

Overnight urinary isoprostanes as a marker of oxidative stress in OSA

Chris D. Turnbull^{1,2}, Ioannis Akoumianakis³, Charalambos Antoniades³, John R. Stradling²

1. Oxford Centre for Respiratory Medicine, Oxford Universities NHS Foundation Trust, Oxford, United Kingdom

2. NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom

3. Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

Corresponding author: Dr Christopher D Turnbull

Oxford Centre for Respiratory Medicine

Churchill Hospital

Old Road

Headington

Oxford

OX3 7LJ

christopher.turnbull@ouh.nhs.uk

Tel. 01865 226767

Word count: 1117

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 published in this journal, 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 two weeks.[1] 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, which had been collected in a subset of patients from our original trial. This would allow 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 also 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 local ethics committee (NRES Committee South West – Exeter, UK Ref 12/SW/0254). We report data from 14 patients included in our original study and from 9 further patients undergoing the same protocol. Patients all had OSA with an original oxygen desaturation index > 4% (ODI) of > 20/ h and had been on CPAP for > 1 year with compliance of > 4 h/n. Patients underwent one week of screening oximetry and had an ODI of < 10/ h during 3 nights on CPAP, and an ODI > 20/ h on at least 1 of 4 nights off CPAP. Patients then went back onto their usual CPAP for at least two weeks prior to being randomised to either continue CPAP for two weeks (control), or to sham CPAP (CPAP-withdrawal) for two 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 two week follow-up visit. Non-acidified 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 by ELISA technique (Abcam, Cambridge, UK, Cat# ab175819) and urine creatinine was measured from the same samples. The treatment effect of CPAP withdrawal versus control on two week urinary isoprostanes levels was modelled using multivariable linear regression, adjusting for the baseline urinary isoprostanes levels, age, gender, BMI, smoking status, antihypertensive usage and statin usage. Data are expressed as mean \pm standard deviation, median (1st quartile, 3rd quartile) or number (%).

Twenty-three patients from Oxford completed the trial with 11 randomised to continue CPAP and 12 randomised to CPAP-withdrawal. Patients were of similar age (CPAP group 59.9 \pm 6.7, CPAP-withdrawal group 61.1 \pm 8.9 years), BMI (CPAP 36.8 \pm 7.4, CPAP-withdrawal 36.9 \pm 7.5 kg/m²), and had similar severity OSA during pre-trial screening off CPAP (CPAP 35.2 (23.7, 53.3), CPAP-withdrawal 34.4 (25.8, 64.6) /h). There were similar numbers of men in both groups (CPAP = 10

(91%), CPAP-withdrawal = 10 (83%)), current smokers (CPAP = 1 (9%), CPAP-withdrawal = 1 (8%)), patients on antihypertensive therapy (CPAP = 5 (46%), CPAP-withdrawal = 7 (58%)) and patients on statin therapy (CPAP = 6 (55%), CPAP-withdrawal = 7 (58%)).

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

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

Table 1: Overnight urinary F2-isoprostanes, non-corrected and corrected. CPAP: Continuous positive airways pressure. Data expressed as mean (standard deviation). 95%CI=95% confidence interval.

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 also presented non-corrected levels and no consensus for reporting has been established.[6]

There were non-significant increases in urinary volumes and non-significant decreases urinary creatinine levels with CPAP-withdrawal. OSA causes increased atrial natriuretic peptide and decreased anti-diuretic hormone production 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 of this study. Patients' OSA severity was monitored by 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 two 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 two weeks, suggesting 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, total antioxidant capacity), and here we report no changes in overnight creatinine-corrected isoprostanes. Similarly, a recent study found no changes in overnight creatinine-corrected urinary F2-isoprostanes during CPAP treatment, compared with sham CPAP.[8] In our original study we also found increased superoxide dismutase levels. Any increase in tissue oxygen radical generation appears to be eliminated by the increased superoxide dismutase via a "pre-conditioning-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.

References

1. Stradling JR, Schwarz EI, Schlatzer C, Manuel AR, Lee R, Antoniadou C, Kohler M. Biomarkers of oxidative stress following continuous positive airway pressure withdrawal: data from two randomised trials. *Eur Respir J*. 2015; 46: 1065–71.
2. Monneret D, Bonnefont-Rousselot D. Paradoxical decrease in isoprostane and increase in superoxide dismutase following CPAP withdrawal in OSA. *Eur Respir J*. 2016; 47: 1012–4.
3. Lee R, Margaritis M, Channon KM, Antoniadou C. Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Curr Med Chem*. 2012; 19: 2504–20.
4. Basu S. Metabolism of 8-iso-prostaglandin F2 α . *FEBS Lett*. 1998; 428: 32–6.
5. Warley AR, Stradling JR. Abnormal diurnal variation in salt and water excretion in patients with obstructive sleep apnoea. *Clin Sci Lond Engl* 1979. 1988; 74: 183–5.
6. Antoniadou C, Lee R, Kohler M, Stradling J. Paradoxical decrease in isoprostane and increase in superoxide dismutase following CPAP withdrawal in OSA. *Eur Respir J*. 2016; 47: 1014–5.
7. Ichioka M, Hirata Y, Inase N, Tojo N, Yoshizawa M, Chida M, Miyazato I, Tanai S, Marumo F. Changes of circulating atrial natriuretic peptide and antidiuretic hormone in obstructive sleep apnea syndrome. *Respir Int Rev Thorac Dis*. 1992; 59: 164–8.

8. Kohler M, Steowhas AC, Ayers L, Senn O, Block KE, Russi EW, Stradling JR. Effects of continuous positive airway pressure therapy withdrawal in patients with obstructive sleep apnea: a randomized controlled trial. *Am J Respir Crit Care Med*. 2011; 184: 1192-9.
9. Paz Y Mar HL, Hazen SL, Tracy RP, Strohl KP, Auckley D, Bena J, Wang L, Walia HK, Patel SR, Mehra R. Effect of Continuous Positive Airway Pressure on Cardiovascular Biomarkers: The Sleep Apnea Stress Randomized Controlled Trial. *Chest*. 2016; 150: 80–90.