The minimum detectable levels of IL-6, sTNF- α RI, and sTNF- α RII determined with the above-mentioned ELISA kits were 0.2 pg/mL, less than 1.5 pg/mL, and less than 1 pg/mL, respectively. The interassay coefficient of variation was 2.7% to 6.9%, and the intra-assay coefficient of variation was 3.7% to 8.8%.

Statistical analysis

ANOVA and analysis of covariance for repeated measures with polynomial trend analysis was used to assess the effects of time across days (days 1-4 of the 88-hour period), TSD versus PSD condition, and interactions among these on immune measures. Day 1 measures were treated as covariates in the analysis so that all groups

started at the same mean value. Polynomials were fitted separately to each subject's measures across time to assess the effects of time and sleep deprivation level within individuals and minimize individual differences at baseline. The 4 daily values were also averaged across time of day to produce a single value for each day.

A log transformation was applied to IL-6 data before analysis because of the lack of normality. The Lomb-Scargle method of periodogram analysis^{30,31} was used to investigate the presence of circadian rhythms in the 5-day time series of each cytokine. This technique is robust for occasional missing values in a time series. The statistical test of Fisher³² was used to distinguish circadian rhythmicity from noise.

RESULTS

Complete time-series profiles were obtained on sTNF- α RI and sTNF- α RII in all 42 subjects. Subsets of subjects had complete data sets for IL-6, soluble IL-2 receptor, IL-10, and TNF- α (Table I). TSD and PSD numbers in Table I represent the pooled total number of subjects taking low-dose caffeine (0.3 mg·kg⁻¹·h⁻¹) and those taking placebo hourly. Pooling the treatment group was justified by the absence of any significant differences between caffeine and placebo groups for any of the cytokine-cytokine receptors analyzed. This is illustrated for the plasma levels of sTNF- α RI in the comparison of the TSD plus placebo subjects versus the TSD plus caffeine subjects (Fig 1). Although an apparent elevation of plasma sTNF- α RI during sleep and decline during daytime wakefulness can be seen in Fig 1 during both the baseline (day 1) and recovery (day 5) periods, this sleep-wake mediated rhythmicity in sTNF- α RI was not evident in sleep deprivation (days 2-4). Periodogram analyses across the 5 days revealed no statistically reliable circadian rhythmicity in any of the 6 cytokine-cytokine receptors analyzed. However, as expected, circadian variation was evident across sleep deprivation in plasma levels of cortisol ($P = .0001$, data not shown). Average 24-hour cortisol levels were not significantly different during the 88-hour period of sleep deprivation between TSD and PSD conditions, and they did not increase or decrease across days of sleep deprivation within the TSD and PSD conditions.

Although circadian variation was not found in cytokine-cytokine receptors, there was an interaction between the effects of time and sleep deprivation level detected for sTNF- α RI ($P = .05$), with an increase observed in the TSD condition but not in the PSD condition. Controlling for day 1, the 2 groups were significantly different at days 2 ($P = .04$) and 3 ($P = .01$) but not day 4 ($P = .15$), and the average change within the TSD and PSD conditions across days 1 to 4 was different ($P = .01$, Fig 2). In contrast, there were no statistically significant effects of time or sleep deprivation level in plasma sTNF- α RII over the 4-day period (data not shown).

An interaction between the effects of time and sleep deprivation level ($P = .04$) was detected for plasma IL-6, with a linear increase observed from day 2 through day 5 in the TSD condition but not in the PSD condition. A difference that began to appear between the 2 groups on day 3 ($P = .07$) reached statistical significance on day 4 ($P = .04$, Fig 3). An overall linear time increase in plasma sol-

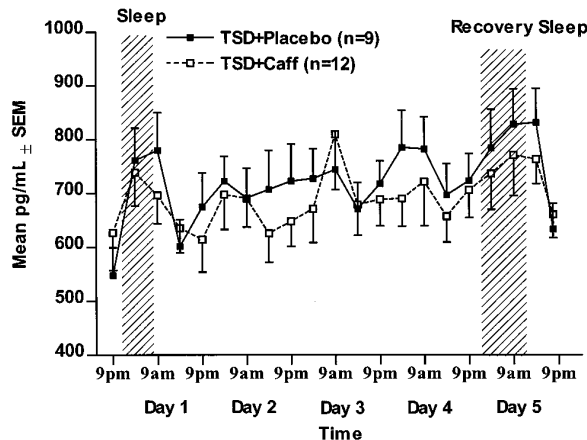


FIG 1. Sequential 6-hour plasma sTNF- α RI levels from 21 healthy young adult men undergoing 88 hours of TSD bounded by baseline and recovery nights of sleep. Mean $\pm$ SEM values are shown for 9 subjects randomized to TSD plus hourly placebo pill and 12 subjects randomized to TSD plus 0.3 mg/kg hourly caffeine (Caff) pill (beginning just before 9 AM on day 2 and continuing until just after 9 PM on day 4).

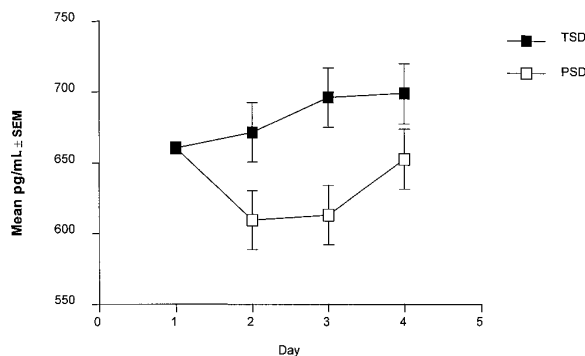


FIG 2. Sequential daily plasma sTNF- α RI levels from 21 healthy young adult men undergoing 88 hours of TSD from the morning of day 1 through day 4 and from 21 healthy adult men undergoing 88 hours of PSD on days 1 to 4, which consisted of two 2-hour nap opportunities per day (ie, every 12 hours at 2:45 PM to 4:45 PM and 2:45 AM to 4:45 AM). Because the TSD and PSD conditions were not different until day 2 (naps began), the day 1 data points were averaged to a single value, and the subsequent data points (days 2-4) were given as single data points (either TSD or PSD), with adjustment of values for the normalization of data in day 1. Daily means $\pm$ SEM represent the average of plasma levels at 4 time points each day (9 PM, 3 AM, 9 AM, and 3 PM). See text for results of statistical analyses.

uble IL-2 receptor was detected across days ($P = .05$). This increase was not associated with the level of sleep deprivation (data not shown). There were no statistically significant effects of time or sleep deprivation level for plasma IL-10 and TNF- α (data not shown).

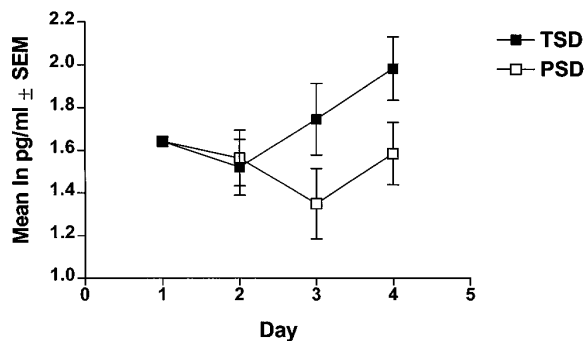


FIG 3. Sequential daily plasma IL-6 levels from 19 healthy young adult men undergoing 88 hours of TSD from the morning of day 1 through day 4 and from 21 healthy adult men undergoing 88 hours of PSD on days 1 to 4, which consisted of two 2-hour nap opportunities per day (see the "Methods" section). IL-6 data were normalized by using a natural log (*ln*) transformation. Day 1 data points were further normalized to a single value, and the subsequent data points (days 2-4) were given as single data points (either TSD or PSD), with adjustment of values for the normalization of data in day 1. Daily means $\pm$ SEM represent the average of plasma levels at 4 time points each day (9 PM, 3 AM, 9 AM, and 3 PM). See text for results of statistical analyses.

TABLE I. Number of subjects randomized to the 2 sleep deprivation conditions for which plasma was available for cytokine-cytokine receptor measurements

Sleep deprivation conditions	sTNF- α RI	sTNF- α RII	IL-6	sIL-2R	IL-10	TNF- α
TSD	21	21	19	10	10	9
PSD	21	21	21	13	13	14

All subjects went through an identical, normal, 24-hour period of sleep and waking (baseline evaluation) before being randomized to the 2 experimental conditions.

sIL-2R, Soluble IL-2 receptor.

DISCUSSION

The results of this study support previous findings linking TNF- α RI and IL-6 to human sleep and sleepiness²³⁻²⁵ and implicate the TNF- α and IL-6 cytokine systems as being sensitive to sleep homeostasis. Increased peripheral levels of both sTNF- α RI and IL-6 were observed during TSD relative to PSD, suggesting that they reflected the differential homeostatic drive for sleep between the TSD and PSD conditions. The differences were not likely to have been the result of other stressors because they occurred without evidence of significant differences across days in 24-hour mean levels of plasma cortisol between the 2 conditions. Moreover, they did not show evidence of circadian variation, which was apparent in cortisol measurements from both conditions. Plasma levels of sTNF- α RI and IL-6 also showed dose-dependent increases within subjects across days of TSD, although the sTNF- α RI concentrations did level off at the fourth day (ie, after 64 hours without sleep) of sleeplessness. In contrast, PSD produced transito-

ry decreases in IL-6 and sTNF- α RI levels before returning to baseline. It is not known whether these decreases would be consistent with the effects of a control condition involving a full night's sleep. It is possible that the two 2-hour naps per day were sufficient to prevent the immune changes seen in the TSD condition, suggesting that naps may in fact present a valuable countermeasure to alterations in immune function associated with prolonged sleep loss.

The results are consistent with major experiments on human cellular responses during sleep deprivation. Monocytes, which make up about 5% of white blood cell counts and are the major source of cytokine production in circulating peripheral blood, have been found to increase significantly during 39 hours and 64 hours of TSD.^{6,9} The fact that both increased numbers of monocytes and elevated sTNF- α RI and IL-6 levels are detected in plasma as a function of the magnitude of homeostatic sleep drive during TSD makes the lack of increased TNF- α levels during this condition intriguing. However, it is important to remember that sTNF- α RI plays a key role in affecting TNF- α -induced sleep,^{17,33} and therefore the increased presence of sTNF- α RI may reflect a potentiation of TNF- α action and increased effectiveness in sleep induction. It remains to be determined whether TSD, which elevated plasma levels of sTNF- α RI, would have similar effects on cytokine release through in vitro stimulation of immune cells.

The possibility that the IL-6 results are attributable simply to the presence of an indwelling catheter for many hours must be considered. It has been shown that IL-6 levels in particular increase by approximately 100% through 10 hours of frequent blood draws. In one study IL-6 levels measured in needle stick samples obtained at the end of a 10-hour period were not different from levels measured in baseline catheter samples.³⁴ The increases seen in the catheter condition were probably attributable to local endothelial cells activated by the presence of the catheter rather than to systemic changes. Although local endothelial cytokine production cannot be excluded in our study, this cannot explain the differential response to the experimental manipulations and certainly runs counter to the drops seen in IL-6 during PSD.

For a mission to Mars, it is estimated that astronauts will not return to Earth for up to 3 years. In shorter duration spaceflights, in which sleep has been investigated during or after flight, chronic partial sleep loss has been common,¹⁻⁵ although the precise causes for it remain unknown. Chronic sleep restriction can result in escalating homeostatic sleep drive to levels equivalent to TSD.³⁵ The current results suggest that chronic insufficient sleep in a long-duration spaceflight could lead to an amplified alteration in immune function. These changes could be so great that they would, in fact, produce a state of secondary immunodeficiency (space-flight immunodeficiency), potentially leading to reactivation of latent viral infections and development of malignancy. Epstein-Barr latent virus infections have, for example, become reactivated during bone marrow stem cell transplantation and transformed B lymphocytes into oligoclonally and monoclonally derived lymphomas.^{36,37} Elevations of plasma cytokine levels are

known to affect the target cell surface expression of viral receptors. Examples of this upregulation are seen in AIDS, where investigations have shown elevations of plasma IL-1 β , TNF- α , IL-6, and IL-10 that facilitate entry of HIV-1 into target cells through chemokine receptors and lead to rapid disease progression.³⁸ Thus in astronauts exposed to prolonged sleep deprivation plus other stressors of interplanetary travel that alter chemokine patterns (isolation, containment, microbial contamination, and solar radiation), it is possible that reactivated latent virus (eg, herpes family and polyomavirus) will trigger a state of chronic infection and possibly malignant transformation of target cell types.

Although this ground-based experiment included conditions of sleep loss, isolation, and confinement that are likely to be present during long-duration spaceflight, it did not model other key aspects of prolonged habitability in space (eg, microgravity, fluid shifts, and muscle unloading) that could have additional effects on immune modulation. Similarly, the effectiveness of intermittent nap sleep in preventing the cytokine elevations found in this study will need to be tested in conditions of spaceflight.

In summary, we have demonstrated that sleep deprivation of 88 hours in normal human subjects produces dysregulation of sleep regulatory cytokines and cytokine receptors. In as much as this ground-based model of sleep deprivation mimics some of the conditions that could prevail during long-duration spaceflight, it is reasonable to suspect that prolonged sleep deprivation on interplanetary space travel may create an imbalance in inflammatory cytokine levels sufficient to depress immunity. Because the increased cytokine levels induced by TSD were prevented by regularly spaced naps, a spaceflight countermeasures program involving regular sleep periods might be envisioned to reduce the changes of harmful effects of sleep deprivation in astronauts.

We thank Michele M. Carlin, Sheelu Samuels, Shiv Kapoor, PhD, and the nurses and staff of the General Clinical Research Center of the Hospital of the University of Pennsylvania for their contributions to study completion. We also thank the volunteer subjects for their cooperation.

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