The effect of obstructive sleep apnoea on hypoxic and inflammatory markers during CPAP withdrawal: Further evidence from three randomised control trials.

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Summary at a glance

Intermittent hypoxia, endothelial dysfunction and adipose tissue mediated inflammation have been proposed as mechanisms of the development of cardiovascular disease in OSA. We explored changes in associated biomarkers after CPAP withdrawal. Return of OSA led to a small reduction in adrenomedullin with no significant change in other cardiovascular markers.

ABSTRACT

Background and objective

Obstructive sleep apnoea (OSA) is associated with cardiovascular disease. Intermittent hypoxia, endothelial dysfunction and adipose tissue mediated inflammation have all been linked to cardiovascular disease in OSA. We have therefore explored the effect of OSA on relevant associated blood markers; adrenomedullin, endocan, endothelin-1, resistin and vascular endothelial growth factor.

Methods

Patients with OSA, established on and compliant with continuous positive airways pressure (CPAP) therapy for > 1 year were included from three randomised controlled trials, conducted at two centres. Patients were randomised to either continued therapeutic CPAP or sham CPAP (CPAP withdrawal) for two weeks. Blood markers were measured at baseline and at 14 days, and the treatment effect between sham CPAP and therapeutic CPAP was analysed.

Results

109 patients were studied (therapeutic CPAP n=54, sham CPAP n=55). Sham CPAP was associated with a return of OSA (between-group difference in ODI +36.0/h, 95%CI +29.9 to +42.2, p<0.001). Sham CPAP was associated with a reduction in adrenomedullin levels at 14 days (-26.0 pg/ml, 95%CI -47.8 to -4.3, p=0.02), compared to therapeutic CPAP. Return of OSA was not associated with changes in endocan, endothelin-1, resistin or vascular endothelial growth factor.

Conclusions

Whilst CPAP withdrawal was associated with return of OSA, it was associated with an unexpected significant reduction in the vasodilator adrenomedullin and not with expected increases in hypoxia-induced markers, markers of endothelial function, or resistin. We propose that the vascular effects occurring in OSA may be brought about by other mechanisms, perhaps partly through a reduction in adrenomedullin.

Key words: Cardiovascular diseases; Sleep Apnoea.

INTRODUCTION

Obstructive sleep apnoea (OSA) syndrome is a common condition affecting approximately 4% of middle aged adults.[1] OSA is recognised as an independent risk factor for arterial hypertension,[2,3] and is associated with cardiovascular disease.[4] The underlying mechanisms for the association of OSA with cardiovascular disease are not fully understood. There is a clear association between OSA and endothelial dysfunction,[5,6] but the relative roles of chronic intermittent hypoxia and inflammation in the development of cardiovascular disease remain unclear.[7]

Blood markers of cardiovascular disease have previously been explored in OSA. Cohort studies have explored differences in cardiovascular markers induced by hypoxia, via hypoxia inducible factor (HIF) activation. Both adrenomedullin (ADM), an endothelial-derived vasodilator,[8,9] and endothelin-1 (ET-1), an endothelium-derived vasoconstrictor,[10] have been reported to be increased in OSA. Counter intuitively the increased ADM levels found in OSA have been proposed as an adaptive mechanism protective against cardiovascular disease.[8] The effects of OSA on vascular endothelial growth factor (VEGF), an angiogenesis stimulator, are not clear.[11-14]

Endocan, an endothelial derived protein, is thought to play a role in endothelium-dependent pathology and is a surrogate marker of endothelial dysfunction.[15] Increased levels of endocan have been reported in OSA,[16,17] and associated with endothelial dysfunction.[17] Resistin, an adipose derived cytokine, is produced in adipose tissue mediated inflammation and is linked with endothelial dysfunction and cardiovascular risk.[18] Resistin has been proposed as a marker of cardiovascular disease in OSA.[19]

CPAP withdrawal has been used previously as a model to explore the physiological effects of OSA. It has been shown to lead to dramatic return of OSA associated with a considerable rise in blood pressure (especially early morning), catecholamine excretion, and reduced endothelial function.[5] CPAP withdrawal is a powerful model for evaluating the effects of OSA by overcoming most of the problems of confounding variables.

We hypothesised that return of OSA during CPAP withdrawal would adversely change blood markers of cardiovascular disease. Therefore, we aimed to explore the effects of OSA in a CPAP withdrawal model on the following blood markers of cardiovascular disease; hypoxic regulated proteins (ADM, ET-1, and VEGF), on a marker of endothelial dysfunction (endocan), and on an adipose tissue derived protein (resistin).

METHODS

Trial design

These data come from three randomised controlled trials with similar protocols. Full methodology and primary outcome data for these trials have been published previously.[5,20,21] All three trials randomised patients with known OSA, established on and compliant with CPAP therapy, to either continued therapeutic CPAP or sham CPAP (CPAP withdrawal) for 14 days. The trials were registered (controlled trials.com ISRCTN 93153804, ISRCTN 73047833 and clinicaltrials.gov NCT 01797653) and approved by the local ethics committee (University Hospital Zurich REC Ref EK-1600, NRES Committee South West – Exeter Ref 12/SW/0254, Zurich Cantonal EC Ref KEK-ZH-NR.2012-0511). The trials were conducted in accordance with the amended Declaration of Helsinki. Written informed consent was taken from all participants.

Patients

Patients with OSA were recruited from the sleep database of the Sleep Disorders Centre of the University Hospital Zurich, Switzerland and the Oxford Centre for Respiratory Medicine of the Churchill Hospital, UK. Patients were eligible if they were; aged 20-75 years, had OSA and an oxygen desaturation index (ODI) >10/h,[5] or 20/h,[20,21] on their original sleep study, had been treated with CPAP for > 12 months, and had an average CPAP usage of ≥ 4 hours/night. Following written informed consent, patients underwent pre-trial overnight pulse oximetry during withdrawing CPAP for four nights to determine eligibility. In one trial patients were included if their ODI was > 10/h during the initial pre-trial period off CPAP.[5] In the other two trials

patients were included if their ODI > 20/h during the initial pre-trial period off CPAP.[20,21] Patients were excluded if they had previous ventilatory failure, Cheyne-Stokes breathing, unstable coronary artery disease, unstable peripheral arterial disease, severe arterial hypertension (>180/110mmHg), severe arterial hypotension (<90/60 mmHg), were a professional driver, had a history of sleep related accidents, or had an acute upper respiratory tract infection.

Intervention, randomisation and blinding

After the initial pre-trial screening week, patients returned onto CPAP therapy for at least 7 days in one trial,[5] and at least 14 days in the other two.[20,21] Following baseline assessments patients were then randomised in a 1:1 ratio to either continue therapeutic CPAP or to sham CPAP for 14 days (CPAP withdrawal). Methods of randomisation have previously been described.[5,20,21] Outcome assessors and patients were blinded to treatment allocation.

Therapeutic CPAP and sham CPAP

Those randomised to therapeutic CPAP had received a new trial CPAP machine delivering a therapeutic pressure. Patients allocated to sham CPAP received a device set to the lowest pressure (4 cmH_2O), the delivered pressure was lowered further by inserting 6 extra holes in the mask end of the CPAP tubing and fitting a flow restricting device at the machine end of the tubing. The mask pressure delivered by sham CPAP was ≤1cmH_2O.

Sleep studies

Sleep studies were performed the night prior to randomisation whilst on CPAP and on the 14th night of the trial either on therapeutic CPAP or sham CPAP. Sleep studies were performed either at home (Oxford; Black Shadow, Stowood Scientific Instruments, Oxford, UK) or in-hospital (Zurich; Alice 5, Philips Respironics AG, Zofingen, Switzerland). The oxygen desaturation index (ODI) was defined as dips in oxygen saturations >4%.

Trial outcomes: blood cardiovascular markers

The main outcomes were the change in the following cardiovascular blood markers: ADM, endocan, ET-1, resistin, and VEGF. Other outcomes were changes in EPO levels (as a comparator that is known to be by hypoxically regulated), the change in ODI, the change in Epworth sleepiness score (ESS), the change in blood pressure and the change in heart rate. Changes from baseline to follow-up were compared, sham CPAP *versus* therapeutic CPAP. Blood samples were collected in the early morning at baseline and at 14 day follow-up and the samples were stored at -80^{O}C for later analysis. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for analysis: ADM (Caltag Medisystems #E-EL-H0275), ET-1 (R&D-Systems #QET00B), endocan (Aviscera #SK00318-01), EPO (R&D Systems #DEP00), resistin (BioVendor #RD191016100) and VEGF (R&D Systems #DVE00). EDTA plasma was used for all tests except for VEGF, which was performed on serum. Samples were measured in duplicate, except for VEGF which was measured in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation where normally distributed, and median (1^{st} quartile, 3^{rd} quartile) when not. Treatment effects (Sham CPAP *versus* therapeutic CPAP) on follow-up blood protein levels were modelled using linear regression (SPSS, Version 20: IBM, Portsmouth, UK), controlling for baseline blood protein level, age, gender, BMI, smoking status, recruiting centre and comorbidities. Change in blood marker levels were approximately normally distributed where baseline and follow-up blood marker levels were not.

RESULTS

The trial profile is shown in *Figure 1*. 109 patients were included in this study, 86 from Zurich and 23 from Oxford. 55 patients were randomised to sham CPAP (CPAP withdrawal) and 54 patients to therapeutic CPAP. Baseline characteristics are reported in *Table 1*.

There was a significant effect of CPAP withdrawal on ODI of +36.0/h (95%CI +29.9 to +42.2, p<0.001) and ESS of +3.9 (95%CI +2.7 to +5.0, p<0.001) at 14 days compared to therapeutic CPAP. CPAP withdrawal had significant effects on morning systolic blood pressure (mean difference +4.6mmHg, 95% CI +0.2 to +9.0, p=0.04), morning diastolic blood pressure (mean difference +5.0, 95% CI +1.8 to +8.3, p=0.002) and morning heart rate (mean difference +6.2mmHg, 95% CI +2.9 to +9.6, p<0.001) compared to therapeutic CPAP.

There was a significant effect of CPAP withdrawal on ADM levels of -26.0 pg/ml (95%CI -47.8 to -4.3, p=0.02) at 14 days, versus therapeutic CPAP (*Figure 2*). No other protein marker significantly changed during CPAP withdrawal at 14 days, versus therapeutic CPAP (*Table 2*).

DISCUSSION

We present data from three randomised controlled trials showing that return of OSA during CPAP withdrawal for 14 days was not associated with changes in hypoxia regulated proteins, protein markers of endothelial dysfunction, or in resistin, an adipocyte derived protein. However, we found an unexpected small reduction in adrenomedullin levels, an endothelial-derived vasodilator, with CPAP withdrawal. These results differ from sustained hypoxia, models of intermittent hypoxia and cohort studies of patients with OSA.

The effect of chronic intermittent hypoxia in OSA on blood markers

Obstructive apnoeas and hypopnoeas typically lead to intermittent arterial oxygen desaturations. Murine models of intermittent hypoxia led to arterial hypertension,[22-24] atherosclerosis,[25] endothelial dysfunction,[26] and oxidative stress.[27] However, these animal experiments often only model longer-cycle intermittent hypoxia and differ from OSA in causing hypocapnic hypoxia and as they do not simulate the asphyxia of upper airway obstruction. It is therefore important to study the mechanism of vascular damage in patients with OSA.

We explored the effects of OSA during CPAP withdrawal on ADM, ET-1 and VEGF, which are activated via HIF during hypoxic conditions. Cohort studies have suggested that these blood proteins play a role in cardiovascular disease in OSA.[8-12] We found no increases in these markers during CPAP withdrawal. The intermittent hypoxia of OSA may not be severe enough to cause an upregulation of these markers or may differ from sustained hypoxia in the pathways it activates. Indeed, to support this, EPO levels

(used as a “control”, known to be upregulated in plasma in response to sustained hypoxia in humans),[28] did not significantly change.

Unexpectedly we found a small significant reduction (but with a high degree of variability) in ADM levels in patients following CPAP withdrawal. ADM levels have previously been reported to be increased in untreated OSA patients compared with patients without OSA,[8,9] and activation by intermittent hypoxia in OSA has been proposed to have a protective role against cardiovascular disease. Our results suggest that intermittent hypoxia seen in OSA may not be responsible for the increases in ADM previously reported. Rather, the reduction in ADM levels we observe after CPAP withdrawal are likely brought about by other mechanisms other than intermittent hypoxia. While the mechanism responsible is not known, reductions in levels of the vasodilator ADM could have a deleterious effect by contributing to hypertension and worsening atherosclerosis.[29]

Our findings from the CPAP withdrawal model showing no changes in VEGF in response to OSA recurrence are in concordance with a previous CPAP withdrawal trial.[30] Our study differs from this previous CPAP withdrawal trial by including a control arm of therapeutic CPAP and withdrawing CPAP for 14 days instead of 7 days.

Endocan

Endothelial dysfunction is thought to be an early and important step in the development of cardiovascular disease.[31] Reduced endothelial function has been shown to be associated with OSA; endothelial function improves with CPAP therapy,[6,32] and worsens with withdrawal of CPAP.[5] Endocan is an endothelial-derived protein and

has been proposed to both contribute to the development of, and act as a surrogate marker of endothelial dysfunction and cardiovascular disease.[15] In observational studies, endocan levels have been reported to be increased in OSA patients compared to healthy control subjects,[16,17] and elevated levels were associated with endothelial dysfunction and atherosclerosis.[17] In a cohort study, CPAP treatment led to decreases in endocan levels in OSA patients.[17] We have not found differences in endocan levels, despite our previous finding of significant endothelial dysfunction in response to CPAP withdrawal as assessed by flow-mediated dilatation using the same model.[5] Although a significant proportion of patients had undetectable endocan levels, a post hoc sensitivity analysis looking at just those with detectable levels did not change this result (treatment effect +0.8ng/ml, 95%CI -2.0, +3.7, p=0.54). This suggests that endocan is not necessarily useful as a marker of endothelial dysfunction in this group.

Resistin

Resistin, an adipose derived cytokine, has been suggested as an important link between OSA, obesity and inflammation.[19] This link is not certain, and others reported no difference in resistin levels in OSA patients,[33] or found a reduction in resistin levels.[34] Reductions in resistin levels have been reported with CPAP treatment,[19] whilst others report no change in resistin levels with CPAP therapy.[35,36] Our results show that resistin levels are not changed during return of OSA seen in CPAP withdrawal suggesting that it does not contribute to endothelial dysfunction seen with CPAP withdrawal.

Limitations

This study does have some important limitations to consider. The data presented here are from three trials with primary outcomes published elsewhere and the outcomes from this study are thus exploratory and hypothesis-generating. CPAP was only withdrawn for 14 days, and markers of cardiovascular risk in OSA may require longer exposure to OSA to change. However, 14 days is sufficient time to see a large relevant rise in blood pressure, increased sympathetic activity and endothelial dysfunction to develop.[5] Patients prepared to undergo CPAP withdrawal are a selected population as during pre-trial screening a significant proportion either did not have sufficient return of OSA, or were unable to continue due to being unable to tolerate being off CPAP. The inclusion of patients with mild to moderate OSA may mean that treatment effects observed only in severe OSA may have been missed. However, almost half of our patients (51.9% in the sham CPAP group and 42.6% in the therapeutic CPAP group) had severe OSA (ODI>30/h). Patients were well matched by randomisation and did not have the confounding effect of variable compliance seen when studying CPAP naïve patients.

Conclusions

Intermittent hypoxia, endothelial dysfunction and adipose tissue derived inflammation are thought to be important processes in the development of cardiovascular disease in OSA. Whilst CPAP withdrawal was associated with return of OSA and blood pressure rises we found no significant increases in hypoxia-induced markers and endothelial-derived inflammatory and vasoconstrictive substances. CPAP-withdrawal was associated with an unexpected decrease in the vasodilatory protein ADM. This

suggests that the particular pattern of IH in OSA does not have the same effects as sustained hypoxia and that the endothelial dysfunction seen in CPAP withdrawal is not reflected in changes in these endothelial markers. We propose that the vascular effects occurring in OSA may be brought about by other mechanisms, perhaps partly through a reduction in the endothelium derived vasodilator ADM.

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	Therapeutic CPAP (n=54)	Sham CPAP (n=55)
Age*	62.2±7.9	63.5±7.9
Male gender #	48 (88.9%)	49 (89.1%)
BMI kg/m^2 *	33.8±6.2	34.2±6.0
ODI at diagnosis *	41.9±19.1	46.2±22.2
ODI during pre-trial screening‡	26.7 (20.9, 34.2)	31.5 (20.4, 45.4)
Coronary artery disease #	7 (13.0%)	5 (9.1%)
Hypertension #	30 (55.6%)	39 (70.9%)
Diabetes #	11 (20.4%)	10 (18.2%)
Smoking status		
Current #	8 (14.8%)	8 (14.5%)
Ex #	17 (31.5%)	24 (43.6%)
Never #	29 (53.7%)	23 (41.8%)

Table 1: Baseline characteristics. Data are presented as mean ± standard deviation, median (1st quartile, 3rd quartile)‡, or number (%)#. ADM=adrenomedullin, BMI=body mass index, CPAP=continuous positive airways pressure, EPO=erythropoietin, ET-1=endothelin-1, ODI=oxygen desaturation index ≥4%, VEGF=vascular endothelial growth factor.*

	Therapeutic CPAP (n=54)		Sham CPAP (n=55)		Unadjusted treatment effect (TE)		Adjusted treatment effect (TE)	
	Baseline ǂ	Follow-up ǂ	Baseline ǂ	Follow-up ǂ	TE (95%CI)	p	TE (95%CI)	p
ADM (pg/ml)	154.7 (82.4, 237.7)	173.1 (105.2, 247.0)	146.0 (99.5, 189.6)	134.2 (79.0, 206.8)	-27.5 (-48.3, -6.8)	0.01*	-26.0 (-47.8, -4.3)	0.02*
Endocan (ng/ml)	0.0 (0.0, 1.2)	0.0 (0.0, 1.7)	0.0 (0.0, 0.2)	0.0 (0.0, 0.2)	+0.3 (-0.5, +1.0)	0.50	+0.1 (-0.7, +0.8)	0.87
EPO (mIU/ml)	11.6 (9.3, 14.2)	12.5 (10.3, 16.2)	10.9 (8.9, 16.2)	10.9 (8.4, 15.5)	-1.2 (-2.7, +0.3)	0.13	-1.3 (-2.8, +0.3)	0.12
ET-1 (pg/ml)	1.8 (1.4, 2.5)	2.0 (1.7, 2.6)	2.1 (1.7, 2.5)	2.0 (1.6, 2.4)	-0.2 (-0.4, +0.0)	0.16	-0.2 (-0.5, +0.0)	0.09
Resistin	4.6	4.7	4.4	4.4	+2.3	0.32	+2.1	0.39

(ng/ml)	(3.9, 5.3)	(3.7, 5.4)	(3.8, 5.4)	(3.5, 5.1)	(-2.3, +7.0)		(-2.8, +7.1)	
VEGF (pg/ml)	259.2 (171.1, 444.8)	205.7 (128.2, 384.8)	304.0 (155.9, 462.5)	299.1 (192.9, 407.0)	+24.7 (-58.4, +107.7)	0.56	+26.0 (-64.1, +116.2)	0.57

*Table 2: Adjusted treatment effect of sham CPAP versus therapeutic CPAP on levels of blood markers of cardiovascular disease. *p<0.05. Adjusted treatment effect is adjusted for baseline blood protein level, age, gender, BMI, smoking status, recruiting centre and comorbidities. ǂ Baseline and follow-up blood marker levels expressed as median (1st quartile, 3rd quartile). ADM=adrenomedullin, CPAP=continuous positive airways pressure, EPO=erythropoietin, ET-1=endothelin-1, VEGF=vascular endothelial growth factor, 95%CI=95% confidence interval.*

Figure 1: Trial Profile/ CONSORT flow diagram. 110 eligible patients with OSA were randomly allocated to either therapeutic continuous positive airways pressure (CPAP) or to withdraw CPAP by the use of sham CPAP. One patient from the sham CPAP group withdrew during the trial but all other patients received the intended treatment and completed the study.

Figure 2: Box and whisker plot of the change in adrenomedullin levels from baseline to follow-up by treatment allocation. Solid black line represents the median, box edges represent the 1st and 3rd quartiles, whiskers extend to 1.5 time the IQR from the 1st and 3rd quariltes, and black diamonds represent the means. CPAP=Continuous positive airways pressure. Adjusted treatment effect p=0.02.