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Effect of Sleep Loss on C-Reactive Protein, an Inflammatory Marker of Cardiovascular Risk

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OBJECTIVES We sought to investigate the effects of sleep loss on high-sensitivity C-reactive protein (CRP) levels.

BACKGROUND Concentrations of high-sensitivity CRP are predictive of future cardiovascular morbidity. In epidemiologic studies, short sleep duration and sleep complaints have also been associated with increased cardiovascular morbidity. Two studies were undertaken to examine the effect of acute total and short-term partial sleep deprivation on concentrations of high-sensitivity CRP in healthy human subjects.

METHODS In Experiment 1, 10 healthy adult subjects stayed awake for 88 continuous hours. Samples of high-sensitivity CRP were collected every 90 min for 5 consecutive days, encompassing the vigil. In Experiment 2, 10 subjects were randomly assigned to either 8.2 h (control) or 4.2 h (partial sleep deprivation) of nighttime sleep for 10 consecutive days. Hourly samples of high-sensitivity CRP were taken during a baseline night and on day 10 of the study protocol.

RESULTS The CRP concentrations increased during both total and partial sleep deprivation conditions, but remained stable in the control condition. Systolic blood pressure increased across deprivation in Experiment 1, and heart rate increased in Experiment 2.

CONCLUSIONS Both acute total and short-term partial sleep deprivation resulted in elevated high-sensitivity CRP concentrations, a stable marker of inflammation that has been shown to be predictive of cardiovascular morbidity. We propose that sleep loss may be one of the ways that inflammatory processes are activated and contribute to the association of sleep complaints, short sleep duration, and cardiovascular morbidity observed in epidemiologic surveys. (J Am Coll Cardiol 2004;43:678–83) © 2004 by the American College of Cardiology Foundation

Sleep complaints and short sleep duration are associated with increased cardiovascular morbidity in a series of epidemiologic surveys (1–6) and in a recent case-control study of overtime work and insufficient sleep (7). Although the mechanism for this association is not known, it is of particular interest that experimental sleep deprivation in healthy adults has been found to lead to increased peripheral circulation of leukocytes (8,9) and interleukin (IL)-6 (10). This suggests that sleep loss itself may contribute to inflammation and, if chronic, to cardiovascular risk.

C-reactive protein (CRP) is a pentameric hepatocyte protein with a half-life of 15 to 19 h (11) and is the major marker of the "acute-phase response," or the formation of plasma proteins in response to an inflammatory stimulus, in humans (12). Synthesis of CRP in the liver is largely controlled by interleukin (IL)-6 and also by tumor necrosis factor-alpha and IL-1 (13). The production of CRP is thought to reflect the activity of these cytokines, particularly IL-6 (14). Although IL-6 levels display circadian variability, CRP levels have been shown to be quite stable across 24 h (15) and, in the absence of disease, are quite reproducible, even over weeks and months (16).

In recent years, since the advent of high-sensitivity technology permitting measurement of CRP levels as low as 0.007 mg/dl from the previous detection limits of 3 to 5 mg/dl (17), epidemiologic studies have pointed to CRP as a predictor of both a long- and short-term risk of stroke and myocardial infarction in men and women (18,19). C-reactive protein has been shown to promote secretion of inflammatory mediators by vascular endothelium (20,21) and opsonizes low-density lipoprotein for uptake by mac-

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Abbreviations and Acronyms

ANOVA = analysis of variance
BP = blood pressure
GCRC = General Clinical Research Center
HR = heart rate
CRP = C-reactive protein
IL = interleukin
PSD = partial sleep deprivation
TSD = total sleep deprivation

rophages in atherosclerotic plaque (22). These data suggest that CRP may be directly implicated in the development of atherosclerotic lesions.

We hypothesize that inflammatory processes are activated by inadequate sleep. C-reactive protein was recently reported to be elevated in patients with obstructive sleep apnea, raising the possibility that deprivation of sleep, due to its disruption via untreated apnea, contributes to inflammation (23). The current investigation directly examined this hypothesis in two experiments in healthy adults exposed to three nights of total sleep loss and 10 consecutive nights of partial sleep loss. It was hypothesized that if sleep loss contributed to the inflammatory response associated with elevated cardiovascular risk, it would not only upregulate CRP but do so in a dose-dependent manner related to the magnitude of sleep lost.

METHODS

Experiment 1: acute total sleep deprivation (TSD). Subjects included 10 healthy male volunteers between the ages of 22 and 37 years (average 27.2 years), who gave signed, informed consent to participate in the research protocol, approved by the Institutional Review Board of the University of Pennsylvania, before undergoing screening tests. Inclusion in the study required that subjects be healthy and free of signs and symptoms of active infection. Inclusion further required complete blood cell counts and chemistry tests within normal limits on all parameters in one or more prestudy assessments; toxicology reports free of medications and drugs; no medical history of cancer, hepatitis, or other serious conditions; age-appropriate normal limits of physical and psychological health; and evidence of a stable sleep/wake pattern defined by a habitual nocturnal sleep duration of 6.5 to 9.0 h and habitual morning awakening between 6:00 and 9:00 AM. Sleep/wake patterns were verified using wrist actigraphy for at least one week before the laboratory study.

PROTOCOL. Subjects spent 10 days (24-h periods) in the National Institutes of Health (NIH) General Clinical Research Center (GCRC) of the Hospital of the University of Pennsylvania. After three baseline days with bed periods between 11:30 PM and 7:30 AM, subjects underwent 88 h of TSD, as a placebo-control group in a double-blind trial of sustained low-dose caffeine. During the period of blood collection and sleep deprivation, subjects were confined to a temperature-controlled, time-isolated hospital room in <50 lux of ambient light. They were prohibited from exercise, maintained on a balanced diet (caffeine, tobacco, alcohol, and other psychoactive substances were prohibited), and behaviorally monitored to ensure they remained awake throughout the 88-h vigil. The GCRC nurses made daily measurements of heart rate (HR) and blood pressure (BP). Subjects were instrumented for continuous physiologic monitoring of electroencephalographic, electrooculographic, and electrocardiographic signals, as well as rectal body temperature, and were required to perform a computerized neurobehavioral test battery (performance, mood, symptoms) for approximately 30 min every 2 h throughout all waking periods. After the 88-h vigil, subjects remained in the laboratory for three days for recovery sleep and testing.

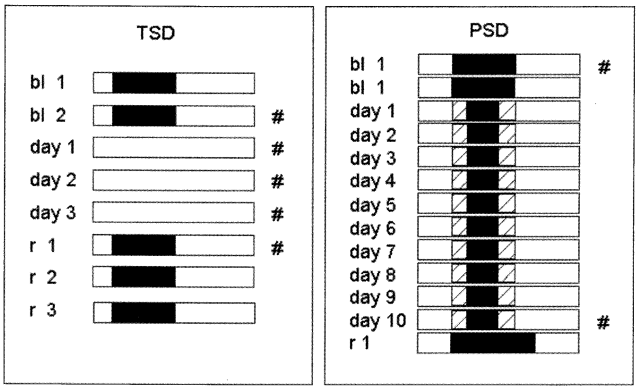

Figure 1. Schematic of acute total sleep deprivation (TSD) protocol **(left panel)** and short-term partial sleep deprivation (PSD) protocol **(right panel)**. **Bars** represent 24-h periods from 9:00 PM to 9:00 PM for TSD and 5:00 PM to 5:00 PM PSD. **Solid bars** = sleep periods for the acute TSD subjects **(left panel)** and for the PSD subjects **(right panel)**. **Hatched bars** = sleep periods for the 8.2-h control group in the PSD experiment **(right panel)**. bl = baseline; r = recovery; # = frequent blood sampling days.

For a five-day period from the second baseline night of sleep until the end of the first recovery day, blood samples were obtained at 90-min intervals via an indwelling venous forearm catheter (Fig. 1, left panel). Eight samples per day were assayed for CRP levels, as described subsequently.

Experiment 2: short-term partial sleep deprivation (PSD). Ten healthy volunteers (6 men and 4 women; age 26 to 38 years [average 30.1 years]) participated in the study. The Institutional Review Board of the University of Pennsylvania approved the research protocol, and subjects gave written, informed consent before going through screening procedures, as in Experiment 1.

PROTOCOL. Subjects spent 14 days (13 nights) in <50 lux ambient light in a time-isolated, temperature-controlled Sleep and Chronobiology Laboratory, which is a satellite of the NIH GCRC of the Hospital of the University of Pennsylvania. They had a scheduled 8.2-h time in bed for the first two nights in the protocol and were randomly assigned in balanced order to one of two experimental conditions. Daily assessment of HR and BP was made by

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GCRC nurses. Five subjects were allocated to each the short-term PSD and control condition. Group assignment was comparable for age, gender, and body mass index (21 to 31.1 kg/m^2 [median 24.6 kg/m^2]). One subject in the sleep-restriction condition was excluded because of difficulty obtaining blood. Subjects in the sleep-restriction condition had 4.2 h of available time in bed (between midnight and 4:00 AM) for sleep each night for the next 10 consecutive nights. Subjects in the control condition continued with 8.2 h (between 10:00 PM and 6:00 AM) per night for the next 10 nights of study. After the 10 days, subjects in both conditions were permitted 14 h of recovery sleep. Throughout all wake periods, subjects were behaviorally monitored to ensure that they remained awake.

Outside of blood collection days, throughout scheduled wake periods, subjects were tested, as in experiment 1, on a computerized neurobehavioral battery of tests. Ambulatory electroencephalograms and electrooculograms and rectal body temperature were also monitored. Nutritionally balanced meals were provided throughout the protocol, at appropriate times for breakfast, lunch, and dinner (caffeine, tobacco, alcohol, and other psychoactive substances were prohibited). As in Experiment 1, subjects were not permitted to have visitors during the protocol.

Blood samples were collected on the first baseline day and on the day 10 (end) of the experimental period, via an indwelling forearm nonthrombogenic catheter and line using a pump system (ConFlo, Stockholm, Sweden). Subjects remained in bed (except for bathroom breaks) throughout blood collection. Integrated samples were collected every 15 min (Fig. 1, right panel) and assayed for CRP at hourly intervals.

SPECIMEN PROCESSING. Blood samples from Experiment 1 were stabilized with ethylenediamine tetraacetic acid (EDTA) and aprotinin (300 kallikrein-inhibiting U/ml blood). Blood samples from Experiment 2 were stabilized with EDTA. All blood samples were set on ice for 5 min before centrifugation at 2,600*g* for 7 min at 4°C. After centrifugation, plasma was pipetted into polypropylene tubes and frozen at −70°C until thawed for analysis.

ASSAYS. Determination of high-sensitivity CRP concentrations was performed using an ultrasensitive latex-enhanced immunoassay (Dade Behring, Newark, Delaware) (16). Day-to-day imprecision for the CRP assay at concentrations of 0.06 and 1.26 mg/dl were 8.8% and 4.3%, respectively. Concentrations of CRP below the detection level of the CRP assay (<0.02 mg/dl) were assigned a value of 0.01 mg/dl. Missing values were replaced by interpolated values. Missing values accounted for 45 of 400 data points in Experiment 1 and 21 of 450 data points in experiment 2.

Statistical analysis. As the distribution of CRP was positively skewed, data were log-transformed before analysis. For ease of interpretation, where reported, the mean value ± SEM was transformed from the log-scale back to the usual scale (mg/dl) (i.e., geometric mean).

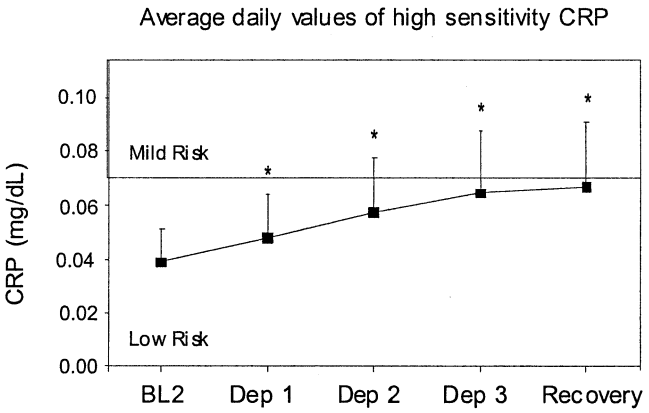

Figure 2. Data were log-transformed before analysis. For ease of interpretation, the mean values ± SEM were transformed from the log scale back to the usual scale (mg/dl) in 10 subjects undergoing 88 h of acute total sleep deprivation. Overall $p < 0.03$ by mixed-models analysis of variance on log-transformed data; each subsequent day is significantly elevated (each $p < 0.05$) from baseline. The upper portion of the graph represents the boundary separating the low- and mild-risk quintiles of C-reactive protein (CRP) derived from analysis of >5,000 apparently healthy Americans (16). BL = baseline; Dep = deprivation.

In Experiment 1 (acute TSD), daily averages of CRP were calculated (from samples taken at 3-h intervals for a total of 8 time points per day) for each subject and then log-transformed. The log CRP concentrations over time, HR, and BP were analyzed using mixed-models analysis of variance (ANOVA); if the effect of time was statistically significant ($p < 0.05$), then model contrasts were used to compare subsequent days with baseline. The p values were not adjusted for multiple comparisons.

In Experiment 2 (short-term PSD), daily averages of CRP were calculated for baseline and day 10 of PSD and then log-transformed. The log CRP concentrations, HR, and BP were analyzed using mixed-models ANOVA with factors for study day, sleep condition, and the interaction; if the interaction was statistically significant ($p < 0.10$), then model contrasts were used to compare day 10 with baseline for each sleep condition. The p values were not adjusted for multiple comparisons.

Baseline values of log CRP concentration, HR, and BP were pooled from Experiments 1 and 2, and Pearson correlation coefficients of log CRP with HR and BP were calculated. The statistical analysis was performed using SAS Version 8.0 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Experiment 1: acute TSD. The CRP concentrations increased steadily and significantly over the five-day period, as depicted in Figure 2. The daily averages were 0.039 ± 0.013 mg/dl at baseline; 0.048 ± 0.016 mg/dl for the first, 0.058 ± 0.020 mg/dl for the second, and 0.065 ± 0.023 mg/dl for the third sleep deprivation days; and 0.066 ± 0.024 mg/dl at recovery. The fixed effect for time was significant: $F(4,9) = 4.43$, $p < 0.03$. Pairwise comparisons showed that CRP concentrations were significantly increased over baseline

Table 1. Heart Rate and Blood Pressure During Total Sleep Deprivation

Baseline	BL (n = 8)	Dep 1 (n = 8)	Dep 2 (n = 7)	Dep 3 (n = 8)	Recovery 1 (n = 7)	Significance
Heart rate (beats/min)	63.0 ± 3.5	65.1 ± 3.7	70.1 ± 3.3	70.9 ± 2.8	69.7 ± 2.9	$F(4,7) = 3.3$, $p < 0.10$
Systolic BP (mm Hg)	121.1 ± 3.8	122.5 ± 2.7	125.4 ± 6.4	128.9 ± 3.0	130.0 ± 4.2	$F(4,7) = 313.3$, $p < 0.0001$
Diastolic BP (mm Hg)	68.1 ± 2.9	70.0 ± 2.4	72.0 ± 4.0	72.9 ± 2.8	71.4 ± 3.6	$F(4,7) = 3.4$, $p < 0.10$

Data are presented as the mean value ± SEM.
BL = baseline; BP = blood pressure; Dep = deprivation (day).

levels on each of the sleep deprivation days and remained elevated on the recovery day (each $p < 0.05$) (Fig. 2).

Table 1 provides the daily HR and systolic and diastolic BP measurements for TSD subjects on each day of blood collection. There was a significant increase in systolic BP through the five-day protocol. Diastolic BP and HR showed trends in the same direction.

Experiment 2: short-term PSD. The CRP levels increased significantly from 0.051 ± 0.020 mg/dl at baseline (8.2 h of time in bed/night) to 0.265 ± 0.131 mg/dl ($t[7] = 2.45$, $p < 0.05$) on the tenth day of short-term PSD (4.2 h of time in bed/night) in experiment 2 (Fig. 3). Subjects in the 8.2-h condition (8.2 h in bed per night throughout the study) went from 0.136 ± 0.047 mg/dl to 0.110 ± 0.049 mg/dl, which was not significant ($t[7] = -0.36$, $p = 0.72$). The interaction between study day and sleep condition approached significance: $F(1,7) = 4.28$, $p < 0.08$.

Table 2 provides the daily HR and systolic and diastolic BP measurements for PSD subjects on the baseline day of blood collection and on the tenth day of the experimental period. The only significant interaction was for HR, which was found to increase significantly in the PSD group. There was no significant interaction for systolic or diastolic BP.

Data from Experiments 1 and 2 were subsequently pooled for the baseline measures. Heart rate and systolic and diastolic BP were correlated with the log-transformed CRP data. We found a correlation of 0.52 ($p < 0.05$) with systolic and 0.49 ($p < 0.10$) with diastolic BP. There was insufficient data to examine correlations in each experiment separately, after sleep deprivation.

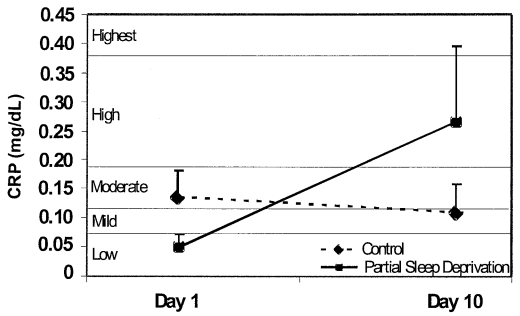

Figure 3. Data were log-transformed before analysis. For ease of interpretation, the mean values ± SEM were transformed from the log scale back to the usual scale (mg/dl) in subjects undergoing 10 consecutive days of short-term partial sleep deprivation (n = 4, **squares**) and control subjects (n = 5, **diamonds**). Significance of difference in change from baseline to day 10 between groups ($p < 0.08$ for interaction) by mixed-models analysis of variance on log-transformed data: the change from baseline to day 10 for the short-term partial sleep deprivation group was significant ($p < 0.05$), whereas the change from baseline to day 10 in the control subjects was not significant ($p = 0.72$). The **horizontal lines** indicate risk boundaries (mild to highest) of C-reactive protein (CRP) quintiles derived from analysis of >5,000 apparently healthy Americans (16).

DISCUSSION

The results of these two experiments demonstrate that both acute TSD and short-term PSD raise basal concentrations of CRP in healthy volunteers, without signs or symptoms of infection. Furthermore, the 60% increase relative to baseline, seen across 88 h of TSD, appeared to be linear and dose-dependent. The CRP levels climbed during the 88-h vigil, through the low prediction zone (0.01 to 0.07 mg/dl), and approached levels associated with mild risk (0.07 to 0.11 mg/dl) for myocardial infarction and stroke (19). In the PSD study, subjects moved from levels consistent with mild risk to levels representing values seen in the highest risk quintile of CRP concentrations (0.38 to 1.50 mg/dl) derived from population studies (19). In this condition, from day 1 to 10, there was an increase of more than fourfold. When the one outlying observation is eliminated from this group (on day 10, CRP = 1.24 mg/dl), there is still a three-fold increase, from an average of 0.051 on day 1 to 0.158 mg/dl on day 10 of PSD.

Although this study is the first to describe basal increases in high-sensitivity CRP in relation to sleep loss, subclinical effects of sleep restriction on inflammatory and immunologic parameters have been reported earlier. Sleep deprivation for between 36 and 64 h (8,9) has been shown to increase the number of leukocytes in the peripheral circulation. More recently, increased IL-6 plasma concentrations were found after sleep deprivation for 24 h (24) and 88 h (10). Neurobehavioral signs and symptoms of fatigue and sleepiness are induced in subjects undergoing acute TSD (8) and short-term PSD (25). Furthermore, a low-dose IL-6 injection leads to increased subjective estimates of fatigue (26). These results are consistent with studies of patients with excessive daytime sleepiness, in whom these symptoms have been associated with increased systemic IL-6 concentrations (27,28). They also support the hypothesis that one way in which CRP is elevated in patients with untreated obstructive sleep apnea is through deprivation of restorative sleep (23).

In several epidemiologic studies, sleep complaints were independently associated with an increased likelihood of cardiovascular morbidity or death. Appels et al. (1) reported that difficulty falling asleep or maintaining sleep, as assessed

Table 2. Heart Rate and Blood Pressure During Partial Sleep Deprivation

Baseline	BL	Dep 10	Condition by Day Interaction	BL vs. Day 10
HR in sleep (beats/min)	75.0 ± 4.1	79.5 ± 7.3	$F(1,7) = 5.89$, $p < 0.05$	NS
HR in PSD (beats/min)	75.2 ± 2.6	97.0 ± 4.8		$t(7) = 4.59$, $p < 0.01$
SBP in sleep (mm Hg)	127.8 ± 6.2	137.3 ± 3.5	NS	
SBP in PSD (mm Hg)	113.6 ± 6.7	135.2 ± 9.4		
DBP in sleep (mm Hg)	73.5 ± 7.4	86 ± 2.9	NS	
DBP in PSD (mm Hg)	68.6 ± 3.0	85.4 ± 4.8		

Data are presented as the mean value ± SEM.
DBP = diastolic blood pressure; HR = heart rate; PSD = partial sleep deprivation; SBP = systolic blood pressure; other abbreviations as in Table 1.

by a questionnaire in middle-aged women, significantly increased the risk for nonfatal myocardial infarction or cardiovascular death, even after adjustment for multiple risk factors of coronary heart disease. Similar results were found in middle-aged men in the Framingham population (2) and in older subjects in the Piedmont Health Survey (3) and the Cardiovascular Health Study (4). Furthermore, it has recently been reported that individuals sleeping for short durations are at increased risk of myocardial infarction (5,6).

One hypothesis for the link between increased cardiovascular risk and elevated CRP is that vascular shear stress exacerbated by increased BP leads to inflammation in the vascular wall. A quantitative positive relationship has been shown between increasing BP and levels of IL-6 and intercellular adhesion molecule-1 in apparently healthy men (29). The association between elevated CRP and sleep deprivation in our subjects may also be related to BP increases. We found that systolic BP was increased in our acute TSD subjects, and there were trends toward increased diastolic BP and HR. This confirms findings from another study of normal, healthy controls in whom there was an increase in BP after a single night of sleep deprivation (30). Altered cardiovascular output has also been previously reported in PSD after four nights of sleep for 4 h per night (31). In that study, systolic BP, but not HR, was found to increase. The potential clinical importance is suggested by the finding that a single night of 50% reduced sleep resulted in increased systolic and diastolic BP and HR (32).

Sleep loss may be an important contributing factor in as yet unexplained associations between inflammation and disease. Experimentally induced acute TSD (33) and cumulative partial sleep loss (34) have been associated with decreased glucose sensitivity and insulin resistance, respectively. Furthermore, a self-reported short sleep length is associated with an increased risk of the development of diabetes, and increased weight gain may mediate this relationship (35). Small elevations in inflammatory mediators have also been associated with the syndrome of insulin resistance and type II diabetes mellitus, independent of the relationship of these conditions with adiposity (36), and baseline CRP concentrations in nondiabetic subjects predict the development of type II diabetes mellitus (37). Statin-type drugs, which are known to decrease CRP (38), decrease the incidence of cardiovascular events and have been associated with a decrease in the development of diabetes (39), suggesting that inflammation may be involved in mediating insulin resistance. In sleep-disordered breathing, sleep is frequently fragmented or otherwise reduced and evidence for a strong relationship between obstructive sleep apnea and cardiovascular risk or cerebrovascular disease (40,41) is mounting. Recently, evidence of a link between obstructive sleep apnea and elevated CRP has been demonstrated (23). The current data raise the possibility that sleep loss alone may be an important contributing factor in the observed elevations in CRP.

Other changes associated with sleep loss may have also contributed to the increases in CRP encountered in our experiments. In vitro studies have suggested an influence of steroids in the synthesis of CRP (13). Nonlinear mixed-models regression found significant increases in daily cortisol levels during the 88-h vigil of Experiment 1 ($t = 2.82$, $p = 0.018$). Furthermore, the circadian nadir in cortisol concentrations has been shown to increase during sleep deprivation (42,43), and evening cortisol levels were also elevated in a PSD study (34).

Similarly, increased body mass has been shown to predispose to cytokinemia due to the synthesis and release of IL-6 from adipose tissue (44). However, diets were designed to maintain body weight, and therefore weight remained stable throughout the study. Body mass index was similar in both the experimental and control groups in the PSD condition and, in general, was well within the range for an age-matched U.S. population. However, body mass index was correlated with baseline CRP levels in subjects pooled from both studies (Spearman's $r = 0.60$, $n = 19$), and individual differences in the response to sleep deprivation, as a function of adiposity, warrant further study.

The participants in the current studies were normal sleepers who underwent sleep deprivation for relatively brief periods of time; therefore, the results cannot be generalized to populations of habitually short-duration sleepers. The present experiments also did not assess the CRP levels after full recovery from the sleep deficit induced by TSD and PSD, so it is not known how long the CRP elevations persisted, but current studies are underway to investigate this issue.

Conclusions. Our findings support the hypothesis that failure to obtain adequate amounts of healthy sleep promotes low-level systemic inflammation. These studies raise

the possibility that apparently healthy individuals may potentiate a major marker of inflammation associated with an increased risk of cardiovascular illness through the accumulation of a sleep deficit.

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