Inflammatory Markers and Sleep Disturbance in Major Depression

Sarosh J. Motivala, PhD, Avishay Sarfatti, BS, Luis Olmos, BS, and Michael R. Irwin, MD

Objective: This study was conducted to determine whether immune activation occurs in major depression, and to evaluate the associations between disordered sleep and markers of inflammation in patients with major depressive disorder. **Methods:** All-night polysomnography was obtained in patients with acute Diagnostic and Statistical Manual of Mental Disorders, 4th edition major depressive disorder ($n = 22$) and age-, gender-, and body weight-matched comparison controls ($n = 18$). After the onset of sleep, nocturnal serum levels of interleukin-6 (IL-6), soluble intercellular adhesion molecule (sICAM), monocyte chemotactic protein (MCP-1), and IL-6 soluble receptor (IL-6sR) were sampled. **Results:** As compared with matched controls, depressed patients showed significant ($p < .05$) nocturnal elevations of circulating levels of IL-6 and sICAM. Both sleep latency and rapid eye movement (REM) density had moderate correlations with IL-6 and sICAM (r's ≥ 0.30). Backward regression analyses indicated that sleep latency ($\beta = 0.34$, $p < .05$) and REM density ($\beta = 0.27$, $p = .09$) were better predictors of IL-6 than depressive status. Similarly, sleep latency ($\beta = 0.27$, $p = .06$) and REM density ($\beta = 0.32$, $p = .02$) were also better predictors of sICAM. **Conclusion:** These findings support the hypothesis that sleep disturbance is associated with elevated levels of the inflammatory markers IL-6 and sICAM. This relationship was not accounted for by other confounding factors such as age and body weight. These findings suggest that the elevations in inflammatory markers found in depressive subjects may be partially the result of disturbances of sleep initiation found in this population. **Key words:** major depression, sleep, inflammation, neuroimmunology.

IL-6 = interleukin-6; **ICAM** = intercellular adhesion molecule; **MCP** = monocyte chemotactic protein; **IL-6sR** = IL-6 soluble receptor; **UCSD** = University of California, San Diego; **MHCRC** = UCSD Mental Health Clinical Research Center; **DSM-IV** = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; **HDRS** = Hamilton Depression Rating Scale; **REM** = rapid eye movement.

INTRODUCTION

Major depression is prospectively associated with cardiovascular disease morbidity and mortality (1–3), and it is suggested that this risk is especially true for depressed males (4,5). Other studies demonstrate an increased risk of coronary artery disease morbidity and death in association with insomnia and sleep complaints (6,7), which are prominent symptoms of depression (8). Indeed, prospective data show that impaired sleep and, in particular, difficulties in sleep initiation are linked to greater cardiovascular morbidity and mortality (9,10). Moreover, difficulties in sleep initiation are found to predict coronary artery disease mortality in males, even after controlling for depression and traditional risk factors (7). The mechanisms that explain the associations among depression, sleep disturbance, and cardiovascular disease are not known, although recent interest has focused on the role of inflammation in the development of coronary artery disease (11) and its potential association with depression (12–14) and disordered sleep (15–17).

Activation of a complex cascade of inflammatory signals is thought to be involved in the development of atherosclerosis (18). Accumulation of oxidized lipoproteins in the arteries, for example, stimulates overlying endothelial cells to express intercellular adhesion molecule (ICAM) and monocyte chemotactic protein (MCP-1), which together lead to the adherence, recruitment, and migration of monocytes and T cells. Indeed, animals deficient in soluble ICAM (sICAM) or MCP-1 show reduced atherosclerotic lesions (19,20). With extracellular lipid accumulation by monocytes, proinflammatory cytokines such as interleukin-6 (IL-6) are secreted inducing further recruitment of inflammatory cells and formation of an atherosclerotic plaque. Importantly, for cell types not responsive to IL-6, coexpression of the IL-6 soluble receptor (IL-6sR) combines with IL-6 to serve as an agonist signal in mediating the action of this proinflammatory cytokine.

In this study, we examined the relationship between major depression and serum levels of four inflammatory risk factors (ie, sICAM, MCP-1, IL-6, and IL-6sR), which are putatively related to the development of coronary disease (11). In addition, we evaluated whether sleep disturbance and/or clinical depression status were more closely associated with inflammatory markers, given the independent association between difficulties initiating sleep and cardiovascular disease risk (7), the prominence of sleep complaints in depressed patients (8), and the effects of sleep loss on the expression of IL-6 (16,17,21). Sleep was measured by all-night polysomnography, and nocturnal serum levels of inflammatory markers were obtained proximal to sleep initiation (ie, 2 hours after lights out). Prior work has found that levels of IL-6 plateau at approximately 2 hours after sleep onset (22,23). Moreover, nocturnal blood sampling can be carried out without the confounding influences of daytime physical activity (24) or mental stress (25), which can alter levels of inflammatory markers. Our hypotheses were that nocturnal levels of sICAM, MCP-1, IL-6, and IL-6sR would be elevated in major depression, and that severity of sleep disturbance, in particular difficulties with sleep initiation (ie, prolonged sleep latency), would correlate with abnormal nocturnal elevations of these inflammatory markers independent of depression status and potential confounders associated with markers of inflammation.

From the Cousins Center for Psychoneuroimmunology, UCLA Neuropsychiatric Institute, University of California, Los Angeles, Neuropsychiatric Institute, Los Angeles.

Address correspondence and reprint requests to Sarosh J. Motivala, PhD, Cousins Center for Psychoneuroimmunology, UCLA Neuropsychiatric Institute, 300 Medical Plaza, Suite 3160A, Los Angeles, CA 90095-7057. E-mail: smotivala@mednet.ucla.edu.

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METHODS AND MATERIALS

Subjects

A total of 40 men were included in the present study, forming 2 groups: control subjects ($n = 18$) and depressed subjects ($n = 22$). All subjects gave informed consent under University of California, San Diego (UCSD) protocol 96095. Control subjects were identified and recruited by the UCSD Mental Health Clinical Research Center (MHCRC) in response to community educational outreach efforts (ie, lectures, meetings with community groups, and so on) or to advertisements placed in local newspapers or UCSD campus publications. Depressed subjects were self-referred from the San Diego community to the UCSD MHCRC for evaluation of depressive symptoms and inclusion in nonintervention studies before entry into a treatment protocol.

A number of behavioral, demographic, and medical variables have been related with inflammatory makers and have been found to account partially for the relationships between depression and these markers (13,14,25,26). Among the leading potential confounders are age, gender, ethnicity, body weight, presence of medical comorbidities, socioeconomic status (eg, educational level), alcohol dependence, and tobacco smoking status. To minimize the effects of these confounders on potential group differences in inflammatory markers, the following subject selection criteria were used. First, the two groups were matched ± 5 years on age and on body weight. To control for gender differences in expression of sICAM and IL-6 (14,27) and because sleep complaints have been found to independently predict coronary artery disease mortality in males, but not in females (7), only males were included in the study. Evidence also suggests that blacks show higher circulating levels of IL-6 (28,29); thus, control subjects were identified who were of similar ethnicity as the depressed subjects. The confounding effect of medical comorbidity was addressed by including only those subjects who were in good medical health. Socioeconomic status has also been associated with levels of IL-6, although the relationship does not appear to be linear and higher levels of IL-6 are found only in those with low socioeconomic status; people with middle to high socioeconomic status have similar levels of IL-6 (30). In the present study, subject recruitment occurred in the La Jolla community yielding a sample of middle to upper class socioeconomic status participants as evidenced by a high education level in both groups (approximately 15 years of education). Subjects with evidence of current or history of alcohol dependence were excluded, because alcohol dependence, but not moderate alcohol use, is associated with increased levels of IL-6 and other inflammatory markers. Lastly, tobacco smoking status is reported to lead to higher levels of inflammatory markers such as IL-6 and sICAM (14). Because the prevalence of tobacco smoking is elevated in association with major depressive disorder, whereas never mentally ill comparison controls show low rates of smoking status (31), the impact of smoking status on inflammatory markers was examined within the depressed group.

To evaluate medical health and ascertain psychiatric diagnoses, subjects received a comprehensive evaluation by a psychiatric fellow-physician that included a structured sleep disorders interview and a structured clinical interview based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (32), medical history and review of systems, physical examination, and screening laboratory tests (chemistry panel, complete blood count, thyroid function tests, and HIV test) to confirm that subjects had no underlying medical condition that might influence sleep disturbance or depression. No subject reported recent (<10 days) viral infections that could affect the immune measures. None had chronic medical conditions such as diabetes mellitus, cancer, or chronic obstructive pulmonary disease. None of the subjects were hypertensive or taking antihypertensive medications that might alter sleep or inflammatory markers. None of the subjects were routinely taking aspirin, and subjects were asked to refrain from aspirin use for 7 days before immune assessment. Laboratory tests (chemistry panel, complete blood cell count, and thyroid tests) were all within normal limits. All subjects were negative for HIV antibodies. For 2 weeks before electroencephalographic (EEG) sleep assessment, sleep–wake activity was assessed by diary to confirm that all subjects followed a habitual sleep–wake schedule that was similar to the study protocol. Psychiatric diagnoses were obtained in a consensus meeting of MHCRC psychiatrists, research fellows, and nursing staff. For the control subjects, none had a lifetime history of a mood disorder by DSM-IV and did not report any persistent sleep difficulties. Depressed subjects fulfilled DSM-IV criteria for current major depressive disorder. Severity of depressive symptoms was evaluated on the day before beginning the sleep protocol using the 17-item Hamilton Depression Rating Scale (HDRS; test–retest reliability of 0.94 on total scores) (33). At the time of evaluation, depressed subjects were not using antidepressants or other psychotropic medications that could affect sleep structure.

Procedures

Laboratory sleep studies included 2 nights of polysomnography during which subjects adhered to their habitual sleep schedules with lights out between 10 PM and midnight. During the first night of adaptation to the laboratory, recordings of pulse oximetry for oxygen desaturation were obtained to exclude subjects with sleep apnea (>10 apneas or hypopneas per hour of sleep). Tibial limb movements were also recorded and scored. None of the subjects showed evidence of sleep apnea or nocturnal myoclonus.

During the second night, all-night polysomnography was completed with placement of electrodes for EEG (C3 or C4), electrooculography, and submental electromyography recordings. EEG sleep records were visually scored according to the criteria of Rechtschaffen and Kales (34) as previously described (35). Sleep onset was defined as the first minute of stage 2 or rapid eye movement (REM) sleep followed by at least 8 minutes of sleep in the next 9 minutes. A REM period was defined by not less than 3 consecutive minutes of REM sleep. REM density was scored as the number of eye movements during the first REM period. Sleep architecture was defined as the duration and relative percent of time spent asleep in stages 1 and 2, delta, and REM. Sleep research technicians show high scoring reliability: 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 ($r = 0.99$).

For nocturnal blood sampling, an intravenous catheter was inserted into a forearm vein at 9 PM. The intravenous catheter was connected to a long, thin plastic tube that enabled blood collection from an adjacent room without disturbing the subjects' sleep. We have previously reported in a sample of 78 subjects that the introduction of an intravenous catheter does not induce a lightening of sleep (36). In those analyses, no differences were found between a night without blood sampling and a night with blood sampling for measures of sleep continuity (ie, total sleep time, sleep efficiency, sleep latency), sleep architecture (ie, duration of stages 1–4 sleep and REM sleep), and REM measures (ie, REM latency, REM density, and REM duration). For the current study, lights out occurred at approximately 11 PM and blood was collected at 1 AM. All subjects were in the supine position and were asleep at the time of blood collection, thus controlling for the effects of physical activity that could alter expression of inflammatory markers (37). In 1 depressed patient, the blood sample could not be obtained until 1:30 AM. Blood samples were not obtained before sleep, because the focus of this study was on the relationship of disordered sleep, particularly sleep initiation, on inflammatory markers. Sampling at 1 AM was used, because IL-6 reaches a stable nocturnal plateau before this time (22,23). We have previously shown that placement of intravenous catheter does not alter circulating levels of IL-6 (23), although levels of IL-6 increase 30 minutes immediately after sleep onset (23). After blood sampling, serum was separated and stored at −80°C.

Inflammatory Markers

For all assays of inflammatory markers, matched controls and depressed patients were run together so that each enzyme-linked immunosorbent assay plate had similar numbers of depressed and control subjects. Levels of MCP-1 were quantified using Quantikine human MCP-1 kits (R&D Systems, Inc., Minneapolis, MN) with an intraassay coefficient of variation of 5% and an interassay coefficient of variation of 5%. The minimal detectable dose of MCP-1 is 31.2 pg/mL. Levels of sICAM-1 were measured using Parameter human sICAM-1 kits (R&D Systems, Inc.) with an intraassay coefficient of variation of 3%. The minimal detectable dose of soluble ICAM-1 is 2.73 ng/mL. In the case of sICAM, kits from different lots were used, and a standard curve was generated on each plate to calibrate plate differences with an interassay coefficient of <3%. Serum levels of IL-6 were measured using Quantikine High Sensitivity human IL-6 kits (R&D Systems, Inc.) 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. Levels of IL-6 soluble receptor were measured using Quantikine human IL-6sR immunoassay kits (R&D Systems, Inc.) with an intraassay coefficient of variation of 2% and an interassay coefficient of variation of 4%. The minimal detectable dose of IL-6sR is 31.2 pg/mL. Two subjects were missing IL-6 values because of limited serum volume.

Statistical Analyses

All data were entered and analyzed in SPSS (38). Inflammatory markers and nearly all sleep variables demonstrated normality, except for sleep latency. Raw values for 2 subjects in the depressed group had values >72 minutes, which were greater than 3 standard deviations from the mean; these 2 values were adjusted to be 1 unit above the highest nonoutlier values, which resulted in a nearly normal distribution.

Differences between depressed and control subjects on continuous variables, including background, clinical, EEG sleep, and inflammatory risk marker variables, were tested by analyses of variance (ANOVA). Differences on categorical variables were tested using chi-squared tests. Appropriate assumption checks were conducted for the ANOVAs, including normality and homogeneity of variance (criterion of Hartley's F_{max} <3.0, per Keppel, 1992).

Regressions were used to test the primary hypotheses that 1) depression status accounted for a significant amount of the variance among inflammatory cytokines and 2) measures of sleep would be a better predictor of inflammatory cytokines than depression status when allowed to enter the regression in a backward manner. Efforts were made in the experimental design and subject selection criteria to minimize group differences in confounding variables such as age, gender, ethnicity, education, medical comorbidity, and alcohol dependence. An a priori approach was used to identify key covariates to be included in the regression analyses. Because of the linear relationships between age and inflammatory markers and between body weight and IL-6 (14), both variables were retained as a priori confounders in the regression analyses. Smoking status was also not included as a covariate in the overall regression analyses, because smoking status was only found in the depressed group; both the depressed and control groups must contain smokers to properly assess the impact of smoking on the relationships between depression and inflammatory markers (39). The effects of smoking status on inflammatory markers were assessed within the depressed group in a separate set of analyses.

In the first regression model, confounder variables were tested along with depression status to predict those inflammatory markers that differed between the groups. If the p value for a given independent variable was greater than .10, it was removed and the model was tested again. This process continued until only variables associated with the inflammatory marker were left in the model. The second set of backward elimination regression analyses included EEG sleep variables along with the confounder variables and depression. Selection of sleep variables to include was based on their relationship to the inflammatory marker. Only sleep variables with a moderate effect size (r = 0.30) were included. Each regression was examined for appropriate fit with pertinent assumptions.

Finally, a subsequent set of analyses were conducted to test whether depressive symptom severity (HDRS score) was related to inflammatory cytokines. Depression status is not a simple dichotomization of depressive severity, and it has been argued that both aspects should be examined when appropriate data are available (40). Depressive severity was analyzed through correlation between total HDRS scores and inflammatory markers (IL-6, sICAM, IL-6sR, and MCP-1). Additional correlations were performed partialing out the variance associated with insomnia according to the sleep dimension found in the HDRS because the HDRS has been noted to be overly influenced by sleep items (41,42). Correlations were reviewed for proper assumptions, including normality criterion of skewness z score and kurtosis z score <3.0 (43) and linearity (criterion of linear relationship through examination of the scatterplot).

RESULTS

Subject Characteristics

Clinical characteristic and depressive symptom severity measures are presented in Table 1. Consistent with our matching procedures, the depressed and control groups were similar in age and body weight. For ethnicity, education level, and alcohol consumption histories, the groups showed marginally significant differences. The depressed group had more European Americans, was less educated, and reported lower alcohol consumption. Furthermore, ethnicity was related to sICAM (F[1,38] = 7.22, p <.05), but not any of the other inflammatory variables. Education was associated with sICAM (r = −0.32, p <.05) and IL-6 (r = 0.39, p <.05). Alcohol consumption was not correlated with inflammatory variables.

The 2 groups differed on the presence of current smoking status, and all smokers were in the depressed group. As noted previously, none of the subjects were currently taking aspirin, beta-blocker medications, or antidepressants. As expected, the depressed subjects showed significant elevations in depressive symptoms measured by the total scores on the HDRS. In addition, sleep disturbance, as measured by the insomnia items of the HDRS, was more common in the depressed patients than controls with depressed patients reporting difficulty falling asleep, early waking with the inability to return to sleep, and difficulty maintaining sleep through the night. None of the controls reported difficulty falling asleep or with awakening too early in the morning, and only 1 control subject reported mild difficulty with maintaining sleep through the night.

TABLE 1. Characteristics and Depressive Severity of Controls and Patients With Major Depressive Disorder

	Controls (n = 18)	Depressed (n = 22)	F	p
Age (yrs.)	40.3 ± 9.2	44.4 ± 7.5	2.39	.13
Weight (lbs.)	175.9 ± 16.2	179.8 ± 30.0	.23	.64
Ethnicity (African American/Euro American)	6/12	2/20	χ^2 = 2.27	.13
Education Level (# of years)	15.7 ± 1.5	14.5 ± 2.2	3.78	.06
Alcohol Consumption (drinks/week)	1.9 ± 1.4	1.2 ± 1.8	1.49	.14
Current tobacco smoking (smoker/nonsmoker)	0/18	13/9	χ^2 = 13.18	<.0001
HDRS	1.2 ± 1.3	19.3 ± 4.2	315.18	<.0001

HDRS = Hamilton Depression Rating Scale, 17 item.
Values in group columns reflect mean ± standard deviation, except for ethnicity and current tobacco use.

Group Differences in Electroencephalographic Sleep and Inflammatory Markers

Polysomnographic recordings showed disturbances of sleep with decreases of total sleep time, prolonged sleep latency, and decreases of stage 2 sleep in the depressed patients as compared with controls (Table 2). Other EEG sleep architecture or REM measures did not differ between the groups.

Table 2 also shows the mean values of the 4 inflammatory markers. As compared with controls, depressed subjects had higher mean serum levels of IL-6 and sICAM. Levels of IL-6sR and MCP-1 did not differ between the groups.

Regression Analyses Testing Associations of Depressed Group Status and Sleep on Interleukin-6 and Soluble Intercellular Adhesion Molecule

Two sets of backward elimination regression models were tested to evaluate: 1) whether major depression was associated with elevated levels of IL-6 or sICAM independent of age, body weight, education, and ethnicity; and 2) whether these relationships were attenuated when the EEG sleep variables were added to the model. In determining which sleep variables to include, only variables related to IL-6 and sICAM with a moderate effect size ($r = 0.30$) were included in these analyses. Of the sleep variables, only sleep latency and REM density met this criteria. Sleep latency correlated with IL-6 ($r = 0.32$) and with sICAM ($r = 0.30$). REM density correlated with IL-6 ($r = 0.31$) and with sICAM ($r = 0.34$). Because age and weight were considered as a priori confounders, they were included in the regression analyses. In addition, because education and ethnicity were at least marginally associated with depression and inflammatory markers, they were also included in the regression analyses.

For the first model tested, backward elimination regression analyses indicated that education and major depression status were significantly associated with IL-6; age, weight, and ethnicity were not significant predictors (Table 3). In the second model, when sleep variables were incorporated in the regression analyses along with depression status, as well as the confounder variables, major depression was no longer a significant predictor. Level of IL-6 was significantly associated with sleep latency and body weight and marginally associated with REM density (Table 3). To explore whether increases of IL-6 are found in depressed subjects with prolonged sleep latency, the interaction term of sleep latency and depression group status was included in a third model. Results indicated that the interaction term was not a significant predictor.

For sICAM, in the first regression model, backward elimination regression analyses indicated that only ethnicity and education were significantly associated with sICAM; age, weight, and depression status were not significant predictors (Table 3). In the second model, with inclusion of sleep latency and REM density into the backward elimination regression, both sleep latency and REM density, along with weight and ethnicity, remained in the model. Depression group status was not a significant predictor. Furthermore, when the interaction term (sleep latency × depressive group status) was tested in a third model, it was not a significant predictor of sICAM level.

Cigarette Use and Inflammatory Markers in the Depressed Patients

To evaluate the influence of cigarette smoking on elevated levels of IL-6 and sICAM in depression, the depressed group was stratified into smokers ($n = 13$) and nonsmokers ($n = 9$). IL-6, IL-6sR, and MCP-1 were not significantly different between smokers and nonsmokers. However, there was a

TABLE 2. EEG Sleep Variables and Inflammatory Markers of Controls and Patients With Major Depressive Disorder

	Controls (n = 18)	Depressed (n = 22)	*F*	*p*
EEG sleep variables				
Total Sleep Time (min)	397 ± 48	340 ± 64	9.81	.003
Sleep Efficiency (%)	86.9 ± 7.9	81.2 ± 12.4	2.87	.098
Sleep Latency (min)	10.5 ± 6.5	22.3 ± 18.9	6.36	.016
Stage 1 (min)	33.4 ± 15.6	27.5 ± 11.8	1.86	.18
Stage 2 (min)	241 ± 37.9	202 ± 47	8.24	.007
Stage 3 (min)	24.4 ± 21.1	22.4 ± 17.8	0.10	.75
Stage 4 (min)	10.2 ± 14.7	5.0 ± 8.8	1.89	.18
Delta (min)	34.5 ± 33.8	28.0 ± 23.0	0.53	.47
REM (min)	88.0 ± 27.6	82.4 ± 34.2	0.32	.58
REM Latency (corrected; min)	62.0 ± 24.2	61.5 ± 41.2	0.002	.96
REM Density	1.42 ± 0.47	1.63 ± 0.62	1.13	.29
REM Duration (1st period; min)	16.9 ± 8.0	21.9 ± 9.6	2.99	.09
Inflammatory markers, serum levels				
IL-6 (pg/ml)	2.33 ± 0.9	3.46 ± 1.6	6.75	.01
sICAM (ng/ml)	219 ± 49	275 ± 86	5.97	.02
MCP-1 (pg/ml)	222 ± 132	253 ± 119	0.23	.63
IL-6sR (ng/ml)	20.9 ± 4.5	21.6 ± 4.9	0.51	.48

REM = rapid eye movement sleep; IL-6 = interleukin-6; SICAM = soluble intercellular adhesion molecule; MCP-1 = monocyte chemotactic protein; IL-6sR = IL-6 soluble receptor.
Values in group columns reflect mean ± standard deviation.

TABLE 3. Backward Elimination Regression Analyses of Correlates of Serum Levels of sICAM and IL-6 in Controls and Patients With Major Depressive Disorder

IL-6

Model 1 (R^2 = .23)					Model 2 (R^2 = .29)				
Correlate	β	t	p	$r_{partial}$		β	t	p	$r_{partial}$
Age	.15	.95	.35	.17	Age	.17	1.08	.29	.20
Weight	.13	.81	.42	.14	**Weight**	**.32**	**2.17**	**.04**	**.24**
Ethnicity	−.16	−1.03	.31	−.18	Ethnicity	−.18	−1.13	.27	−.21
Education	**−.29**	**−1.82**	**.08**	**−.28**	Education	−.21	−1.23	.24	−.22
Sleep Latency	N/A				**Sleep Latency**	**.34**	**2.21**	**.03**	**.30**
REM Density	N/A				**REM Density**	**.27**	**1.75**	**.09**	**.22**
Major Depression	**.30**	**1.90**	**.07**	**.29**	Major Depression	.16	.91	.37	.17

sICAM

Model 1 (R^2 = .30)					Model 2 (R^2 = .44)				
Correlate	β	t	p	$r_{partial}$		β	t	p	$r_{partial}$
Age	.08	.52	.61	.09	Age	.06	.39	.69	.07
Weight	.11	.79	.43	.14	**Weight**	**.26**	**1.99**	**.06**	**.25**
Ethnicity	**.45**	**3.21**	**.003**	**.42**	**Ethnicity**	**.43**	**3.18**	**.003**	**.46**
Education	**−.35**	**−2.49**	**.02**	**−.30**	Education	−.17	−1.11	.28	−.20
Sleep Latency	N/A				**Sleep Latency**	**.27**	**1.99**	**.06**	**.24**
REM Density	N/A				**REM Density**	**.32**	**2.42**	**.02**	**.35**
Major Depression	.16	.99	.33	.17	Major Depression	.07	.45	.65	.08

In backward elimination analyses, all variables were entered into the model and if a variable did not at least indicate a trend for significance ($p < .10$), it was removed. In model 1, age, weight and depression group status were tested as correlates. In model 2, two sleep variables found associated with inflammatory markers, namely sleep latency and REM density, were incorporated into the backward elimination analysis. Variables retained in final model are in bold. Partial correlations ($r_{partial}$) reflect the correlation between the given predictor variable and the outcome variable when all other variables are controlled. Beta weights reflect either weights in the final model (in bold), or weights in the initial model. R^2 reported is from the final model.
IL-6 = interleukin-6; sICAM = soluble intercellular adhesion molecule; REM = Rapid Eye Movement; N/A = not in regression for Model 1.

significant difference for sICAM (F[1,20] = 11.4, $p < .01$), with depressed smokers showing significantly higher levels (317 ± 89; mean ± standard deviation) than depressed nonsmokers (214 ± 20).

Depressive Symptom Severity and Inflammatory Cytokines

In addition to depression status, associations of depressive severity (HDRS scores) with inflammatory markers were also tested. Correlations showed that total HDRS scores were correlated with IL-6 ($r = 0.40$, $p = .01$) and with sICAM ($r = 0.36$, $p = .02$) but not with IL-6sR ($r = 0.06$, $p = .760$) or MCP-1 ($r = 0.14$, $p = .422$). To test the relationship between depressive symptom severity and inflammatory markers independent of measures of subjective sleep disturbance, partial correlations between inflammatory markers and HDRS scores were done, extracting the variance associated with the three insomnia-related items on the HDRS. Results indicated that when the insomnia items were partialed out, depressive symptoms did not correlate with any of the inflammatory markers, including IL-6 ($r = 0.16$, $p = .413$), sICAM ($r = 0.22$, $p = .253$), IL-6sR ($r = -0.25$, $p = .189$), and MCP-1 ($r = 0.18$, $p = .357$). In contrast, the early insomnia item from the HDRS correlated with IL-6 ($r = 0.34$, $p = .04$), but the middle and late insomnia items did not.

DISCUSSION

Along with complaints of insomnia and prolonged sleep latency, clinical depression is accompanied by elevated nocturnal levels of sICAM and IL-6. Moreover, the present study extends prior work linking depression, endothelial activation, and inflammation (12–14) and demonstrates an association between difficulties in sleep initiation and elevations in levels of the proinflammatory cytokine IL-6 and endothelial activation marker sICAM. Furthermore, sleep latency and REM density were better predictors of IL-6 and sICAM than depression status. Finally, when insomnia items were partialed out, severity of depressive symptoms was no longer correlated with IL-6 and sICAM. Together, these data support the hypothesis that problems in sleep initiation are related to elevated levels of a proinflammatory cytokine and are partially responsible for the link between major depression and IL-6.

Recent observations indicate a bidirectional interaction between sleep and proinflammatory cytokines that is particularly visible when examining sleep continuity variables. In animals, when IL-6 is administered before the onset of the sleep cycle, sleep fragmentation occurs (44). We have suggested that IL-6 might contribute to disordered sleep in humans (15) and found in alcoholic men and healthy male controls that circulating levels of IL-6 before sleep onset correlate with prolonged

sleep latency (29). Furthermore, when there is loss of sleep (eg, total night sleep deprivation) or increases of REM sleep, expression of IL-6 as measured by circulating levels and stimulated production are reported to increase during the day (17,21,23,45). Thus, given the reciprocal interactions between sleep and IL-6, we speculate that sleep disturbance in depressed patients is associated with increases of inflammatory markers. In turn, increases of IL-6 might delay sleep onset, which perpetuates an abnormal cycle of inflammation and disordered sleep. Further study using a longitudinal design or an experimental manipulation of sleep and/or cytokines (eg, administration of a cytokine antagonist) is needed to evaluate the roles of inflammatory markers in disordered sleep among depressed patients.

The factors underlying the high levels of sICAM and IL-6 in depressed patients and the associations with disordered sleep are not known. It has been shown that acute sympathetic and β-adrenergic activation increase immune cell chemotaxis, cellular adhesion molecule expression (46), and production of proinflammatory cytokines (47). Depressed patients have been shown to have high sympathetic tone at rest (48), and either acute or chronic loss of sleep leads to nocturnal elevations of circulating catecholamines and sympathetic activity (49,50). Alternatively, parasympathetic outflow contributes to the alterations in expression of inflammatory markers; in animal models, activation of cholinergic receptors on immune cells and/or increases of parasympathetic outflow are associated with decreased production of proinflammatory cytokines (51). Thus, disordered sleep in depressed patients could lead to increases in markers of inflammation and endothelial activation through a shift of sympathovagal balance toward sympathetic dominance.

Our findings linking sleep and IL-6 and sICAM may have important health implications. Recent epidemiologic data show that self-reported difficulty initiating sleep is a predictor of cardiovascular disease mortality (7). Similarly, objective measures of difficulty initiating sleep (ie, prolonged sleep latency) yields a 2-fold elevated risk of death in a healthy older adult population (52). Prospective data further indicate that sICAM and IL-6, directly implicated in atherosclerotic disease processes, predict risk for myocardial infarction independent of cholesterol levels, smoking status, and obesity (53,54). Likewise, in patients recovering from an acute coronary artery event, comorbidity for depression is associated with elevated levels of sICAM independent of smoking status, obesity, and other traditional risk markers (14). Thus, in future studies, examination of disordered sleep along with inflammation and endothelial activation may provide insight about whether these biobehavioral mechanisms contribute to excess cardiovascular mortality in people with depression.

High levels of MCP-1 and sIL-6 receptor have been found in untreated patients with major depression (12,55). Thus, we expected that levels of MCP-1 and IL-6 receptor would also be related to depression and severity of disordered sleep. However, in the one study that examined levels of MCP-1, the depressed sample was on average 10 years younger and predominantly female as compared with the present sample of middle-aged males. If confirmed in future research, depression in women might be more broadly associated with increases in markers of endothelial activation and inflammation consistent with their increased risk for inflammatory disorders (56,57).

For interpretation of these findings, there are a few considerations. First, depressed subjects reported more sleep complaints, including difficulty initiating sleep, than controls, and these complaints were consistent with polysomnographic findings. Previous work indicates that the nature of sleep dysregulation in depression can vary, although sleep continuity variables show the most consistent findings (58,59). In the current study, depressed subjects had poorer sleep continuity, reflected by longer sleep latency and less total sleep time. Depressed subjects did not differ from controls on other sleep measures such as REM latency; this measure is also influenced by age, depressive severity, and inpatient/outpatient status (59).

Second, levels of inflammatory markers are associated with a number of variables that include but are certainly not limited to medical health status, medication use, alcohol dependence, cigarette use, and whole-body adiposity. In the current study, all subjects were medically healthy and were not taking medications known to affect inflammatory markers (eg, statins). Furthermore, individuals with a present or past history of alcohol dependence were excluded. Whereas smoking use was not associated with IL-6 levels, sICAM was higher in the depressed smokers than the depressed nonsmokers. Tobacco smoking may contribute to elevated levels of sICAM in depression. Nevertheless, Lesperance and colleagues (14) found that depression was associated with higher sICAM independent of smoking. Regarding adiposity, prior studies indicate that it is partially responsible for elevated levels of IL-6 in depression (13), and in the present study, backward elimination regression analyses indicated that sleep disturbance and body weight were better correlates of IL-6 than depression. Larger studies are warranted to determine whether sleep difficulties act as mediators of the elevations in this cytokine in depression.

Third, the prominent relationships between sleep latency and IL-6 may partially be accounted for nocturnal sampling of this marker at the beginning of the night, proximal to sleep onset. Sampling later in the night or in the morning might reveal associations with other measures of sleep or late-night aspects of sleep (eg, REM amounts). For example, in alcoholics, we have found that circulating levels of IL-6 at 3 AM correlate with amounts of REM sleep in the late part of the night (29), and REM sleep amounts are associated with elevated stimulated production of IL-6 in the morning (45). The cross-sectional nature of the study precludes determination of the direction of the association between disordered sleep and inflammation, or whether elevated levels of inflammatory markers persist once abnormal sleep normalizes. Lastly, given that the sample included only men, future studies are needed to determine whether these findings generalize to women.

In conclusion, medically healthy patients with acute major depressive disorder show elevated levels of sICAM and IL-6, and these differences were not accounted for by age or body weight. In addition, severity of sleep disturbance as measured by sleep latency and REM density was related to nocturnal elevations of sICAM and IL-6. The findings suggest that disturbance of sleep initiation contributes to the elevations of IL-6 seen in major depression. Interventions that target disordered sleep may have potential salutary effects on inflammatory processes. To the extent that improvements in sleep are maintained over time, this could foster declines in cardiovascular morbidity and mortality that are commonly seen in people with clinical depression.

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