C-reactive Protein is Associated With Sleep Disordered Breathing Independent of Adiposity

Naresh M. Punjabi, MD; Brock A. Beamer, MD

Johns Hopkins University Department of Medicine, Baltimore, MD

Study Objectives: It is well established that medical conditions such as obesity and cardiovascular disease are associated with increased levels of inflammatory biomarkers such as C-reactive protein (CRP). Prior studies have produced inconsistent results regarding the association between sleep disordered breathing (SDB) and CRP, possibly due to the confounding effects of obesity or medical comorbidity. The present study examined the association between degree of SDB and level of CRP independent of prevalent medical conditions and obesity.

Design: Cross-sectional study.

Subjects and Setting: University-based clinical sample referred for diagnostic polysomnography.

Measurements and Results: The study sample consisted of 69 men (mean age 40 years; mean BMI of 31.2 kg/m²) free of prevalent medical conditions including hypertension, diabetes mellitus, and cardiovascular disease. Measurements of morning and evening CRP levels were performed along with full-montage polysomnography. Confounding due to obesity was assessed by adjustments for body mass index, waist circumference, and percent body fat. A strong association was found between degree of SDB and serum levels of CRP, with or without adjustment for age and several measures of adiposity. Between the lowest and highest quartiles of apnea-hypopnea index (AHI) the mean difference in adjusted level of CRP was 3.88 μg/ml (P < 0.001). Moreover, an independent association between serum CRP levels and nocturnal hypoxia was also observed, whereas no association was noted with parameters of sleep architecture.

Conclusions: While more research is needed to elucidate causal pathways involving the effects of sleep-related hypoxia on low-grade systemic inflammation, the results of this study suggest that mechanisms other than adiposity per se could contribute to the inflammatory state seen in adults with SDB.

Keywords: Sleep disordered breathing, obstructive sleep apnea, inflammation, C-reactive protein

Citation: Punjabi NM; Beamer BA. C-reactive protein is associated with sleep disordered breathing independent of adiposity. SLEEP 2007;30(1):29-34.

INTRODUCTION

SLEEP DISORDERED BREATHING (SDB) IS A CHRONIC CONDITION CHARACTERIZED BY REPETITIVE COLLAPSE OF THE UPPER AIRWAY DURING SLEEP. The resulting decrease or cessation of airflow often leads to periods of intermittent hypoxemia and recurrent arousals from sleep. It is estimated that approximately 5% of adults in the general population meet the diagnostic criteria for SDB. A growing body of evidence from clinic- and population-based studies indicates that SDB is associated with a plethora of adverse health conditions. Causal relationships between SDB, hypertension, diabetes mellitus, and cardiovascular disease have become areas of intense research, and numerous studies now implicate SDB as a contributory factor to these conditions independent of conventional risk factors such as obesity.

Although the mechanisms by which SDB may lead to such complications are not completely defined, sympathetic activation due to intermittent hypoxemia and/or sleep fragmentation has been implicated in the putative causal pathway. There is also increasing recognition that low grade systemic inflammation may be yet another mechanism linking SDB to cardiovascular disease. Inflammation plays an important role in arterial plaque formation, plaque rupture, and vascular thrombosis thereby increasing the susceptibility to myocardial ischemia and infarction. In fact, whether in high-risk or in general populations, serum levels of circulating inflammatory markers are predictive of incident hypertension and cardiovascular events. C-reactive protein (CRP), an acute phase reactant secreted by the liver, is one of the most actively studied biomarkers of low-grade inflammation. Studies examining the effects of SDB on serum CRP levels have provided conflicting results, with some studies demonstrating an independent association with disease severity and others showing no relationship.

Adipose tissue is an important source of inflammatory cytokines and produces a significant proportion of the circulating interleukin-6 (IL-6), the principal cytokine that induces CRP. The well-established association between obesity, particularly central obesity, and systemic inflammation raises the possibility that the inflammatory marker profile in SDB merely reflects the confounding effects of obesity. Furthermore, the high prevalence of medical conditions in SDB such hypertension, diabetes mellitus, and cardiovascular disease — conditions that are independently correlated with higher CRP levels — introduces the added concern for confounding in delineating whether SDB is related to systemic inflammation. Recognizing the potential limitations of previous work, the current investigation sought to determine whether SDB and its physiologic consequences of nocturnal hypoxemia and sleep disruption were related to CRP levels independent of measures of body mass and fat distribution in the absence of coexisting medical conditions. We also examined whether systemic inflammation in SDB is further influenced acutely by the overnight physiologic stress imposed by SDB-related inter-
mittent hypoxemia and sleep fragmentation. It was hypothesized that SDB severity would be associated with serum CRP levels independent of the confounding influences of age and measures of body composition and that the overnight physiologic stress imposed by SDB would acutely increase CRP levels.

METHODS

Study Sample

Consecutive male patients undergoing clinical polysomnography were initially screened for inclusion in the current investigation. Women were not included in the study sample because the dramatic effects of hormonal differences on CRP levels necessitates recruitment of pre- and post-menopausal women (on and off oral contraceptive and hormonal therapy) in subgroups of a size that were beyond the scope of the current study. Men with a previous history of hypertension, stable or unstable angina, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass surgery, congestive heart failure, and stroke were considered ineligible. Other exclusionary criteria included diabetes mellitus (prior diagnosis or fasting glucose ≥ 126 mg/dl), chronic obstructive lung disease, renal or hepatic dysfunction, and history of upper airway surgery. Finally, patients using anti-inflammatory agents (e.g., steroids or other immunosuppressive agents), supplemental oxygen, or positive airway pressure therapy at any time in the past were also considered ineligible. Approval for the study protocol was acquired from the local institutional review board, and informed consent was obtained from all study participants.

Body Composition Analysis

Anthropometric measures included height, weight, and waist circumference. Height was measured to the nearest 0.1 kg and height was measured using a portable stadiometer to the nearest 0.5 cm. Waist circumference was measured midway between the lower rib margin and the iliac crest with the subject in the standing position at the end of a normal expiration. Foot-to-foot bioimpedance analysis was conducted to estimate percent body fat using a Tanita Scale (Tanita Corporation of America, Inc, Arlington Heights, IL). Previous studies have found excellent concordance of Tanita estimates with measures of body fat derived from dual energy X-ray absorptiometry (DXA). Subjects were measured standing erect with bare feet on the analyzer footpads. Percent body fat was estimated using the programmed height, weight, age, and sex-specific equation.

Polysomnography

The overnight polysomnogram included the following physiologic recordings: C3-A2 and C3-O1 electroencephalograms, right and left electrooculograms, and submental and bilateral anterior tibialis surface electromyograms. Respiration was monitored throughout the night with a nasal pressure transducer, thermocouples at the nose and mouth, and with thoracic and abdominal strain gauges. Continuous recording of the oxyhemoglobin saturation (\(\text{SaO}_2\)) was obtained with an oximeter (Ohmeda 3700; Englewood, CO). Physiologic signals were digitized (Embla recordings systems, Medcare, Buffalo, NY) for offline analysis of sleep and breathing patterns. Sleep stage scoring was performed on 30-second epochs according to standard criteria. Apnea was defined as complete cessation of airflow for at least 10 seconds. Hypopnea was defined as any reduction in airflow that was associated with an electroencephalographic arousal or a 4% drop in the \(\text{SaO}_2\). The apnea-hypopnea index (AHI) was calculated as the total number of apneas and hypopneas per hour of total sleep time. The average degree of oxyhemoglobin desaturation (\(\Delta\text{SaO}_2\)) associated with each disordered breathing event was determined and used an index of hypoxicemic stress during sleep.

Blood Collection and Biochemical Analysis

A fasting blood sample (10 ml) was obtained from the anterior cubital vein in a resting and fasting state shortly after the conclusion of the overnight sleep recordings. In addition, a blood sample was also obtained just prior to the onset of the sleep study. All venous samples were centrifuged immediately and placed at 4°C prior to serum separation. After centrifugation at 3000 rpm for 20 minutes, serum was separated into multiple aliquots and stored at -80°C. High sensitivity (hs)-CRP levels were measured in duplicate for each eligible patient using commercially available ELISA kits (ALPCO, Windham, NH). The hs-CRP assay kit has a reported linear range of 1.0 – 150.0 ng/ml and sensitivity of 0.5 ng/ml. The intra- and inter-assay coefficients of variation are 6.33% and 2.20%, respectively.

Statistical Analysis

All results are reported as means along with the standard deviation (SD) or standard error (SEM). The dependent variable of interest was the serum level of CRP. Bivariate associations between the morning and evening CRP levels and indices of SDB severity were initially examined using scatter plots and Pearson’s correlation coefficients. Because the primary objective of the analysis was to determine the independent association between CRP level and SDB severity, multivariable linear regression methods were used to account for the confounding effects of age, BMI, waist circumference, and percent body fat. To minimize the influence of outlying CRP values, log-transformed CRP levels were also modeled. Inferences derived with such transformations were no different that those based on modeling serum CRP levels directly. Thus, for ease of interpretation, figures display the results from the linear modeling of CRP levels. Furthermore, while continuous and categorical forms of each independent variable (e.g., AHI, \(\Delta\text{SaO}_2\)) were used in the model construction, results from the categorical analyses are presented to avoid the assumption of a linear association between the dependent and independent variables. In addition to modeling the morning CRP levels, the difference and the ratio between the morning and evening levels were also examined. Statistical significance was determined by the two-sided test of each regression coefficient. Model fit was assessed by examining the distribution of residuals from each multivariable model as a function of the covariates included in the model. All statistical analyses were conducted using the Stata 7.0 statistical software package.

RESULTS

The study sample consisted of 69 men with an average age of 40.2 years and BMI of 31.2 kg/m². The anthropometric, body composition, and sleep characteristics of the sample are shown...
in Table 1. As expected, there was a moderate degree of correlation between BMI and AHI \((r = 0.45, 95\% \text{ CI}: 0.24–0.62)\). BMI was also correlated with other measures of body composition including waist circumference \((r = 0.91, 95\% \text{ CI}: 0.87–0.94)\) and percent body fat \((r = 0.79, 95\% \text{ CI}: 0.68–0.86)\). Further bivariate analyses revealed that morning and evening CRP serum levels were most correlated with AHI \((r = 0.48, 95\% \text{ CI}: 0.27–0.64)\) for morning levels, \(R = 0.50, 95\% \text{ CI}: 0.30–0.66\) for evening levels. In contrast, the unadjusted associations between CRP and measures of body composition were only modest. The correlation coefficients between morning CRP levels, BMI, waist circumference, and percent body fat were 0.30 \((95\% \text{ CI}: 0.07–0.50)\), 0.34 \((95\% \text{ CI}: 0.11–0.53)\), and 0.26 \((95\% \text{ CI}: 0.02–0.47)\), respectively. No significant differences in morning and evening CRP levels were noted (absolute difference = 0.03, 95% CI: -0.13–0.20) in unadjusted and adjusted analyses. Thus, all subsequent models were based on the morning CRP levels.

Figure 1 shows the unadjusted and adjusted CRP levels across quartiles of AHI. Compared to patients with minimal or no SDB (AHI < 6.5, first quartile), CRP levels were higher in patients with moderate (AHI: 19.3–50.0, third quartile) and severe SDB (AHI>50.0, fourth quartile). Compared to the first AHI quartile, the absolute differences in CRP levels for the third and fourth AHI quartiles were by 1.94 µg/ml (third vs first quartile, \(P<0.04\)) and 4.00 µg/ml (fourth vs first quartile, \(P<0.0001\)), respectively. Adjustments for variables including age, BMI, waist circumference, percent body fat, and the amount of total sleep time on the overnight sleep study led to some attenuation in the association between CRP and AHI. Nevertheless, individuals with severe SDB (fourth quartile) maintained a significantly large difference in CRP levels (\(\Delta = 3.88, P<0.002\)) compared to patients with no SDB, and there remained a progressive increase in CRP levels with increasing AHI (\(P<0.0001\) for linear trend).

Figure 2 shows the unadjusted and adjusted CRP levels as a function of the average oxyhemoglobin desaturation \((\Delta \text{SaO}_2)\) associated with occurrence of apneas and hypopneas. With increasing degree of oxyhemoglobin desaturation \((\Delta \text{SaO}_2)\), CRP levels increased independent of age and measures of adiposity (i.e., BMI, waist circumference, percent body fat). Compared to patients that had an average \(\Delta \text{SaO}_2\) of less than 3.7% (first category), patients with \(\Delta \text{SaO}_2\) greater than 5.7% (third category) had higher CRP levels (\(\Delta = 2.58, P<0.016\)).

Analyses relating nocturnal sleep stage distribution to morning CRP levels showed that percentages of stage 1 and stage 2 sleep were associated with CRP levels in unadjusted analyses with correlation coefficients of 0.24 \((P<0.05)\) and -0.29 \((P<0.01)\). However, after adjustments for body composition, the associations between CRP levels and sleep stage percentages were no longer significant (data not shown).

**DISCUSSION**

The results of the present study demonstrate that, in the absence of confounding medical conditions, SDB is associated with elevated levels of CRP independent of age, BMI, waist circumference, and percent body fat. In a clinic-based sample of men free of medical comorbidity, a dose-response relationship was identified between serum CRP levels, the frequency of disordered breathing events, and the degree of nocturnal hypoxemia. However, an independent association between CRP levels and the distribution of sleep stages in SDB was not observed. Finally, comparisons of morning and evening CRP levels revealed no significant change after a night of directly observed sleep.
The potential impact of SDB on subclinical inflammation has been examined in several studies (Table 2). In agreement with our findings, SDB has been independently associated with higher CRP levels in children and adolescents—study samples with minimal concerns for underlying comorbidities that could confound the associations of interest. Although earlier reports in adults also raised the possibility that SDB may independently contribute to low-grade systemic inflammation, a number of studies have been unable to verify such an association. In fact, in the largest clinical sample reported to date, Guilleminault et al. noted that obesity (i.e., BMI) was the primary correlate of serum CRP levels and that CRP did not relate to SDB severity. Similarly, Minoguchi et al. noted that obesity (i.e., BMI) was the primary correlate of serum CRP levels, the association between obesity and inflammation is ascribed to the fact that adipose tissue produces pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6 that upregulate the hepatic synthesis of acute-phase proteins. In fact, it is estimated that approximately 30% of circulating IL-6 is produced by adipose tissue. There is also evidence to indicate that visceral adipose tissue secretes more TNF-α and IL-6 than subcutaneous adipose tissue. Thus, in assessing whether SDB can elicit low grade systemic inflammation, factors such as body mass, body fat distribution, and medical comorbidity must be thoroughly considered. The exclusion of all medical comorbidity in the current study and the use of multiple obesity measures allowed for a focused examination of serum CRP levels in SDB in a sample across the spectrum of SDB severity. The present study extends previous work by demonstrating that, despite multiple and collinear adjustments for obesity, the AHI and the degree of oxyhemoglobin saturation were positively associated with serum CRP levels. A unique feature of the analyses presented is the identification of an association of serum CRP levels with nocturnal hypoxemia but not sleep stage distribution. Moreover, although it was anticipated that the physiologic stress imposed by intermittent hypoxemia and recurrent arousals due to SDB would acutely perturb systemic inflammation, differences in morning and bedtime CRP levels were not noted—a finding that is consistent with previous work in adolescents. The lack of overnight change in CRP levels could be attributed to the relatively long half-life (~19 hours) of CRP, ineffectiveness of acute but not chronic bouts of physiologic stress to increase CRP levels, or both.

Assuming that the reported associations reflect a causal effect of SDB on systemic inflammation, a relevant question is whether the effect is due to SDB-related hypoxemia and/or sleep fragmentation. At present, the biological mechanisms underlying a potential causal association are not known. Experimental data on the physiologic responses to high altitude suggest that a nonspecific systemic inflammatory response can be induced by hypoxic exposure, usually within 2 days. Moreover, in vitro studies using various cell lines show that low oxygen tension stimulates the

---

**Table 2—Studies on the association between sleep-disordered breathing and serum CRP levels.**

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>N</th>
<th>Study sample</th>
<th>Comparison group</th>
<th>Measures used to adjust for obesity</th>
<th>Prevalent medical conditions</th>
<th>Association between CRP and SDB (i.e., AHI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shamsuzzaman (2002)</td>
<td>42</td>
<td>SDB patients</td>
<td>BMI and age-matched controls</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td>Yokoe (2003)</td>
<td>44</td>
<td>SDB patients</td>
<td>Obese controls</td>
<td>BMI</td>
<td>Included</td>
<td>Yes</td>
</tr>
<tr>
<td>Teramoto (2003)</td>
<td>80</td>
<td>SDB patients</td>
<td>Age and BMI-matched controls</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td>Barcelo (2004)</td>
<td>65</td>
<td>SDB patients</td>
<td>Healthy men</td>
<td>BMI</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Guilleminault (2004)</td>
<td>239</td>
<td>SDB patients</td>
<td>Community subjects</td>
<td>BMI, neck</td>
<td>Hypertension</td>
<td>No</td>
</tr>
<tr>
<td>Saletu (2005)</td>
<td>147</td>
<td>SDB patients</td>
<td>Patients with AHI &lt; 5</td>
<td>BMI</td>
<td>Excluded</td>
<td>No</td>
</tr>
<tr>
<td>Akashiba (2005)</td>
<td>96</td>
<td>SDB patients</td>
<td>Subjects with AHI &lt; 5</td>
<td>BMI</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Kokturk (2005)</td>
<td>151</td>
<td>SDB patients</td>
<td>Subjects with CAD</td>
<td>None</td>
<td>CAD</td>
<td>Yes</td>
</tr>
<tr>
<td>Minoguchi (2005)</td>
<td>52</td>
<td>SDB patients</td>
<td>Obese male subjects</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td>Boudjeltia (2005)</td>
<td>49</td>
<td>SDB patients</td>
<td>---</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td>Can (2006)</td>
<td>92</td>
<td>SDB patients</td>
<td>Subjects with AHI &lt; 1</td>
<td>BMI</td>
<td>Excluded</td>
<td>No</td>
</tr>
<tr>
<td>Hayashi (2006)</td>
<td>60</td>
<td>SDB patients</td>
<td>Male subjects</td>
<td>BMI</td>
<td>Included</td>
<td>Yes</td>
</tr>
<tr>
<td>Kageyama (2006)</td>
<td>30</td>
<td>Patients with CAD/SDB</td>
<td>Healthy subjects</td>
<td>---</td>
<td>Included</td>
<td>Yes</td>
</tr>
<tr>
<td>Current study (2006)</td>
<td>69</td>
<td>SDB patients</td>
<td>Patients with AHI &lt; 6</td>
<td>BMI, waist, % body fat</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pediatric Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tauman (2004)</td>
<td>81</td>
<td>Children referred for SDB</td>
<td>Patients with AHI &lt; 1</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
<tr>
<td>Kaditis (2005)</td>
<td>141</td>
<td>Children referred for SDB</td>
<td>Healthy children</td>
<td>BMI</td>
<td>Excluded</td>
<td>No</td>
</tr>
<tr>
<td>Larkin (2005)</td>
<td>143</td>
<td>Clinic and community-based adolescents</td>
<td>Subjects from a non clinical cohort</td>
<td>BMI</td>
<td>Excluded</td>
<td>Yes</td>
</tr>
</tbody>
</table>
expression and production of IL-6, the principal initiator of the hepatic acute-phase response. It is also plausible that disturbance in sleep may increase CRP level. Experimental work with acute total and short-term partial sleep deprivation shows that sleep loss can elevate CRP concentrations. However, in the present study no such association was observed between serum CRP levels, total sleep time and the distribution of sleep stages.

There are several strengths and limitations of the current study that merit discussion. Limitations include the possibility of uncontrolled or unknown factors (e.g., subclinical cardiovascular disease) that could confound the association between SDB and systemic inflammation. However, the exclusion of prevalent medical conditions and the adjustments for adiposity with multiple covariates limits the degree to which residual confounding could potentially bias the results. Another limitation is the fact that only indirect measures of obesity (i.e., BMI, waist circumference, and measurement of body fat by bioimpedance) were available. It is well established that the total body fat and the amount of visceral fat are important determinants of circulating CRP levels. Studies that incorporate methods which directly measure total body fat (DXA scanning) and visceral fat accumulation (MRI or CT) will be essential to determine whether the observed associations between SDB and CRP persist after accounting for such measures. Though more difficult, measures of fatty deposition within the liver and skeletal muscle also would define further the effect of fat distribution on CRP levels. Another significant limitation is that the study sample, which included only men referred for clinical polysomnography, necessitates great caution in generalizing these findings, particularly to women. Examining the association between SDB and CRP levels in women requires consideration of factors such as the effects of the menstrual cycle and the use of oral contraceptives and hormonal replacement therapy on CRP levels. In normal premenopausal women, there is a 2-fold increase in CRP levels concurrent with the hormonal changes from the follicular to the luteal phase.

As seen in Figure 1, this magnitude of change is greater than observed between any adjacent quartile of AHI. Moreover, the use of oral contraceptives in premenopausal women and hormone replacement therapy in postmenopausal women has also been shown to influence CRP levels. Finally, because of the cross-sectional design of the current study, no definitive statement regarding causality can be made. Unfortunately, studies that have examined CRP levels before and after treatment with continuous positive pressure therapy have yielded conflicting results, thereby limiting inferences regarding cause and effect. Studies following CRP levels prospectively in epidemiological and clinical samples are needed to further elucidate the role of SDB in mediating low-grade systemic inflammation.

There are several public health and clinical implications if SDB is found to be a cause of systemic inflammation. CRP levels that are well below the “upper limit of normal” level of 3 mg/dl levels may portend an increased risk for future cardiovascular events and add prognostic information to traditional cardiovascular risk factors. Since more than 25% of the sample in the current study exceeded the 3mg/dl cut-point, these data would suggest that a state of low-grade inflammation is present in SDB and may act as an intermediary in the causal pathway to cardiovascular disease.

ACKNOWLEDGMENTS

Supported by National Institutes of Health Grants HL7578 and AG025553.

REFERENCES

8. Taramoto S, Yamamoto H, Ouchi Y. Increased C-reactive protein and increased plasma interleukin-6 may synergistically affect the progression of coronary atherosclerosis in obstructive sleep apnea syndrome. Circulation 2003; 107(5):E40.