

# The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults

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## Abstract

Inflammatory cytokines are released in response to stress, tissue damage, and infection. Acutely, this response is adaptive; however, chronic elevation of inflammatory proteins can contribute to health problems including cardiovascular, endocrine, mood, and sleep disorders. Few studies have examined how sleep deprivation acutely affects inflammatory markers, which was the aim of the current study. Nineteen healthy men and women aged  $28.05 \pm 8.56$  (mean  $\pm$  SD) were totally sleep deprived for 40 h under constant routine conditions. Pro-inflammatory markers: intracellular adhesion molecule-1 (ICAM-1), E-selectin, vascular adhesion molecule-1 (VCAM-1), c-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-1 $\beta$  (IL-1 $\beta$ ), and the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1ra) were assayed in plasma. Daytime levels during baseline (hours 1–15 of scheduled wakefulness) were compared to daytime levels during sleep deprivation (hours 25–39 of scheduled wakefulness), thus controlling for circadian phase within an individual. Repeated measures ANOVA with planned comparisons showed that 40 h of total sleep deprivation induced a significant increase in E-selectin, ICAM-1, IL-1 $\beta$ , and IL-1ra, a significant decrease in CRP and IL-6, and no significant change in VCAM-1. Alterations in circulating levels of pro- and anti-inflammatory cytokines and cell adhesion molecules during sleep deprivation were consistent with both increased and decreased inflammation. These findings suggest that one night of sleep loss triggers a stress response that includes stimulation of both pro- and anti-inflammatory proteins in the healthy young subjects tested under our experimental conditions.

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**Keywords:** Cell adhesion molecules; Cytokines; Inflammation; Sleep loss

## 1. Introduction

The acute phase response (APR) describes specific immune responses to tissue damage/infection and is aimed at promoting healing and recruitment of host defenses. The APR is also responsive to stress (e.g., psychosocial stress and exercise). One aspect of the APR includes stimulation of acute phase proteins (APPs), such as interleukin-6 (IL-6) released by immune cells and c-reactive protein (CRP)

released by the liver (Black and Garbutt, 2002). APPs then stimulate the production of pro-inflammatory cytokines, cell adhesion molecules, and other inflammatory mediators that are important for tissue repair and host defense. While acute increases in these inflammatory markers are important for health, chronic elevations of inflammatory proteins have been implicated in the development and/or progression of health problems such as cardiovascular, endocrine, mood, and sleep disorders (Cesari et al., 2003; DeSouza et al., 1997; El-Solh et al., 2002; Maes et al., 1997; Okun et al., 2004; Ridker et al., 1997). A common problem associated with such disorders is disrupted sleep. Whether disrupted sleep contributes to the elevation of inflammatory

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proteins observed in these disorders has received little attention.

Sleep and sleep loss have been reported to be associated with alterations in immune cell production of inflammatory markers (Irwin, 2002). For example, Irwin et al. (2006) reported that a night of sleep restricted to 4 h increased pro-inflammatory cytokine gene expression. In addition, they reported that monocyte production of IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in response to lipopolysaccharide was greater following the night of sleep restriction. Dimitrov and colleagues reported that sleep increases levels of the soluble IL-6 receptor (Dimitrov et al., 2006) and that sleep is associated with alterations in balance between Th1 and Th2 cytokines (Dimitrov et al., 2004). Few studies have examined how sleep loss per se influences circulating inflammatory markers (Irwin, 2002). Circulating levels of IL-6 have been reported to be increased in healthy adults following 7 nights of 6-h vs. 8-h scheduled sleep (Vgontzas et al., 2004) and in African American male alcoholics during one night of 3.5-h vs. 7.5-h scheduled sleep (Irwin et al., 2004). Meier-Ewert et al. (2004) reported increased CRP levels following 10 nights of 4.2-h vs. 8.2-h scheduled sleep. Redwine et al. (2004) reported an increase in the cell adhesion molecule L-selectin during one night of 3.5-h vs. 7.5-h scheduled sleep. In several studies the effect of total sleep deprivation on IL-6 levels was examined and results from these studies are inconsistent. Specifically, Dinges et al. (1995) and Born et al. (1997) reported that IL-6 levels did not change during 15–63 h of total sleep deprivation; whereas, Vgontzas et al. (1999b) reported increased IL-6 levels during 40 h of total sleep deprivation. Shearer et al. (2001) also reported that IL-6 levels were increased across 88 h of total sleep deprivation compared to partial sleep deprivation consisting of a 2-h nap opportunity every 12 h. However, Haack et al. (2002) reported that IL-6 levels were reduced during sleep deprivation compared to levels during sleep. Meier-Ewert et al. (2004) reported increased CRP levels during 64 h of total sleep deprivation; whereas, Dimitrov et al. (2006) reported that one night of sleep loss did not significantly alter CRP levels.

To our knowledge, no study to date has examined the effect of total sleep deprivation on cell adhesion molecules or anti-inflammatory cytokines. The aim of the current study, therefore, was to determine whether acute total sleep deprivation per se increases circulating levels of inflammatory markers in healthy young participants when sleep deprivation occurred under controlled laboratory constant routine conditions. We hypothesized that acute total sleep deprivation is a sufficient stimulus to trigger an increase in circulating inflammatory proteins (IL-6, CRP, interleukin-1 $\beta$  (IL-1 $\beta$ ), ICAM-1, VCAM-1, and E-selectin). In addition, due to recent interest in the role of anti-inflammatory cytokines in cardiovascular health (Frostegard et al., 1999; Heeschen et al., 2003) and the balance between pro- and anti-inflammatory cytokines, we examined circulating levels of IL-1ra as a secondary analysis. We also examined

salivary cortisol levels and subjective stress ratings during sleep deprivation.

## 2. Methods

Nineteen healthy individuals (9 females, 10 males) aged  $28.05 \pm 8.56$  (mean  $\pm$  SD) participated. Participants were free of any medical and psychiatric conditions determined by medical history, physical and psychological exams, blood and urine chemistries, electrocardiogram, and toxicology screens for drug use at screening and admission to the laboratory. In addition, participants had a normal body mass index ( $18.5\text{--}24.5 \text{ kg/m}^2$ ), were non-smokers, and were free of medication use. Participants reported no shift work for the past three years or travel across more than one time zone in the past three months. Participants maintained a consistent sleep-wakefulness schedule of 8-h sleep per night at a regular clock time ( $\pm 30$  min) for three weeks prior to the laboratory procedures to minimize any effect of prior sleep loss on the results. The regular sleep-wakefulness schedule was verified by sleep logs, phone calls of bed times, and wake times to a time stamped voice recorder, and one week of wrist actigraphy (Mini Mitter, Bend, OR). Institutional Human Subjects Review Boards approved the procedures for the study, and all participants gave written informed consent. The investigation was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 2000.

Following the screening procedures, participants lived for three baseline days and nights on a General Clinical Research Center consisting of scheduled sleep for 8 h per night at habitual bedtimes. A 40 h constant routine (Duffy and Wright Jr., 2005) and 8-h recovery sleep opportunity followed (Fig. 1). Constant routine conditions included constant posture ( $\sim 35^\circ$  head-up bed rest), dim light exposure ( $\sim 1.5$  lux in the angle of gaze), and hourly nutrition intake (200 mEq Na<sup>+</sup>, 100 mEq K<sup>+</sup>, 2500 cc fluids isocaloric, no caffeine, spaced equally in hourly aliquots). Staff members continuously monitored participants to ensure wakefulness. Subjective stress ratings were assessed using a 100 mm visual analog scale (the extremes of this dimension were 100 mm = stressed, 0 mm = relaxed), every 2 h throughout sleep deprivation. Blood was sampled every 30 min through an indwelling 18-gauge intravenous catheter throughout the 40 h of sleep deprivation. Heparinized saline (0.45% sodium chloride, 10 U of heparin/ml) was infused at a rate of 5–10 ml/h between samples. Saliva was collected by having participants spit into polystyrene tubes every hour. Saliva collection began after 2 h of wakefulness and ended after 38 h of wakefulness.

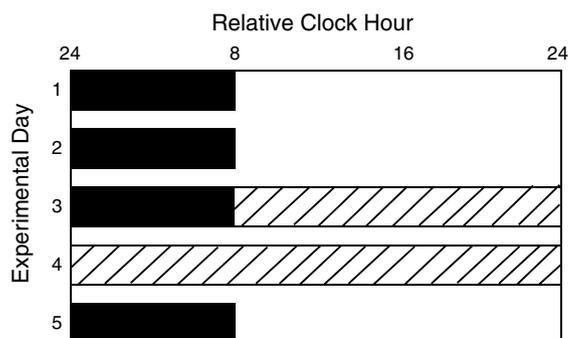


Fig. 1. Protocol figure. Data are plotted to a relative clock hour with wake time arbitrarily assigned a value of 08:00 h and all other times referenced to this value, modulo 24 h (e.g., breakfast scheduled 1.5 h after awakening would be reported as occurring at a relative clock hour of 09:30 h). Successive days of the study are plotted beneath each other. Participants were scheduled to sleep for 8 h at their habitual bedtime for three baseline days and for a recovery sleep episode on experimental day 5 (black boxes). Beginning with experimental day 3, participants were sleep deprived under constant routine conditions for 40 h (diagonal filled boxes).

### 2.1. Plasma and saliva data

After plasma and saliva samples were collected and centrifuged at 4 °C, they were frozen at –80 °C to maintain stability until assayed. Plasma samples were combined into hourly aliquots and assayed using commercially available ELISA kits (high-sensitivity IL-6 (hsIL-6), IL-1ra, soluble ICAM-1 (sICAM-1), soluble E-selectin (sE-selectin), soluble VCAM-1 (sVCAM-1)—R&D Systems, Minneapolis, MN; high-sensitivity CRP (hsCRP)—Alpco Diagnostics, Windham, NH) (Aziz et al., 2003; Mahmud and Feely, 2005). Salivary cortisol was assayed using an enzyme linked immunoassay (EIA) (Diagnostics Systems Laboratories, Webster, TX) (Noteboom et al., 2001). In a subset of participants ( $n = 7$ ), who had sufficient sample remaining for analysis, IL-1 $\beta$  was assayed (R&D Systems, Minneapolis, MN).

The lower limits of detection were 0.35 ng/ml, 2.00 ng/ml, 0.10 ng/ml, 0.039 pg/ml, 0.00124 mg/L, < 22.00 pg/ml, and 0.10 pg/ml for sICAM-1, sVCAM-1, sE-selectin, hsIL-6, hsCRP, IL-1ra, and IL-1 $\beta$  ELISAs, respectively; average inter-assay coefficients of variation were 1.80%, 4.34%, 4.50%, 6.01%, 8.40%, 3.00%, and 6.48%, respectively; and average intra-assay coefficients of variation were 6.74%, 9.73%, 7.84%, 6.73%, 9.62%, 7.46%, and 18.25%, respectively. The lower limit of detection was 0.011  $\mu$ g/dl for the cortisol EIA and the average inter- and intra-assay coefficients of variation were 19.37% and 7.67%, respectively.

### 2.2. Statistical analyses

Comparisons were made between samples collected at baseline (hours 1–15 of scheduled wakefulness for plasma markers and hours 3–14 of scheduled wakefulness for saliva due to missing data at hour 2 of wakefulness for sampling in some subjects) vs. sleep deprivation (hours 25–39 of scheduled wakefulness for plasma markers and hours 27–38 of scheduled wakefulness for saliva). These sample times were analyzed to control for the influence of circadian phase within individuals by examining daytime levels 24 h apart. Unavailable sample (e.g., blood sampling difficulties or insufficient sample volume available for assay) occurred for < 2% of samples for the inflammatory markers studied except IL-1 $\beta$  for which 5% of samples were unavailable. Missing data for these samples were replaced with a linear interpolation between the sample immediately prior to and following the missing sample for that subject. Missing data for one subject reduced the sample size for CRP to 18 subjects. Plasma and saliva data were log transformed to correct for violations of homogeneity of variance. A  $2 \times 15$  ( $2 \times 12$  for salivary cortisol) repeated measures ANOVA was used to test for differences between baseline and sleep deprivation by time of day. All significant main effects and interactions are reported. For all analyses, planned comparisons were made between each hour at baseline vs. sleep deprivation.

## 3. Results

Statistical results for plasma and saliva markers are presented for log transformed data; however, the raw data is presented in the figures to show the observed levels. Fig. 2a shows increases in sE-selectin during sleep deprivation compared to baseline (main effect of sleep deprivation  $F(1, 18) = 4.074$ ,  $p = 0.059$ ), and planned comparisons indicated significantly higher sE-selectin levels occurred in the afternoon and late evening. No significant main effects or interactions for sICAM-1 or sVCAM-1 levels were observed across sleep deprivation (Figs. 2b and c). However, planned comparisons indicated significantly increased sICAM-1 at one time point in the early afternoon. Figs. 3a and b show that sleep deprivation significantly decreased CRP (main effect of sleep deprivation  $F(1, 17) = 6.211$ ,  $p < 0.05$ , main effect of time of day  $F(14, 238) = 3.143$ ,

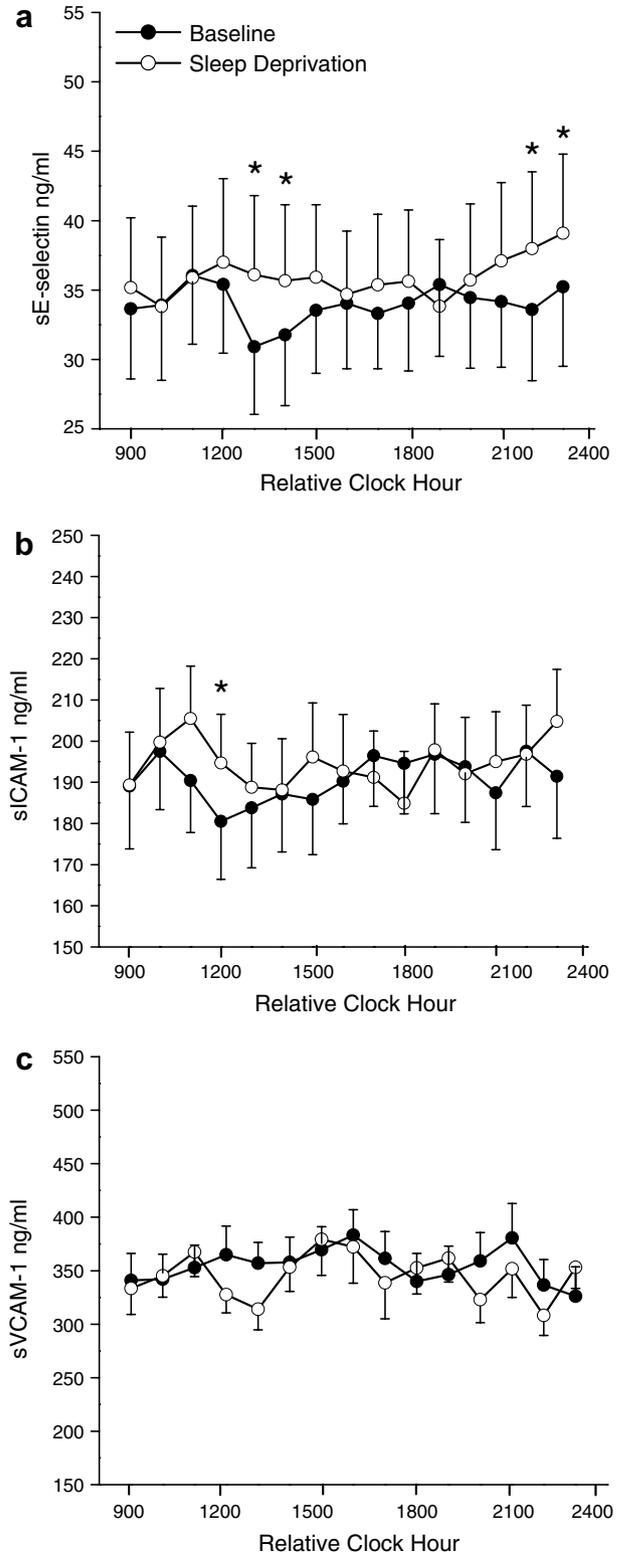


Fig. 2. Effects of 40 h of total sleep deprivation on cell adhesion molecules. (a–c) Non-transformed plasma levels of sE-selectin, sICAM-1, sVCAM-1, respectively, for baseline hours 1–15 of scheduled wakefulness (closed circles) and sleep deprivation hours 25–39 of scheduled wakefulness (open circles). Data are plotted in relative clock hour (see Fig. 1). Error bars represent SEM. \* indicates planned comparisons that are significantly different between baseline and sleep deprivation for log transformed data,  $p < 0.05$ .

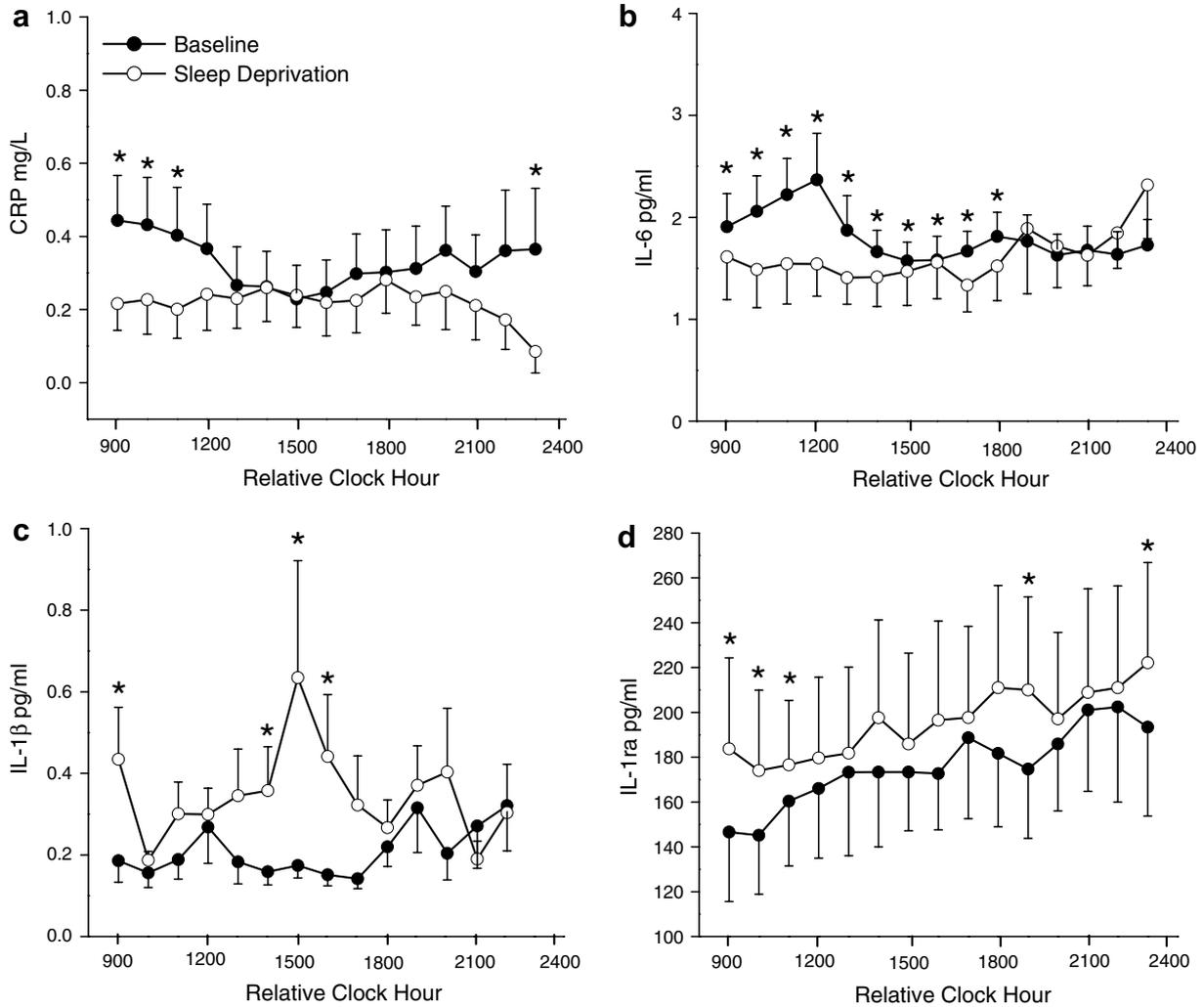


Fig. 3. Effects of 40 h of total sleep deprivation on inflammatory markers. (a–d) Non-transformed plasma levels of CRP, IL-6, IL-1 $\beta$ , and IL-1ra, respectively, for baseline hours 1–15 of scheduled wakefulness (closed circles) and sleep deprivation hours 25–39 of scheduled wakefulness (open circles). Data are plotted in relative clock hour (see Fig. 1). Error bars represent SEM. \* indicates planned comparisons that are significantly different between baseline and sleep deprivation for log transformed data,  $p < 0.05$ .

$p < 0.05$ , and sleep deprivation  $\times$  time of day interaction  $F(14, 238) = 2.057$ ,  $p < 0.05$  and IL-6 (main effect of sleep deprivation  $F(1, 18) = 4.774$ ,  $p < 0.05$ , main effect of time of day  $F(14, 252) = 1.976$ ,  $p < 0.05$ , and sleep deprivation  $\times$  time of day interaction  $F(14, 252) = 3.108$ ,  $p < 0.05$ ). Planned comparisons indicated significantly decreased CRP levels during sleep deprivation in the morning and evening and significantly decreased IL-6 levels during sleep deprivation across most of the day.

Approximately 50% of IL-1 $\beta$  samples were below the lower limit of detection, and these samples were assigned the lower limit of detection value of 0.10 pg/ml. Of the samples that were below the lower limit of detection, ~62% occurred during baseline and ~38% during sleep deprivation, which is consistent with findings from a prior study in which it was reported that “IL-1 $\beta$  was often undetectable, but this was less so after a night without sleep” (Dinges et al., 1995). Due to missing data at hour 39 for one participant, hours 1–14 and 25–38 for IL-1 $\beta$  were ana-

lyzed. A non-significant trend for increased IL-1 $\beta$  was observed during sleep deprivation (main effect of sleep deprivation  $F(1, 6) = 4.470$ ,  $p = 0.08$ ). Planned comparisons indicated that IL-1 $\beta$  levels (Fig. 3c) were significantly higher during sleep deprivation in the morning and afternoon. Fig. 3d shows a significant increase in IL-1ra during sleep deprivation (main effect of sleep deprivation  $F(1, 18) = 5.457$ ,  $p < 0.05$ , main effect of time of day  $F(14, 252) = 10.133$ ,  $p < 0.05$ , and sleep deprivation  $\times$  time of day interaction  $F(14, 252) = 2.047$ ,  $p < 0.05$ ). Planned comparisons indicated that significantly higher IL-1ra levels occurred in the morning and evening. No significant main effects or interaction for salivary cortisol levels were observed across sleep deprivation. However, planned comparisons indicated that salivary cortisol levels were significantly decreased at two time points measured in the early afternoon and early evening (Fig. 4). Fig. 5 shows that participants reported significantly higher stress levels across sleep deprivation (main effect of sleep deprivation

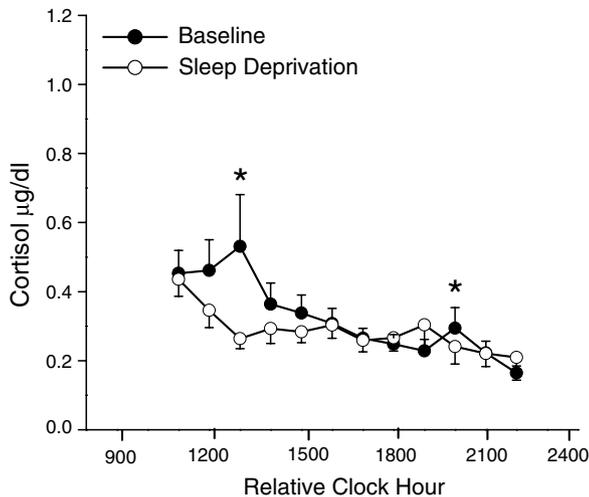


Fig. 4. Effects of 40 h of total sleep deprivation on salivary cortisol. The ordinate represents non-transformed salivary cortisol ( $\mu\text{g}/\text{dl}$ ) levels for baseline hours 3–14 of scheduled wakefulness (closed circles) and sleep deprivation hours 27–38 of scheduled wakefulness (open circles). Data are plotted in relative clock hour (see Fig. 1). Error bars represent SEM. \* indicates planned comparisons that are significantly different between baseline and sleep deprivation for log transformed data,  $p < 0.05$ .

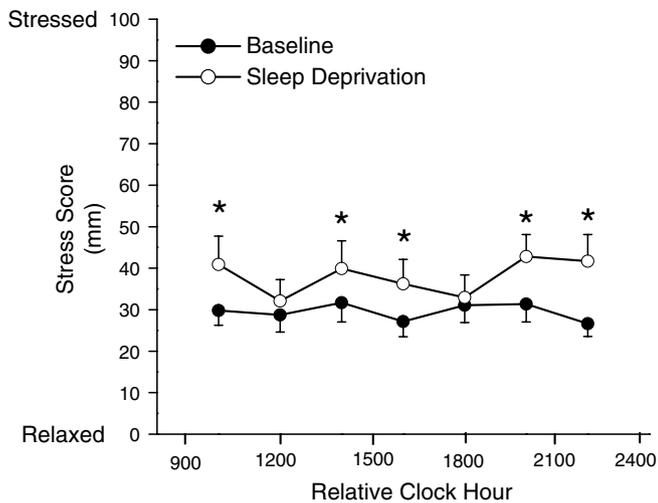


Fig. 5. Effects of 40 h of total sleep deprivation on subjective stress ratings. The ordinate represents visual analog scale in mm (100 mm = stressed, 0 mm = relaxed) for baseline hours 2–14 of scheduled wakefulness (closed circles) and sleep deprivation hours 26–38 of scheduled wakefulness (open circles). Data are plotted in relative clock hour (see Fig. 1). Error bars represent SEM. \* indicates planned comparisons that are significantly different between baseline and sleep deprivation,  $p < 0.05$ .

$F(1, 18) = 9.610$ ,  $p < 0.05$ ). Planned comparisons indicated significant increases in subjective stress ratings across the day.

#### 4. Discussion

Our findings demonstrate that one night of total sleep deprivation in healthy young participants during strictly

controlled constant routine conditions of bed rest, inactivity, dim light, and hourly nutrition intake, significantly altered circulating levels of pro- and anti-inflammatory cytokines and cell adhesion molecules. However, findings were mixed with some inflammatory markers significantly increasing and others significantly decreasing during sleep deprivation. Specifically, we found a significant increase in pro-inflammatory sE-selectin and sICAM-1 levels but no significant change in sVCAM-1 levels. In addition, we found a significant decrease in CRP and IL-6. IL-1 $\beta$  levels were increased, indicative of an increase in the inflammatory response, but we also found a significant increase in IL-1ra levels, indicative of an increase in the anti-inflammatory response. Taken together, these findings suggest that the challenge of 40 h of total sleep deprivation may have been met with a balance of pro- and anti-inflammation in these participants under constant routine conditions.

Findings from our study are the first to demonstrate that total sleep deprivation increases circulating levels of sE-selectin, sICAM-1, and IL-1ra. In a subset of participants in the current study, we measured IL-1 $\beta$ , and observed increases across sleep deprivation, which is consistent with prior findings of increased plasma levels of IL-1 $\beta$  by 39 h of total sleep deprivation (Dinges et al., 1995). Our finding in humans of an increase in IL-1ra levels in response to total sleep deprivation is consistent with that for sleep deprived mice (Hu et al., 2003). The primary role of IL-1ra is to inhibit interleukin-1 (IL-1), which is an important pro-inflammatory cytokine involved in promoting sleep and fever (Arend, 2002). Injection of IL-1ra in the CNS of animals results in a reduction of IL-1 induced sleep (Imeri et al., 1993), likely by attenuating IL-1 induced suppression of firing rates of wakefulness related neurons in the preoptic area of the hypothalamus/basal forebrain (Alam et al., 2004). In humans, IL-1ra levels have been reported to be positively correlated with sleep onset latency (Krouse et al., 2002). Thus, it is possible that the increase in IL-1ra observed in the current study may be a response of the immune system to counter the sleep promoting effects of IL-1 (Alam et al., 2004; Imeri et al., 1993; Krouse et al., 2002), and may also serve to maintain cytokine balance during 40 h of acute total sleep deprivation.

As noted, increased cell adhesion molecules are reported to be involved in the pathogenesis and progression of cardiovascular disease (Black and Garbutt, 2002; Cesari et al., 2003; DeSouza et al., 1997; Libby and Ridker, 2004; Ridker et al., 1997). However, increased sE-selectin levels observed in our healthy subjects were below the levels observed in patients with hypertension, coronary artery disease, or sleep apnea (DeSouza et al., 1997; El-Solh et al., 2002). In addition, the increase in sICAM-1 was significant at only one time point examined. Future research is needed to examine the possibility that increased sympathetic activation (Spiegel et al., 1999) and systolic blood pressure (Meier-Ewert et al., 2004) during sleep deprivation

induces shear stress that results in shedding of cell adhesion molecules.

As noted, total sleep deprivation has been reported to increase (Shearer et al., 2001; Vgontzas et al., 1999b), decrease (Haack et al., 2002) or induce no measurable change (Born et al., 1997; Dinges et al., 1995) in IL-6 levels. Our finding of a significant decrease in IL-6 during 40 h of total sleep deprivation is consistent with the study by Haack et al. (2002). Taken together, the effects of sleep deprivation on circulating levels of IL-6 appears equivocal, which may in part be related to both pro- and anti-inflammatory roles of IL-6 (Xing et al., 1998). Continuous use of an indwelling IV across 24 h has been reported to be associated with an increase in IL-6 levels regardless of sleep or sleep deprivation (Haack et al., 2002). The reported increase in IL-6 was hypothesized to be due to local inflammation at the IV site as higher IL-6 levels were reported for samples that were difficult to obtain, especially during sleep. Our finding of a decrease in IL-6 during sleep deprivation across the biological day is inconsistent with the hypothesis that local inflammation in response to the IV in our study causes changes in IL-6 during sleep deprivation. Sleep deprivation has been reported to increase (Meier-Ewert et al., 2004) or induce no measurable change (Dimitrov et al., 2006) in CRP levels. Our finding of a decrease in CRP levels during 40 h of sleep deprivation is inconsistent with these prior findings. Several methodological differences among the studies cited above and our study may contribute to the inconsistent findings for IL-6 and CRP, including the duration of sleep deprivation, circadian phase, control over posture and light exposure, blood sampling frequency, the timing and type of nutrition, previous limits of assay sensitivity, and differences in subjects' BMI; obesity increases pro-inflammatory markers (Panagiotakos et al., 2005).

Several other studies have examined the effect of total sleep deprivation on cortisol, with some reporting an increase (Leproult et al., 1997; Mullington et al., 1996; Weitzman et al., 1983), others reporting a decrease (Akerstedt et al., 1980; Dinges et al., 1994; Vgontzas et al., 1999a), and others reporting no change (Dzaja et al., 2004; Follenius et al., 1992; Gary et al., 1996; Gonzalez-Ortiz et al., 2000; Haack et al., 2002; Heiser et al., 2000; Ozturk et al., 1999). We found a significant decrease in salivary cortisol during sleep deprivation. Factors that may contribute to prior inconsistent findings are the method of cortisol assessment (e.g., plasma versus saliva), as well as the circadian time, and the frequency of sampling. Further research is required to determine which factors influence the cortisol response to acute total sleep deprivation.

Our sleep deprivation protocol has a number of strengths and limitations. Sleep deprivation was conducted under constant routine conditions; and thus, we controlled for possible behavioral and environmental influences on inflammatory markers. In addition, our study is the only study to combine controlled constant routine conditions with frequent blood sampling. Our findings are generaliz-

able only to missing one night of sleep in young healthy men and women. Furthermore, we only examined 40 h of total sleep deprivation during the biological day when melatonin levels are low; and thus, we did not assess the influence of total sleep deprivation on inflammatory markers during the biological night when melatonin levels are high. Additional research is needed to characterize changes in inflammatory markers during the biological night with and without sleep and to examine the association between sleep and circadian changes in inflammatory and endocrine variables. It is possible that different findings may have occurred if sleep deprivation was extended beyond 40 h, and/or occurred under different conditions (e.g., the addition of other stressors) or in different populations (e.g., older adults or patients with medical or psychiatric disorders who may already have some underlying inflammation). In addition, we did not assess the influence of sleep deprivation on *in vitro* stimulated immune cell cytokine production (Irwin et al., 2006), which may show different findings from circulating levels. As with all studies that measure circulating levels of pro- and anti-inflammatory markers, without more invasive procedures it is not possible to determine the mechanism of production or the specific tissues responding to the stress of sleep deprivation. Another methodological strength of our study was the maintenance of participants on their habitual sleep-wakefulness schedule in lieu of scheduling participants to sleep at the same clock hour. Previously, we and others have reported that alteration of the relationship between habitual sleep time and internal biological time alters human physiology and behavior (Wright Jr. et al., 2006).

It is common for people to forgo one night of sleep. The results of our study indicate that 40 h of total sleep deprivation in young healthy adults alters both pro- and anti-inflammatory markers, but that changes in levels of these markers were below the levels reported to be associated with increased risk of cardiovascular, mood, endocrine, and sleep disorders (Cesari et al., 2003; DeSouza et al., 1997; El-Solh et al., 2002; Maes et al., 1997; Okun et al., 2004; Ridker et al., 1997). While it remains unclear what underlying mechanisms contribute to the changes we observed in circulating levels of inflammatory markers during sleep loss, our findings suggest that one night of sleep deprivation appears to have significant but small effects on inflammatory markers in healthy young adults.

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