Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials

G V Robinson, J C T Pepperell, H C Segal, R J O Davies, J R Stradling

Background: Obstructive sleep apnoea (OSA) is associated with high cardiovascular morbidity and mortality and is an independent risk factor for hypertension. Novel circulating cardiovascular risk markers enabling a more accurate prediction of cardiovascular risk have been identified. Examination of these markers may clarify the increased risk in OSA and contribute to an analysis of the benefits of treatment.

Methods: Plasma levels of total cholesterol and triglyceride and activated coagulation factors XIIa and VIIa, factors VII, VIII, XII, fibrinogen, thrombin-antithrombin (TAT), von Willebrand factor antigen (vWFAg), soluble P-selectin (sP-sel), and homocysteine were measured before and after treatment for 1 month with therapeutic or subtherapeutic (control) continuous positive airways pressure (CPAP) in 220 patients with OSA.

Results: Levels of activated coagulation factors XIIa, VIIa, TAT and sP-sel were higher in OSA patients at baseline than in unmatched controls, but did not fall with 1 month of therapeutic CPAP treatment. The raised sP-sel levels correlated only with body mass index (p = 0.002). There was a trend towards a significant fall in total cholesterol with therapeutic CPAP (p = 0.06) compared with the control group. In the therapeutic group there was a clinically significant mean fall in total cholesterol of 0.28 mmol/l (95% confidence interval 0.11 to 0.45, p = 0.001) which may reduce cardiovascular risk by about 15%.

Conclusion: A number of activated coagulation factors are increased in untreated OSA patients, potentially contributing to vascular risk, but they do not fall with 1 month of CPAP treatment. Nasal CPAP may produce a clinically relevant fall in total cholesterol level, potentially reducing cardiovascular risk, but this needs to be verified in a larger prospective study.
controlled trials. The unit is a regional referral centre which takes patients referred from the surrounding area. Referrals come from general practitioners (36%), ear, nose and throat surgeons (41%), and other hospital consultants (23%).

Patients
The details of these two trials are described elsewhere. In brief, patients were eligible if they were male, aged 30–75 years, with excessive daytime sleepiness (Epworth Sleepiness Score (ESS) >9) and proven OSA on overnight respiratory polysomnography, defined as more than 10 dips of >4% oxygen desaturation per hour due to OSA. Patients were excluded if they required urgent CPAP treatment because of associated respiratory failure or to prevent job loss through excessive daytime sleepiness, declined to participate, or were unable to give informed consent. Previous cardiovascular events or the presence of any cardiovascular risk factor were not determinants in offering or declining a patient entry to the trials. The studies were approved by the Central Oxford Research Ethics Committee and all participants gave written informed consent.

Procedures
OSA was diagnosed from a one night respiratory polsomnographic study. Patients’ body movements, heart rate, and transient falls in pulse transit time were recorded as measures of arousal from sleep. These indices are accurate in diagnosing and quantifying the severity of OSA.

The severity of sleep apnoea was then quantified numerically as the number of dips in oxygen saturation of >4% for every hour of the study. This index is one of the best predictors of response to nasal CPAP, correlates well with conventional apnoea-hypopnea index measurements, and is the most consistent index between repeat studies of patients with OSA. Patients assessed their subjective daytime sleepiness using the ESS. Objective sleepiness was quantified with the Osler test. Triglyceride and cholesterol were measured using automated routine methods (Abbott Aeroset Analyser, Maidenhead, UK) and homocysteine was measured by fluoroscopic labelling (Drew Scientific, Barrow in Furness, UK). Plasma coagulation levels of fibrinogen, factor VII, factor VIII, and factor XII were measured by automated routine methods using an MDA 40 (Organon Technica). Activated coagulation factor VII (VIIla) was assayed by recombinant soluble tissue factor assay (STACLOT, Diagnostica, France) and activated factor XII (XIIa) was measured by ELISA (Shields Diagnostics, Dundee, UK). Von Willebrand factor antigen (vWFAg) was measured using an in-house ELISA. Soluble P-selectin (sP-sel) was measured by human SP-Selectin Immuonassay ELISA using recombinant human SP-selectin antibody (R&D Systems, Abingdon, UK). Thrombin-antithrombin complexes (TAT) were measured by enzyme immunoassay (Dade Behring, Marburg, Germany). Inter-assay and intra-assay variation for all the techniques used was <10%.

All blood samples were taken in the non-fasting state. Subjects were not given specific information about exercise or diet during the study period. All the cardiovascular markers were measured again 1 month after randomisation. After the baseline assessments, patients were admitted for a further night and, after education, were randomised to receive therapeutic or subtherapeutic nasal CPAP (as described elsewhere). Patients assigned to therapeutic CPAP received a standard overnight titration. Patients assigned to receive subtherapeutic (placebo) CPAP used a specially adapted machine, designed to deliver less than 1 cm H2O pressure (but above zero), which is insufficient to hold open the pharynx. In all other respects the subtherapeutic CPAP machine is identical to the therapeutic CPAP machine and neither improves nor worsens the OSA.

Follow up
Patients were discharged home following the night of CPAP titration. A specialist nurse team assisted all patients with telephone advice and further mask readjustment if necessary over the following month. At 4 weeks, patients retitrated for repeat blood tests, ESS, and Osler test. Hour meters on the CPAP machines were read to assess mean nightly use during the trial. At the end of the study period all patients had their CPAP retitrated to establish their subsequent long term therapeutic airway pressure.

Sample handling
Blood samples were taken between 11.00 and 13.00 hours into 0.105 M sodium citrate and centrifuged at 2000g at room temperature for 20 minutes. Assays were performed as soon as possible.

A smaller group of normal control subjects established the normal ranges for VIIa, sP-sel, TAT, and XIIa. These normal healthy subjects had a mean (SD) age of 35.3 (8.8) years and were on no regular medication including aspirin. All the controls had a BMI within the normal range and were free from co-morbidity.

Data analysis
Pre-treatment levels were compared with those of the normal controls (unpaired t tests) and Pearson’s correlation was used to explore the relationship with baseline OSA severity in patients. Changes between the two patient groups were compared with unpaired t tests.

RESULTS
Two hundred and twenty patients entered the studies. The baseline characteristics of the two groups were well matched (table 1). There was a higher nightly compliance with CPAP in the group assigned therapeutic CPAP (mean (SD) 5.0 (1.9) hours per night v 4.1 (2.4) hours per night; difference 0.9 hours, 95% confidence interval (CI) 0.33 to 1.47, p<0.005).

Of the subjects treated with therapeutic CPAP, one had ischaemic heart disease, 12 were hypertensive and one had diabetes. Of the subjects treated with subtherapeutic CPAP, three had ischaemic heart disease, 11 were hypertensive, and one had diabetes. None were being treated with statins. Activated coagulation factors were measured in subsets of the 220 subjects because of variable assay availability. Fibrinogen, factor VII, factor VIII, factor XII, TAT, and activated factor XII (FXIIa) were measured in 46 patients, activated factor VII (FXIIa) in 44, homocysteine and triglyceride in 101, vWFAg in 90, and sP-sel in 93. Total cholesterol was measured in 213 patients.

<table>
<thead>
<tr>
<th>Table 1 Baseline patient characteristics before CPAP treatment</th>
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<tbody>
<tr>
<td>Subtherapeutic CPAP (n = 112)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Oxygen saturation dips &gt;4% (per hour of sleep)</td>
</tr>
<tr>
<td>CPAP compliance score</td>
</tr>
<tr>
<td>Baseline ESS</td>
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</tbody>
</table>

Values are mean (SD). CPAP, continuous positive airway pressure; ESS, Epworth Sleepiness Score. *p<0.005.
Comparison with normal controls

Plasma levels of fibrinogen, factors VII, VIII, and XII, vWF Ag, homocysteine, total cholesterol, and triglyceride were the same as in the normal control subjects or within laboratory normal ranges at baseline (table 2). Plasma levels of activated factors XIIa and VIIa, s-Psel, and TAT were higher at baseline than in the unmatched controls (table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Baseline blood levels before CPAP treatment</th>
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<tbody>
<tr>
<td><strong>Cardiovascular marker</strong></td>
<td><strong>Pre subtherapeutic CPAP (n = 112)</strong></td>
</tr>
<tr>
<td>FXIa (ng/ml)†</td>
<td>2.0 (1.2), n = 19</td>
</tr>
<tr>
<td>vWF Ag (IU/dl)</td>
<td>111.2 (40.8), n = 18</td>
</tr>
<tr>
<td>s-Psel (ng/ml)</td>
<td>53.0 (32.3), n = 46</td>
</tr>
<tr>
<td>TAT (μg/l)†</td>
<td>12.3 (24.1), n = 20</td>
</tr>
<tr>
<td>vWF Ag (IU/dl)</td>
<td>120.8 (50.5), n = 46</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.6 (0.6), n = 20</td>
</tr>
<tr>
<td>FXII (IU/dl)</td>
<td>111.0 (50.5), n = 46</td>
</tr>
<tr>
<td>FXII (IU/dl)</td>
<td>124.1 (59.3), n = 20</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>9.7 (13.6), n = 49</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.7 (1.1), n = 107</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.6 (1.9)*, n = 49</td>
</tr>
</tbody>
</table>

Values are mean (SD) with 95% confidence interval (CI).
†No laboratory reference range as new test.
‡Compared with all OSA patients before randomisation.
*Non-fasting levels.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Blood levels after therapeutic and subtherapeutic CPAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change after therapeutic CPAP</strong></td>
<td><strong>Change after subtherapeutic CPAP</strong></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>3.9–7.8*</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.55–1.9* (fasting)</td>
</tr>
<tr>
<td>FXIa (ng/ml)†</td>
<td>0.80 (0.87)</td>
</tr>
<tr>
<td>FVIII (IU/dl)</td>
<td>0.29 (0.76)</td>
</tr>
<tr>
<td>s-Psel (ng/ml)†</td>
<td>4.23 (4.25)</td>
</tr>
<tr>
<td>TAT (μg/l)†</td>
<td>9.31 (4.35)</td>
</tr>
<tr>
<td>vWF Ag (IU/dl)</td>
<td>50–200*</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>5.05–13.9*</td>
</tr>
<tr>
<td>FXII (IU/dl)</td>
<td>50–200*</td>
</tr>
<tr>
<td>vWF Ag (IU/dl)</td>
<td>50–200*</td>
</tr>
<tr>
<td>FVIII (IU/dl)</td>
<td>50–200*</td>
</tr>
<tr>
<td>FXII (IU/dl)</td>
<td>50–200*</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>5–15*</td>
</tr>
</tbody>
</table>

Values are mean (SD) with 95% confidence interval (CI).
*Laboratory reference range
†No laboratory reference range as new test
*Non-fasting levels
$Significance of the difference between the change in the two treatment groups (unpaired t test).
Correlation of markers with OSA severity

There were no correlations between the baseline raised levels of VIIa, XIIa, and TAT, with BMI, age, severity of OSA (as determined by 4% oxygen saturation dip rate), and baseline subjective and objective sleepiness (as determined by the ESS and Osler test, respectively). Under multiple linear regression, only BMI was an independent predictor of baseline sP-sel levels (p<0.002). OSA severity was not a predictor of sP-sel levels after correction for BMI.

Change with treatment: coagulation factors

The levels of coagulation factors VIIa and XII fell in the group treated with subtherapeutic CPAP but not in those treated with therapeutic CPAP. None of the other markers reflecting increased haemostatic activation had fallen after 1 month of treatment with therapeutic CPAP.

Cholesterol and triglyceride

Mean baseline levels of total cholesterol were within the normal laboratory range in both groups. There was a difference in the fall in total cholesterol in those treated with therapeutic CPAP compared with the control group which showed a trend towards significance (mean difference in change 0.2 mmol/l, 95% CI −0.12 to 0.41, p = 0.06). When analysed separately there was a highly statistically significant fall in plasma total cholesterol in the group treated with therapeutic CPAP (mean (SD) change −0.28 (0.88) mmol/l, 95% CI −0.45 to −0.11, p = 0.001) but no significant change in the group treated with subtherapeutic CPAP (mean (SD) change −0.07 (0.68) mmol/l, 95% CI −0.06 to 0.21, p = 0.24).

DISCUSSION

This study reports changes in circulating cardiovascular risk factors in patients with moderate to severe OSA from the two Oxford randomised placebo controlled trials of OSA treatment. We have shown a fall in cholesterol levels with therapeutic CPAP (with a trend towards significance) and raised baseline levels of activated coagulation factors XIIa, VIIa, sP-sel, and TAT which neither fell with CPAP treatment nor were correlated with OSA severity.

Accumulating evidence suggests an increased prevalence of coronary artery and cerebrovascular disease in patients populations with OSA. Until relatively recently, cardiovascular disease prevention concentrated on the identification and modification of traditional risk factors such as diet and obesity, cigarette smoking, hypertension, and hyperlipidaemia. However, recent evidence suggests that this approach may be inadequate. Up to 30% of myocardial infarctions occur in patients without traditional risk factors. As a consequence, attention is shifting towards the identification of newer markers of atherosclerotic disease including those involving the fibrinolytic and coagulation systems, lipoprotein metabolism, and inflammation. Many studies have found hypercoagulable states (as determined by increased clotting factor levels, impaired fibrinolysis, and increased platelet activity) in ischaemic stroke, coronary artery disease, and hypertension. OSA is associated with an increased risk of atherosclerotic disease, so knowledge of the effects of OSA on these markers and their response to treatment is important in elucidating the pathogenesis.

Factors raised at baseline in patients with OSA

Activated factors VIIa and XIIa

Activated factor XII is an initiator of intrinsic coagulation and fibrinolysis and influences the activity of the coagulation pathway. Previous studies in normal and diabetic subjects have confirmed its role as a marker of endothelial activation or dysfunction. Activated factor VII plays a central role in clot formation and is important in inducing the thrombo-genic potential of atherosclerotic plaque. No previous studies have examined the relationship between OSA and these two markers. The finding of raised baseline levels of these two markers in untreated OSA patients suggests a potential mechanism for the increased vascular risk in this patient population, via activation of the coagulation pathways and clot generation.

Soluble P-selectin (sP-sel)

Soluble P-selectin is a cell surface molecule involved in leucocyte rolling and attachment, and is hypothesised to play a role in the initiation of atherosclerosis. A study in apparently healthy women has shown that the future risk of cardiovascular events increases with, and correlates with, increasing levels of sP-sel, independent of hypertension, obesity, exercise, diabetes, and hyperlipidaemia. At least seven previous small studies (one with 94 subjects, the others with 18 or less) have examined the relationship between platelet activation and OSA. However, the results are not consistent, with some studies showing a fall in sP-sel with nasal CPAP treatment and some not.

Not all of these studies used matched controls and none had a placebo controlled treatment arm. The mechanism for the increased platelet activation in OSA is unexplained (but has several potential causes), as platelet activation is increased by sympathetic activation, hypoxaemia and obesity, all of which are seen in OSA. Our findings suggest that patients with OSA have increased platelet activation (in agreement with some previous reports), and this was not reduced by the treatment regime used in this study. Despite the increase in platelet activation, there was no correlation with OSA severity. Only BMI correlated with levels of sP-sel and not with the measures of OSA severity. This suggests that the mechanism may be via associated obesity and high leptin levels, which can activate platelets in their own right, rather than a direct consequence of OSA itself. OSA has also been shown to be associated with high leptin levels, which may also explain the lack of response to treatment.

Thrombin-antithrombin complex (TAT)

TAT is a marker of thrombin turnover, indicating increased thrombin activity. A previous study has shown higher TAT levels in patients with OSA, although the effect was confined to patients with additional hypertension and similar increases in TAT have been found in patients with non-apnoeic hypertension which suggests that the mechanism may be via associated hypertension. Twenty five of our patients were hypertensive, but an insufficient number of those in whom TAT was measured were hypertensive to enable further investigation of this point. A previous population based study found lower vWFAg levels in hypertensive than normotensive subjects and suggested that, at the initial stages of hypertension where there is endothelial dysfunction rather than damage, endothelial synthesis of vWFAg might be impaired.

The most likely explanation for the failure of the plasma levels of sP-sel, TAT, and activated factors VIIa and XIIa to fall following 1 month of CPAP treatment is because they are related to associated obesity, hypertension, and pre-existing atherosclerotic disease, and are not a direct consequence of OSA itself.

Factors with normal levels at baseline

We did not find any difference in plasma levels of factors VII, VIII, XIII, vWFAg, homocysteine, cholesterol, triglyceride, and...
fibrinogen between patients with OSA and unmatched controls.

**Fibrinogen**

Fibrinogen is an independent risk factor for cardiovascular disease and is also raised in subjects with pre-existing atherosclerotic disease. Levels of fibrinogen and factor VIII are raised in overweight normotensive and hypertensive subjects, with BMI correlating with fibrinogen and factor VIII levels. Small uncontrolled studies have shown raised fibrinogen levels in patients with OSA, with higher morning fibrinogen levels falling with CPAP treatment, but we have been unable to confirm these findings.

**vWFαG, factors VII, VIII, and XII**

vWFαG is a marker of endothelial function and an independent predictor of target organ damage. In prospective studies of patients with underlying atherosclerotic disease, vWFαG levels are independently associated with a risk of future thrombotic events. There are no previously published data concerning the relationship between vWFαG, factors VII, VIII, XII and OSA. The results of this study suggest that these factors are normal in patients with OSA.

**Homocysteine**

Homocysteine levels are associated with an increased cardiovascular risk and may be a predictor of long term prognosis following premature myocardial infarction. Homocysteine levels have previously been reported to be raised in patients with OSA, but only in patients with associated ischaemic heart disease and/or hypertension. The results of our study support the conclusion that OSA is not independently associated with raised blood homocysteine levels.

**Factors changing with treatment**

**Cholesterol**

This study has shown a fall in total cholesterol following treatment for 1 month with therapeutic CPAP and no change with subtherapeutic control treatment. No previous studies have shown a fall in cholesterol levels in subjects with OSA following treatment with CPAP. Before randomisation to treatment groups, the same number of subjects in each group had total cholesterol levels over the optimum of 5.5 mmol/l (54/107 in the subtherapeutic CPAP group and 55/106 in the therapeutic CPAP group). Following treatment the number of subjects in the subtherapeutic CPAP group with raised total cholesterol was unchanged (55/107) compared with 41/106 in the group treated with therapeutic CPAP, suggesting a clinically relevant fall. This suggests that four patients need to be treated with therapeutic nasal CPAP to move one subject into the optimum cholesterol range, a clinically significant effect.

A fall in total cholesterol of 0.28 mmol/l is also likely to represent a significant vascular risk reduction in this group of subjects who have a significantly raised baseline cardiovascular risk. Previous studies have quantified the reduction in cardiovascular risk seen with a fall in cholesterol, with a greater protective effect seen at younger ages. A fall in cholesterol of 0.6 mmol/l at age 40 equates to a 50% fall in the risk of ischaemic heart disease, with a similar fall in cholesterol equating to a fall of about 20% in the risk of ischaemic heart disease at the age of 70. The fall of 0.28 mmol/l in this study is less than that expected with statin therapy (about 1.8 mmol/l), but is similar to that expected with an alteration in diet (about 0.3 mmol/l) which is predicted to lead to a 15% reduction in the risk of ischaemic heart disease at the age of 60. Assuming that 1% of UK and US adult men have OSA of the severity described in these studies, a 15% reduction in the risk of a vascular event would be expected to prevent about 2000 vascular events per year.

The reason for the fall in total cholesterol levels in our patients with OSA is not clear. It is possible that cholesterol is one of the first molecules to respond to the reduction in oxidative stress seen with treatment of OSA. An alternative explanation is that the patients treated with therapeutic CPAP altered their diet and increased their activity levels following resolution of their daytime sleepiness, leading to the fall in cholesterol level. However, there was no change in the patients’ weight following therapeutic CPAP treatment, and no parallel fall in the non-fasting triglyceride level, which might be expected to fall further than total cholesterol with a change in diet. It is also possible that these results may have arisen from our exploratory analysis approach, and this potentially important clinical observation should be prospectively tested as a primary end point in a future trial.

The current study, with many end points and small numbers in several of the cardiovascular markers assessed, may lead to problems with type I and type II errors. The small numbers also mean that the power of the study is small. Based on these figures, a prospective randomised controlled trial to assess fall in cholesterol as a primary end point would need a sample size of 700 subjects (90% power, α = 0.05). Clearly this will be a major undertaking, but it is the only way to clarify the current findings.

**Study limitations**

The major limitation of this study lies in its exploratory analysis approach with multiple end points and, as a consequence, small subsets of patients with each cardiovascular marker have been assessed. This reduces the power of any positive finding and potentially leads to type I and type II errors.

A further limitation lies in the short CPAP treatment time of 1 month. This was because of the placebo controlled nature of the study; it is unethical to treat patients with moderate to severe OSA with subtherapeutic (placebo) treatment for sustained periods. It is possible (though not likely) that this may have resulted in our failure to show a fall in the raised baseline levels of factors XIIa, VIIa, sP-sel and TAT. An alternative more likely explanation is that the raised markers are secondary to one or a combination of hypertension, obesity, and pre-existing atherosclerotic disease and are not a direct consequence of OSA itself.

An additional potential limitation lies in the fact that the blood samples were taken in the non-fasting state. There are no data to inform whether or not this may have affected the majority of the circulating cardiovascular risk factors measured, although it is possible that cholesterol levels may have been affected. Epidemiological screening studies support the measurement of cholesterol in the fasting and non-fasting state to identify those at highest risk of cardiovascular disease, with close agreement (for HDL cholesterol) between levels taken in both states, which do not alter decisions about preventative treatment.

**Conclusion**

The demonstration of several raised circulating cardiovascular risk markers in OSA (compared with an unmatched control population) adds to the evidence that OSA is associated with higher levels of cardiovascular disease. There was no correlation between any of the raised markers and OSA severity. Treatment for 1 month with therapeutic CPAP led to a potentially clinically significant fall only in the total cholesterol level which should be assessed in further trials. Other risk factors did not change. This suggests that most of the increase in these factors may be the result of
associated factors such as obesity and pre-existing cardiovascular disease rather than the OSA itself.

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