Effects of short-term continuous positive airway pressure withdrawal on cerebral vascular reactivity measured by blood oxygen level-dependent magnetic resonance imaging in obstructive sleep apnoea: a randomised controlled trial

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Withdrawing continuous positive airway pressure therapy and the resulting recurrence of obstructive sleep apnoea did not result in a significant reduction in cerebral vascular reactivity, despite clinically relevant increases in blood pressure http://ow.ly/T2QD30mU6L2

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ABSTRACT Impaired cerebral vascular reactivity (CVR) increases long-term stroke risk. Obstructive sleep apnoea (OSA) is associated with peripheral vascular dysfunction and vascular events. The aim of this trial was to evaluate the effect of continuous positive airway pressure (CPAP) withdrawal on CVR.

41 OSA patients (88% male, mean age 57±10 years) were randomised to either subtherapeutic or continuation of therapeutic CPAP. At baseline and after 2 weeks, patients underwent a sleep study and magnetic resonance imaging (MRI). CVR was estimated by quantifying the blood oxygen level-dependent (BOLD) MRI response to breathing stimuli.

OSA did recur in the subtherapeutic CPAP group (mean treatment effect apnoea–hypopnoea index +38.0 events·h^{-1}, 95% CI 24.2–52.0; p<0.001) but remained controlled in the therapeutic group. Although there was a significant increase in blood pressure upon CPAP withdrawal (mean treatment effect +9.37 mmHg, 95% CI 1.36–17.39; p=0.023), there was no significant effect of CPAP withdrawal on CVR assessed *via* BOLD MRI under either hyperoxic or hypercapnic conditions.

Short-term CPAP withdrawal did not result in statistically significant changes in CVR as assessed by functional MRI, despite the recurrence of OSA. We thus conclude that, unlike peripheral endothelial function, CVR is not affected by short-term CPAP withdrawal.

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This study is registered at clinicaltrials.gov with identifier NCT02493673. Data are available from the Dept of Pulmonology, University Hospital Zurich, after approval from the Local Ethics Committee Zurich, Switzerland, and from the applicants' own institution, and with agreement from the PI of the study, M. Kohler. Due to ethical reasons and the privacy of the patients, the data are available upon request. Requests for the data may be sent to the corresponding author.

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Introduction

In population-based studies, the prevalence of moderate-to-severe sleep-disordered breathing in the middle-aged population is estimated to be between 9% and 23% in women and between 17% and 50% in men [1, 2]. Obstructive sleep apnoea (OSA), characterised by complete or partial obstruction of the upper airway, causes intermittent hypoxia, hypercapnia, increased sympathetic nervous system activity, surges in blood pressure (BP) and impaired peripheral vascular function [3, 4]. OSA is associated with increased risk of stroke, cardiovascular events, heart failure and impaired neurocognitive function [5–7]. However, the underlying pathophysiological mechanisms are poorly understood. Contrasting findings exist about cerebral vascular reactivity (CVR) and cerebral blood flow (CBF) deregulation in OSA patients. How these impairments contribute to the risk of stroke remains to be clarified. The use of different imaging techniques and statistical thresholds and the lack of standardised measurement methods make it difficult to compare results of previous studies [8–17].

Functional magnetic resonance imaging (MRI) during respiratory challenges allows estimation of CVR and CBF [18]. Blood oxygenation level-dependent (BOLD) MRI is able to detect magnetic field variations induced by changes in oxyhaemoglobin and deoxyhaemoglobin concentrations. The BOLD signal responds to changes in the arterial gas concentration induced by the administration of gas mixtures or breath holding [18]. While pure oxygen inhalation mostly induces a change in the deoxyhaemoglobin concentration and, in turn, a BOLD signal increase, the administration of gas mixtures containing carbon dioxide (CO_2) results in an additional vascular modulation of the BOLD response [19].

Continuous positive airway pressure (CPAP) therapy is sometimes interrupted, *e.g.* during upper airway infections or during holidays. These interruptions, and thus recurrence of OSA, might impair CVR and CBF by several possible mechanisms, *e.g.* impaired endothelial function, augmented sympathetic activity and an increase in oxidative stress due to intermittent hypoxia as well as reduced cerebral tissue oxygenation [3, 20]. During apnoeic episodes, increases in intracranial pressure, correlating with systemic BP fluctuations, could result in an excess of flow in brain vessels following apnoea termination, leading to capillary damage, because brain tissue is sensitive to rapid reperfusion [21]. Information on CVR in OSA patients assessed *via* BOLD MRI is sparse and originates mainly from small and mostly case–control studies [8, 11, 15, 17].

We conducted a 2-week CPAP withdrawal randomised controlled trial in patients with moderate-to-severe OSA to examine the link between OSA, CVR and brain perfusion, respectively. We hypothesised that CPAP withdrawal would result in a reduction of daytime CVR and CBF.

Methods

Trial design

This randomised, double-blind, placebo-controlled, parallel-group trial (therapeutic *versus* subtherapeutic CPAP) included 41 patients with moderate-to-severe OSA. Patients had been treated with CPAP for at least 1 year. Dynamic MRI acquisitions were performed between 07:00 h and 09:00 h during the inhalation of medical air (MA) (*i.e.* 21% O_2), oxygen (99.5% O_2) and carbogen (5% CO_2 + 95% O_2). We refer to the conditions induced by MA, oxygen and carbogen as "normoxia", "hyperoxia" and "hypercapnia", respectively. Gases were administered in blocks of 3 min each (MA-oxygen-MA-oxygen-MA-carbogen-MA). Participants wore a mask with a one-way valve and a 0.5-L reservoir bag; the mask covered the mouth and nose completely and was tightly adjusted to the face. We requested that the subjects breathed normally; gas flow rates were set to 10 $L \cdot min^{-1}$ each (supplementary figure e1).

Subjects

Participants were eligible if they met the following inclusion criteria: 1) age between 20 and 75 years; 2) apnoea–hypopnoea index (AHI) and/or oxygen desaturation index ($ODI_{4\%}$) $\geqslant$20 events$\cdot h^{-1}$ in their in-laboratory sleep study at the time of diagnosis; 3) treated with CPAP for at least 1 year with high

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compliance (device usage ⩾4 h per night on at least 80% of the past 365 days with a current AHI ⩽10 events·h^{-1} on treatment, measured from the CPAP machine download data); and 4) an ODI4% ⩾15 events·h^{-1} from current nocturnal pulse oximetry studies during a preliminary 5-night period off CPAP treatment. The trial was approved by the local ethics committee (KEK-ZH-No.2014-0684), and all procedures in this trial involving human participants were performed in accordance with Good Clinical Practice guidelines. The trial was registered prior to commencement (ClinicalTrials.gov identifier: NCT02493673). See supplementary material for details.

Patient evaluation and follow-up

Recruitment started in June 2015 and the last follow-up was completed in December 2017. Once the persistence of relevant OSA was confirmed (number of oxygen desaturations ⩾4% (ODI4%) ⩾15 events·h^{-1}) by home overnight pulse oximetry (Pulsox-300i; Konica Minolta Sensing Inc., Osaka, Japan) during the preliminary 5-night period off CPAP, patients resumed CPAP therapy for at least 2 weeks. An MS-DOS program (MINIM, London, UK) allocated participants using two minimisation criteria: ODI4% <30 or >30 events·h^{-1} and body mass index <35 or >35 kg·m^{-2}. Baseline in-laboratory assessments were performed on all subjects using therapeutic CPAP. Follow-up assessments were performed after 2 weeks on either therapeutic (control arm) or subtherapeutic (intervention arm) CPAP settings. Participants as well as outcome assessors remained blinded to the treatment assignment until completion of the data analysis.

Sleep studies and CPAP devices

In-hospital respiratory polygraphies (Alice 5 Diagnostics System; Philips Respironics, Pittsburgh, PA, USA) were scored manually according to the American Academy of Sleep Medicine task force criteria [22]. The severity of OSA was quantified using the AHI and the ODI4%. Patients in both groups received the same CPAP device (REMstar Auto A-Flex; Philips Respironics). In the therapeutic group, pressure and mode were set according to the previous individual's settings. In the withdrawal group, subtherapeutic pressure was generated by setting the CPAP device to the lowest pressure, inserting a flow-restricting connector at the machine outlet, and inserting six extra holes in the collar of the tube at the end of the mask to prevent rebreathing of CO_2 (supplementary figures e1 and e2).

Primary outcome

The primary outcome was CVR in response to hyperoxia and hypercapnia of grey matter (GM), white matter (WM) and the whole brain assessed by functional MRI as measures of cerebral endothelial function. See supplementary material for details.

Secondary outcome measures

Before gas administration, CBF was assessed using arterial spin labelling (ASL) MRI.

Participants measured their BP and heart rate (HR) in triplicate every morning; the average of three measurements was used for further analysis.

Subjective sleepiness was assessed using the Epworth Sleepiness Score. See supplementary material for details.

Statistical methods/data analysis

Normally distributed data are expressed as mean±SD, unless stated otherwise. For all outcomes, we calculated an effect size and 95% confidence intervals with a linear regression analysis adjusting for treatment group and baseline measurements of the outcome. A multivariable linear regression adjusting for several variables (*e.g.* sex, age, BP at home and in hospital, AHI, ODI) was also performed to generate and adjust effect size; a two-sided significance level of <0.05 was used to determine statistical significance. The statistical analysis was performed in R (version 3.4.4; R Core Team, Vienna, Austria). See the supplementary material for more information (supplementary table e1).

Results

Trial profile and patient characteristics

The trial flow chart is presented in figure 1. 49 patients were randomised and allocated to therapeutic (n=27) or subtherapeutic (n=22) CPAP for 2 weeks. The two trial arms were similar regarding baseline patient characteristics (table 1).

Effects of CPAP withdrawal on cerebral vascular reactivity

There was no significant effect of short-term CPAP withdrawal on CVR assessed *via* BOLD MRI in GM, WM or whole brain under either hyperoxic or hypercapnic conditions (table 2, supplementary table e2,

supplementary figures e3–e5). As expected, the quantitative BOLD response analysis showed an effect on the signal pattern depending on the applied stimulus and tissue type (figures 2 and 3).

Multivariable linear regression modelling to calculate the treatment effect size, adjusting for baseline measurements as well as age, sex, systolic BP, diastolic BP, HR, AHI and ODI, did not change the results significantly (supplementary tables e1 and e2).

Effects of CPAP withdrawal on secondary outcomes

Cerebral blood flow

A 2-week CPAP withdrawal was not associated with a significant change in CBF assessed *via* functional MRI over GM (mean treatment effect +4.20 mL·100 g·min^{-1}, 95% CI −0.96–9.36; p=0.110) or WM (mean treatment effect −0.62 mL·100 g·min^{-1}, 95% CI −3.90–2.66; p=0.700) (table 3).

Subjective sleepiness

CPAP withdrawal led to a statistically significant increase in the Epworth Sleepiness Score compared with continuing CPAP (mean treatment effect +3.29, 95% CI 0.87–5.72; p=0.009) (table 3).

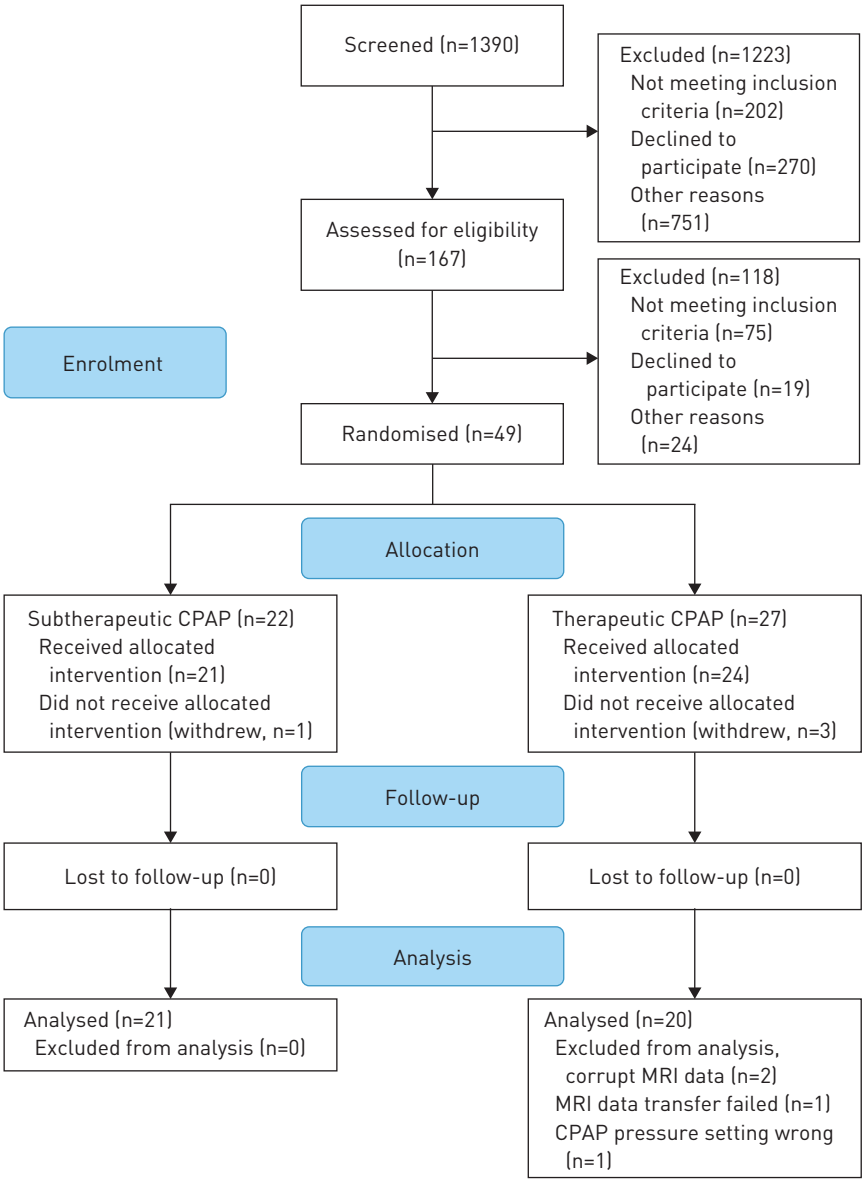

FIGURE 1 CONSORT flow diagram. CPAP: continuous positive airway pressure; MRI: magnetic resonance imaging.

TABLE 1 Baseline characteristics of the trial population

	Subtherapeutic CPAP	Therapeutic CPAP
Subjects n	21	20
Age years	56.2±9.2	57.6±11.1
Male sex	20 (95)	16 (80)
BMI kg·m^{-2}	35.1±5.6	32.8±7.2
Comorbidities		
Hypertension	10 (48)	10 (50)
Diabetes mellitus	6 (29)	4 (20)
Dyslipidaemia	2 (10)	2 (10)
Obesity	17 (81)	14 (70)
Medications		
ACE inhibitor	3 (14)	6 (30)
AT 2 antagonist	5 (24)	2 (10)
Calcium channel blocker	5 (24)	5 (25)
Diuretics	4 (19)	6 (30)
Anit-diabetic drugs	6 (29)	4 (20)
Statins	4 (19)	5 (25)
Other	10 (48)	15 (75)
OSA severity and CPAP usage		
ODI 5 nights off CPAP events·h^{-1}	42.3±23.6	37.0±16.2
AHI under CPAP events·h^{-1}	2.9±2.0	2.1±2.2
CPAP usage % days in 1 year	94.8±6.9	96.0±5.0
CPAP usage h	6.1±1.3	6.4±1.3

Data are presented as n (%) or mean±SD, unless otherwise stated. CPAP: continuous positive airway pressure; BMI: body mass index; ACE: angiotensin converting enzyme; AT 2: angiotensin II blocker; OSA: obstructive sleep apnoea; AHI: apnoea–hypopnoea index; ODI: oxygen desaturation index.

Ambulatory blood pressure and heart rate

Discontinuation of CPAP for 2 weeks compared with continuing CPAP led to a statistically significant increase in systolic BP (mean treatment effect +9.37 mmHg, 95% CI 1.36–17.39; p=0.023) and diastolic BP (mean treatment effect +7.61 mmHg, 95% CI 1.40–13.83; p=0.018). There was a trend towards an increase in HR in the subtherapeutic CPAP group (mean treatment effect +4.01 bpm, 95% CI −0.37–8.40; p=0.071) (table 3).

Effects of CPAP withdrawal on obstructive sleep apnoea

Withdrawal of CPAP was associated with a return of OSA as demonstrated by a significant increase in AHI (mean treatment effect +38.0 events·h^{-1}, 95% CI 24.2–52.0; p<0.0001) and ODI (mean treatment effect +38.0 events·h^{-1}, 95% CI 23.1–53.0; p<0.0001) at 2 weeks (table 3).

TABLE 2 BOLD MRI signal changes during hyperoxic and hypercapnic challenges

BOLD signal change %		Subtherapeutic CPAP group[#]			Therapeutic CPAP group[¶]			Treatment effect[+]	p-value
		Baseline	Follow-up	Change	Baseline	Follow-up	Change		
Grey matter	First O_2	2.61±1.13	2.57±0.87	−0.04±1.14	2.59±0.86	3.11±1.01	0.52±1.02	−0.54 (−1.10–0.02)	0.056
	Second O_2	2.57±0.97	2.57±1.00	0.00±1.24	2.56±1.09	2.85±1.12	0.30±1.28	−0.29 (−0.94–0.37)	0.38
	CO_2	3.49±1.37	3.47±1.21	−0.02±1.18	3.89±2.00	3.52±1.12	−0.37±2.00	0.07 (−0.61–0.75)	0.84
Overall brain	First O_2	2.36±0.81	2.52±0.89	0.16±0.66	2.75±1.53	2.72±0.50	−0.03±1.56	−0.12 (−0.57–0.32)	0.58
	Second O_2	2.24±0.82	2.30±0.79	0.06±0.81	2.47±1.03	2.47±0.72	0.00±1.11	−0.11 (−0.56–0.35)	0.64
	CO_2	2.96±0.99	3.19±1.00	0.22±0.93	3.18±1.14	3.15±0.70	−0.03±1.23	0.10 (−0.42–0.62)	0.70
White matter	First O_2	1.56±0.55	1.58±0.98	0.02±0.86	1.45±0.66	1.64±0.76	0.19±0.97	−0.11 (−0.65–0.43)	0.69
	Second O_2	1.65±0.86	1.68±0.97	0.03±1.05	1.41±0.84	1.36±0.61	−0.05±0.88	0.24 (−0.26–0.75)	0.33
	CO_2	1.90±0.71	2.02±0.96	0.12±1.09	1.87±1.07	1.81±0.92	−0.07±1.36	0.21 (−0.39–0.81)	0.48

Data are presented as mean ±SD or mean (95% CI). BOLD: blood oxygen level-dependent; MRI: magnetic resonance imaging; CPAP: continuous positive airway pressure. [#]: n=21; [¶]: n=20; [+]: treatment effect (mean follow-up measurement in the subtherapeutic CPAP arm minus mean follow-up measurement in the therapeutic CPAP arm), adjusted for baseline of subtherapeutic CPAP.

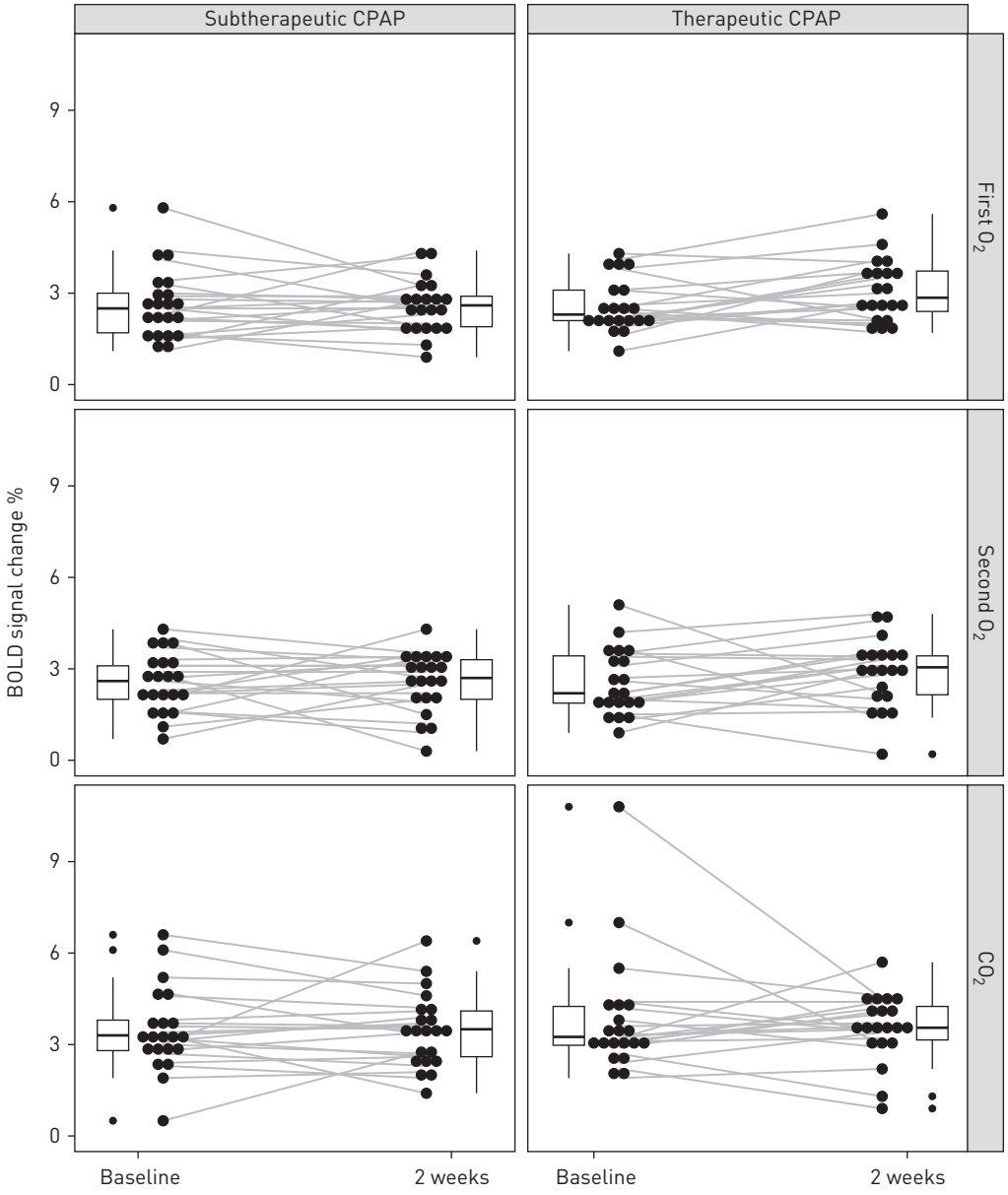

FIGURE 2 Percentage blood oxygen level-dependent (BOLD) signal change in grey matter. The subtherapeutic continuous positive airway pressure (CPAP) group is shown on the left and the therapeutic CPAP group on the right. Boxplots are shown for the first and second hyperoxic stimulus (O_2) as well as the hypercapnic stimulus (CO_2) at baseline and follow-up after 2 weeks.

Discussion

This randomised controlled trial investigated the possible changes in CVR and CBF induced by short-term withdrawal of CPAP in patients with moderate-to-severe OSA. The recurrence of OSA upon CPAP withdrawal was documented by a return of sleep-disordered breathing as well as increased sleepiness and BP. Contrary to our hypothesis, there was no significant effect of short-term CPAP withdrawal on daytime CVR as a measure of cerebral endothelial function or on daytime CBF assessed by functional MRI.

A causal relationship between OSA, peripheral endothelial dysfunction and BP has been demonstrated; these measures of cardiovascular risk improve with CPAP treatment [3, 4]. Further evidence from a randomised controlled trial indicated a protective effect of CPAP against severe nocturnal cerebral hypoxia that was similar in magnitude and duration to values causing cerebral dysfunction during unilateral carotid artery clamping during neurosurgery, assessed *via* near-infrared spectroscopy [20]. Observational studies have also described that the use of CPAP, especially among patients with high treatment adherence, is associated with lower incidence and relative risk reduction of stroke [23, 24]. However, a recent randomised controlled trial did not show a reduction in major cardiovascular endpoints in OSA patients

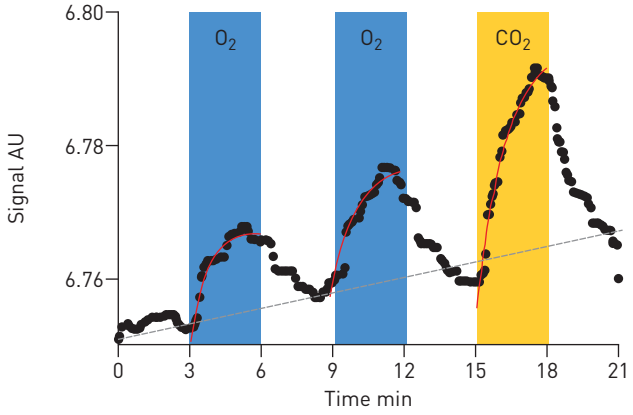

FIGURE 3 Example of a blood oxygen level-dependent (BOLD) signal curve of a random participant. The grey line indicates shift correc-tion of the scanner. The hyperoxic stimuli (O_2) are presented in blue and the hypercapnic stimulus (CO_2) in yellow. The red lines are the mathematically fitted curves. The black dots represent the BOLD signal acquisition. The signal change is shown on the y-axis in arbitrary units (AU).

with manifest cardiovascular disease allocated to CPAP [25]. Therefore, the beneficial effect of CPAP treatment on major cardiovascular outcomes has been subject to extensive discussion [26, 27].

Several techniques have been used to assess CVR, such as transcranial Doppler ultrasonography (TCD) and positron emission tomography (PET) or single photon emission computed tomography [28–30]. However, MRI allows the entire brain to be mapped with high spatial resolution without the use of radiation, and it has higher reliability compared to ultrasound methods [31, 32]. The use of BOLD and ASL MRI enables the investigation of two separate but complementary aspects of vascular regulation. Baseline CBF is age-dependent and higher in the GM than the WM owing to differences in metabolic demands, neural activity and vascular anatomy. Although hyperoxia is believed to be a mild vasoconstrictor, the absence of negative BOLD signal changes implies that O_2-induced vasoconstriction plays a minor role in brain perfusion in contrast to CO_2, which is a strong vasodilator [33–35]. In the literature, the average BOLD response to hyperoxia induced by breathing of 100% O_2 in healthy controls is ~1% in the WM and ~3% in the GM. The overall signal change induced by hypercapnia in the brain, on the other hand, has a mean magnitude of 2–3% [33, 36, 37]. Our results are in line with the literature concerning the dependence of the response on the stimulus and tissue type (table 2, figures 2 and 3).

Assuming that autonomic impairments could contribute to cerebral injury, Macey and colleagues [11, 38] performed different tasks (handgrip, cold stimulus, Valsalva manoeuvre) in treatment-naïve OSA patients based on previously reported time-lagged and weaker HR responses to BP changes. Interestingly, they did not find a significant BOLD signal change during the Valsalva manoeuvre in the OSA group compared to healthy controls. Although HR changes occurred during the challenge in the latter study, the BOLD signal response was not delayed, suggesting that cerebral autoregulatory mechanisms may adopt faster than peripheral autonomic regulatory pathways [11]. Thus, our finding of BOLD responses with a mean

TABLE 3 Secondary outcomes

	Treatment effect after 2 weeks CPAP withdrawal	Adjusted p-value[#]
CBF grey matter mL·100 g·min^{-1}	4.20 (−0.96–9.36)	0.110
CBF white matter mL·100 g·min^{-1}	−0.62 (−3.90–2.66)	0.700
Systolic morning BP mmHg[¶]	9.37 (1.36–17.39)	0.023
Diastolic morning BP mmHg[¶]	7.61 (1.40–13.83)	0.018
Heart rate bpm	4.01 (−0.37–8.40)	0.071
Apnoea–hypopnea index events·h^{-1}	38.0 (24.2–52.0)	<0.001
Oxygen desaturation index events·h^{-1}	38.0 (23.1–53.0)	<0.001
Epworth Sleepiness Scale[+]	3.29 (0.87–5.72)	0.009

Data are presented as mean (95% CI). CPAP: continuous positive airway pressure; CBF: cerebral blood flow; BP: blood pressure; bpm: beats per minute. [#]: adjusted for baseline; [¶]: BP data from 14 days' home measurements; [+]: Epworth Sleepiness Scale has a maximum of 24 points.

magnitude comparable to responses described in healthy controls, as a surrogate measure of CVR, could reflect an underlying regulatory mechanism that includes the cerebral vasculature to ensure blood supply and CVR, hitherto not studied in OSA patients.

In contrast to these results, others have described reductions in CVR in the brainstem during swallowing in OSA patients [8]. This finding was interpreted as a potential contributor to the pathogenesis of OSA because altered brainstem CVR may be involved in the control of upper airway muscles. Prilipko *et al.* [13] described significantly higher CVR in several brain regions in healthy subjects compared to age-matched OSA patients. They found that CPAP treatment in the OSA group led to an improvement in CVR but was not associated with a change of CBF after 2 months. However, the observed changes were not homogeneous and did not follow major vascular territories.

GM CBF values reported in two prior studies were comparable with our results [10, 16]. In addition, decreased as well as increased CBF values in various brain areas of awake, untreated OSA patients assessed by ASL MRI have been described [9, 10, 12–14, 16]. We chose to perform ASL measurements assessing CBF in the absence of evoked responses or challenges. Measuring CBF in this setup is comparable with previous studies [9, 10, 12, 13, 16]. To further refine detection of brain blood flow changes, taking measurements using ASL during respiratory challenges could provide additional information [17].

When interpreting the BOLD signal response, several important considerations need to be taken into account. CO_2 is an important modulator of vascular tone and influences systemic BP *via* the activation of the sympathetic nervous system, which may in turn affect CVR [39, 40]. To address this issue in TCD studies, the cerebrovascular conductance index has been introduced. This index takes the BP into account by dividing the cerebral artery velocity through the mean arterial BP (MAP) [41]. Ryan *et al.* [40] described normal hypercapnic cerebrovascular conductance in OSA patients and, in particular, no overnight decline in conductance CVR. Moreover, they did not find any difference between the OSA and healthy control group regarding the MAP response [40]. Others have stated that interpretation of the CVR results after correcting for BP changes is more accurate in TCD measurements; therefore, continuous monitoring of BP during the scan is desirable [42]. However, it is important to mention that TCD studies themselves are limited in that they measure blood flow velocity, rather than volumetric flow, which is only representative of blood flow if the diameter of the insonated vessel remains constant [43, 44]. To date, all studies assessing OSA and CVR *via* BOLD MRI lack continuous BP recording during the scan [8, 11, 13, 15, 17].

Furthermore, the relaxation of vascular smooth vessels and increase in CBF is not always characterised by a linear relationship, but rather a sigmoidal one, with attenuated responses at the extremes, presuming that BP is constant [18]. When vessels are maximally dilated in response to low systemic BP (*e.g.* hypovolaemia), the vascular response to hypercapnia is subdued. Once the vasodilation mediated through CO_2 has reached its limit, increases in perfusion pressure could lead to passive CBF increases [18, 41].

TCD measurements in OSA subjects using the Duffin rebreathing method showed that the maximum end-tidal CO_2 tension (P_{ETCO_2}) achieved did not differ between OSA and healthy controls [40]. Although rises in P_{ETCO_2} up to 57 mmHg were achieved, most of the responses were not suitable for a sigmoidal fitting [40].

Preliminary results on BOLD signal modelling and vascular resistance describe the possibility of differentiating multiple CBF response patterns based on CO_2 stimuli [45]. While this is an interesting research approach, these models assume that MAP, neural activation and metabolism are constant, which is not the case in a clinical setting [46].

There are several further factors potentially contributing to the complexity of this topic, *e.g.* viscosity and composition of the blood, the role of cardiac output and the rate of cerebral local oxygen consumption [47]. Indeed, direct assessment of CBF, arterial BP and other parameters using invasive methods such as thermal diffusion are only employed in critical ill patients.

We did not measure and adjust for gas concentrations delivered to, or expired by, the subjects. Ponsaing *et al.* [17] measured end-tidal gas concentration during breathing of 5% CO_2-enriched air and breath-holding BOLD MRI. They found no difference between OSA patients and controls in the BOLD response to hypercapnia. Even when adjusting for the change in O_2 and CO_2 levels of the hypercapnia BOLD CVR response, the result was still not significant. However, for any future studies on this topic, we suggest that concentrations of gases delivered to and expired by the subject should be monitored using computerised gas control systems, which provide precise and repeatable sequences of P_{ETCO_2} and P_{ETO_2} [17]. Given that MRI measurements were performed in the morning, we cannot exclude the possibility of CBF or CVR impairment during nocturnal apnoeic episodes. Another possible limitation is the withdrawal period of 2 weeks, which might not be sufficient to show the full extent of OSA recurrence and its consequences on CVR and CBF. Furthermore, the current trial population only consisted of a selected

group of patients with vascular risk factors (*i.e.* hypertension, dyslipidaemia, diabetes) and optimal therapy compliance, but without any known major cerebral vascular pathologies or history of stroke. Thus, there might be a different response to CPAP withdrawal in patients with previous cerebrovascular events.

In conclusion, despite the recurrence of OSA and its immediate effects on BP, we found no effect of CPAP withdrawal on CVR or CBF assessed by functional MRI.

In particular, daytime CVR did not show any significant reduction after 2 weeks of CPAP withdrawal, assuming that CVR regulation outlasts other pathophysiological effects of OSA in the short term. We suggest that other mechanisms besides changes in CVR must be considered to contribute to the increased risk of stroke.

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