Cardiovascular Risk Markers in Obstructive Sleep Apnoea Syndrome and Correlation with Obesity.

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Running title: CRP and Homocysteine in OSAS.
Abstract

Background: Elevated C-reactive protein (CRP) and homocysteine levels are risk factors for cardiovascular disease. Some, but not other, previous studies have reported increased levels of CRP and homocysteine in patients with obstructive sleep apnoea syndrome (OSAS). We investigated levels of these factors in carefully selected OSAS patient and matched normal control cohorts.

Methods: We measured CRP and homocysteine levels in 110 subjects following polysomnography (PSG). Non-OSAS patients [G1] were compared to two patient groups (mild/moderate [G2] and severe OSAS [G3]), group-matched for body mass index (BMI), and a fourth group of severe OSAS patients who were more obese [G4]. All were free of other disease and similar in age, smoking habits and cholesterol levels. 50 suitable patients were commenced on continuous positive airway pressure therapy (CPAP) after PSG and 49 reassessed 6 weeks later.

Results: CRP levels were similar in all 3 BMI-matched groups (median[IQR] 1.11[0.76,2.11]mg/l [G1] vs 1.82[1.20,3.71] [G2] vs 2.20[1.16,3.59] [G3]; p=0.727 by Kruskal-Wallis testing) but significantly higher in G4 than in all other groups (5.36[2.42,9.17] mg/l, p<0.05 by individual group comparisons). In multivariate analysis of all subjects, BMI was an independent predictor for CRP levels (Beta=0.221; p=0.006), whereas AHI and other measures of OSAS were not. There was no difference in homocysteine levels between all four groups (p=0.101). Furthermore, CPAP therapy did not alter CRP (2.29[1.32,4.10] vs 2.84[1.13,5.40] mg/l; p=0.145) or homocysteine levels (8.49±3.66 vs 9.90±4.72µmol/l; p=0.381).

Conclusion: CRP and homocysteine levels were not associated with OSAS severity in men but CRP is independently associated with obesity.

Key Words: obstructive sleep apnoea syndrome, C - reactive protein, homocysteine, cardiovascular disease, obesity
Introduction

Obstructive Sleep Apnoea Syndrome (OSAS) is a highly prevalent disorder affecting about 4% of adults (1) and is associated with repetitive episodes of transient oxygen desaturation during sleep. The predominant physical morbidity of the disorder is cardiovascular and OSAS is an independent risk factor for a number of cardiovascular diseases, particularly systemic arterial hypertension (2, 3) but also coronary artery disease, congestive cardiac failure and cerebrovascular events (4). Therapy with nasal continuous positive airway pressure (nCPAP) decreases cardiovascular morbidity and mortality (5, 6). However, the mechanisms underlying cardiovascular complications in OSAS remain unclear.

In recent years, the identification of novel cardiovascular risk factors has significantly enhanced our understanding of the development of cardiovascular diseases in general and greatly improved risk stratification. These include factors of haemostasis and thrombosis, such as homocysteine, fibrinogen, lipoprotein-a and inflammatory markers such as selectins P and E, cellular adhesion molecules, tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and C-reactive protein (CRP) (7). Among them, markers like homocysteine and CRP have attracted specific attention as assays to measure their levels are widely available in general hospitals rather than specific research laboratories. In particular, CRP, an acute-phase reactant, has been closely linked to cardiovascular diseases. It is the prototypic marker of inflammation and numerous prospective studies have identified that an elevated level of CRP is a strong predictor of future cardiovascular risk (8-10). On the other hand, mildly elevated levels of the amino acid homocysteine in the plasma have been shown to correlate with the incidence of premature coronary artery disease, peripheral vascular disease and stroke (11, 12).

Whether or not CRP and homocysteine levels are elevated in OSAS patients is still under debate. Some reports have identified increased levels of CRP in OSAS patients (13-18) whereby others did not (19, 20). Also unclear is the impact of CPAP therapy on CRP levels with one study reporting a favourable outcome (14) and others who did not find a significant change in levels with effective CPAP therapy (19, 21). A major pitfall of evaluating CRP levels is the fact that this marker is directly associated with obesity (22, 23) and many previous reports have been influenced by small numbers, inadequately matched populations, particularly for body mass index (BMI) and inclusion of patients with established cardiovascular or metabolic diseases. Similarly, the role of plasma homocysteine levels in OSAS is unclear with some studies reporting higher levels only in OSAS patients suffering from pre-existing cardiac disease (24, 25) and other reports identifying homocysteine levels to be independently associated with OSAS (18, 26). Only one study of 12 OSAS patients has evaluated the effect of CPAP therapy after a variable time point and found a reduction in homocysteine levels (27). However, many of the reports on homocysteine in OSAS may have been influenced by similar methodological limitations as mentioned above.

We performed a prospective controlled study in tightly selected groups of OSAS patients and control subjects to evaluate the relationship of OSAS with CRP and homocysteine levels, with a specific focus on the impact of obesity as a possible confounder.

Methods
Subjects

Consecutive males with suspected OSAS based on symptoms such as heavy and loud snoring, witnessed apnoeas and/or daytime sleepiness and no other medical disorder were considered for the study, which was approved by the St. Vincent’s University Hospital Ethics Committee. Also recruited from the general population outside the hospital environment were normal males who were group-matched in age and BMI to the patient cohort. Based on our general sleep clinic population, and the requirement to be free of all co-morbidity, we predicted that we needed healthy controls between 30 and 40 years and a BMI between 28 and 34 kg/m2. Over the course of the study, demographics of the patient population were carefully monitored to allow us to make slight adjustments in the criteria for the control population. These criteria were set purely on subject’s demographic variables and analysis of data was made independently and in a blinded fashion.

All subjects gave written, informed consent and no subject fitting the inclusion criteria refused participation. Each subject underwent clinical assessment, testing for full blood count, liver and kidney function, cardiac enzymes including troponin T and lipid profile. Furthermore, determination of vitamin B12 and folate were done in all subjects to ensure that low levels of these vitamins did not represent potential secondary causes for higher homocysteine concentrations. Each subject also completed the Epworth Sleepiness Scale (ESS) and supine blood pressure was measured while awake at least three times during the daytime. Overnight polysomnography (PSG) was performed as previously described (28). Apnoeas were defined as a complete cessation of airflow for at least 10 seconds and hypopnoeas as a reduction of respiratory signals for at least 10 seconds associated with oxygen desaturation of >4% and/or arousal. Sleep studies were performed in the sleep laboratory and supervised throughout by an experienced sleep technician.

CPAP Treatment

Fifty suitable subjects with moderate or severe OSAS and who had agreed to possible CPAP therapy prior to diagnostic sleep study initiated nasal CPAP therapy within one week after PSG. One patient dropped out due to intolerance of CPAP. The remaining 49 patients underwent repeat of their sleep study after 6 weeks of therapy. All were evaluated for symptoms and side effects, and objective compliance data were downloaded from the devices.

CRP and homocysteine measurements

Venous blood samples were obtained from all subjects while fasting at the same time (7 am) following initial PSG and again from patients commenced on CPAP after 6 weeks therapy and serum or plasma was stored at -80°C until further analysis. Serum for CRP levels was measured utilizing nephelometric technique which measures the light scattered onto complexes of samples containing CRP and monoclonal antibodies to CRP.

For homocysteine determinations, blood samples were collected into EDTA-containing specimen tubes and immediately placed on ice. Concentrations were determined by fluorescence polarisation immunoassay (FPIA) on an Abbott IMx analyser.
Statistical analysis

The expected difference in CRP and homocysteine levels between groups which might be clinically important and the pooled standard deviation were specified on the basis of the previous published studies. The required sample size to detect a difference of 3.2 mg/l in CRP and 7.5 µmol/l in homocysteine between groups of different AHI (which is the comparison of interest) with 90% power at the 5% significance level was 27 subjects in each group.

Subject baseline characteristics and CRP and homocysteine findings are expressed as mean ± standard deviation or median [interquartile range] depending on their distribution and compared using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test with post-hoc pairwise comparison to assess for differences between groups for independent samples and the paired t-test or Wilcoxon Signed Rank for paired samples. Categorical variables were compared using the χ² test. In addition, levels of cardiovascular risk factors among the groups were evaluated by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. To assess the correlation between CRP or homocysteine, respectively, with baseline and PSG variables, we employed the Pearson’s or Spearman’s correlation analysis. To identify potential independent predictors of CRP and homocysteine levels, we used a stepwise backward linear regression model with CRP or homocysteine as the dependent variables and age, BMI, smoking status, total cholesterol, triglyceride, LDL-Cholesterol, HDL-Cholesterol, ESS, apnoea/hypopnoea index (AHI), desaturation index (DI), basal oxygen saturation (SaO₂), min SaO₂ and percent total sleep time (TST) <90% as independent factors with group selection as covariates. To rule out a possible confounding effect of the non-BMI-matched group on the results of the CRP predictors, a separate General Linear Model to assess for Analysis of Covariance (ANCOVA) was employed with the different groups as factors and BMI as covariate. Statistical analysis was performed using a commercial software package (SPSS Version 11.0, Chicago, IL).

Results

Subjects

Baseline characteristics of the study population are described in table 1. Subjects were classified into three groups according to their apnoea/hypopnoea frequency as non-OSAS [G1] (AHI ≤ 5), mild to moderate OSAS [G2] (AHI >5, ≤ 30) or severe OSAS (AHI>30) [G3]. The non-OSAS group consisted of 22 normal control subjects, recruited from the general population, who were not complaining of habitual snoring or excessive daytime sleepiness (EDS) (ESS 5 ± 2) and 8 individuals describing clinical features of OSAS including significant EDS (ESS 16 ± 3) and snoring but who demonstrated no objective findings of sleep-disordered breathing on PSG studies. Also included was a fourth group of patients with severe OSAS (AHI>30) but who were significantly more obese [G4]. The 4 groups were similar with regards to age, smoking status, total cholesterol, LDL-cholesterol, HDL-cholesterol, folate and vitamin B12 levels. Non-OSAS subjects had significantly lower triglyceride level than all other groups. The BMI and neck size in the very obese, severe OSAS group were significantly higher than in all other groups but there was no difference in BMI in the remaining three groups. No subject had clinical evidence of any other medical disorder and detailed biochemical
profile, including liver and renal function, cardiac enzymes and resting electrocardiogram were within normal limits in all subjects. No individual had elevated troponin T levels.

Table 1. Baseline characteristics of study population. Values represent mean±standard deviation or median [interquartile range] depending on the distribution (* = body mass index; † = blood pressure; ‡ = Epworth Sleepiness Scale; § = apnea/hypopnea index; ll = desaturation index; ** = oxygen saturation; †† = per cent of total sleep time < 90%; ^ = p-value<0.05 vs. non-OSAS, # = p-value<0.05 vs. mild to moderate OSAS, ¶ = p-value<0.05 vs. severe, BMI-matched OSAS)

<table>
<thead>
<tr>
<th></th>
<th>Non-OSAS</th>
<th>Mild to moderate OSAS</th>
<th>Severe, BMI-matched OSAS</th>
<th>Severe, obese OSAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>30</td>
<td>35</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Age</td>
<td>41±6</td>
<td>42±8</td>
<td>43±9</td>
<td>39±9</td>
</tr>
<tr>
<td>BMI† (kg/m²)</td>
<td>30.7±3.1</td>
<td>32.9±6.03</td>
<td>32.1±3.5</td>
<td>42.5±4.8^#¶</td>
</tr>
<tr>
<td>Neck size (cm)</td>
<td>43.1±1.8</td>
<td>44.6±2.9</td>
<td>44.1±2.7</td>
<td>48.1±3.0^# ¶</td>
</tr>
<tr>
<td>Current smokers</td>
<td>9 (30%)</td>
<td>13 (37%)</td>
<td>12 (39%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.3±0.8</td>
<td>5.2±0.8</td>
<td>5.3±0.8</td>
<td>5.2±1.0</td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>1.02±0.25</td>
<td>1.03±0.21</td>
<td>1.00±0.18</td>
<td>0.92±0.18</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L)</td>
<td>3.57±0.72</td>
<td>3.29±0.67</td>
<td>3.47±0.73</td>
<td>3.34±0.91</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.45±0.74</td>
<td>1.96±1.1^</td>
<td>1.91±0.9^</td>
<td>2.00±0.87^</td>
</tr>
<tr>
<td>BP † (mmHg)</td>
<td>131/82±18/9</td>
<td>128/81±13/7</td>
<td>135/86±18/12</td>
<td>139/84±15/9</td>
</tr>
<tr>
<td>Folate (µg/l)</td>
<td>7.9[6.3,11.8]</td>
<td>7.5[5.9,12.2]</td>
<td>8.3[5.8,10.3]</td>
<td>7.6[4.3,13.4]</td>
</tr>
<tr>
<td>ESS ‡</td>
<td>6[5,14]</td>
<td>12[6,16]</td>
<td>16[12,19]^#</td>
<td>14[9,18]^</td>
</tr>
<tr>
<td>AHI §</td>
<td>1.1[0.4,1.9]</td>
<td>14.0[9.0;21.9]^</td>
<td>50.9[37.1,73.2]^#¶</td>
<td>74.1[67.3,98.3]^#¶</td>
</tr>
<tr>
<td>DI ll</td>
<td>1.5[0.0,3.0]</td>
<td>13[8,22]^</td>
<td>49[34,71]^#</td>
<td>69[55,80]^#¶</td>
</tr>
<tr>
<td>Basal SaO2 **</td>
<td>93.8[92.8,95.5]</td>
<td>93.8[92.8,94.4]</td>
<td>92.6[90.5,94.7]^</td>
<td>89.8[86.7,92.7]^#¶</td>
</tr>
<tr>
<td>Min SaO2</td>
<td>88.9[87.2,90.8]</td>
<td>84.7[81.4,86.9]^</td>
<td>77.5[67.7,81.5]^#</td>
<td>66.9[57.5,77.8]^#¶</td>
</tr>
<tr>
<td>%TST&lt;90% ††</td>
<td>0[0,0]</td>
<td>1[0,6]^</td>
<td>15[4,45]^#</td>
<td>31[12,80]^#</td>
</tr>
</tbody>
</table>

No subject was taking any regular medication. AHI and oxymetry data as well as ESS were significantly different between the groups. All subjects except one individual in the
very obese group demonstrated daytime awake oxygen saturation >92%. 50 suitable patients with moderate or severe OSAS and who had agreed to possible CPAP therapy prior to diagnostic sleep study initiated nasal CPAP therapy within one week after PSG. They were 40±8 years of age and their BMI was 34±6 kg/m². One patient dropped out due to intolerance of CPAP. Following 6 weeks of CPAP therapy in the 49 CPAP-treated OSAS patients, AHI fell from 37[14;73] to 7[4;11] (p<0.001 compared to pre-CPAP levels), desaturation index fell from 38[15,67] to 5[2;8] and all showed SaO₂ levels above 90% during sleep (% total sleep time < 90% 0%[0,0]; min SaO₂ 93%[91,94]). ESS improved from 15±5 to 7±5 (p<0.001) and daytime blood pressure from 134/84±17/11 to 127/80±15/10 (p=0.01). Objective recordings from CPAP machines revealed a nightly compliance of 4.6 ± 1.3 hours (mean ± SD).

**CRP levels**

There were no significant differences between CRP levels in the non-OSAS group G1 (1.11[0.76,2.11] mg/l), mild to moderate OSAS G2 (1.82[1.20,3.71]) and the severe OSAS/BMI-matched group G3 (2.20[1.16,3.59]) (Kruskal-Wallis: p=0.727). Levels were higher in the severe OSAS/obese group (5.36[2.42,9.17] mg/l) than in all other groups (p<0.001 vs G1, p=0.006 vs G2, p=0.005 vs G3) (figure 1).

As severe OSAS, higher BMI patients had significantly worse PSG variables as the severe OSAS, BMI-matched group, we adjusted both groups in AHI and oxymetry variables by excluding 9 subjects in the BMI matched group who had an AHI between 30 and 40 per hour. These two groups were now similar in demographic but also PSG parameters. BMI was significantly different (33.7±2.5 vs. 42.5±4.8; p<0.001) and CRP levels remained higher in the very obese group (3.01±1.71 vs 5.66±3.89 mg/l, p=0.027).

Spearman’s correlation analysis showed that CRP levels correlated positively with BMI (r=0.484, p<0.001), neck size (0.413, p<0.001), ESS (r=0.295, p=0.002), total Cholesterol (r=0.194, p=0.048) AHI (r=0.423, p<0.001), DI (r=0.456, p<0.001), and %TST <90% (r=0.421, p<0.001) and negatively with the basal SaO₂ (r=-0.312, p<0.001) and minimal SaO₂ (r=-0.414, p=0.001). CRP level did not show any significant correlation with age, smoking status, HDL-Cholesterol, LDL-Cholesterol and triglyceride level.

Stepwise backward linear regression analysis including all subjects identified the BMI as the strongest and the total cholesterol as a further independent predictor of CRP level (table 2).

### Table 2. Stepwise multiple regression analysis of the relationship between CRP levels and various independent variables. B represents parameter estimates and CI the 95% confidence interval of the parameter estimates. Groups are included as covariates († = body mass index; € Epworth Sleepiness Scale; § = apnoea/hypopnoea index; ||= per cent of total sleep time < 90%; **) = desaturation index)

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>95% CI (Lower Bound)</th>
<th>95% CI (Upper Bound)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI †</td>
<td>0.221</td>
<td>0.065</td>
<td>0.376</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
To assess the potential impact of neck size rather than general obesity we subsidized the BMI with neck size in the multivariate analysis. There was a strong correlation between these two variables ($r=0.781, p<0.001$). In this second multivariate analysis, neck size was the strongest (Beta: 0.405, $p=0.003$) and cholesterol a further independent predictor (Beta: 0.235, $p=0.020$) similar to the model described in table 2.

As group 4 represented patients with significantly higher BMI, which could have potentially influenced the results, we performed an ANCOVA to assess for a possible confounding effect. As shown in table 3, the different groups did not show a significant relationship with CRP levels.

**Table 3. Analysis of Covariance to assess the effect of the different groups and BMI on CRP. Presented are the parameter estimates with the 95% confidence interval (CI) and the significance values.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>95% CI (Lower Bound)</th>
<th>95% CI (Upper Bound)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.290</td>
<td>0.145</td>
<td>0.436</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 1</td>
<td>-0.0197</td>
<td>-2.751</td>
<td>2.711</td>
<td>0.989</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.168</td>
<td>-2.414</td>
<td>2.751</td>
<td>0.897</td>
</tr>
</tbody>
</table>
Homocysteine levels

Homocysteine levels in all patient and control groups were within the normal laboratory reference range of 5-15 µmol/l. No significant difference in homocysteine levels were detected between non-OSAS (8.30±2.12 µmol/l), mild-moderate OSAS (7.16±1.58 µmol/l), BMI-matched, severe OSAS (8.40±2.98 µmol/l) and obese, severe OSAS subjects (9.16±4.87 µmol/l) (ANOVA: p=0.101).

Pearson’s correlation analysis only identified a significant relationship between homocysteine levels and HDL-cholesterol (r=-0.212, p=0.028) which remained independently associated in stepwise multiple linear regression analysis (table 4). There was no correlation between homocysteine and age, smoking status, BMI, total cholesterol, triglyceride, LDL-cholesterol, ESS and all PSG variables.

Table 4. Stepwise multiple regression analysis of the relationship between homocysteine levels and various independent variables. B represents the parameter estimates and CI the 95% confidence interval of the parameter estimates. Groups are included as covariates († = body mass index; ‡ = Epworth Sleepiness Scale; § = apnoea/hypopnoea index; ll= per cent of total sleep time < 90%; ** = desaturation index)

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>95% CI (Lower Bound)</th>
<th>95% CI (Upper Bound)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-Cholesterol</td>
<td>-2.756</td>
<td>-5.382</td>
<td>-0.130</td>
<td>0.040</td>
</tr>
<tr>
<td>Age</td>
<td>0.016</td>
<td>-0.057</td>
<td>0.089</td>
<td>0.590</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>-0.057</td>
<td>-0.697</td>
<td>0.582</td>
<td>0.500</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.374</td>
<td>-0.197</td>
<td>0.944</td>
<td>0.562</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>-0.129</td>
<td>-0.863</td>
<td>0.605</td>
<td>0.629</td>
</tr>
<tr>
<td>BMI †</td>
<td>-0.058</td>
<td>-0.174</td>
<td>0.058</td>
<td>0.642</td>
</tr>
<tr>
<td>ESS ‡</td>
<td>0.041</td>
<td>-0.606</td>
<td>0.143</td>
<td>0.469</td>
</tr>
<tr>
<td>AHI §</td>
<td>-0.028</td>
<td>-0.059</td>
<td>0.003</td>
<td>0.775</td>
</tr>
<tr>
<td>%TST&lt;90% ll</td>
<td>-0.630</td>
<td>-3.395</td>
<td>2.134</td>
<td>0.792</td>
</tr>
<tr>
<td>Basal SaO₂</td>
<td>0.103</td>
<td>-0.092</td>
<td>0.297</td>
<td>0.736</td>
</tr>
<tr>
<td>Min SaO₂</td>
<td>0.078</td>
<td>0.007</td>
<td>0.150</td>
<td>0.557</td>
</tr>
</tbody>
</table>
Effect of CPAP therapy on CRP and homocysteine levels

Patients with OSAS, who were commenced on nasal CPAP therapy after their diagnostic PSG, were re-evaluated 6 weeks later. There was no change in BMI, no other diseases were diagnosed or medications introduced. Treatment with nasal CPAP did not alter CRP (2.29[1.32,4.10] vs 2.84[1.13,5.40] mg/l; p=0.145) or homocysteine (8.49±3.66 µmol/l vs 9.90±4.72 µmol/l; p=0.381) levels.

Discussion

In the present study we identify an independent relationship between levels of C-reactive protein with the body mass index but not with the severity of sleep disordered breathing in male subjects who were free of cardiovascular disease. Homocysteine levels were neither determined by OSAS severity or demographic variables. Effective CPAP therapy of 6 weeks duration had no influence on either CRP or homocysteine levels.

OSAS is associated with the development of cardiovascular diseases, particularly systemic arterial hypertension (2, 3) but also coronary artery disease, congestive cardiac failure and stroke (4). CPAP therapy significantly reduces cardiovascular morbidity and mortality (5, 6). The pathophysiology underlying cardiovascular complications in OSAS remains unclear. The pathogenesis is most likely multifactorial and potential mechanisms include sympathetic excitation, inflammation, vascular endothelial dysfunction and metabolic dysregulation. CRP levels are widely recognised as potent, independent predictors of future cardiovascular events among apparently healthy subjects (8) as well as in subjects with known cardiovascular disease (9, 10). Moreover, recent data suggest a direct active role in the pathogenesis of atherosclerosis (29). CRP is mainly produced in the liver in response to interleukin-6 (IL-6). Adipose tissue is a potent source of IL-6 production which leads to increased CRP levels in obese subjects (22, 23).

The question of CRP levels being elevated in OSAS patients is still uncertain. After the index report in 2002 on a pilot trial suggesting that CRP level might be related to OSAS severity (13), various other studies have addressed this issue with different conclusions (14-20). These different findings may have been contributed to by a number of methodological factors such as small subject numbers, inadequately matched study populations, particularly in terms of BMI, and inclusion of patients with pre-existing cardiovascular or metabolic diseases. Yokoe et al. observed in a cohort including 30 OSAS patients and 14 controls elevated CRP levels in the patient group, however OSAS patients had significantly higher BMI and the populations also included subjects with hypertension, coronary artery disease, stroke and diabetes mellitus (14). Further studies suggested an association of CRP with OSAS but with similar methodological limitations (15-17). However, recent studies have suggested that obesity rather than OSAS per se is the best predictor of CRP (19, 20). A recent study by Can et al. found higher CRP levels than matched controls, but only patients with mild-moderate disease were included and no direct correlation to PSG parameters were observed (18). Furthermore, the impact of CPAP therapy on CRP levels is still unclear. Whereby Yokoe et al. found an
improvement in levels after one month of CPAP therapy (14), two subsequent studies failed to confirm these results (19, 21). In particular, Akashiba and co-workers followed 96 OSAS patients on CPAP therapy for 9 month and CRP did not change significantly (21).

The present study has a number of strengths in comparison to some previous reports. First, we took great care in patient selection to exclude any with a cardiovascular or other medical disorder and none were taking any regular medication. Groups were matched in all demographic parameters and to study specifically the impact of BMI on CRP levels we included a further group of patients with severe OSAS but significantly higher BMI. This group had higher CRP levels than all other groups with or without OSAS, even after adjustment for AHI. Furthermore, 6 weeks of effective CPAP therapy had no effect on CRP levels and no PSG variable showed a significant independent relation to CRP. BMI was an independent predictor of CRP levels in multivariate analysis and even after separate analysis allowing for the potential confounding effect by selecting a non-BMI-matched group this relationship was still present. Thus, we conclude from our study that CRP levels are not elevated in OSAS patients independent of obesity.

These findings underline the complexity of the process leading to cardiovascular complications in OSAS. There is growing evidence that inflammation plays a role in this process and, as one possible explanation, we have identified a selective activation of inflammatory over adaptive pathways by intermittent hypoxia in a cell culture model (28). As a consequence, we among others, found elevated circulating levels of tumour necrosis factor alpha (TNF-α) in OSAS patients which fall with short-term CPAP therapy (30-32). Other inflammatory factors have been found to be elevated in OSAS including interleukin-8 (IL-8) and intercellular adhesion molecule-1 (ICAM-1) (33). Differences in the relation of inflammatory markers to obesity might be explained by findings from studies suggesting that adipose tissue is a potent source of IL-6, the precursor of CRP, but less so of TNF-α (34). Various studies have shown a direct relationship between BMI and CRP levels (22, 23) and these interactions seem to dominate this stage of inflammation whereby OSAS itself might potentially influence the production of other inflammatory cytokines particularly TNF-α.

A further finding of the present study is that patients with OSAS had similar homocysteine levels as control subjects and multivariate analysis revealed no association of these levels with OSAS severity. Homocysteine is an intermediate amino acid formed during the metabolism of methionine. The proposed mechanisms of development of atherosclerosis in subjects with higher homocysteine levels are oxidative stress and depletion of nitric oxide leading to endothelial dysfunction (35). Prospective studies have shown a correlation of mildly elevated homocysteine concentrations with premature coronary artery disease, peripheral vascular disease and stroke (11, 12). Previous studies assessing homocysteine levels in OSAS patients have reported different findings (18, 24-26) but similar to previous studies of CRP levels in OSAS, these reports may have been influenced by small numbers and potential limitations in study design. Lavie et al. have conducted a large study and only detected elevated homocysteine levels in OSAS patients with co-existing cardiovascular disease (24). Only one report so far has addressed the impact of CPAP therapy on homocysteine levels (27). However, in this study only 12 subjects were included, patients were suffering from various cardiovascular diseases and the time of re-evaluation varied.
One potential limitation of our study is the inclusion only of men. We designed the study specifically in this way to avoid gender differences which could influence the analysis. However, as a consequence, our data cannot automatically extrapolate to female patients with OSAS. Furthermore, the non-OSAS group of 30 subjects included 8 subjects with suspected OSAS but who failed to demonstrate objective abnormalities on their PSG. None of these subjects described symptoms of narcolepsy, periodic limb movements or any other sleep disorder associated with excessive daytime sleepiness so that upper airway resistance syndrome (UARS) may represent the underlying diagnosis. This group is therefore strictly not a normal control population. However, we do not believe this to have influenced the results as CRP and homocysteine levels did not differ between true controls and sleepy non-OSAS patients and also between sleepy non-OSAS and BMI-matched, OSAS patients (data not shown).

In conclusion, the present data provide evidence that OSAS is neither related to CRP nor homocysteine levels. However, CRP levels are strongly linked to obesity. Other cardiovascular risk markers such as TNF-$\alpha$ might therefore be better targets in predicting cardiovascular risk in subjects with OSAS.

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Legend to Figure.
Serum level of high-sensitivity C-reactive protein in subjects without OSAS, mild to moderate OSAS, severe OSAS/BMI-matched and severe OSAS/obese. Boxes represent values within the interquartile range and whiskers the data range, lines across the boxes represent the median values. Kruskal-Wallis represents the comparison of non-OSAS, mild-moderate OSAS and severe BMI-matched groups (Groups 1-3); ** represents the statistically significant difference between the more obese severe OSAS group (Group 4) when compared with Groups 1-3 by individual group comparison (p<0.05 for all comparisons).
References


CRP in mg/l

p=0.727 (Kruskal-Wallis)