

Endothelial Function and Arterial Stiffness in Minimally Symptomatic Obstructive Sleep Apnea

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Rationale: Moderate–severe obstructive sleep apnea (OSA) is associated with endothelial dysfunction, increased arterial stiffness, and hypertension. It is not known whether minimally symptomatic OSA is also associated with impaired vascular function.

Objectives: To determine whether minimally symptomatic OSA is associated with impaired vascular function.

Methods: In 64 patients (7 females) with minimally symptomatic OSA (oxygen desaturation index, 23.1 [SD, 15.6]; Epworth Sleepiness Scale score, 8 [SD, 3.8]), and 15 matched control subjects without OSA, endothelial function was assessed by ultrasonographic measurement of flow-mediated dilatation, and by applanation tonometry–derived pulse wave analysis (forearm ischemia and salbutamol-induced changes in augmentation index, AI_x). Arterial stiffness was assessed by AI_x and ambulatory blood pressure (ABP) was measured over 1 week.

Measurements and Main Results: In patients with OSA, flow-mediated dilatation was significantly lower than in control subjects (5.0% [SD, 2.7%] and 7.5% [SD, 3.3%], respectively; $P = 0.003$). AI_x was significantly higher in the OSA group compared with the control group (26.0% [interquartile range (IQR), 19.0–29.5%] and 21.0% [IQR, 8.0–27.0%], respectively; $P = 0.04$). Change in AI_x after both forearm ischemia and salbutamol was significantly smaller in patients with OSA (–2.0% [IQR, –5.0 to +4.0%] and –3.0% [IQR, –7.0 to 0.0%], respectively), than in control subjects (–6.0% [IQR, –8.0 to –5.0%] and –7.0% [IQR, –10.0 to –3.0%]; $P = 0.005$ and $P = 0.04$, respectively). ABP was similar (97.6 mm Hg [SD, 7.9 mm Hg] and 94.8 mm Hg [SD, 7.4 mm Hg], OSA and control groups, respectively; $P = 0.21$).

Conclusions: In patients with minimally symptomatic OSA, diverse properties of endothelial function are impaired and arterial stiffness is increased. Although this was not associated with a significantly increased ABP, the findings suggest that patients with minimally symptomatic OSA are at increased cardiovascular risk.

Keywords: obstructive sleep apnea; endothelial function; atherosclerosis; arterial stiffness; vascular reactivity

Obstructive sleep apnea (OSA) is characterized by repetitive apneas/hypopneas during sleep, associated with oxygen desaturations and sleep disruption. It has been estimated that between 2 and 4% of the adult population in Western countries experience moderate–severe OSA with daytime symptoms, and it is becoming more prevalent as the average population body weight rises (1). The prevalence of minimally symptomatic OSA among middle-aged adults has been shown to be as high as 26% (1),

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Moderate–severe obstructive sleep apnea (OSA) is associated with endothelial dysfunction and increased arterial stiffness. It is not known whether minimally symptomatic OSA has the same negative effect on vascular function.

What This Study Adds to the Field

In patients with minimally symptomatic OSA, diverse properties of endothelial function are impaired and arterial stiffness is increased. These findings suggest that patients with minimally symptomatic OSA are at increased cardiovascular risk.

making OSA one of the most frequent disorders and thus of epidemiologic interest.

Cross-sectional and prospective studies have implicated OSA as an important causal factor in the development of cardiovascular disease (2). The mechanisms underlying the association between OSA and cardiovascular disease are currently not fully understood, and indeed may not be causal. Multiple causal factors leading to vessel wall damage and atherosclerotic plaques have been proposed, including reflex sympathetic activation, consequent increases in blood pressure, endothelial dysfunction, systemic inflammation, and reactive oxygen species (3–5).

Endothelial dysfunction and increased arterial stiffness play a central role in the initiation of atherosclerosis and are associated with cardiovascular risk factors (6–9). As a result, there is growing interest in early detection of these measures of cardiovascular risk with the anticipation that an intervention will benefit patients who are at increased risk for future cardiovascular events.

Moderate to severe OSA has been associated with endothelial dysfunction, increased arterial stiffness, and hypertension, all of which have been shown to improve after continuous positive airway pressure (CPAP) therapy (5, 10, 11). In contrast to the fall in blood pressure seen with CPAP in hypersomnolent patients with OSA, blood pressure did not decrease after therapy in nonsleepy hypertensive patients with OSA (12). However, there are no data on endothelial dysfunction and arterial stiffness, potentially reflecting earlier changes in vascular function in patients with minimally symptomatic OSA. More importantly, because of the absence of daytime symptoms, these patients might not be treated with CPAP.

To investigate the hypothesis that vascular function is impaired in patients with minimally symptomatic OSA, we performed a controlled cross-sectional study investigating different aspects of endothelial function, arterial stiffness, and ambulatory blood pressure in patients with minimally symptomatic OSA.

Some of the results of this study have been previously reported in the form of an abstract (13).

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METHODS

Patients

Patients with possible obstructive sleep apnea were referred to the Oxford Sleep Unit, Oxford Centre for Respiratory Medicine (Oxford, UK), by general practitioners; ear, nose, and throat surgeons; or other hospital consultants, usually because of severe snoring or witnessed apneas. Patients were eligible for the study if they were between 45 and 75 years of age, had proven obstructive sleep apnea with a severity defined as more than 7.5 oxygen desaturations exceeding 4% per hour (oxygen desaturation index [ODI] > 7.5/h), and no history of excessive daytime sleepiness or any other daytime symptoms of OSA that would have justified CPAP therapy. All eligible patients were offered participation in the study.

Control subjects were mainly identified and recruited from a general practitioners' database and were eligible for the study if they were between 45 and 75 years of age with no history of OSA, had fewer than five oxygen desaturations exceeding 4% per hour (ODI < 5/h), and an apnea/hypopnea index less than 5 per hour (AHI < 5/h). Control subjects were matched to patients with OSA for sex, age, body mass index, waist-to-hip ratio, and cardiovascular comorbidities. The study was approved by the Oxford Research Ethics Committee (record no. 05/Q1604/159), and written informed consent was obtained from all participants.

Sleep Study and Assessment of Sleepiness

In patients, OSA was diagnosed from a one-night in-hospital respiratory polygraphic sleep study. Patients' body movements, heart rate, and pulse transit time changes were recorded as measures of arousal from sleep. Pulse oximetry, snoring, and increases in the respiratory swing in pulse transit time were used as markers of breathing pattern and respiratory effort (Win-Visi monitoring system; Stowood Scientific Instruments, Oxford, UK) as previously described and validated (14, 15).

The results of the sleep study were scored automatically, with manual review to ensure accuracy of the data. OSA was diagnosed from review of all data, and the severity was quantified as the number of oxygen desaturations exceeding 4% per hour of study (ODI). In control subjects, OSA was excluded by home sleep studies using the ApneaLink device (ResMed; MAP Medicine Technology, Martinsried, Germany). The device records the patient's nasal respiratory pressure signal and finger oximetry during sleep; it has been validated as an accurate instrument with which to detect snoring, apnea/hypopnea, and oxygen desaturations (16). The results of the sleep study were scored automatically with dedicated software (ResMed; MAP Medicine Technology), with manual review to ensure accuracy of the data. Apneas were defined as a cessation of airflow lasting more than 10 seconds, and hypopneas as a reduction in airflow of at least 50% lasting more than 10 seconds, associated with a drop in oxygen saturation exceeding 4%.

Subjective sleepiness was assessed with the Epworth Sleepiness Scale, which assesses the tendency to fall asleep during eight typical daytime situations (17). Objective sleepiness was measured with one sleep resistance challenge (Oxford Sleep Resistance [OSLER] test), which tests the ability to stay awake in a darkened and sound-isolated room (18).

Cardiovascular Risk Score

A cardiovascular risk score (Framingham index) was used to objectively assess an individual's 5-year risk of death due to cardiovascular events (19). The risk score is based on 11 factors including age, sex, systolic blood pressure, serum total cholesterol concentration, height, serum creatinine concentration, cigarette smoking, diabetes, left ventricular hypertrophy, and history of stroke and myocardial infarction. The risk score is an integer, with points added for each factor according to its association with risk. The sum score and the corresponding risk of a fatal cardiovascular event were derived from individual patient data according to Pocock and coworkers (19).

Flow-mediated Dilatation

Patients were asked to abstain from alcohol, tobacco, or caffeine on the day measurements were taken. Room temperature and lighting were set

at the same level for all measurements. Flow-mediated dilatation (FMD) measurements were performed by ultrasound scanning according to the method originally described by Celermajer and coworkers (8). Longitudinal images of the brachial artery were obtained with a high-frequency (10.0-MHz) ultrasound scanning probe (Acuson CV70; Siemens Medical Solutions, Inc., Camberley, Surrey, UK) proximal to the antecubital fossa. Two-dimensional images, acquired with electrocardiogram gating, were obtained at baseline with Doppler ultrasound imaging to assess arterial diameter and flow velocity. Reactive hyperemia was then induced by inflation of a pneumatic tourniquet around the forearm to 200 mm Hg for 5 minutes, and repeated arterial diameter and flow velocity measurements were made at maximal dilatation 60 seconds after cuff deflation. To assess endothelial-independent vasodilation, we measured maximal brachial artery diameter 3 minutes after a single sublingual dose of nitroglycerin (NTG, 0.5 mg). All measurements were stored digitally and analyzed offline. Brachial artery diameter was measured automatically at the onset of the R wave with dedicated software (Vascular Research Tools 5; Medical Imaging Applications LLC, Coralville, IA) as previously described and validated (20, 21). The mean values of at least three cardiac cycles were averaged for each time point and results of endothelial-dependent (FMD) and endothelial-independent (NTG) vasodilation were expressed as percent change in arterial diameter from the baseline diameter.

Pulse Wave Analysis

Radial artery pulse waveforms were recorded with a pressure tonometer and designated software as previously described (SphygmoCor; At-Cor Medical, Sydney, Australia) (www.atcormedical.com) (22). Briefly, mean values of approximately 10 radial pulse waves are used to generate a corresponding central aortic pressure waveform with a validated mathematical transfer function (23). The software uses an algorithm to determine the inflection point of the aortic pressure waveform, which corresponds to the onset of the reflected wave returning from peripheral arteries, and divides the aortic pressure wave into an early and late systolic peak. The augmentation index (AI_x) quantifies augmentation of central aortic pressure (due to the reflected component of the pulse pressure waveform), which typically increases with age as the arteries become stiffer (or less compliant) (24). AI_x is calculated as the difference between the second (P2) and first systolic peak pressure (P1), expressed as percentage of the central pulse pressure (PP): $AI_x (\%) = [(P2 - P1) / PP] \times 100$. As heart rate influences AI_x , all values of AI_x were corrected to 75 beats/minute as previously described (25). In essence, the faster the pulse wave returns from the periphery, the stiffer the arteries must be, and the higher the calculated augmentation index.

First, a baseline measurement of AI_x was performed as a measure of arterial stiffness. To assess an endothelium-dependent change of AI_x , reactive hyperemia was induced by inflation of a pneumatic tourniquet around the forearm to 200 mm Hg for 5 minutes and a repeated measurement of AI_x was performed between 120 and 180 seconds after cuff deflation. A sublingual dose of 0.5 mg of NTG was then administered for 3 minutes to assess endothelium-independent vascular reaction, followed by a repeated measurement of AI_x after 4 minutes. Previous studies have shown that 20 minutes is sufficient for hemodynamic changes after NTG to return to baseline (26). Therefore, after a 30-minute break, 400 μ g of salbutamol was administered by inhalation as an alternative method to induce endothelium-dependent vascular dilatation (26), and AI_x was remeasured after 10 minutes; at this time the peak plasma concentration of salbutamol occurs (26).

A minimum of 10 radial artery pulse waveforms is required by the software to calculate the AI_x and also to derive an operator index (indicating the quality and reproducibility of the arterial signal) (SphygmoCor; At-Cor Medical). Several estimates of AI_x were performed on each occasion and the measurement with the highest operator index was used for statistical analysis.

Blood Pressure

All patients had office blood pressure measured in the sitting position, after a period of rest for 5 minutes, with a standard digital automatic monitor (Omron Healthcare Co., Kyoto, Japan). The mean value of three readings was used for analysis.

Participants were asked to measure their ambulatory blood pressure at home three times per day in triplicate on seven continuous days

with a standard digital automatic monitor (Omron Healthcare Co.) in the sitting position after a period of rest for 5 minutes.

The mean value of the 7 days of readings was used for analysis.

Data Analysis

Data are expressed as means (SD) if data were normally distributed and as medians (interquartile range) if data were not normally distributed. All statistical analyses were performed with Statistica version 6.0 (StatSoft, Tulsa, OK). Differences between the OSA and control groups in terms of patients characteristics, blood pressure, endothelial function, and arterial stiffness were compared by independent *t* tests if data were normally distributed, or by the Mann-Whitney U test if data were not normally distributed. For comparison of frequencies, the χ^2 test of independence was used. Spearman's rank test was used for correlation analysis. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

Subject Characteristics

Sixty-four patients with minimally symptomatic OSA and 15 control subjects without OSA were recruited. Control subjects were well matched for age, sex, body mass index, waist-to-hip ratio, and cardiovascular risk profile. As expected, patients with OSA had a higher ODI, a greater neck circumference, and tended to have a higher score on the Epworth Sleepiness Scale although objective sleepiness assessed by the OSLER test was similar in the two groups (Table 1).

Blood Pressure

Mean office blood pressure, and blood pressures averaged from the 7-day period of ambulatory measurements, were similar in patients with OSA and in control subjects (Table 2). Systolic office blood pressure, and averaged 7-day systolic blood pressure, tended to be higher in patients with minimally symptomatic OSA than in control subjects (Table 2).

TABLE 1. PATIENT CHARACTERISTICS

	Patients with OSA (n = 64)	Control Subjects without OSA (n = 15)	P Value
Age, yr	57.9 (6.8)	58.1 (6.9)	0.91
BMI, kg/m ²	32.9 (6.2)	31.7 (2.6)	0.74
Waist-to-hip circumference ratio	0.97 (0.06)	0.97 (0.06)	0.39
Neck circumference, cm	43.7 (4.2)	40.8 (3.0)	0.01
Females, %	10.9	13.3	0.79
Current smokers, %	12.5	13.3	0.93
Ex-smokers,* %	40.6	33.3	0.66
Hypertension,* %	40.6	46.7	0.67
Diabetes,* %	15.6	6.7	0.37
CAD,* %	9.4	13.3	0.65
Cardiovascular risk score,† %	2.5 (3.8)	3.0 (4.6)	0.68
Total cholesterol, mmol/L	5.4 (1.2)	5.2 (1.2)	0.68
Fasting glucose, mmol/L	5.9 (1.2)	6.0 (0.8)	0.80
Antihypertensive medication, %	40.6	40.0	0.97
Cholesterol-lowering medication, %	32.8	40.0	0.60
Glucose-lowering medication, %	14.1	6.7	0.44
Oxygen saturation dips >4% (per h of sleep)	23.1 (15.6)	2.9 (1.1)	<0.0001
Minimal nocturnal oxygen saturation, %	78.5 (8.0)	90.0 (2.1)	<0.0001
ESS	8.5 (3.8)	6.5 (3.4)	0.06
OSLER test, min	32.6 (10.8)	35.2 (10.2)	0.41

Definition of abbreviations: BMI = body mass index; CAD = coronary artery disease; ESS = Epworth Sleepiness Scale; OSA = obstructive sleep apnea; OSLER = Oxford Sleep Resistance.

Values represent means (SD).

* Defined by medical history.

† Cardiovascular risk score estimates the risk of death (in percent) in the next 5 years due to a cardiovascular event.

TABLE 2. BLOOD PRESSURE MEASUREMENTS AND BASELINE AUGMENTATION INDEX

	Patients with OSA (n = 64)	Control Subjects without OSA (n = 15)	P Value
Office systolic BP	140.4 (18.0)	131.7 (17.5)	0.09
Office diastolic BP	84.3 (9.0)	81.3 (8.2)	0.23
Office mean BP	102.0 (11.2)	98.1 (10.2)	0.12
7-d systolic BP	130.6 (10.1)	125.2 (13.2)	0.08
7-d diastolic BP	81.1 (7.9)	79.6 (5.8)	0.49
7-d mean BP	97.6 (7.9)	94.8 (7.4)	0.21
AI _x baseline* (%)	26.0 (19.0 to 29.5)	21.0 (8.0 to 27.0)	0.04

Definition of abbreviations: AI_x = augmentation index; BP = blood pressure (mm Hg); OSA = obstructive sleep apnea.

Values represent means (SD) unless otherwise indicated.

* Medians (interquartile range); 60 patients with OSA and 15 control subjects could be assessed. AI_x is corrected to 75 beats/minute.

Flow-mediated Dilatation

Mean baseline brachial artery diameter was 4.7 (0.8) mm in patients with OSA and 4.3 (0.6) mm in the control group (mean difference, +0.4 mm; 95% confidence interval [CI], -0.04 to +0.84 mm; *P* = 0.13). Endothelial function, as assessed by measurement of flow-mediated dilatation of the brachial artery, was impaired in patients with OSA (mean FMD, 5.0 [2.7]%) compared with control subjects (mean FMD, 7.5 [3.3]%) (mean difference, 2.5%; 95% CI, -4.1 to -0.9%; *P* = 0.003) (Figure 1). As would be expected, mean NTG-induced endothelium-independent vasodilation was not different between patients with OSA and control subjects: 14.1 (5.0)% and 15.7 (6.1)%, respectively (mean difference, -1.6%; 95% CI, -4.6 to 1.4%; *P* = 0.28). Flow-mediated dilatation was correlated with the cardiovascular risk score (*r* = -0.36; *n* = 79; 95% CI, -0.54 to -0.15; *P* = 0.001), but there was no significant correlation between FMD and ODI.

Pulse Wave Analysis

Pulse wave analysis could be performed in 60 patients with OSA (measurements were not possible in 4 patients with atrial fibrillation) and 15 control subjects. Arterial stiffness, assessed by AI_x, was higher in patients with OSA compared with matched control subjects (Table 2). The endothelial-dependent changes in AI_x after ischemia-induced hyperemia and application of salbutamol were greater in control subjects than in patients with OSA (Table 3). As expected, the endothelial-independent response to

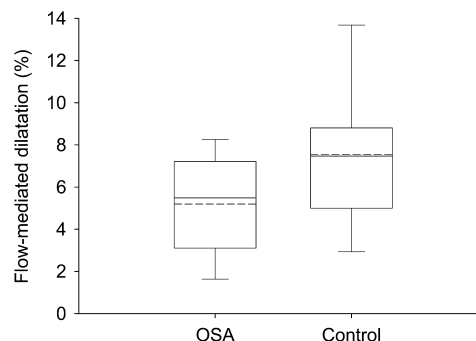


Figure 1. Endothelial function, assessed by flow-mediated dilatation of the brachial artery, was significantly impaired in patients with minimally symptomatic obstructive sleep apnea (OSA) compared with matched control subjects without OSA. Box extremities, 25th and 75th percentiles; error bars, 10th and 90th percentiles; dashed line in box, mean; solid line, median.

TABLE 3. CHANGES IN AUGMENTATION INDEX AFTER FOREARM ISCHEMIA, NITROGLYCERIN, AND SALBUTAMOL

	Patients with OSA (n = 60)	Control Subjects without OSA (n = 15)	P Value
ΔAI_x after ischemia, %	-2.0 (-5.0 to +4.0)	-6.0 (-8.0 to -5.0)	0.005
ΔAI_x after NTG, %	-15.0 (-21.0 to -10.0)	-17.0 (-22.0 to -10.0)	0.97
ΔAI_x after salbutamol, %	-3.0 (-7.0 to 0.0)	-7.0 (-10.0 to -3.0)	0.04

Definition of abbreviations: ΔAI_x = change in augmentation index from baseline; NTG = nitroglycerin; OSA = obstructive sleep apnea.

Values represent medians (interquartile range).

All values of AI_x are corrected to 75 beats/minute.

NTG was not different between the two groups (Table 3). AI_x at baseline was correlated with ODI ($r = 0.30$; 95% CI, 0.08 to 0.50; $P = 0.01$).

DISCUSSION

To our knowledge, this is the first well-controlled, cross-sectional study on endothelial function, arterial stiffness, and blood pressure in patients with minimally symptomatic obstructive sleep apnea. We found that patients with minimally symptomatic obstructive sleep apnea had impaired endothelial function, assessed by flow-mediated dilatation and pulse wave analysis measurements, and increased arterial stiffness, compared with matched control subjects without obstructive sleep apnea. However, both office and averaged 7-day mean ambulatory blood pressure were similar in patients with minimally symptomatic OSA and control subjects.

We found that endothelial function assessed by two different techniques was impaired in patients with minimally symptomatic OSA compared with well-matched control subjects without OSA. The average flow-mediated dilatation (FMD) was 5% in the OSA group, which is lower than the reported FMD in a healthy control population (8). Moreover, the observed difference in FMD between the patients with OSA and matched control subjects was 2.5% (see Figure 1) in our study; the size of this difference has been shown to be of clinical significance in studies looking at the association between flow-mediated dilatation and subsequent cardiovascular events (27, 28). In agreement with our study, Ip and coworkers (5) found that FMD was similarly impaired (FMD was 5.3%) in 28 patients with moderate-severe OSA without comorbidities, as was the observed difference in FMD (3%) between subjects with and without OSA. One of the potential underlying mechanisms for endothelial dysfunction in patients with OSA seems to be a downregulation of endothelial nitric oxide (NO) synthase as a result of increased oxidative stress (29), excessive arterial wall shear stress caused by recurrent surges in blood pressure during apneic events (3), as well as increased endothelial cell apoptosis (30), and increased levels of coagulation factors and cholesterol (31).

The augmentation index, a measure of central arterial stiffness and pressure wave reflection, independently predicts cardiovascular events in high-risk populations (32). In our study, the augmentation index was significantly higher in patients with minimally symptomatic OSA compared with matched control subjects. The observed difference is comparable in size to the effect seen after 4 weeks of CPAP therapy in patients with moderate-severe OSA (11). The mechanism responsible for the increase in arterial stiffness is speculative at present. A higher sympathetic activity, due to recurrent sleep fragmentation or intermittent hypoxia, may contribute to the observed increase in

arterial stiffness (11). Alternatively, the augmentation index has been shown to increase with the plasma level of asymmetric dimethylarginine (an endogenous inhibitor of endothelial NO synthase), suggesting that impaired endothelial function secondary to a lower bioavailability of NO may be a further cause for increased arterial stiffness in patients with OSA (5).

Consistent with an impaired FMD, we found that endothelial function assessed by pulse wave analysis (change in augmentation index [AI_x] induced by both salbutamol and reactive hyperemia) was worse in patients with minimally symptomatic OSA than in matched control subjects. In contrast to FMD measurements, the augmentation index reflects the vascular function of a composite of the whole arterial tree (33), and therefore the decreased endothelial-dependent change in AI_x we found in OSA reflects impaired endothelial function of differently sized arteries compared with FMD. To our knowledge, there are no other published studies that have investigated endothelial function in OSA by the same technique.

In contrast to the observed differences in endothelial function and arterial stiffness (measures representing early and sensitive changes in vascular function), we found no statistically significant difference in either office blood pressure or ambulatory blood pressure (measured during a period of 1 wk) between patients with minimally symptomatic OSA and matched control subjects; although there was a trend toward higher systolic blood pressure in patients with OSA (Table 2). This negative finding is supported by our previous work, in which we found that CPAP did not reduce blood pressure in nonsleepy hypertensive patients with moderate-severe OSA (12). However, the current study was not powered to look at blood pressure differences as a primary outcome, and therefore studies including a larger number of patients with minimally symptomatic OSA are needed to clarify this point.

It must be mentioned that although our control group was meticulously matched, neck circumference was greater in patients with OSA although waist-to-hip circumference was not; this might suggest that subtle differences in fat distribution could be a possible confounder as it has been shown that neck circumference may be a better measure of upper body obesity in some circumstances and is strongly correlated with cardiovascular risk (34).

In conclusion, this controlled cross-sectional study has shown that in patients with minimally symptomatic OSA, diverse properties of endothelial function are impaired, and arterial stiffness is increased, when compared with well-matched control subjects without OSA. Although this was not associated with significantly increased blood pressure, the findings of this study suggest that patients with minimally symptomatic OSA are at increased cardiovascular risk, as has been demonstrated in more severe disease. However, final proof that this association is causal, and not due to confounders, must await a randomized and controlled intervention study.

Conflict of Interest Statement: M.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.J.O.D. has no personal conflict of interest. ResMed UK made an unrestricted charitable donation to support research work in the Oxford Sleep Unit in 2006 and provides CPAP machines for a currently running multicenter trial. J.R.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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