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

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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, XI, 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.

Obstructive sleep apnoea (OSA) is the second most common chronic respiratory disorder, affecting 2–4% of adult men in the developed world. In the UK approximately 0.5–1.5% of men have moderate or severe disease, causing significant daytime symptoms and potentially warranting treatment with continuous positive airways pressure (CPAP).

Patients with OSA have increased cardiovascular morbidity and mortality, but how much of this is due to OSA rather than to its association with factors such as upper body obesity, insulin resistance, increasing age, alcohol and caffeine consumption, and cigarette smoking has been difficult to determine. Cross sectional epidemiological studies have implicated OSA as a probable independent risk factor for arterial thrombotic disease and a raised blood pressure, which has been shown to fall with effective OSA treatment.

The combined stroke and coronary event risk for an average untreated patient with moderate to severe disease (estimated from conventional risk factors) is high at about 3% per year. Furthermore, any additional effect of OSA on cardiovascular morbidity in the community is likely to be increasing, as the prevalence of OSA is increasing in association with the rising prevalence of obesity.

Interest in circulating cardiovascular risk markers has contributed to the discussion as to whether OSA may be an independent risk factor for cardiovascular disease by providing plausible linking mechanisms, some of which are predictable from the known effects of OSA on sympathetic drive and oxidative stress. However, few large randomised controlled studies establishing an independent relationship of these factors with OSA have been performed, and study results have been inconsistent.

The pathogenesis of arterial thrombotic disease involves a large number of genetic and environmental factors related to both atherosclerosis and haemostasis. The measurement of circulating cardiovascular risk factors (including several new phenotypic markers of cardiovascular disease) enables a more accurate prediction of cardiovascular risk to be made, as there are clearly established relationships between levels of various circulating haemostatic risk factors and a subsequent cardiovascular event.

This analysis uses new data from two published randomised placebo controlled trials of CPAP treatment in OSA. We have measured a number of circulating cardiovascular risk factors in cohorts of patients with OSA before and after 1 month of standard treatment with nasal CPAP compared with a control arm. The aims of the study were (1) to establish whether OSA is associated with raised baseline levels of these circulating markers (compared with normal controls), (2) to establish whether a relationship exists between the severity of OSA and levels of these circulating markers, and (3) to assess any reversibility with treatment. The identification of high levels of circulating cardiovascular risk markers in patients with OSA (shown previously in uncontrolled studies), would confirm the increased cardiovascular risk in this patient group by correlation with indices of OSA severity or, more convincingly, by reversal with CPAP treatment. This would then suggest a possible causal relationship with OSA.

METHODS
Design and setting
This study is an analysis of the cardiovascular risk data collected during the Oxford parallel, double blind, placebo controlled trials of CPAP treatment in OSA.

Abbreviations: BMI, body mass index; CPAP, continuous positive airways pressure; ESS, Epworth Sleepiness Score; OSA, obstructive sleep apnoea; sP-sel, soluble P-selectin; TAT, thrombin-antithrombin complex; vWFAg, von Willebrand factor antigen
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 polysomnographic 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 Oslor test.

Triglyceride and cholesterol were measured using automated routine methods (Abbott Aeroset Analyser, Maidenhead, UK) and homocysteine was measured by fluorescence 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 (VIIa) was assayed by recombinant soluble tissue factor assay (STACLLOT, Diagnostica, France) and activated factor XII (XIIa) was measured by ELISA (Sheild Diagnostics, Dundee, UK). Von Willebrand factor antigen (vWF:Ag) was measured in 213 patients. 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 either therapeutic airway pressure.

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 reattended 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 (FVIIa) in 44, homocysteine and triglyceride in 101, vWF:Ag in 90, and sP-sel in 93. Total cholesterol was measured in 213 patients.

Table 1 Baseline patient characteristics before CPAP treatment

<table>
<thead>
<tr>
<th></th>
<th>Subtherapeutic CPAP (n = 112)</th>
<th>Therapeutic CPAP (n = 108)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>49.1 (10.3)</td>
<td>49.7 (10.3)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>35.9 (6.3)</td>
<td>35.6 (7.6)</td>
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<tr>
<td>Oxygen saturation dips &gt;4% (per hour of sleep)</td>
<td>38.5 (20.3)</td>
<td>38.9 (21.1)</td>
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<tr>
<td>CPAP compliance (hours per night)</td>
<td>4.1 (2.4)</td>
<td>5.0 (1.9)</td>
</tr>
<tr>
<td>Baseline ESS</td>
<td>16.2 (3.3)</td>
<td>16.3 (3.3)</td>
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Values are mean (SD). CPAP, continuous positive airway pressure; ESS, Epworth Sleepiness Score. *p<0.005.
were the same as in the normal control subjects or
vWFAg, homocysteine, total cholesterol, and triglyceride
Plasma levels of fibrinogen, factors VII, VIII, and XII,
Comparison with normal controls
Circulating cardiovascular risk factors in OSA 779

<table>
<thead>
<tr>
<th>Cardiac marker</th>
<th>Pre subtherapeutic CPAP</th>
<th>Pre therapeutic CPAP</th>
<th>Normal controls</th>
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<tbody>
<tr>
<td>FVIIa (ng/ml)†</td>
<td>2.0 (1.2), n = 19</td>
<td>2.1 (1.0), n = 26</td>
<td>1.7 (0.3), n = 21</td>
</tr>
<tr>
<td>vWF Ag (IU/dl)</td>
<td>111.2 (40.8), n = 18</td>
<td>93.7 (50.7), n = 26</td>
<td>32.0 (14.0), n = 32</td>
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<tr>
<td>TAT (ng/l)†</td>
<td>53.0 (32.3), n = 46</td>
<td>58.2 (41.9), n = 47</td>
<td>29.5 (10.5), n = 22</td>
</tr>
<tr>
<td></td>
<td>12.3 (24.1), n = 20</td>
<td>12.3 (25.1), n = 26</td>
<td>2.6 (1.4), n = 29</td>
</tr>
</tbody>
</table>

Values are mean (SD). tNo laboratory reference range as new test.
£Compared with all OSA patients before randomisation.
*Non-fasting levels.

Comparison with normal controls
Plasma levels of fibrinogen, factors VII, VIII, and XII, vWFAg, 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-P-sel, and TAT were higher at baseline than in the unmatched controls (table 2).

<table>
<thead>
<tr>
<th>Table 3 Blood levels after therapeutic and subtherapeutic CPAP</th>
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<tr>
<td>Change after therapeutic CPAP</td>
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<td>-----------------------------</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
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<tr>
<td>n = 106</td>
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<tr>
<td>Triglyceride (mmol/l)</td>
</tr>
<tr>
<td>0.55–1.9*</td>
</tr>
<tr>
<td>FXIIa (ng/ml)†</td>
</tr>
<tr>
<td>n = 52</td>
</tr>
<tr>
<td>FVIIa (mU/ml)</td>
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<tr>
<td>2.6 (7.8)</td>
</tr>
<tr>
<td>s-Psel (ng/ml)†</td>
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<tr>
<td>n = 47</td>
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<tr>
<td>TAT (ng/l)†</td>
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<tr>
<td>n = 47</td>
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<tr>
<td>vWF Ag (IU/dl) 50–200*</td>
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<tr>
<td>n = 44</td>
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<tr>
<td>Fibrinogen (g/l) 1.5–4.0*</td>
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<tr>
<td>n = 26</td>
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<tr>
<td>FVII (IU/dl) 50–200*</td>
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<tr>
<td>n = 26</td>
</tr>
<tr>
<td>FXII (IU/dl) 50–200*</td>
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<tr>
<td>n = 26</td>
</tr>
<tr>
<td>Homocysteine (µmol/l) 5–15*</td>
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<tr>
<td>n = 52</td>
</tr>
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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, $p = 0.24$).

A larger study with cholesterol change as a primary end point is needed to examine this further. Non-fasting triglyceride levels were not raised at baseline and did not change with treatment (table 3).

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 patient 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 thrombogenic 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, XII, 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.

**vWFAG, factors VII, VIII, and XII**
vWFAG is a marker of endothelial function and an independent predictor of target organ damage. In prospective studies of patients with underlying atherosclerotic disease, vWFAG levels are independently associated with a risk of future thrombotic events. There are no previously published data concerning the relationship between vWFAG, 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 homocytene 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. 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. 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.

**A fall in total cholesterol of 0.28 mmol/l is also likely to 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.

**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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REFERENCES