Overnight urinary isoprostanes as a marker of oxidative stress in obstructive sleep apnoea

To the Editor:

Oxidative stress is thought to be of importance in the development of cardiovascular disease in obstructive sleep apnoea (OSA). However, our recent study [1] using data from two centres showed that markers of oxidative stress were not increased by the return of OSA caused by continuous positive airways pressure (CPAP) withdrawal for 2 weeks. Unexpectedly, we found decreased levels of urinary isoprostanes in early morning urine specimens and this, combined with increased levels of blood superoxide dismutase, raised the possibility that oxidative stress might be decreased by OSA.

Following on from concerns raised by Monneret and Bonnefont-Rousselot [2], we took the opportunity to look at the levels of urinary isoprostanes in overnight urine samples, rather than early morning samples, which had been collected in a subset of patients from our original trial. This allowed us to address two concerns, the timing of our original samples, and the possible effects of urine dilution on the levels of urinary isoprostanes (by correcting for both creatinine levels and urine production). Isoprostanes are a stable biomarker of oxidative stress [3], but their half-life is short in blood (minutes) and longer in urine (hours) [4]. This additional analysis allowed us to investigate the possibility that urinary isoprostanes might have been increased overnight, with the reductions observed in early morning spot urines reflecting a secondary and compensatory fall.

Here we present data from one of the two centres involved in the original study, a randomised control trial, which was prospectively registered (ISRCTN73047833) and approved by the local ethics committee (National Research Ethics Service committee South West, Exeter, UK; ref 12/SW/0254). We report data from 14 patients included in our original study and from nine further patients undergoing the same protocol. Patients all had OSA with an original oxygen desaturation index (ODI) >4% of >20 events·h^{-1} and had been on CPAP for >1 year with compliance of >4 h·night^{-1}. Patients underwent 1 week of screening oximetry and had an ODI of <10 events·h^{-1} during 3 nights on CPAP, and an ODI >20 events·h^{-1} on at least one out of 4 nights off CPAP. Patients then went back onto their usual CPAP for ⩾2 weeks prior to being randomised to either continue CPAP for 2 weeks (control), or to sham CPAP (CPAP-withdrawal) for 2 weeks.

Patients completed overnight urine collections on the night prior to their baseline visit and on the final night of their treatment period, prior to their 2-week follow-up visit. Nonacidified urine was collected and the start and end times for collection, along with the total urine volume were recorded. Aliquots of urine were then stored at −80°C for later use. Urinary F2-isoprostanes were measured using ELISA (ab175819; Abcam, Cambridge, UK) and urine creatinine was measured from the same samples. The treatment effect of CPAP withdrawal *versus* control on 2-week urinary isoprostane levels was modelled using multivariable linear regression, adjusting for the baseline urinary isoprostane levels, age, sex, body mass index (BMI), smoking status, antihypertensive usage and statin usage. Data are expressed as mean±SD, median (interquartile range) or n (%).

23 patients from Oxford (UK) completed the trial, with 11 randomised to continue CPAP and 12 randomised to CPAP-withdrawal. Patients were of similar age (CPAP group 59.9±6.7 years, CPAP-withdrawal group 61.1±8.9 years), BMI (CPAP 36.8±7.4 kg·m^{-2}, CPAP-withdrawal 36.9±7.5 kg·m^{-2}) and had similar severity OSA during pre-trial screening off CPAP (CPAP 35.2 (23.7–53.3) events·h^{-1}, CPAP-withdrawal 34.4 (25.8–64.6) events·h^{-1}). There were similar numbers of males (CPAP 10 (91%), CPAP-withdrawal 10 (83%)), current smokers (CPAP 1 (9%), CPAP-withdrawal 1 (8%)), patients on

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TABLE 1 Overnight urinary F2-isoprostanes, noncorrected and corrected

	CPAP		CPAP-withdrawal		Treatment effect (95% CI)	p-value
	Baseline	2 weeks	Baseline	2 weeks		
Subjects n	11		12			
Non-corrected F2-isoprostanes ng·mL^{-1}	3.1±1.3	3.4±1.4	3.2±1.4	2.2±0.8	−1.3 (−2.4–−0.1)	0.03
Creatinine mmol·L^{-1}	13.8±10.5	13.8±10.6	11.3±4.3	8.4±2.9	−3.0 (−8.0–1.9)	0.21
Urine volume mL	814±497	889±569	699±312	988±335	205 (−88–499)	0.16
Urinary excretion rate mL·h^{-1}	78.1±43.1	83.9±53.2	57.0±31.1	86.7±28.5	18.9 (−12.3–50.1)	0.22
Corrected F2-isoprostanes/creatinine ratio ng·µmol^{-1}	0.31±0.18	0.37±0.19	0.29±0.11	0.27±0.07	−0.1 (−0.23–0.03)	0.11
F2-isoprostanes production corrected by urine excretion rate ng·h^{-1}	216.6±120.7	251.7±145.8	168.2±116.3	182.8±78.7	−56.9 (−161.0–47.2)	0.26

Data are presented as mean±SD, unless otherwise stated. CPAP: continuous positive airway pressure.

antihypertensive therapy (CPAP 5 (46%), CPAP-withdrawal 7 (58%)) and patients on statin therapy (CPAP 6 (55%), CPAP-withdrawal 7 (58%)) in both groups.

While there was a significant effect of CPAP-withdrawal on noncorrected overnight urinary F2-isoprostanes, there was no significant effect of CPAP withdrawal on corrected F2-isoprostanes, when corrected either by creatinine, or by rate of urine production (table 1). The return of OSA increased overnight urine production, as has been observed previously [5].

Our results show that the return of OSA with CPAP-withdrawal does not lead to an increase in oxidative stress as measured by corrected levels of overnight urinary isoprostanes. By correcting overnight urinary isoprostanes excretion for urine production or urinary dilution, in this smaller subgroup of patients, we did not reproduce our previous finding of decreased urinary F2-isoprostanes levels in early morning spot urines following CPAP withdrawal [1]. In this previous study we did not correct for urinary creatinine, as we were using early morning spot urines in which correction for urinary creatinine can introduce further variability. Others have presented noncorrected levels, and no consensus for reporting has been established [6].

There were nonsignificant increases in urinary volumes and nonsignificant decreases in urinary creatinine levels with CPAP-withdrawal. OSA causes increased production of atrial natriuretic peptide and decreased production of antidiuretic hormone, leading to increased urinary production and urinary dilution [7]. Therefore, collection of overnight urine and correction for urine creatinine, or rate of urine production, seems sensible in future OSA trials measuring isoprostanes to allow for any effect of urinary dilution.

There are limitations to this study. Patient OSA severity was monitored using oximetry and therefore flow-limited events leading to arousal, but not desaturation, could have been missed. However, our hypothesis was that intermittent hypoxia was inducing oxidative stress and this is adequately assessed by oximetry. Secondly, CPAP was only withdrawn for 2 weeks and this may be insufficient time to observe the deleterious consequences of OSA. However, we have observed a compensatory increase in superoxide dismutase at 2 weeks, suggesting that changes in oxidative stress are occurring [1], along with clear changes in blood pressure, heart rate, catecholamines and endothelial function [8].

In our original study we found no changes in key biomarkers of oxidative stress (malondialdehyde, lipid hydroperoxides and total antioxidant capacity), and here we report no changes in overnight creatinine-corrected isoprostanes. Similarly, another study found no changes in overnight creatinine-corrected urinary F2-isoprostanes during CPAP treatment, compared with sham CPAP [9]. In addition, in our original study, we found increased superoxide dismutase levels. Any increase in tissue oxygen radical generation appears to be eliminated by the increased superoxide dismutase *via* a "preconditioning-type effect", which may explain why systemic markers of oxidative stress do not change. The findings of this current study are different to our original conclusion, that oxidative stress may be reduced by CPAP withdrawal. However, our current findings provide further evidence that either oxidative stress is not increased overnight by OSA, or that a compensatory increase in superoxide dismutase more than compensates for any increased reactive oxygen species generation in the tissues. Therefore, other mechanisms may be responsible for any development of cardiovascular disease in OSA.

Chris D. Turnbull[1,2], Ioannis Akoumianakis[3], Charalambos Antoniades[3] and John R. Stradling[2]
[1]Oxford Centre for Respiratory Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK. [2]NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK. [3]Division of Cardiovascular Medicine, Radcliffe Dept of Medicine, University of Oxford, Oxford, UK.

Correspondence: Chris D. Turnbull, Oxford Centre for Respiratory Medicine, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LJ, UK. E-mail: christopher.turnbull@ouh.nhs.uk

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