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Conflict of interest: None declared.

References

1 Stradling JR, Schwarz EI, Schlatzer C, *et al.* Biomarkers of oxidative stress following continuous positive airway pressure withdrawal: data from two randomised trials. *Eur Respir J* 2015; 46: 1065–1071.
2 Del Ben M, Fabiani M, Loffredo L, *et al.* Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea and the effect of continuous positive airway pressure treatment. *BMC Pulm Med* 2012; 12: 36.
3 Alonso-Fernández A, García-Río F, Arias MA, *et al.* Effects of CPAP on oxidative stress and nitrate efficiency in sleep apnoea: a randomised trial. *Thorax* 2009; 64: 581–586.
4 Minoguchi K, Yokoe T, Tanaka A, *et al.* Association between lipid peroxidation and inflammation in obstructive sleep apnoea. *Eur Respir J* 2006; 28: 378–385.
5 Monneret D. Isoprostane as oxidative stress biomarker in the VARIAFIT study: analytical and methodological considerations. *Diabetes Metab* 2014; 40: 316–317.
6 Mamo LB, Suliman HB, Giles BL, *et al.* Discordant extracellular superoxide dismutase expression and activity in neonatal hyperoxic lung. *Am J Respir Crit Care Med* 2004; 170: 313–318.
7 Klawitter J, Haschke M, Shokati T, *et al.* Quantification of 15-F2t-isoprostane in human plasma and urine: results from enzyme-linked immunoassay and liquid chromatography/tandem mass spectrometry cannot be compared. *Rapid Commun Mass Spectrom* 2011; 25: 463–468.
8 Arab A, Wang J, Bausch K, *et al.* Transient hyperoxic reoxygenation reduces cytochrome C oxidase activity by increasing superoxide dismutase and nitric oxide. *J Biol Chem* 2010; 285: 11172–11177.
9 Kinnula VL, Crapo JD. Superoxide dismutases in the lung and human lung diseases. *Am J Respir Crit Care Med* 2003; 167: 1600–1619.
10 Chen CF, Tsai SY, Ma MC, *et al.* Hypoxic preconditioning enhances renal superoxide dismutase levels in rats. *J Physiol* 2003; 552: 561–569.

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From the authors:

We thank Monneret and Bonnefont-Rousselot for their commentary. Our controlled trial design overcomes most of their concerns; any failure of assay techniques would not have produced a biased result, but in fact would have decreased the chances of finding any between-group effects. In our study we observed a statistically significant decrease in urinary F2-isoprostanes following the withdrawal of continuous positive airway pressure (CPAP) treatment in patients with obstructive sleep apnoea (OSA), in a randomised, 2-week trial, where those involved in the assays were entirely blind to group allocation [1]. In addition to this unexpected result, we observed no significant change in any plasma marker of oxidative stress we measured in this population (malondialdehyde and lipid hydroperoxides) or even the production of superoxide radicals by peripheral blood mononuclear cells. It is important to note that these were morning measurements.

Regarding the methods of F2-isoprostane analysis, we used ELISA to measure F2-isoprostane because immunoassays are a widely used alternative for isoprostane analysis. The assay we chose for this measurement (Oxford Biomedical, Oxford, UK) is validated against gas chromatography–mass spectrometry (GC-MS), and shows high correlation between the two methods [2]. Further, measurement by GC-MS is not required, particularly in the setting of interventional controlled clinical trials where paired samples are analysed.

Regarding the correction of F2-isoprostanes for urinary creatinine excretion this is important in measurements of 24 h (or overnight) urine collections to account for dehydration of the participants, and it is less relevant in spot urine measurement of F2-isoprostanes, where measurement of creatinine could on its own introduce further variability. To date, relatively few studies have evaluated biomarker performance by both absolute and normalised concentration, and there is no consensus on how data should be reported [3]. It has also been shown that normalising to creatinine may briefly amplify the biomarker signal soon after a reduction in glomerular filtration rate [4]. Non-urinary creatinine-corrected F2-isoprostane levels are also reported in the literature, as highlighted by another randomised clinical trial which also measured F2-isoprostane in serial urine samples [5].

Regarding the estimation of superoxide dismutase (SOD), this can be derived from the measurement of its enzymatic activity (which is a highly variable assay and it is preferred when assessing acute interventions) or from the measurement of its total concentration (when testing the effects of non-acute interventions that modulate gene expression, such as in our study). In any case, SOD protein levels in plasma may be more reflective of the protein levels in the tissues of interest, rather than its enzymatic activity in the plasma.

The hypoxic/hyperoxic preconditioning mechanism we propose is only advanced as a hypothesis, as this study was not designed to specifically answer this question. As detailed in the paper, this was an exploratory analysis

and thus it should be investigated further with properly designed mechanistic studies where this is the primary outcome. Although there was a simultaneous increase of plasma concentration of SOD and fall in urinary F2-isoprostanes in this trial, we cannot claim causality between the two. These are relatively crude biomarkers and do not reflect the oxidation state at the level of specific tissue types (*e.g.* the lungs or the heart).

To conclude, despite the general belief that OSA increases oxidative stress and CPAP reduces it, the randomised and controlled clinical trials assessing this concept in patients with OSA are limited, and are either uncontrolled or include smaller numbers of subjects, and the results are variable. The "unexpected" results of our randomised and controlled interventional 2-week study are clear, and the actual assay results not in our view "questionable". The most important limitation we fully acknowledge in this (and all other clinical trials using systemic oxidative stress as a readout), is the absence of any reliable circulating or urinary biomarkers of oxidative stress that accurately describe tissue oxidation [6, 7]. In the OSA model, for example, oxidation in the lung tissue may be completely different to systemic oxidation in the cardiovascular system or the kidneys, and this may also explain the discrepancy between the various studies. Finally, as we fully acknowledged in the paper, this was a 2-week study and the situation may well be different in the longer term.

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Paradoxical decrease in isoprostane and increase in superoxide dismutase following CPAP withdrawal in OSA http://ow.ly/UcgYP

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References

1 Stradling JR, Schwarz EI, Schlatzer C, *et al.* Biomarkers of oxidative stress following continuous positive airway pressure withdrawal: data from two randomised trials. *Eur Respir J* 2015; 46: 1065–1071.
2 Callewaert DM, Sloan C. Enzyme immunoassay of isoprostanes. *Methods Mol Biol* 2010; 610: 435–449.
3 Ralib AM, Pickering JW, Shaw GM, *et al.* Test characteristics of urinary biomarkers depend on quantitation method in acute kidney injury. *J Am Soc Nephrol* 2012; 23: 322–333.
4 Waikar SS, Sabbisetti VS, Bonventre JV. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. *Kidney Int* 2010; 78: 486–494.
5 Billings FT 4th, Petracek MR, Roberts LJ 2nd, *et al.* Perioperative intravenous acetaminophen attenuates lipid peroxidation in adults undergoing cardiopulmonary bypass: a randomized clinical trial. *PLoS One* 2015; 10: e0117625.
6 Oxford Biomedical Research. Isoprostanes: Biomarkers for Oxidative Stress. www.oxfordbiomed.com/node/2582 Date last accessed: November 11, 2015. Date last updated: July 5, 2012.
7 Lee R, Margaritis M, Channon KM, *et al.* Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Curr Med Chem* 2012; 19: 2504–2520.